Cancer (Molecular) | 12 - 2PM Monday, May 7th | OHSU Auditorium

Nathan Donley | Chromosome-wide control of DNA replication and genome integrity

Worapol Ngamcherdtrakul | SiRNA-based nanoparticle platform for the treatment of HER2 positive breast cancer

Alison Macleod | Combination Therapy to target KIT and PDGFRA mutant cells

Alain Silk | Tumor Heterogeneity from Spontaneous Heterotypic Fusion

James Korkola | Microenvironment Microarrays for the Study of Cancer Growth and Metastasis

Sunjong Kwon | Quantitative Imaging of Individual mRNAs and Co-Imaging of Protein to Follow AKT Signaling Activation in Breast Cancer Cells

Lina Gao | The Androgen Receptor Promotes Androgen-Independent Prostate Cancer Cell Survival through Up-regulation of c-Myc

Robynn Schillace | Do breast cancer stem cells express the estrogen receptor providing a target for tamoxifen?

Dmitri Rozanov | Exclusively non-toxic TRAIL-based approach to target various cancer cell types

Frances Lee-Lin | What do Chinese American immigrant women think about breast cancer? Findings from Focus Groups

Cancer (Treatment) | 3 - 4:15 PM Monday, May 7th | OHSU Auditorium

Chad Burk | ALL Progression Induces PD1 on T Cells and Blockade of PD1 Enhances Adoptive Immunotherapy

Joshua Walker | Effects of Total Body Irradiation on T-Cell and B-Cell Subsets as Well as Macrophage in Rhesus Macaque

Oleg Sostin | Impact of Real-time Tumor Tracking and Fraction Size on Treatment-related Morbidity in Prostate Cancer Patients Treated with Intensity-modulated Radiotherapy

Leonel Kahn | I-125 Plaque Brachytherapy for Choroidal Melanoma: mature single-institution outcomes
Renato Luna | Neoadjuvant therapy influences lymph node ratios and overall survival without decreasing total node harvest.

Jared Fischer | Phenotypic plasticity allows Apc-deficient intestinal stem cells to avoid chemotherapy

Dermatology | 4:15 - 5 PM Monday, May 7th | OHSU Auditorium

Stephen Hyter | ET-1 is a transcriptional target of p53 in epidermal keratinocytes and in a non-cell autonomous manner controls UV radiation-induced melanocyte homeostasis in vivo

Zhixing Wang | Selective ablation of keratinocytic Ctip2/Bcl11b triggers AD-like skin inflammatory responses in adult mice

Renato Goreshi | Double-blind, randomized, bilateral comparison of skin irritancy following application of the combination acne products clindamycin/tretinoin and benzoyl peroxide/adapalene

Amala Soumyanath | Piperine and melanoma: a crucial issue for future clinical trials in vitiligo

Stroke | 12 - 12:50 PM Monday, May 7th | Old Library 217

Parisa Javedani | Dabigatran Etxilate: Management in Acute Ischemic Stroke

Keri Vartanian | LPS preconditioning redirects TLR signaling following stroke: TRIF-IRF3 plays a seminal role in mediating tolerance to ischemic injury

Raffaella Gesuete | Poly-ICLC Preconditioning Protects Blood-Brain Barrier Functions Against Ischemic Injury

Frances Bahjat | Changes in spontaneous activity assessed by accelerometry predict treatment efficacy and correlate with extent of cerebral ischemia-reperfusion injury in the nonhuman primate

Cardiovascular System | 12:50 - 2:20 PM Monday, May 7th | Old Library 217

Quin Denfeld | Projected Survival and Self-Care Behaviors Among Adults with Heart Failure

Andrew Thomas | Prognosis and Secondary Shockable Rhythms in Out-of-Hospital Cardiac Arrest

Devon Scott-Drechsel | Hyperglycemia slows embryonic growth through suppression of cell cycle

Shobana Ganesan | Clopidogrel resistance: Role for unbound concentrations of active metabolite
Cheryl Maslen | An Excess of Deleterious Variants in VEGF-A Pathway Genes in Down Syndrome-Associated Atrioventricular Septal Defects

David Farrell | Genome-Wide Association Study of Gamma' Fibrinogen Levels in the Framingham Offspring Cohort

Cheryl Maslen | The National Registry of Genetically Triggered Thoracic Aortic Aneurysms (GenTAC): Registry Progress and Research Successes

Health Promotion, Improving Outcomes, Health Disparity | 3 - 4PM Monday, May 7th | Old Library 217

Aisling Fernandez | NAMCS/NHAMCS: National Ambulatory Health Care Data, 2009

Sadee Saithong | Caregiver preparedness over a 10-year period: Parkinson's disease Spouses

Mellisa Pensa | Using Vital Statistics to Estimate the Frequency of Elective Early-Term Deliveries: Implications for Hospital Improvement

Wael Sabbah | Oral Health as a Risk Factor for Mortality in Middle-Aged Men: the Role of Socioeconomic Position and Health Behaviours

Benjamin Sun | Randomized Evaluation of an Emergency Department Observation Syncope Protocol (EDOSP)

Karen Lyons | Predicting Covarying Depressive Symptoms in Lung Cancer Dyads over Time

Andrea Cedfeldt | A Comparison Between Physicians and Demographically Similar Peers in Accessing Personal Healthcare

Cell and Molecular Biology | 12 - 2 PM Tuesday, May 8th | OHSU Auditorium

Branden Tarlow | Isolation and characterization of primary adult human liver progenitor cells

Ekaterina Placzek | 3-Iodothyronamine Undergoes Clathrin-mediated Endocytosis in Cells.

Brian Conti | Dynamic Structural Rearrangement of Translocon Proteins Sec61, TRAP, and OST Coincides with Nascent Chain Entry into the ER Lumen

Shefali Chauhan | Unraveling Long Distance Electron Transfer Mechanism: A Substrate Mediated Approach
Kelly Chacon | The green pathway toward purple assembly of Thermus thermophilus CuA

Allison Stickles | Old drugs, new chemistry: unbridged, aryl-4-quinolone esters as novel antimalarials

David Gibbs | Peptides, Proteins and Networks: Removing obstacles and getting on the road to systems integration.

Jack Ferracane | Phosphoric acid released dentin matrix components affect pulp cell behavior

Dennis Koop | Novel Use of One Dried Blood Spot for Tacrolimus and Creatinine Determination in Kidney Transplant Recipients

Larry David | Examination of βB2/βB3 crystallin heterodimer conformation using hydrogen/deuterium exchange

Alcohol Abuse | 2 - 3 PM Tuesday, May 8th | OHSU Auditorium

John Crabbe | Mice that Drink Too Much, Too Fast

Amanda Barkley-Levenson | Ethanol reward and aversion in a high drinking selected mouse line

Ovidiu Iancu | High Drinking in the Dark (HDID) Selected Lines and Brain Gene Networks

Josh Kaplan | Cellular mechanisms underlying the alcohol-induced increase in GABAA inhibition of cerebellar granule cells in low alcohol preferring DBA/2J mice and suppression of GABAA inhibition in high alcohol preferring C57BL/6J mice

Emily Young | D1 But Not D2 Receptors in the Nucleus Accumbens are Necessary for Acquisition of Ethanol Conditioned Place Preference in DBA/2J Mice

Noah Gubner | Varenicline Does Not Attenuate the Expression of Ethanol-Induced CPP in DBA/2J Mice

Marcia Ramaker | Effect of Ganaxolone and THIP in Limited-Access Ethanol Intake in Mice

Psychology and Behavioral Neuroscience | 12 - 1 PM Tuesday, May 8th | Old Library 217

Amy Wagner | Behavioral Activation as an Alternative Treatment for PTSD among Returning Veterans

Antony Abraham | SKF 81297, a dopamine D1 receptor agonist, enhances extinction of contextual fear
Elinor Sullivan | Maternal high-fat diet consumption suppresses serotonergic system signaling in juvenile nonhuman primate offspring resulting in increased anxiety, decrease social interactions and perturbations in energy balance regulation

Taciana Costa Dias | Functional connectivity patterns of the reward system inform distinct communities in youth with and without ADHD

**Drugs of Abuse | 1 - 2 PM Tuesday, May 8th | Old Library 217**

Emily Eastwood | Opioid Sensitivity in Mice Selectively Bred to Consume or Not Consume Methamphetamine

John Harkness | Effects of sodium butyrate on methamphetamine sensitized locomotor activity

Gabriel Searcy | Impulsivity related to subjective nicotine withdrawal

Laura Carim Todd | Does yoga improve smoking cessation outcomes? A systematic review of the literature

**Developmental Biology | 3 - 4:15 PM Tuesday, May 8th | Old Library 217**

Matthew McCarroll | Graded levels of Pax2a and Pax8 regulate cell fate during sensory placode formation

Molly Harding | FGF-Ras-MAPK signaling drives apical constriction via apical positioning of Rho-kinase during mechanosensory organ formation.

Kateryna Kyrylkova | Integration of BCL11B into the FGF Signaling Network in the Mouse Incisor

Jenna Ramaker | Interactions between Amyloid Precursor Proteins and the heterotrimeric G protein Goα may regulate neuronal migration.

Katie Kindt | Kinocilia mediate mechanosensitivity in developing zebrafish hair cells

Biliana Veleva | A Role for Pseudokinases During Development of the Cerebral Cortex

**Trauma and Critical Care | 1 - 2:15 PM Wednesday, May 9th | OHSU Auditorium**

Judith Baggs | Who is Attending? End-of-Life Decision Making in the ICU

Tim Lee | Hypertonic reconstituted lyophilized plasma is an effective low volume hemostatic resuscitation fluid for trauma
Loic Fabricant | Cryopreserved Deglycerolized Blood is Safe and Achieves Superior Tissue Oxygenation Compared to Standard Liquid Red Blood Cells

Mark Piedra | Timing of Cranioplasty after Decompressive Craniectomy for Trauma

Rose Merten | Treatment of severe extremity injury and compartment syndrome using autologous bone marrow mononuclear cells in a large animal model

Kathleen Carlson | Re-Injury among Veterans with Traumatic Brain Injury Discharged from VA Polytrauma Rehabilitation Centers

**Surgery** | 3 - 5 PM Wednesday, May 9th | OHSU Auditorium

Farbod Khaki | Soft tissue shadow on lateral cervical spine radiograph does not predict development or severity of chronic dysphagia

Farbod Khaki | Correction of lumbar hypolordosis with Smith-Petersen osteotomy and transforaminal interbody fusion

Gabriel Andeen | Comparative Effectiveness of Robotic vs Conventional Total Laparoscopic Hysterectomy

Jia Ooi | Non-Visual CT Data is useful in the Differentiation of Jaw Lesions

Jeff Crawford | Routine Completion Axillary Lymph Node Dissection for Positive Sentinel Node in Mastectomy Patients is not Associated with improved local control

Jeffrey Barton | INR Fails to Accurately Portray Coagulation Status Following Liver Resection

Jana Childes | Use of Technology for Communication after Laryngectomy: Three Patterns of Utilization

Marcus Kret | Compliance with Long-term Surveillance Recommendations following Endovascular Aneurysm Repair or Type B Aortic Dissection

Phong Dargon | Surgical intervention for radial artery catheter-associated ischemic complications

Andras Gruber | Contribution of Factor XI Activation to the Pathomechanism of Polymicrobial Abdominal Sepsis

**Children and Adolescents** | 1 - 2 PM Wednesday, May 9th | Old Library 217

Anna Cedar | Validity of a web 2.0 instrument designed to assess needs of medical students and residents in diagnosing and managing pediatric respiratory emergencies

Megan Herting | Differences in brain activity during a verbal associative memory-encoding task in high and low-active adolescents
Cindy Mcevoy | Increased Pregravid Body Mass Index is Associated with Subsequent Bronchodilator Prescriptions in Early Childhood.

Nichole Hildebrandt | Nurturing Healthy & Empowered Youth thru filmmaking

**Aging | 3 - 4 PM Wednesday, May 9th | Old Library 217**

Deniz Erten-Lyons | Neuropathologic basis of white matter hyperintensity accumulation

Andrew Palmer | How Do Communication Difficulties Impact the Social Lives of Older Adults?

Mattie Gregor | Social Engagement As a Means to Improve Cognitive Reserve

Jeffrey Kaye | Technology & Aging: New Approaches to Understanding Change

Donna Graville | The Impact of Age on Outcomes after Supracricoid Laryngectomy

**Public Health & Healthcare Perceptions | 4 - 5 PM Wednesday, May 9th | Old Library 217**

Daniel Bristow | Television and the social idealization of medicine

Cathy Gordon | PCORI Expert Interviews Project

Arpita Tiwari | Effectiveness of Public reporting of health care quality information as a quality improvement strategy

Valerie King | Oregon State Guidelines: Development of a Statewide, Multi-stakeholder Guidelines Program

Cinda Hugos | Fatigue: Take Control: A VA Multi-Center Randomized Controlled Trial

**Informatics | 12 - 1:15 PM Thursday, May 10th | OHSU Auditorium**

Kyle Ambert | Virk: An Active Learning System for Optimally Identifying Biomedical Community Database Contributions

James McCormack | Clinician Perspectives on the Quality of Patient Data Used for Clinical Decision Support: A QUAL Study
Paul Gorman | Is Perceived Quality in Primary Care Associated with Practice Size and/or Use of Health Information Technology?

William Hersh | Identifying Patients for Clinical Studies from Electronic Health Records: The TREC Medical Records Track

Melissa Haendel | CTSAconnect: A Linked Open Data approach to represent clinical and research expertise, activities, and resources

Shahim Essaid | Semantic Linking of Biospecimen Resources

**Hearing | 1:15 - 2 PM Thursday, May 10th | OHSU Auditorium**

Lourdes Quintanilla-Dieck | Expression of Inflammatory Cytokines and Ion Homeostasis Genes in the Cochlea Is Up-regulated by Lipopolysaccharide-induced Sepsis

Sripriya Ramamoorthy | Potentiometric measurement of the transmembrane potential distribution along isolated outer hair cells in an external electrical field

Lina Reiss | Plasticity in pitch perception with cochlear implant experience

**Neuroscience | 2 - 4 PM Thursday, May 10th | OHSU Auditorium**

Karen Tonsfeldt | Estrogen regulation of the M-current in GnRH neurons

Daniel Cleary | Opposing respiratory effects of two analgesic drugs in the brainstem revealed using a novel method of plethysmography

Mandy Cook | The neurodegenerative Drosophila melanogaster AMPK mutant loe interferes with the RHO pathway and actin dynamics

David Morton | Using fruit flies to model the role of TDP-43 in Amyotrophic Lateral Sclerosis

Michael Pellegrino | STAT3 activation by NGF and gp130 cytokines stimulates sympathetic axon regeneration

Eric Schnell | Neuroligin involvement in synapse formation during adult neurogenesis

Maria Borisovska | Measuring vesicular release of dopamine from single periglomerular neurons

Julie Saugstad | Regulated Expression of MicroRNAs by Activation of the Group I Metabotropic Glutamate Receptors in Mouse Brain
Jane Nie | Dysfunctional Neuronal M2 Muscarinic Receptor Causes Airway Hyperresponsiveness in Diet-induced Obese Rats

Domenico Tupone | Adenosine A1 receptor activation in rat brain induces a hypothermic and hypometabolic state

**Students 1 | 11 - 12 PM Thursday, May 10th | Old Library 217**

Shreya Bhattacharya | Role of transcriptional regulator COUP-TF-interacting protein 2 (Ctip2) in hair follicle morphogenesis and postnatal hair cycling

Danielle Williamson | Intramolecular chaperones are sufficient to regulate organelle-specific pH-dependent activation of Furin and Proprotein Convertase 1/3

Jessica Martin | A Role for Adenine Nucleotides in the Sensing Mechanism to Purine Starvation in Leishmania donovani

Daniel Coleman | Role of Melanocytic RXRα/b in solar UV induced tissue homeostasis

Daniel Austin | A State-Space Model for Finger Tapping with Applications to Cognitive Inference

**Immunology and Virology | 12 - 1:15 PM Thursday, May 10th | Old Library 217**

Kristen Haberthur | Using SVV infection of aged RM to understand VZV reactivation

Kei Adachi | Rational design of novel adeno-associated virus vectors by a next generation sequencing-based approach (Barcode-Seq) to study virus biology

Susan Murray | NF-kB-inducing kinase interferes with regulatory T cell function and phenotypic stability

Yoshinobu Koguchi | NF-kB inducing kinase negatively regulates invariant natural killer T cell development

Gil Benedek | Binding of partial MHC class II constructs to monocytes reduces CD74 expression and induces both specific and bystander T-cell tolerance

Georgiana Purdy | The big ball of wax: novel insights into the mycobacterial cell wall

**Rare and Genetic Diseases | 1:15 - 2 PM Thursday, May 10th | Old Library 217**
Xuehong Liu | Thermal Instability of ΔF508 CFTR Channel Function: Protection by Single Suppressor Mutations and Inhibiting Channel Activity

Yohei Norimatsu | Molecular Modeling of the CFTR Chloride Channel

David Koeller | Evidence for an association between infant mortality and homozygosity for a carnitine palmitoyltransferase 1a genetic variant

Women’s Health | 2 - 3 PM Thursday, May 10th | Old Library 217

Karen Eden | Evidence-based decision aids in women’s health improve the decision making process

Connie Nguyen-Truong | Factors Associated with PAP Testing Practices Among Vietnamese American Immigrant Women: A Community Based Participatory Research

Rebecca Block | The Fertility Preservation Decision-Making Process of Adolescent and Young Adult Women with Cancer

Jeffrey Jensen | Polidocanol Foam for Female Permanent Contraception

Students 2 | 3 - 4 PM Thursday, May 10th | Old Library 217

William Giardino | Selective involvement of the neuropeptide urocortin-1 in long-term alcohol consumption in mice

Kate Wagner | Mild stress increases sensitivity to pain through the dorsal medial hypothalamus and rostral ventromedial medulla

Irina Fonareva | Salivary alpha amylase (sAA) as a stress biomarker in middle-aged adults.

Johanna Feuerstein | SVM to detect the presence of visitors in a smart home environment

Awards Talks | 4 - 5 PM Thursday, May 10th | Old Library 217

Student Paper of the Year: Daniel T. Lioy

A role for glia in the progression of Rett’s syndrome

Lioy DT, Garg SK, Monaghan CE, Raber J, Foust KD, Kaspar BK, Hirrlinger PG, Kirchhoff F, Bissonnette JM, Ballas N, Mandel G.
Vollum Institute, Oregon Health & Science University, Portland, OR

Rett's syndrome (RTT) is an X-chromosome-linked autism spectrum disorder caused by loss of function of the transcription factor methyl-CpG-binding protein 2 (MeCP2). Although MeCP2 is expressed in most tissues, loss of MeCP2 expression results primarily in neurological symptoms. Earlier studies suggested the idea that RTT is due exclusively to loss of MeCP2 function in neurons. Although defective neurons clearly underlie the aberrant behaviours, we and others showed recently that the loss of MECP2 from glia negatively influences neurons in a non-cell-autonomous fashion. Here we show that in globally MeCP2-deficient mice, re-expression of Mecp2 preferentially in astrocytes significantly improved locomotion and anxiety levels, restored respiratory abnormalities to a normal pattern, and greatly prolonged lifespan compared to globally null mice. Furthermore, restoration of MeCP2 in the mutant astrocytes exerted a non-cell-autonomous positive effect on mutant neurons in vivo, restoring normal dendritic morphology and increasing levels of the excitatory glutamate transporter VGLUT1. Our study shows that glia, like neurons, are integral components of the neuropathology of RTT, and supports the targeting of glia as a strategy for improving the associated symptoms.

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Postdoctoral Paper of the Year: Joe Aslan, PhD

S6K1 and mTOR regulate Rac1-driven platelet activation and aggregation

Aslan JE, Tormoen GW, Loren CP, Pang J, McCarty OJ.

Platelet activation and thrombus formation are under the control of signaling systems that integrate cellular homeostasis with cytoskeletal dynamics. Here, we identify a role for the ribosome protein S6 kinase (S6K1) and its upstream regulator mTOR in the control of platelet activation and aggregate formation under shear flow. Platelet engagement of fibrinogen initiated a signaling cascade that triggered the activation of S6K1 and Rac1. Fibrinogen-induced S6K1 activation was abolished by inhibitors of Src kinases, but not Rac1 inhibitors, demonstrating that S6K1 acts upstream of Rac1. S6K1 and Rac1 interacted in a protein complex with the Rac1 GEF TIAM1 and colocalized with actin at the platelet lamellipodial edge, suggesting that S6K1 and Rac1 work together to drive platelet spreading. Pharmacologic inhibitors of mTOR and S6K1 blocked Rac1 activation and prevented platelet spreading on fibrinogen, but had no effect on Src or FAK kinase activation. mTOR inhibitors dramatically reduced collagen-induced platelet aggregation and promoted the destabilization of platelet aggregates formed under shear flow conditions. Together, these results reveal novel roles for S6K1 and mTOR in the regulation of Rac1 activity and provide insights into the relationship between the pharmacology of the mTOR system and the molecular mechanisms of platelet activation.

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Resident Paper of the Year: Katie Sharff, MD

Safe and effective treatment of high-risk and geographically isolated patients receiving outpatient parenteral antibiotic therapy (OPAT)

Katie A. Sharff1, Kimberly Felder1, Matthew DeHart2, and Penelope Barnes1

1Department of Infectious Disease, Oregon Health & Science University, Portland, OR
2Department of Orthopedic Surgery, Oregon Health & Science University, Portland, OR
**Background:** Patients discharged on outpatient parenteral antibiotics therapy (OPAT) are at risk from line infection, antibiotic toxicities and infection progression. Published OPAT guidelines exclude high-risk patients from discharge with OPAT. For those receiving OPAT, close follow-up with the OPAT center is recommended. In 2007, our hospital established an OPAT program that cared for patients discharged on IV antibiotics irrespective of insurance, geographical distance from the supervising hospital, co-morbid condition or psychiatric disease. Few patients were refused OPAT by the program, and instead we developed a process map to manage high-risk patients in the community. The aim of this study was to examine whether safe and effective OPAT care was provided to patients who were at high risk of OPAT complications and lived long distances from the supervising hospital.

**Methods:** A process map was created for the institutional OPAT program using retrospective chart review. The success of the OPAT process was then evaluated by analysis of clinical complications and outcomes. Clinical outcome was correlated with discharge to home or facility and distance from supervising hospital.

**Results:** 247 patients with bone, spine and brain infection were managed for 9256 OPAT days in 2009. Patients received OPAT at home (56%) or associated with medical facilities (44%). 35% of patients received OPAT more than a one hour drive from the OPAT supervising hospital, and 10% more than four hours. The patients had risks for OPAT complications including 53% with psychiatric disease, 45% lacked adequate funding for home OPAT care and 10% were homeless on admission to hospital. Outcomes analysis showed 99% of patients had their infection controlled at the end of OPAT. 89% of patients (95% CI: 85-93%) completed the OPAT course free from OPAT related readmission and 90% of patients (95% CI: 85-93%) completed their OPAT course free from relapse of infection. 93% of patients maintained the same PICC line during their OPAT course. The all-cause readmission rate was 24% (95% CI: 19-30%) and 33% of patients had an unexpected antibiotic change. Distance from the hospital and discharge to facility or home made little difference to the OPAT complication rate.

**Conclusions:** This study demonstrated that patients with multiple risks for OPAT complications or who live a long distance from the supervising hospital could receive safe and effective OPAT care. A complex process map was designed to manage these patients and highlights the importance of transitions of care and ongoing clinical follow-up.
Neuropathologic basis of white matter hyperintensity accumulation

Deniz Erten-Lyons; Randy Woltjer; Hiroko H Dodge; Sara Stanfield; Lindsay Reese; Huong Tran; Joseph Quinn; Katherine Wild; Barry Oken; Jeffrey Kaye; Lisa Silbert

Department of Neurology, Oregon Health & Science University, Portland, OR

Objective: The neuropathologic basis of white matter hyperintensity (WMH) volume accumulation observed on brain magnetic resonance imaging (MRI) is not known. Few studies have examined the neuropathologic correlates of cross-sectional WMH volumes and longitudinal WMH trajectories.

Methods: Sixty-six elderly participants in the Oregon Brain Aging Study were included for having an autopsy, >1 MRI scan and the last MRI scan within 24 months of death. First, multiple regression analyses, adjusted for age at MRI and duration between MRI and death, were used to examine the association between cross-sectional WMH volume obtained on the MRI most proximal to death, and several neuropathologic measures including: myelin pallor, arteriosclerosis, microvascular disease, small vessel infarcts, large vessel infarcts, atherosclerosis, neurofibrillary tangle (NFT) and neuritic plaque (NP) scores. Second, a mixed-effects model was used to examine the association between longitudinal trajectory of WMH accumulation over time and neuropathologic measures. Time interaction terms for scores of myelin pallor, arteriosclerosis, microvascular disease, small vessel infarcts, large vessel infarcts, and atherosclerosis, NFT and NP scores, and APOE ε4 presence were included. Analysis was adjusted for duration of follow up and age at death.

Results: Mean age at death was 94.54 (SD 5.45) years. Higher burdens of arteriosclerosis, microvascular disease and small vessel infarcts were significantly associated with larger cross-sectional WMH volume. No significant associations were observed with other pathologic measures. In the mixed-effects model, higher burdens of arteriosclerosis and NFT pathology showed more WMH accumulation over time.

Conclusion: These findings suggest that WMH burden observed on MRI scans in very old individuals reflects the degree of small vessel ischemic disease burden, which is best captured by measures of arteriosclerosis on autopsy. Additionally, tau pathology may play a role in the processes leading to accumulation of WMH.
How Do Communication Difficulties Impact the Social Lives of Older Adults?

Andrew D. Palmer*, MS; Jason T. Newsom, PhD; and Karen S. Rook, PhD.

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It is known that communication impairments affect the nature of social relationships in specific populations and it is also known that the size and nature of adults’ social networks change with age. To date, however, there is limited information about the relative impact of communication difficulties in the older population. To examine this relationship, data were analyzed from a representative national sample of community-dwelling adults aged 65 and older living in the continental United States (n=742). All adults had been screened for cognitive impairment prior to inclusion. Results from multiple regressions indicated that communication difficulty was significantly associated with several parameters of social relationships even after controlling for age, gender, partnership status, health, and functional limitations. Communication difficulty was a significant predictor of loneliness (p < .001), fewer positive social exchanges (p < .01), smaller network size (p < .05), and fewer social activities (p < .01) but was not a significant predictor of negative social exchanges. These findings suggest that communication impairments in older adults may have a more significant impact on positive than on negative aspects of social relationships.

Social Engagement as a Means to Improve Cognitive Reserve


The Oregon Center for Aging and Technology, Oregon Health & Science University, Portland, OR

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Faced with an aging population and a growing number of people suffering from dementia, it is critical to find an effective means to slow the process of cognitive decline. Past epidemiological studies have demonstrated that larger social networks are linked to a decreased incidence of Alzheimer’s disease, indicating that social interaction could potentially have a protective effect against the disease. Socialization is a highly cognitively stimulating task; conversations require the executive functions of attention and working memory, as well as social cognition to understand others’ intentions and feelings. Despite accumulating evidence that increasing social interaction could be a promising tool to improve the cognitive well-being of the elderly, few controlled prevention studies using social interaction have been conducted to date. The purpose of the present study is to examine whether conversation-based cognitive stimulation has a positive effect on cognitive functions among older adults with normal cognition or mild cognitive impairment. For a total of six weeks, we are conducting daily face-to-face conversations with participants over the Internet, using personal computers and webcams to allow participants access to social interaction without leaving their homes. Social engagement information is also collected in the form of self-report surveys, and from audio recorders worn by the participants over the course of several days. All participants completed a range of cognitive tests at the beginning of the study, with the hypothesis that participants who receive social interaction will perform better on additional testing after the trial, compared with those who did not receive the intervention. It is hoped that, in addition to informing future research on social engagement and cognition, the results of this study may guide the development of additional interventions to slow the process of cognitive decline in older adults.

Funded by NIH Grants R01AG033581, P30-AG008017, P30-AG024978, R01-AG024059.
Intelligent systems for detection of aging changes


Oregon Center for Aging & Technology, Oregon Health & Science University, Portland, OR

The Oregon Center for Aging & Technology (ORCATECH) is a collaborative research center at the Oregon Health & Science University developing technologies for continuous assessment in the home that will maintain independence and optimize health for our aging population.

Our rapidly aging population will result in an increasing number of people at risk for loss of independence through dementia, frailty and other syndromes of aging. Evolving sensor and other technologies can provide a means of early detection and intervention, minimizing morbidity and cost. Integrated, continuous and unobtrusive home monitoring of activity and function may be able to provide important information for maintaining cognitive and physical health.

ORCATECH is developing a new clinical paradigm for continuous assessment focused on early detection of health problems (and therefore early intervention) in order to help people live in their place of choice as long as possible. Using unobtrusive and scalable technologies, ORCATECH has created a novel in-home assessment system that collects data all the time, yielding valuable information to identify changes that may indicate the start of impairment before it might otherwise be known. Our ultimate aim is to provide key information to diagnose early, treat effectively and promote health.

ORCATECH’s research efforts are centered on the key value of maintaining independence in a person’s home through creating a new model for translational research – the Living Laboratory. The Living Laboratory is a community network of homes where volunteer participants allow scientists to assess aging-in-place in a new way. This not only provides a unique way to study aging, but is also a community-based opportunity for collaborative research between academic and industry partners.

ORCATECH is truly collaborative, engaging partners from academia, industry and community organizations. Through interactive and cross-disciplinary research, ORCATECH seeks to identify and develop technologies to help meet the challenges of aging, especially around two key reasons for loss of independence: impairment in mobility and decline of cognitive function. Technologies can be dazzling, but do they really work? ORCATECH is committed to evidence-based research, where scientific methodology is used to objectively evaluate the relevance and effectiveness of new technologies. ORCATECH has deep experience in community-based aging research, having collected more than 300,000 continuous days of data in more than 450 homes since 2005.

The Impact of Age on Outcomes after Supracricoid Laryngectomy

Donna Graville, PhD, CCC-SLP*; Andrew Palmer, MS, CCC-SLP; Daniel Clayburgh, MD, PhD; Joshua Schindler, MD

NW Clinic for Voice & Swallowing, Dept of Otolaryngology, Oregon Health & Science University, Portland, OR

*Corresponding author email: graville@ohsu.edu
In a retrospective review of 18 patients treated via supracricoid laryngectomy, age was associated with more negative outcomes, including increased duration of tube feedings and poorer subjective voice and swallowing function. We discuss the relative impact of age itself versus age as a proxy indicator of more advanced disease.

**Summary of Proposal:**

Historically, total laryngectomy has been the primary means of surgically managing advanced tumors of the larynx. However, this procedure entails a significant amount of morbidity, including creation of a permanent tracheostoma, and loss of natural voice. Thus, there is considerable interest in preservation of laryngeal function, either via non-surgical modalities such as radiation therapy, chemoradiation therapy, or via alternative surgical techniques. In 1959 supracricoid laryngectomy (SCL) was described as an alternative to total laryngectomy (Lacourreye et al., 1990). First adopted into practice in Europe, this has been demonstrated to be an oncologically sound alternative to total laryngectomy in select patients, and may be used in patients as a salvage procedure after radiation therapy (Lacourreye et al., 1996).

SCL is used for select patients with T1 through T4 supraglottic and glottic carcinoma. This procedure avoids the creation of a tracheostoma via preservation of the hyoid bone, cricoid cartilage, and at least one functional arytenoid unit in order to preserve the airway and maintain laryngeal function. The epiglottis may or may not be resected. Reconstruction is then performed using either cricothyroidopiglottopexy (CHEP) or cricothyroidopexy (CHP). This neoglottis functions via close approximation of the remaining arytenoid mucosa against the epiglottis or base of tongue; this allows for voice generation via vibration of mucosa between these surfaces.

Although the neoglottic mechanism for swallowing and generating voice after SCL more closely resembles the physiology of the normal larynx than that in patients after total laryngectomy, function is not normal (So, Yun, Baek, et al., 2009). Post-operatively patients have chronic problems with swallowing and aspiration (Lewin, Hutcheson, Barringer, et al., 2008) and a perceptually abnormal voice with a breathy, strained, and rough quality (Schindler, Favero, & Nudo, 2005). Many patients require significant voice and swallowing therapy in a specialized center in order to achieve functional outcomes. Functional data following SCPL in the United States is much more limited, consisting of smaller series and shorter follow up times, although excellent voice and swallowing results have been reported (Webster, Samlan, Jones, et al., 2010; Zacharek, Pasha, Meleca, et al., 2001). Despite this, little is known about factors associated with better or poorer voice and swallowing outcomes. We undertook a retrospective review of patients treated with SCL at our institution, to evaluate which prognostic indicators related to voice and swallowing outcomes.

All study variables were summarized descriptively. Functional outcome variables included the duration of hospital stay (days), time to decannulation of tracheostomy tube (days) and duration of tube feedings whether as the primary or a supplementary source of nutrition (days). Subjective voice ratings were measured with the Voice Handicap Index (VHI) and subjective dysphagia ratings were measured with the MD Anderson Dysphagia Inventory (MDADI). In addition, comorbidity was measured using the Charlson Comorbidity Index (CCI).

Of the 18 patients, 13 underwent cricothyroidopiglottopexy (CHEP) and 5 cricothyroidopexy (CHP). Mean follow-up was 607 days. On average, decannulation occurred at 27.4 ± 15.3 days and feeding tube removal at 87.9 ± 43.2 days postoperatively. Sixty-seven percent of patients tolerated an unrestricted diet at follow-up. Subjective outcomes varied quite widely on both the MDADI and the VHI as can be seen from the considerable range on measures. Scores on the MDADI ranged from 44 to 100, although the mean score was relatively high (M = 78.4, SD = 17.8). On the VHI, scores ranged from 14 to 81, with a mean score of 43.2 (SD = 18.8). This would indicate a “moderate voice impairment” according to normative data for this measure (Jacobson, Johnson, Grywalski, et al., 1997).
For the purposes of interpretation, we chose to regard any correlation of .40 or more as being potentially clinically significant. As can be seen, there were six associations that met this criterion. Age > 60 was positively associated with length of stay (r = .40), duration of feeding-tube placement (r = .74), and worse scores on both the MDADI (r = -.72) and the VHI (r = .47). In addition, those whose surgery was a CHP rather than a CHEP tended to have longer durations of tube-feeding (r = .49), and worse scores on the VHI (r = .43). Further examination of these data, however, revealed that age may be a proxy indicator for more extensive disease as well as the cumulative impact of head and neck cancer treatments over time, as older individuals were more likely to have a higher T-stage, prior radiation therapy and were more likely to receive a CHP procedure. Thus, a combined variable was created by simply summing the binary values for a previous history of radiation treatment, higher T-stage, and greater likelihood of CHP and this was also correlated with increased tube feeding duration (r = .60) and worse MDADI score (r = -.50).

To date, there has been some debate about which demographic characteristics, disease variables, and comorbidities are associated with better or worse patient outcomes and how these affect patient selection and post-operative management. Increasingly there is a recognition that certain individuals may be good surgical candidates but are more “complex” and therefore require more careful management post-operatively and are likely to need more prolonged rehabilitation. Our study adds to this literature and contributes to the discussion about pre-operative selection and post-operative management. For example, based on their findings, Benito et al. (2011) now advocate for PEG-tube placement in those who are at higher-risk of aspiration and pulmonary complications, including those who will have 1 arytenoid resected, a CHP procedure performed, are over 70 years of age, or who have had a prior history of radiation, recognizing both the longer and more difficult post-operative course of these groups.

SCL patients require extensive rehabilitation after surgery in order to achieve functional outcomes. Those who have undergone multiple cancer interventions and have more extensive surgery may have a prolonged recovery course and be at-risk for poorer outcomes. These factors may have implications for patient selection criteria and the course of post-operative management.

References


John Crabbe | Mice that Drink Too Much, Too Fast

Amanda Barkley-Levenson | Ethanol reward and aversion in a high drinking selected mouse line

Ovidiu Iancu | High Drinking in the Dark (HDID) Selected Lines and Brain Gene Networks

Josh Kaplan | Cellular mechanisms underlying the alcohol-induced increase in GABAA inhibition of cerebellar granule cells in low alcohol preferring DBA/2J mice and suppression of GABAA inhibition in high alcohol preferring C57BL/6J mice

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Marcia Ramaker | Effect of Ganaxolone and THIP in Limited-Access Ethanol Intake in Mice

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Mice that Drink Too Much, Too Fast

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Genetics influences individual risk for developing alcohol dependence, and many specialized mouse and rat lines have been discovered and/or developed that are genetically predisposed to prefer alcohol solutions to water. These genetic animal models are widely used to explore the neurobiological underpinnings of alcohol sensitivity, tolerance and dependence. One limitation of these models, however, has been that they generally do not show uncontrolled drinking, and seem to regulate their intake to maintain blood non-intoxicating blood alcohol levels. This self-regulation is lost in human alcoholic drinking.

To overcome this limitation, we have selectively bred mice that drink large amounts of alcohol during short periods of access and become intoxicated. This talk will highlight some of the findings from our lab and others studying these mice.

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The High Drinking in the Dark (HDID) line of mice has been selectively bred for high blood ethanol concentrations following the Drinking in the Dark test and is a genetic model of binge-like drinking. HDID mice drink to intoxication during a limited access procedure and tend to drink in larger bouts than the genetic control stock. Differences in voluntary ethanol intake may be related to differing sensitivity to the motivational effects of ethanol, either rewarding or aversive. Previous studies of selected lines and inbred strains have shown a strong negative genetic relationship between home cage drinking and ethanol-conditioned taste aversion. Less consistently seen is a correlation between voluntary ethanol consumption and ethanol-conditioned place preference. This pattern has been demonstrated in the HDID mice, which show a blunted ethanol-conditioned taste aversion relative to unselected controls, while not demonstrating consistent differences in ethanol-conditioned place preference. These findings suggest that the binge-like drinking of the HDID line may be related to a weakened sensitivity to ethanol’s aversive effects, rather than an altered perception of ethanol reward.

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**High Drinking in the Dark (HDID) Selected Lines and Brain Gene Networks**

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Replicate HDID selected lines have been formed from mouse heterogeneous stock (HS) founders (HS/NPT). Details of the selection phenotype are found in Crabbe et al. (2011). HDID-1 (S_{22}), HDID-2 (S_{11}) and HS/NPT controls were used for brain gene expression analysis. Data (N=32 to 40 per line) were collected using the Illumina MouseRef 8 v2.0 arrays in both the ventral striatum (VS) and the occipital cortex (OC). The OC data were used to account for non-specific effects of selection. Data were analyzed using both measures of differential gene expression and the weighted gene covariance network analysis (WGCNA). All animals were genotyped using the Mouse Universal Genotyping Array (Geneseek). The genotyping data revealed evidence of significant genetic drift in both selected lines, with greater drift in the HDID-1 mice. The 9393 gene transcripts showing the greatest consensus variance in the selected lines and controls were used for the WGCNA. The VS data were clustered into 21 gene modules, each of which could be defined by unique gene ontology (GO) annotations. Permutation testing (N=1000) was used to determine the significance of the differences in the gene modules between the HS/NPT controls and the selected lines. One module (Black [color has no meaning]) showed the largest and most consistent (across both selections) connectivity disruption; differential Z-scores were 7 or greater. GO analysis of the Black module revealed a significant enrichment in genes associated neurological system processes ($p < 5 \times 10^{-5}$), glutamate secretion ($p < 8 \times 10^{-6}$) and neurotransmitter transport ($p < 8 \times 10^{-6}$). Eleven gene transcripts within the Black module showed similar significant connectivity changes in both selections; the transcripts were associated *Dgkz, Fam13c, Fgf13, Isl1, Npcd, Sh3r1, Tpbg, Tuba8* and 3 putative genes of unknown function. No member of the Black module showed (across both selections) significant differential gene expression (eBayes, FDR < 0.05). There were however 133 transcripts outside of the Black module that showed significant and consistent
differential expression; however, with few exceptions these changes were very small (< 10%). In the OC, and clustering the gene transcripts as in the VS, the Black module showed only modest and non-significant changes. Overall, the data illustrate that in two independent HDID selections, similar gene network changes were detected. Further, the data suggest a module and specific genes for manipulating ethanol consumption. These targets show almost no overlap with those associated with 2-bottle choice preference drinking.

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**Cellular mechanisms underlying the alcohol-induced increase in GABA_A inhibition of cerebellar granule cells in low alcohol preferring DBA/2J mice and suppression of GABA_A inhibition in high alcohol preferring C57BL/6J mice**

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In humans, low cerebellar sensitivity to alcohol is associated with high risk for alcohol abuse, but the cellular mechanisms underlying such differential sensitivity are unclear. We recorded from cerebellar granule cells (GCs) in slices from high- and low-alcohol preferring C57BL/6J (B6) and DBA/2J (D2) mice to test the hypothesis that low sensitivity to alcohol-induced enhancement of GABA_A inhibition corresponds to high alcohol preference. Contrary to previously published data in Sprague Dawley rats (SDRs) showing that alcohol consistently enhances tonic GABA_A inhibition in GCs, we detected both enhancement and suppression of tonic GABA_A inhibition by 52mM EtOH in mouse GCs. The distribution of response type varied as a function of preference phenotype, with enhancement and suppression of tonic GABA_A inhibition being significantly greater in D2 and B6 mice respectively. The average amplitude of these antipodal responses across all cells resulted in a net increase in GC tonic GABA_A inhibition in D2s but a net decrease in B6s. In parallel studies in GCs from low drinking SDRs and D2s, we determined that the enhancement of tonic GABA_A inhibition is mediated by inhibition of nitric oxide synthase (NOS), since blocking NOS prevented enhancement by EtOH. Although direct inhibition of NOS also caused an increase in GABA_A inhibition in B6 GCs, the magnitude of the enhancement was significantly less than in SDRs and D2s. Immunocytochemistry confirmed reduced expression levels of NOS in the GC layer of B6 mice relative to SDRs. Thus, the lack of enhancement of tonic GABA_A inhibition by EtOH in the majority of B6 GCs results from reduced expression of NOS. In B6 GCs that showed EtOH-induced suppression of tonic GABA_A inhibition, there was no change in sIPSC frequency, and the suppression was not blocked by TTX. Thus, suppression of tonic GABA_A inhibition in B6 GCs may be due to direct actions of EtOH on extrasynaptic GABA_A receptors that mediate tonic inhibition. Together, our study identifies antipodal actions of EtOH on GC tonic inhibition: enhancement mediated by inhibition of NOS, and direct inhibition of extrasynaptic GABA_A receptors. The differential expression of these two opposing mechanisms may be a cellular mechanism underlying differential sensitivity of the cerebellum to EtOH, an important risk factor for alcohol abuse in humans.

**D1 but Not D2 Receptors in the Nucleus Accumbens are Necessary for Acquisition of Ethanol Conditioned Place Preference in DBA/2J Mice**

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Dopamine (DA) receptors within the nucleus accumbens (Acb) have been shown to play a role in ethanol conditioned behaviors, but the specific contributions of the D1 and D2 receptor subtypes have yet to be identified. Previous microinfusion studies have suggested that activation of D1/D2/D3 receptors in Acb plays no role in expression of ethanol conditioned place preference (CPP; Gremel & Cunningham, 2009). Because the brain areas that mediate the acquisition and expression of ethanol-conditioned behavior might differ, it is important to also examine the brain systems underlying the initial learning of the stimulus-ethanol association. Thus, this experiment examined the effects of intra-Acb core D1 and D2 receptor antagonists on acquisition of ethanol-induced CPP. DBA/2J male mice were surgically implanted with bilateral cannula aimed above the Acb core and allowed to recover. Mice were then exposed to an unbiased place conditioning procedure using tactile cues, 5-min conditioning trials and 2 g/kg ethanol (IP). CPP training consisted of 4 conditioning trials (ethanol and saline trials on alternating days). Thirty min before each ethanol trial, mice received bilateral infusions of the selective D1 receptor antagonist SCH23390 (0, 0.05 and 0.5 μg/0.1 μL) or the D2 receptor antagonist raclopride (0, 0.5, 2 and 5 μg/0.1μL) into the Acb core. A sham infusion procedure was used on saline trials. After conditioning, all mice received a 30-min drug-free preference test. Mice infused with SCH23390 during acquisition showed impaired learning of CPP at both doses relative to vehicle infused controls. In contrast, intra-Acb core infusion of raclopride had no effect on acquisition of CPP at any dose examined, even though higher doses reduced ethanol’s locomotor activating effect. These results suggest that D1 but not D2 receptor activation in the Acb was critical for establishing a stimulus-ethanol association. Follow up experiments indicated that intra-Acb core infusions of SCH23390 did not alter acquisition of a lithium chloride-induced conditioned place aversion, and did not produce rewarding or aversive effects on its own. Taken together, these follow up experiments suggest that Acb D1 receptor blockade interfered with the primary rewarding effect of ethanol, rather than a more general interference with associative learning in the CPP procedure. Overall, these studies underscore the importance of Acb D1 receptor activation in ethanol-conditioned behaviors.

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Varenicline does not attenuate the expression of ethanol-induced CPP in DBA/2J mice

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There are limited pharmaceutical options for the treatment of alcohol dependence. One promising new drug is varenicline, which received FDA approval as a smoking cessation aid and has also been found to reduce ethanol consumption in humans and in rodent models. Despite these promising effects on alcohol consumption, there has been limited research on the effects of varenicline on other behaviors relevant to alcohol abuse, which would provide a better understanding of its clinical usefulness for the treatment of alcohol dependence. For example, varenicline could attenuate the conditioned rewarding effects of ethanol that are thought to influence relapse. The current study examined the ability of varenicline to attenuate the expression of an established ethanol-induced conditioned place preference (CPP). DBA/2J mice were conditioned with 2 g/kg ethanol and saline on 8 alternating days, during which each treatment type was paired with a distinct floor type. Next, mice were pretreated with saline or varenicline (0.5, 1 or 1.5mg/kg) and tested for their preference for the two floor types. The pretreatment was given 15 min prior to the 30-min preference test. All pretreatment groups exhibited preference for the ethanol-paired floor, but there was no significant effect of varenicline on floor preference. The 1 and 1.5 mg/kg doses of varenicline significantly reduced
locomotor activity levels on the preference test day, indicating that the doses were used were behaviorally effective. The current results indicate that varenicline may not be effective at reducing the preference for environmental cues that have been previously paired with ethanol and that are thought to influence relapse.

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**Ganaxolone and THIP Alter Limited-Access Ethanol Intake in Mice**


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The present study examined the role of a GABAergic neurosteroid agonist or an extrasynaptic GABA<sub>A</sub> receptor agonist on ethanol intake in a 2-bottle limited-access lickometer procedure. Male C57BL/6J mice (n=24) were allowed 2-hour access to either a 10% (v/v) ethanol solution (10E) or water beginning 2 hours into the dark cycle. In separate groups of mice, intake was measured following a systemic injection of the neurosteroid agonist ganaxolone (GAN; 0, 5, 10 mg/kg; n=12) or the extrasynaptic GABA<sub>A</sub> receptor agonist gaboxadol (THIP; 0, 2, 4, 8, 16 mg/kg; n=12). GAN dose-dependently decreased ethanol intake when compared to its within-subjects vehicle, with 10 mg/kg GAN decreasing intake by 18%. Bin analysis revealed a consistent decrease across the 2-hour span. There was no significant change in any of the bout parameters examined. THIP also dose-dependently decreased ethanol intake compared to its within-subjects vehicle, decreasing intake by 18% with 8 mg/kg THIP and by 83% with 16 mg/kg THIP. This decrease occurred primarily during the first hour of access, and bout analysis revealed that the change in intake was partly attributable to an alteration in bout frequency. Importantly, mice achieved physiologically relevant blood ethanol concentrations (GAN group: 1.15 ± 0.17 mg/ml; THIP group: 1.25 ± 0.21 mg/ml) at the end of the 2-hour session. These data extend previous studies showing an effect of neurosteroids and extrasynaptic GABA<sub>A</sub> receptor activation on ethanol intake in a variety of behavioral paradigms and contribute to literature highlighting extrasynaptic GABA<sub>A</sub> receptors as an important target in ethanol reinforcement.

Funding was provided by grants AA16981 and AA12439 and the Department of Veteran Affairs. MJR was supported by an OHSU Scholarship, and MMF was supported by KO1 AA16849.
A role for glia in the progression of Rett's syndrome

Rett's syndrome (RTT) is an X-chromosome-linked autism spectrum disorder caused by loss of function of the transcription factor methyl-CpG-binding protein 2 (MeCP2). Although MeCP2 is expressed in most tissues, loss of MeCP2 expression results primarily in neurological symptoms. Earlier studies suggested the idea that RTT is due exclusively to loss of MeCP2 function in neurons. Although defective neurons clearly underlie the aberrant behaviours, we and others showed recently that the loss of MECP2 from glia negatively influences neurons in a non-cell-autonomous fashion. Here we show that in globally MeCP2-deficient mice, re-expression of Mecp2 preferentially in astrocytes significantly improved locomotion and anxiety levels, restored respiratory abnormalities to a normal pattern, and greatly prolonged lifespan compared to globally null mice. Furthermore, restoration of MeCP2 in the mutant astrocytes exerted a non-cell-autonomous positive effect on mutant neurons in vivo, restoring normal dendritic morphology and increasing levels of the excitatory glutamate transporter VGLUT1. Our study shows that glia, like neurons, are integral components of the neuropathology of RTT, and supports the targeting of glia as a strategy for improving the associated symptoms.

S6K1 and mTOR regulate Rac1-driven platelet activation and aggregation

Platelet activation and thrombus formation are under the control of signaling systems that integrate cellular homeostasis with cytoskeletal dynamics. Here, we identify a role for the ribosome protein S6 kinase (S6K1) and its upstream regulator mTOR in the control of platelet activation and aggregate formation under shear flow. Platelet engagement of fibrinogen initiated a signaling cascade that triggered the activation of S6K1 and Rac1. Fibrinogen-induced S6K1 activation was abolished by inhibitors of Src kinases, but not Rac1 inhibitors, demonstrating that S6K1 acts upstream of Rac1. S6K1 and Rac1 interacted in a protein complex with the Rac1 GEF TIAM1 and colocalized with actin at the platelet lamellipodial edge, suggesting that S6K1 and Rac1 work together to drive platelet spreading. Pharmacologic inhibitors of mTOR and S6K1 blocked Rac1 activation and prevented platelet spreading on fibrinogen, but had no effect
on Src or FAK kinase activation. mTOR inhibitors dramatically reduced collagen-induced platelet aggregation and promoted the destabilization of platelet aggregates formed under shear flow conditions. Together, these results reveal novel roles for S6K1 and mTOR in the regulation of Rac1 activity and provide insights into the relationship between the pharmacology of the mTOR system and the molecular mechanisms of platelet activation.

Resident Paper of the Year: Katie Sharff, MD

Safe and effective treatment of high-risk and geographically isolated patients receiving outpatient parenteral antibiotic therapy (OPAT)

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Background: Patients discharged on outpatient parenteral antibiotics therapy (OPAT) are at risk from line infection, antibiotic toxicities and infection progression. Published OPAT guidelines exclude high-risk patients from discharge with OPAT. For those receiving OPAT, close follow-up with the OPAT center is recommended. In 2007, our hospital established an OPAT program that cared for patients discharged on IV antibiotics irrespective of insurance, geographical distance from the supervising hospital, co-morbid condition or psychiatric disease. Few patients were refused OPAT by the program, and instead we developed a process map to manage high-risk patients in the community. The aim of this study was to examine whether safe and effective OPAT care was provided to patients who were at high risk of OPAT complications and lived long distances from the supervising hospital.

Methods: A process map was created for the institutional OPAT program using retrospective chart review. The success of the OPAT process was then evaluated by analysis of clinical complications and outcomes. Clinical outcome was correlated with discharge to home or facility and distance from supervising hospital.

Results: 247 patients with bone, spine and brain infection were managed for 9256 OPAT days in 2009. Patients received OPAT at home (56%) or associated with medical facilities (44%). 35% of patients received OPAT more than a one hour drive from the OPAT supervising hospital, and 10% more than four hours. The patients had risks for OPAT complications including 53% with psychiatric disease, 45% lacked adequate funding for home OPAT care and 10% were homeless on admission to hospital. Outcomes analysis showed 99% of patients had their infection controlled at the end of OPAT. 89% of patients (95% CI: 85-93%) completed the OPAT course free from OPAT related readmission and 90% of patients (95% CI: 85-93%) completed their OPAT course free from relapse of infection. 93% of patients maintained the same PICC line during their OPAT course. The all-cause readmission rate was 24% (95% CI: 19-30%) and 33% of patients had an unexpected antibiotic change. Distance from the hospital and discharge to facility or home made little difference to the OPAT complication rate.

Conclusions: This study demonstrated that patients with multiple risks for OPAT complications or who live a long distance from the supervising hospital could receive safe and effective OPAT care. A complex process map was designed to manage these patients and highlights the importance of transitions of care and ongoing clinical follow-up.
Chromosome-wide control of DNA replication and genome integrity

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Chromosomal rearrangements are a common source of disrupted gene regulation and are present, sometimes to an extensive degree, in the majority of human cancers. Current models suggest that an underlying genomic instability is responsible for the rapid accumulation of these genetic rearrangements—thus enhancing the rate that tumor cells acquire malignant properties. Despite being implicated as an important force in cancer progression, the mechanisms that cause genomic instability remain poorly defined. Our lab has shown that certain chromosomal translocations produce derivative chromosomes that display a chromosome-wide delay in replication timing (DRT). A chromosome that displays DRT exhibits a 2-3 hour delay in the onset and completion of DNA replication, while the other chromosomes in the cell replicate at the appropriate time. DRT chromosomes are present in many types of human cancers, both in tumor cell lines and primary tumor samples. Importantly, these chromosomes are very unstable and undergo frequent rearrangements, resulting in an overall increase in the genomic instability of the cell. To gain a better understanding of this phenotype, we developed a method to systematically engineer chromosomes that display DRT \textit{de novo}. Using the Cre/loxP recombinase system, we generated many random, balanced chromosomal translocations and assayed those translocation derivatives for DRT. From these studies, we identified multiple loci that appear to be involved in the
acquisition of the DRT phenotype. Finally, by modifying this strategy to generate intrachromosomal deletions at these loci, we have defined genetic regions on two different chromosomes that, when disrupted, cause DRT in *cis*.

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**SiRNA-based nanoparticle platform for the treatment of HER2 positive breast cancer**

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Human epidermal growth receptor type 2 (HER2) is commonly found in breast and stomach cancer. Patients with HER2 positive cancer (accounting for 20-30% of all breast cancer patients) have poor clinical prognosis. Many are or become resistant to HER2 targeted drugs such as Trastuzumab (Herceptin) and Lapatinib. Small interfering RNA (siRNA) can silence genes in a very specific manner, and hence hold great promise for cancer treatment. We have optimized siRNA against HER2 (siHER2) by systematic narrowing down of 76 siRNA sequences to obtain the most specific and effective sequence for silencing HER2 and inhibiting the cell growth. Many HER2 positive cell lines that were resistant to Herceptin and Lapatinib (with the dose required to inhibit 20% growth (GI20) > 30 µg/mL) were still highly responsive to our best siHER2. Despite its great potential, siRNA based therapy has not been widely used clinically, mainly due to the lack of enabling delivery platform.

In this regard, we have developed nanoparticle platform that meets strict requirements of siRNA delivery; it must: (1) be iv injectable, (2) have sufficiently long blood half-life so that they can seek and accumulate in tumor, (3) protect siRNA against enzymatic degradation, and (4) allow endosomal escape of siRNA to the site of action, cytosol. Our nano-constructs consist of PEG-PEI co-copolymer coated on mesoporous silica nanoparticle core, HER2 antibody, pore forming peptide, and siRNA. The constructs could protect siRNA against enzymatic degradation for at least 24 hrs in blood, while naked siRNA was degraded within minutes. Optimal carrier per siRNA mass ratio was found to be 25. The nano-constructs (carrier + siRNA) could successfully silence HER2 and inhibit growth of HER2 positive breast cancer cell lines (HCC1954, BT474) in vitro while had no effects on HER2 negative cells, indicating the treatment specificity. The in vitro success has been translated well to gene silencing and tumor inhibition in mice. Initial safety studies showed that our nano-constructs would likely be safe for clinical uses.

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**Combination therapy to target KIT and PDGFRA mutant cells**

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Purpose: Gastrointestinal stromal tumors (GIST), the most common abdominal sarcoma, arise from the interstitial cells of Cajal. Ninety percent of GIST tumors harbor activating mutations in the receptor tyrosine kinases KIT or PDGFRA. Despite improvements in GIST treatments with the advent of imatinib and other small molecule KIT/PDGFRα kinase inhibitors, advanced disease remains incurable. Although metastatic disease can be controlled in some cases for years using kinase inhibitors, over time most patients develop drug resistant disease. Currently, there is a need to identify
gene targets whose inhibition is synthetic lethal when combined with biochemical inhibition of the primary GIST oncogenic kinase (KIT or PDGFRA). An RNAi screen in chronic myelogenous leukemia cells (Bcr-Abl+) identified members of the non-canonical Wnt/Ca2+/NFAT pathway that when inhibited, synergized with imatinib to kill tumor cells. My goal is to determine whether a similar phenomenon exists in KIT mutant cell lines, and if so, what mechanisms are mediating this synergy.

Experimental methods: We combined a calcineurin inhibitor (CSA or FK506) with a KIT inhibitor (imatinib or dasatinib) and measured cell proliferation after 48-72 hours in KIT and PDGFRA mutant cell lines. Using this proliferation data, we calculated combination indices using the method of Chou and Talay to determine whether synergy occurred. In addition we used immunoblotting to analyze NFAT phosphorylation and subcellular localization in KIT mutant cells. We also measured the functional activity of NFAT using an NFAT-responsive promoter-reporter model.

Results: We found better than additive effects (synergy) when combining calcineurin inhibitor CSA (or FK506) with an RTK inhibitor in all KIT or PDGFRA-mutant- cell lines tested. The average combination index (CI) values for KIT mutant cells ranged from 0.3-0.5 and the average CI value for the PDGFRA cell line ranged from 0.5-0.8. Contrary to other cellular models, NFAT appears to be constitutively active (dephosphorylated) in these KIT and PDGFRA-mutant cell lines as assessed by immunoblotting. Unexpectedly, a majority of this active NFAT appears to reside in the cytoplasm rather than the nucleus. BEZ-235, a dual PI3K/mTOR inhibitor, is able to partially block the basal transcription activity induced by NFAT in KIT mutant cells and moderately inhibits activation of NFAT following treatment with TPA and ionomycin. Conversely, the MEK inhibitor selumetinib significantly blocks activation of NFAT by TPA and ionomycin, but has no effect on basal NFAT activity. KIT inhibitors – dasatinib and imatinib - are moderately effective at inhibiting both basal transcription activity and induced activity following treatment with TPA and ionomycin.

Conclusions: These data indicate that combination therapy of a calcineurin inhibitor plus an RTK inhibitor results in synergistic killing of KIT or PDGFRA mutant cells. The observed synergy between inhibition of the KIT signaling pathway and the Wnt/Ca2+/NFAT signaling pathway may point toward an escape pathway these cell lines use when KIT or PDGFRA are inhibited. Additionally, the constitutive activation of NFAT in these cell lines indicates aberrant signaling in a pathway that has not previously been implicated in GIST biology- the Wnt/Ca2+/NFAT pathway. NFAT may be regulated differently by the PI3K/AKT pathway and the MAPK pathway as seen by the differential effects of inhibitors of these pathways on NFAT activity. Finally, there may be crosstalk between KIT signaling pathways and NFAT signaling pathways since KIT inhibitors are able to modulate NFAT transcriptional activity. We are currently investigating which pathways need to be inhibited in order to see synthetic lethality in KIT mutant cells. In addition, we are determining which upstream activators in the Wnt/Ca2+/NFAT pathway could account for the constitutive activation of NFAT in these cells.

Tumor Heterogeneity from Spontaneous Heterotypic Fusion

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Heterogeneity within tumor cell populations provides the phenotypic diversity upon which selection pressures act to drive tumor evolution, cancer progression and disease recurrence following therapy. The origin of tumor heterogeneity arises primarily from clonal evolution as a result of genetic instability, including: nucleotide-level mutations, whole
chromosome gains or losses, chromosomal translocations, deletions and amplifications. Here we show that an additional process, namely spontaneous fusion between cancer cells and non-tumor cells present within the tumor microenvironment, can also produce tumor heterogeneity. Specifically, we have found that cancer cell fusion with primary macrophages produces hybrid cells carrying genomic material from both parental cells. As a result of comprising two differentially expressed genomes, fusion-derived cells form a transcriptionally distinct population, with a gene expression pattern both overlapping and unique relative to both parental cell types. Like parental cancer cells, hybrids divide indefinitely in culture and generate tumors when isografted into immunocompetent mice. However, fusion also produces novel phenotypes in hybrid cells, and these phenotypes are retained in hybrid progeny for several weeks post-fusion, with potential influence on tumorigenic capacity. Importantly, we find that the hybrid-cell genome is highly dynamic, and undergoes frequent chromosome loss during division, allowing for phenotypic plasticity. These experiments demonstrate a potentially important contribution of spontaneous cell fusion to tumor heterogeneity and cancer progression.

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**Microenvironment Microarrays for the Study of Cancer Growth and Metastasis**

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The microenvironment has a profound influence on tumor growth, metastatic spread, and response to therapeutic agents. It has been exceedingly difficult to study the effects of the microenvironment on cellular phenotypes because it consists of a complex mixture of growth factors, cytokines, and other extracellular matrix proteins. Furthermore, additional cells such as fibroblasts and immune cells may be present and interact with the tumor cells, eliciting further changes. Attempts to model the microenvironment have used complex mixtures of proteins such as matrigel. While these preparations have proven useful for the study of cells in 3-d cultures, they suffer from lot variation and incompletely defined constituents, making it difficult to determine which protein(s) have are influencing the phenotype under study. In order to more systematically interrogate the effects of specific microenvironment proteins, we have begun to implement microenvironment microarray (MEArray) technology here at OHSU. These arrays consist of recombinant or purified proteins spotted on a solid surface. The approach allows for thousands of different microenvironments to be constructed on a single slide upon which cells can be grown. The MEArrays can then be assessed for diverse effects on cellular phenotypes including ability to bind substrate, enhancement of proliferation, escape from senescence, and response to therapeutic agents. Preliminary data detailing some of these assays will be presented for a small number of cancer cell lines grown on the arrays. We believe that this will be a powerful new approach that will allow us to greatly improve our understanding of how the microenvironment can enhance the growth, metastatic spread, and survival of cancer cells.

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**Quantitative imaging of individual mRNAs and co-imaging of protein to follow Akt signaling activation in breast cancer cells**

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Measuring mRNA level provides key information of gene expression in biological systems. Northern blot, real-time PCR, and RNA-Seq have been used to quantify mRNAs, but those methods provide average gene expression information from bulk transcriptome measurement. To get spatial and heterogeneous information in intact cells and tissues, we developed a fluorescence in situ hybridization (FISH) for imaging individual mRNA molecules in breast cancer cells using multiple probes labeled with single fluorophores (Raj et al, 2008). We acquired images of 0.2 m optical sections using automated widefield microscopy (60X objective, NA=1.42), performed “Deconvolution” to subtract blurred light or to reassign it back to a source, and generated three-dimensional image of whole cell. Each transcript was clearly detected as a particle with a diameter of about 0.25 m, counted by IMARIS software. We successfully performed simultaneous detection of three different mRNA species, Her2, Akt1, and Akt3, in single cells of breast sub-type cell lines, HCC1954, AU565, MDAMB231, and MCF7, using probes labeled with different fluorophores. To simultaneously visualize and quantify mRNAs and proteins, we combined immunocytochemistry and FISH, called as “immunoFISH”. Using this technology, we counted Fra-1 mRNA molecules and simultaneously detected an activated form of Akt protein (pAkt) in MCF7 cells, treated with insulin or EGF on different times. Increased levels of Fra-1 mRNA particles were closely correlated to the pAKT activation with insulin/EGF treatment, suggesting that Fra-1 is an Akt-inducible gene in breast cancer cells. Our method could provide a spatiotemporal profile of both transcripts and proteins along with pathways activation in cells, tissues, and solid tumors in a quantitative manner.

References

The Androgen Receptor Promotes Androgen-Independent Prostate Cancer Cell Survival through Up-regulation of c-Myc

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Prostate cancer is the most common cancer in men in the United States and the second leading cause of cancer death. The androgen receptor (AR) is the central signaling pathway in prostate cancer. Because of this, androgen deprivation therapy (ADT), which involves reducing levels of androgens or interfering with binding of androgens to AR, has been the major therapeutic focus for the past 70 years. However, in many patients there is no benefit from ADT, and all prostate cancers eventually progress. At progression on ADT, the AR is ubiquitously expressed. For this reason, we contend that AR-dependent mechanisms are critical for prostate cancer progression after ADT. Indeed, recent work demonstrates that the AR can function independently of androgens to promote prostate cancer cell survival. However, specific androgen-independent AR target genes that account for this effect were not known and targeting the AR protein, itself, had not been possible. Our work addresses those deficits.

We used a systems biology approach to determine that the AR promotes expression of the c-Myc oncogene that is commonly upregulated in human prostate cancer. While c-Myc is AR-activated, it is not androgen-activated. AR RNAi or c-Myc RNAi each reduces prostate cancer cell survival in castrate conditions, and c-Myc overexpression abrogates the effect of AR RNAi on reducing prostate cancer cell viability. Thus, c-Myc is a critical gene through which AR promotes androgen-independent prostate cancer cell survival. We treated prostate cancer cells with a new selective AR degrading
compound that has recently entered phase I clinical trials. Drug treatment reduced AR expression, c-Myc expression, and androgen-independent prostate cancer cell survival. Our results define a new link between two critical prostate cancer cell survival pathways – AR and c-Myc. Our results also demonstrate the potential of a new therapeutic strategy to suppress AR and c-Myc so that we may control prostate cancer long-term.

Do breast cancer stem cells express the estrogen receptor providing a target for tamoxifen?

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Current treatment for estrogen receptor (ER) positive breast cancer patients includes 5 years of tamoxifen therapy, but research shows that, at 15 years, cancer recurs in 33% of patients. One hypothesis to explain these clinical observations is that stem cells may be responsible for cancer recurrence and that the stem cells may not exhibit the same receptor status as the tumor. We have been isolating stem cells from fresh breast cancer and normal tissue to identify differences in ER expression. Because these cells are rare, the crucial steps in this research have been to develop technology to gain meaningful results from 1000 or fewer cells. We have looked at gene expression using Taqman low density array technology (TLDA) and we are looking at protein expression by ELISA and protein PCR. The goal is to determine if the stem cells isolated from fresh ER positive breast cancer tissue are also ER positive. The findings from this study will guide standards of care for patients with ER positive breast cancer.

Exclusively non-toxic TRAIL-based approach to target various cancer cell types

Dmitri V. Rozanov, Anton Cheltsov, Alexei Y. Savinov, Vladislav S. Golubkov, Alexander E. Aleshin, Stephan Vasile, Eduard Sergienko, and Alex Strongin

The execution of the NIH screening grant resulted in the identification of a compound MLS0092727 which specifically potentiates the extrinsic TRAIL-mediated apoptosis in prostate and breast carcinoma cells but not in normal mammary epithelial cells. We have then identified a protein target of this compound and a combined treatment of one of its analogs (analog 1) has induced 90% and 80% cytotoxicity in TRAIL- and chemotherapy-resistant breast and prostate carcinoma cells, respectively and has not in the least affected the viability of human primary hepatocytes, one of the most sensitive cells to chemotherapy. We have also shown that both activation of the NF-κB and overexpression of the MDR protein do not affect the potency of MLS0092727 and its analog 1 to stimulate TRAIL’s activity in TRAIL-resistant carcinoma cells.

In addition, we have constructed a leucine zipper (LZ)-TRAIL and showed that it is very stable (melting temperature is 81.1 °C and a half-life in mouse blood is in the range of several hours), very active (0.1-10 ng/ ml of LZ-TRAIL is cytotoxic to many cancer cell lines), and safe (30 μg/ml of TRAIL does not induce any cytotoxicity in normal mammary epithelial cells and human hepatocytes). For comparison, as a clinical candidate TRAIL.0 version of TRAIL has been developed and it has been shown that TRAIL.0 has a half-life in the mouse bloodstream equal to 3.5 min. As a consequence of the shortcomings of the clinical version of TRAIL, the results of the clinical trials with TRAIL are not encouraging. Out of 121
patients treated, none had a complete response and only two had a partial response (Johnstone RW et al. Nature Reviews Cancer 2008, 8: 782-798).

However, LZ-TRAIL cannot be used in cancer treatment because the LZ sequence is derived from the yeast gene which means that the LZ-TRAIL can be immunogenic in the human body. To convert LZ-TRAIL into an anti-tumor agent applicable for human cancer therapy the yeast LZ motif should be replaced with a human LZ peptide. We have already selected several human LZ-TRAIL constructs with the stability comparable to that of the yeast LZ-TRAIL. The potent anti-tumor activity of one of the selected human LZ-TRAIL constructs was confirmed in vivo in mice.

We believe that the development of the drug-like lead that specifically potentiates TRAIL-induced apoptosis in cancer cells but not in normal cells when combined with our re-engineered TRAIL will validate our TRAIL-based approach as an effective and exclusively non-toxic cancer therapy.

What do Chinese American immigrant women think about breast cancer? Findings from Focus Groups

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Significance: Despite progress in increasing cancer early detection, morbidity and mortality benefits are not equally distributed among ethnic minority groups. Early detection of cancer through regular screening plays a vital role in reducing mortality from breast cancer (BC).

Problem and Purpose: BC remains the most commonly diagnosed cancer among Asian American women; however, mammography screening rates are well under the Healthy People 2020 projected goals of 81.1%. Asian Americans also consistently have the lowest mammography rate of all races. This presentation describes how a cancer screening research program was launched for Asian Americans, especially for Chinese Americans, the largest Asian population in the US. The purpose of this focus group study was to better understand Chinese American women’s beliefs about BC screening and to refine the development of a targeted, culturally-responsive BC screening education program, which is currently being tested in a randomized controlled trial, for Chinese Americans.

Theoretical / Scientific Framework: The development of the discussion topics was guided by two popular models of health behavior change – the Transtheoretical Model of Change (TTM) and the Health Belief Model (HBM).

Methods and Analysis: Thirty-eight foreign-born women, ages 40 and older, participated in 5 focus groups where discussion was facilitated through a semi-structured discussion guide. Focus group discussions in Chinese were audio taped, transcribed, and translated into English. Data were examined using constant comparison and content analysis. Three primary themes emerged through iterative coding: knowledge and beliefs; support, communication and educational needs; and access to care. Within these categories, several subthemes were also identified. Further, several women were profoundly affected by the mostly negative BC-related experiences of relatives and friends. Some common myths remain about causes and treatment of BC, and may impact adherence to screening.
Findings and Implications: Health care providers in primary care settings should be aware of culturally driven motivations or barriers to mammography among Chinese American women. Such awareness could be the first step in opening a dialogue around BC that is culturally responsive, and that could elicit trust and adherence from the patient. Provider-client interactions should involve more discussion about women’s BC risks and screening harms and benefits.
**Cancer (Treatment) | 3 - 4:15 Monday, May 7th | OHSU Auditorium**

**Chad Burk** | ALL Progression Induces PD1 on T Cells and Blockade of PD1 Enhances Adoptive Immunotherapy

**Joshua Walker** | Effects of Total Body Irradiation on T-Cell and B-Cell Subsets as Well as Macrophage in Rhesus Macaque

**Oleg Sostin** | Impact of Real-time Tumor Tracking and Fraction Size on Treatment-related Morbidity in Prostate Cancer Patients Treated with Intensity-modulated Radiotherapy

**Leonel Kahn** | I-125 Plaque Brachytherapy for Choroidal Melanoma: mature single-institution outcomes

**Renato Luna** | Neoadjuvant therapy influences lymph node ratios and overall survival without decreasing total node harvest.

**Jared Fischer** | Phenotypic plasticity allows Apc-deficient intestinal stem cells to avoid chemotherapy

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**ALL Progression Induces PD1 on T Cells and Blockade of PD1 Enhances Adoptive Immunotherapy**

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**Background:** Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy and, despite tremendous success in therapy over the past 3 decades, remains the primary cause of cancer-related mortality in children. Enthusiasm for the use cellular immunotherapy for ALL has been tempered by the poor response to donor lymphocyte infusions following allogeneic hematopoietic stem cell transplantation. However, ALL blasts are susceptible to T cell and NK cell mediated lysis in vitro suggesting that poor response to in vivo immune interventions may be due to events occurring during the priming of the immune response. Using a murine model of precursor B cell ALL we examined the impact of leukemia progression on T cells in vivo. **Methods:** We developed a transplantable syngeneic model of pediatric ALL derived from transgeneic mice expressing the human E2aPBX1, a recurring translocation present in 5% or pediatric leukemia (Bijil et al, Genes and Development). This murine line displays a precursor B cell phenotype and results in 100% lethality following injection of 100,000 cells (Qin et al, ASH, 2010). Using congenic (CD45.1) B6 recipients, we tracked the early progression of ALL in vivo and examined the T cells in the leukemia-containing compartments by flow cytometry and PCR. **Results:** Using congenic markers, ALL cells can be detected in bone marrow as early as 3 days following intravenous injection of 1,000,000 cells with a sensitivity of 0.01%. Spleen and lymph node involvement was seen later (10 days) followed by the detection of circulating blasts by 2 weeks. E2aPBX1 cells express variable levels of costimulatory molecules in vitro with no change in expression during in vivo progression. Notably, PDL1 and PDL2 are expressed both in vitro and in vivo at higher levels than on non-malignant precursor B cells in leukemia-bearing mice. Remarkably, although PD1+ T cells are not seen in the bone marrow of non-leukemia-bearing mice, PD1 expression on bone marrow T cells was markedly increased during progression such that 60-80% of all bone marrow CD4 and CD8 T cells were positive by 2 weeks following leukemia injection. In addition to expression of PD1, these T Cells also co-expressed Tim3, a phenotype associated with T cell exhaustion. Blockade of PD1 or PDL1 starting 3 days following leukemia injection had no impact on leukemia progression. However, combining PD1 blockade with the
adoptive transfer of T cells from leukemia-primed donors resulted in improved survival compared to primed T cells alone (p=0.0004). **Conclusions:** Early progression of ALL results in the induction of PD1 and Tim3 on T cells in vivo. Combination of PD1 blockade plus adoptive T cell therapy results in therapeutic benefit suggesting that this axis may be an attractive target in ALL.

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**Effects of Total Body Irradiation on T-Cell and B-Cell Subsets as Well as Macrophage in Rhesus Macaque**

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**Purpose/Objectives:**
Anti-tumor immunity is known to play a major role in the control of neoplasms *in-vivo*, and tumor immunotherapy is rapidly becoming a major part of oncology research and evidence-based practice. However, the effects of radiation therapy on the multiple cell types responsible for cellular and humoral immunity are not entirely understood. The specific aim of the present analysis was to determine the acute effects of mini total body irradiation (TBI) on circulating levels of T-cells, B-cells, and monocytes in rhesus macaques (RMs).

**Material/Methods:**
Four female RMs (8-10 years old) were exposed to 2 Gy ionizing radiation to induce immunosuppression, and this state was subsequently maintained with daily tacrolimus and prednisone treatment. T-cell, B-cell, and monocyte frequencies were determined by flow cytometry. In addition, CD4 and CD8 T-cells as well as B-cells were further subdivided into naïve and memory subsets. Relative frequency and proliferative status of these subsets was determined by flow cytometry and by measuring changes in Ki-67 expression levels. The institutional animal care and use committee at ONPRC approved the study.

**Results:**
TBI effectively lymphodepleted the RMs while treatment with tacrolimus and prednisone alone did not. However, B-cells were more effectively eliminated than either CD4 or CD8 T-cells. Interestingly, the levels of circulating macrophage increased dramatically following TBI.

**Conclusions:**
Our preliminary results suggest that TBI has differential effects on the cell types involved with anti-tumor immunity, and may effect humoral immunity and antigen presentation to a greater extent than T-cell mediated immunity.

This work was supported by 8P51 OD011092-53 and NIH R01AG037042.

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**Impact of Real-time Tumor Tracking and Fraction Size on Treatment-related Morbidity in Prostate Cancer Patients Treated with Intensity-modulated Radiotherapy**
Purpose/Objectives: To assess the impact of real-time tumor tracking with Calypso beacons vs. gold fiducials on urinary (UA) symptoms in patients treated with intensity-modulated radiotherapy (IMRT) for prostate cancer. Simultaneously, we compared standard fractionation IMRT (SIMRT) and hypofractionated IMRT (HIMRT) regimens.

Materials/Methods: We retrospectively reviewed symptoms in 4 cohorts of patients definitely treated with IMRT for prostate cancer. 87 patients were treated with standard planning target volume (PTV) margins and gold seed implants to track target position. 174 patients were treated with Calypso® real-time tumor tracking system. 31 and 67 patients in the respective cohorts were treated with HIMRT. Altogether, 163 patients were treated with SIMRT and 98 patients with HIMRT. To evaluate urinary symptoms, we used AUA BPH Symptom Score Index measured before treatment and at follow-up visits. We also recorded nocturia, UA incontinence (of any form), and use of α-blockers. The range of follow-up was 6-42 mo. To account for repeated measures taken on the same subject over time, generalized estimating equation was used to evaluate changes in continuous variables. Binary outcome measures were analyzed using test of proportions.

Results: Tumor tracking modality, and not fraction size, was associated with mean AUA BPH scores; no effect on nocturia was found. Regardless of fraction size, mean AUA BPH scores in Calypso cohort were 2.57 (95% CI: 0.19-4.45) points lower than corresponding scores in gold seeds cohort at baseline. At 6 mo, these differences somewhat decreased but then reached a peak of 3.69 (95% CI: 1.22-6.16) at 12 mo, and later dissipated. While similar at baseline, gold seeds cohort had an 18% (95% CI: 2-34 %) greater prevalence of α-blocker use in the end of treatment and a 27% (95% CI: 13-41 %) greater period prevalence of UA incontinence during follow-up compared to Calypso cohort.

Conclusions: No statistically significant differences in UA toxicity were found between SIMRT and HIMRT. Statistically significant, but clinically insignificant, AUA BPH score differences were found between gold seeds and Calypso cohorts. An 18% difference in prevalence of α-blocker use at the end of treatment and a 27% difference in prevalence of UA incontinence between gold seeds and Calypso patients suggest a lower UA toxicity associated with Calypso system of tumor tracking.

I-125 Plaque Brachytherapy for Choroidal Melanoma: mature single-institution outcomes

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Purpose/Objectives: We report a series of patients treated with I-125 plaque brachytherapy for choroidal melanoma. The specific aims of the analysis were to evaluate local tumor control, overall and metastasis-free survival, loss of visual acuity versus loss of subjective vision, and describe the need for secondary enucleation.

Materials/Methods: We identified 317 patients with choroidal melanoma managed by I-125 plaques from 1991 to 2011. The selected tumors had been prescribed 85 Gy to the prescription point per COMS protocol. We recorded tumor and treatment characteristics, mortality, rate of local recurrence (LR), development of metastatic disease, need and reason for secondary enucleation, changes in visual acuity, and subjective vision loss (new patient-reported loss between previous time point and current time point/total patients at current time point). Kaplan-Meier survival curves were
used to calculate overall and metastasis-free survival. Log-rank analysis was used to determine factors associated with overall survival.

**Results:** Mean tumor thickness was 3.57 mm (range 1-12) and mean maximum basal tumor diameter was 11.00 mm (range 4-20). Ciliary body invasion was seen in 28/317 patients. Using a mean plaque size of 16.91 mm (range 4-24) the mean calculated dose of 87.64 Gy was given over a mean of 144.9 hours. One and 5-year overall survival were 98.7% and 77.4%, respectively. One and 5-year metastasis free survival were 99.7% and 93.4%, respectively. Factors associated with overall survival (p < .05) included age at treatment, maximum basal tumor diameter, retinal detachment. Five-year LR free survival was 97.8%. There were 20 secondary enucleations total. LR was cause for secondary enucleation in 10/317 cases (all LR were enucleated); 3/10 of these LR had pre-treatment invasion into the ciliary body. Pain was the main reason for secondary enucleation in 10/317 cases. Visual acuity in the treated eye of equal or better than 20/200 was seen in 87.9%, 77.3%, 54.1%, 46.7%, and 40% of patients pre-treatment, and 1, 5, 10, and 15 years post-treatment, respectively. Subjective vision loss (within treated eye) was reported in 45.0%, 31.0%, 22.3%, 18.2%, and 16.7% of patients at pre-treatment, 1, 5, 10, and 15 years follow-up.

**Conclusion:** Curative intent I-125 plaque brachytherapy provides a high 5-year disease-free survival and low risk of LR. Enucleation was required in fewer than 6.5% of cases, with pain and local recurrence each accounting for half of the cases. Nearly one third of tumors requiring secondary enucleation due to LR had pre-treatment ciliary body invasion. Visual acuity showed a gradual decline up to 15 years post-treatment, with subjective vision loss noted predominantly in the first 5 years of follow-up.

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**A prospective randomized comparison of pain and inflammatory response between single port and laparoscopic cholecystectomy**

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**Introduction:** The aim of this study was to compare the postoperative inflammatory response and severity of pain between single incision laparoscopic surgery (SILS) cholecystectomy and conventional laparoscopic cholecystectomy.

**Methods and Procedures:** Two groups of 20 patients were prospectively randomized to either conventional laparoscopic cholecystectomy (LC) or SILS cholecystectomy. Serum interleukin-6 (IL-6) levels were assayed prior to surgery and then at 4-6 hours and 18-24 hours after the procedure. Serum C-reactive protein (CRP) levels were also assayed at 18-24 hours after surgery. Pain was measured at three time points after surgery using the visual analogue scale (VAS) and the number of analgesic doses administered in the post-operative period was recorded during first 24h after the procedure. Thirty-day surgical outcomes were also recorded. Student’s t-test and the Mann-Whitney test were used when appropriate. P values <0.05 were considered significant.

**Results:** The groups had equivalent BMI, age and comorbidity distribution. The peak of IL-6 levels occurred 4-6 hours after surgery and the median level was 8.9 pg/ml in SILS and 12.8 pg/ml in LC group (p=0.495). The median CRP level before discharge was 1.6 mg/dl in LC and 1.9 mg/dl in SILS group (p=0.383). In addition, there was no difference in analgesic use or pain intensity as measured on the VAS between two groups (p=0.723). The length of the surgical procedure was significantly longer in the SILS group (p<0.001). No intra operative complications occurred in any group.
Conclusion: SILS does not significantly reduce systemic inflammatory response, post-operative pain or analgesic use when compared to laparoscopic cholecystectomy.

Phenotypic plasticity allows Apc-deficient intestinal stem cells to avoid chemotherapy

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Although driver mutations are present in tumors, the earliest phenotypes of mutant cells may be variable since tumorigenesis requires the accumulation of multiple alterations. To better characterize the early phenotypes of driver mutations, we used a mouse model that stochastically activates Cre in a small percentage of cells within intestinal crypts, thus allowing for fate mapping of genetically altered β-gal" cells. Using this model combined with a conditional Apc allele, we previously showed phenotypic plasticity of Apc−/− cells because both adenomatous and normal appearing β-gal" crypts were observed. Here, we show that this phenotypic plasticity is modulated by the field size of Apc-deficient crypts, and that sulindac can reduce the field size of Apc−/− crypts, presumably by inhibiting crypt fission, but does not eliminate Apc−/− crypts. We combined our mouse model with the ApcCKO/CKO alleles and the KrasG12D allele and found that nearly all mutant foci became transformed in less time. To test if the increased transformation was caused by the crypts being both Apc−/− and Kras+, we used a mouse model of low frequency Cre activation where the mutant crypts are completely isolated. In these mice, >99% of Apc−/− or Apc+/−; Kras+ cells retained normal crypt phenotypes suggesting that the transformation advantage conferred by Kras is due the increased crypt fission following Kras activation. Finally, we administered sulindac at different time points in ApcCKO/CKO mice. Sulindac administration at weaning resulted in adenoma regression, but normal appearing, Apc−/− foci persisted, suggesting that NSAIDs modulate phenotypes but do not directly eliminate Apc−/− stem cells. Sulindac administration throughout life not only inhibited Apc−/− adenomas, but also inhibited the enhanced Apc−/− crypt clonal expansion, consistent with human studies showing that only chronic administration (> 5 years) of low dose NSAIDs is effective in reducing human colorectal cancer risk. Phenotypic plasticity complicates both chemoprevention and clinical screening because mutant clones are not intrinsically linked with tumorigenesis, and thus avoid both detection and therapy while continuing to accumulate new mutations.
**Projected Survival and Self-Care Behaviors among Adults with Heart Failure**

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Background: There is a growing need for tools that guide clinical and self-management practices for patients with heart failure (HF), the fastest growing cardiovascular condition in the U.S. The Seattle Heart Failure Model (SHFM) uses 24 objective clinical variables to calculate one-year survival, but does not account for subjective measures such as patients’ ability to recognize and respond to symptoms when they occur.

**Purpose:** To examine the relationship between SHFM projected one-year survival and self-care behaviors among adults with symptomatic HF.

**Methods:** We completed a secondary analysis of data collected during a study of symptoms among adults with moderate to advanced HF. Projected one-year survival was based on SHFM scores. Self-care behaviors were measured using the Self-Care of Heart Failure Index maintenance, management, and confidence scores, and the European Heart Failure Self-Care Behavior Scale consulting behaviors score. Linear correlations, correcting for multiple comparisons, were quantified between projected one-year survival and HF self-care.

**Results:** The average age of the sample (n=166) was 56±12 years, 61% were male, and the average projected one-year survival was 70.6%±15.7%. There was a relatively weak but significant inverse relationship between one-year survival and self-care maintenance (r= -0.180, p=0.021) and management (r = -0.230, p=0.01), but not self-care confidence or consulting behaviors (both p>0.05).
Conclusion: As projected one-year survival rates increase, there is a weak association of worsening self-care management and maintenance. In the future, it may be worthwhile to incorporate self-care behaviors as part of prognostic tools.

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Prognosis and Secondary Shockable Rhythms in Out-of-Hospital Cardiac Arrest

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Background: Non-shockable arrest rhythms (Pulseless Electrical Activity and Asystole) represent an increasing proportion of reported cases of out-of-hospital cardiac arrest (OHCA). The prognostic significance of conversion from non-shockable to shockable rhythms during the course of resuscitation has been debated in published literature.

Objective: To evaluate whether out-of-hospital cardiac arrest survival with initially non-shockable arrest rhythms is improved with subsequent conversion to shockable rhythms.

Methods: Secondary analysis of the prospectively-collected data in Epistry – Cardiac Arrest, an epidemiologic registry developed and maintained by the Resuscitation Outcomes Consortium (ROC). Ten North American sites contribute data to Epistry, documenting all out-of-hospital cardiac arrests through hospital discharge. The sample for this analysis includes OHCA from December 1, 2005 through May 31, 2007 contributed by six US and two Canadian sites. The investigational cohort includes all EMS-treated adult (18 and older) cardiac arrest patients who presented with non-shockable cardiac arrest rhythms and were treated by EMS personnel. We compared survival to hospital discharge between patients that did versus those who did not develop a shockable rhythm based on receipt of subsequent defibrillation (i.e., presumed conversion to VF/VT). Missing data were handled using multiple imputation. Multivariable logistic regression was used to assess the relationship between subsequent shockable rhythm and survival to hospital discharge after adjusting for potentially confounding variables: age, gender, public location, witnessed status, bystander resuscitation, EMS response interval, and ROC site. Results are reported as odds ratios (OR).

Results: A total of 6,556 adult cardiac arrest cases presented in non-shockable rhythms, were treated by EMS, and had no exclusion criteria. Survival to discharge in patients who converted to a shockable rhythm during out-of-hospital resuscitation was 2.77% similar to the survival in those who did not convert which was 2.72% (p = 0.92). After adjusting for known confounders, the adjusted odds for conversion to a shockable rhythm was not associated with improved survival (OR 0.88, 95% CI: 0.60-1.30).

Conclusion: For OHCA patients presenting in PEA/Asystole, survival to hospital discharge was not associated with conversion to a shockable rhythm during EMS resuscitation efforts.

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Hyperglycemia slows embryonic growth through suppression of cell cycle

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The incidence and prevalence of diabetes mellitus are rising in the US population. In pregnant women the diabetic condition results in a 3-5 fold increased risk for fetal cardiac and valve malformations due to the elevation of maternal blood glucose concentration leading to elevated glucose concentrations in the developing embryo and fetus. The development of the heart in the chicken embryos was studied with and without exposure to hyperglycemia just prior to heart septation and chamber formation. Pulsed hyperglycemia was created by the daily administration of exogenous D-glucose (30 or 50 mM) from days 0 to 3 of egg incubation, which caused daily spikes in the plasma glucose concentration. In a second model, sustained hyperglycemia was induced with a single injection of D-glucose (750 mM) into the yolk on day 0, which elevated plasma glucose concentration from the control level of 70 mg/dl to 200 mg/dl and lead to a reduced gene expression of the glucose transporter, GLUT1. Both models of hyperglycemia reduced embryo size, increased mortality, and delayed development at 96 hours. Within the heart outflow tract, reduced proliferation of myocardial and endocardial cells was associated only with sustained hyperglycemia. Gene expression of key regulators of the cell cycle was altered by sustained hyperglycemia. The cycle inhibitory gene, p21 was significantly increased while cyclin D1, a S to G1 cell cycle promoter, decreased compared to controls. The evidence suggests that hyperglycemia-induced developmental delays are a result of: decreased glucose transports which deprives the cells of nutrition; and slowed cell cycle progression which lead to reduced cellular proliferation in the embryonic chick heart. The suppression of critical development steps of organogenesis may underlie the septal and valve defects observed during later development due to hyperglycemic conditions.

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**Clopidogrel resistance: Role for unbound concentrations of active metabolite**

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**College of Pharmacy, Oregon State University, Corvallis, OR and Oregon Health & Science University, Portland, OR**

**Introduction:** Clopidogrel, either alone or in combination with aspirin, remains the cornerstone of modern antiplatelet strategies. In spite of the inter-individual variability, clopidogrel still remains unsurpassed in its usefulness in reducing vascular ischemic events. Although, being used since late 1990’s the pharmacokinetic (PK) fate of clopidogrel and its metabolites are poorly explained, hence there is a critical need to identify the cause of variability with clopidogrel therapy. The inter-patient variability response is believed to be multi-factorial, caused both by genetic and non-genetic factors. In this study, we examined whether the concentration of inactive metabolite (IM) affects the free concentration of active metabolite (AM), and thus potentially causing inter-individual variability of clopidogrel.

**Methods:** Female subjects (n=28) with stable coronary disease who are not taking clopidogrel were recruited. Serial blood samples were collected at 0, 20, 40, 60, 90, 120, and 240 minutes after administering 300mg of single oral dose of clopidogrel. Plasma was isolated, and quantified for total and free concentrations of AM, and IM using liquid chromatography-tandem mass spectrometry. Inhibition of platelet aggregation was measured using phosphorylated vasodilator stimulated phosphoprotein (VASP) assay.

**Results:** As expected, significant correlation was observed between VASP and both free (r=0.49; p<0.05) and total (r=0.49; p<0.05) concentrations of AM. Surprisingly, we observed a significant correlation to both free (r=0.42; p<0.05) and total (r=0.67; p<0.001) concentrations of IM too. Total concentrations of IM are strongly correlated to total concentrations (r=0.83; p<0.001) and free concentrations (r=0.84; p<0.001) of AM. The free concentrations of AM also correlates with free concentrations of IM (r=0.58; p<0.01). Interestingly, a positive correlation was observed between free fractions of AM and bound fractions of IM (p<0.05).
Conclusions: Earlier studies have shown that the IM does not have any direct effect on platelet activity. However, the positive correlation between total concentrations of IM and unbound concentrations of AM suggests that the IM displaces AM from binding sites. Thus, the IM might increase the unbound concentration of the AM leading to increased platelet inhibition.

An Excess of Deleterious Variants in VEGF-A Pathway Genes in Down Syndrome-Associated Atrioventricular Septal Defects

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About half of people with trisomy 21 have a congenital heart defect (CHD) while the remainder have a structurally normal heart, demonstrating that trisomy 21 is a significant risk factor but is not causal for abnormal heart development. We used a candidate gene approach in a study cohort of individuals with Down syndrome (DS) to determine if rare genetic variants in genes involved in atrioventricular valvuloseptal morphogenesis contribute to atrioventricular septal defects (AVSD) in this sensitized population. We found a significant excess (p<0.0001) of variants predicted to be deleterious in DS with AVSD cases compared to DS with no heart defect controls. The variants with the highest probability of being damaging were found in six genes: COL6A1, COL6A2, CRELD1, FBLN2, FRZB and GATA5. Several of the variants were recurrent in unrelated cases. There were no variants with an equal probability of being damaging in these genes found in controls, demonstrating a highly specific association with AVSD. Of note, all of these genes are in the VEGF-A pathway even though the candidate genes analyzed in this study represented numerous biochemical and developmental pathways, suggesting that rare variants in the VEGF-A pathway may contribute to the genetic underpinnings of AVSD in humans.

Genome-Wide Association Study of Gamma' Fibrinogen Levels in the Framingham Offspring Cohort

Rehana S. Lovely¹, Qiong Yang², Joseph M. Massaro², Ralph B. D'Agostino, Sr.², Christopher J. O'Donnell³, Jackilen Shannon⁴ and David H. Farrell⁴

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Introduction γ' fibrinogen is an isoform of fibrinogen that arises from alternative mRNA splicing. γ' fibrinogen is a newly-emerging biomarker that has been associated with cardiovascular disease (CVD) in case-control studies, but larger community-based studies on the association of γ' fibrinogen with CVD and on the association of γ' fibrinogen with single-nucleotide polymorphisms (SNPs) are lacking.
**Objectives** To investigate the associations between $\gamma'$ fibrinogen levels and CVD and between $\gamma'$ fibrinogen levels and genetic loci.

**Methods** $\gamma'$ fibrinogen levels were measured in 3,300 participants from the Framingham Heart Study Offspring Cohort. Associations of $\gamma'$ fibrinogen with prevalent CVD were examined using multiple logistic regression. A genome-wide association study was performed using data from the SHARE database.

**Results** $\gamma'$ fibrinogen was associated with significant increases in risk of prevalent CVD and myocardial infarction (multivariable-adjusted odds ratio 1.53 (95%CI 1.14-2.05) and 1.76 (95%CI 1.06-2.92), respectively). The adjusted odds ratio for subjects in both the highest tertile of $\gamma'$ fibrinogen and highest tertile of total fibrinogen for prevalent CVD and myocardial infarction was 2.17 (95%CI 1.42-3.32) and 3.08 (95%CI 1.41-6.72), respectively. These odds ratios were higher than those for either marker alone. 46 SNPs in or near the fibrinogen gene locus demonstrated genome-wide significance ($P<5.0\times10^{-8}$) for $\gamma'$ fibrinogen levels. In contrast to previous association studies of total fibrinogen levels, no other loci on other chromosomes reached this threshold for significance. Also in contrast to total fibrinogen levels, $\gamma'$ fibrinogen levels were significantly associated with several SNPs in the PLRG1 locus adjacent to the fibrinogen gene locus. PLRG1 encodes pleiotropic regulator 1, a component of the spliceosome that affects alternative splice site choice.

**Conclusions** These findings suggest that $\gamma'$ fibrinogen has a significant association with CVD and is associated with SNPs that are distinct from SNPs that mediate circulating levels of total fibrinogen.

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**Conclusions** These findings suggest that \( \gamma' \) fibrinogen has a significant association with CVD and is associated with SNPs that are distinct from SNPs that mediate circulating levels of total fibrinogen.

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**The National Registry of Genetically Triggered Thoracic Aortic Aneurysms (GenTAC): Registry Progress and Research Successes**

Cheryl L. Maslen, PhD, on behalf of the GenTAC Consortium

Funded by the National Institutes of Health, the GenTAC Registry is a multicenter, longitudinal, observational cohort study of patients with conditions involving genetically triggered thoracic aortic aneurysm and/or dissection (TAAD). GenTAC was established to provide a biospecimen inventory and bioinformatics infrastructure that will enable research to advance the clinical management of such patients. Primary diagnoses include Marfan syndrome, bicuspid valve with aneurysm and/or a family history of aneurysm, idiopathic TAAD in patients \(<50\) years of age, Turner syndrome, familial TAAD, other congenital heart disease with TAAD, Ehlers-Danlos syndrome, and Loeys-Dietz syndrome. To date GenTAC has recruited 2826 subjects, with a final goal of 4000 registrants. Initial analyses of GenTAC data/specimens have included studies of genetic causes for aortic conditions via gene sequencing, SNP, CNV and genome-wide association studies, potential usefulness of TGF- \( \beta \) blood levels as prognostic or therapeutic marker in Marfan subjects, surgical approaches/outcomes for ascending aortic conditions and gender differences in TAAD. Other studies in progress include cross-sectional and longitudinal data regarding phenotype-genotype correlations of disease risk factors, features, treatment, and outcomes, and analysis of imaging methods/integration of imaging findings with clinical and genetic data. GenTAC phenotyping and imaging cores have been established to facilitate these inquiries. The GenTAC Registry is a resource for anyone in the scientific community interested in advancing our understanding of genetically mediated TAAs and their causes, diagnosis, and optimal treatment. Investigators interested in utilizing GenTAC for ancillary studies should contact the registry at gentac-registry@rti.org or apply at http://gentac.rti.org.
Isolation and characterization of primary adult human liver progenitor cells

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Adult intrahepatic liver progenitor cells are a facultative stem cell with the ability to differentiate into cholangiocytes or hepatocytes. Developing assays to isolate and characterize these cells is important to studies of chronic liver injury and cell therapy. We generated monoclonal antibodies recognizing cell surface antigens to allow isolation of subtypes of epithelium from dispersed human liver tissue that were unsuitable for orthotopic liver transplantation.

Long-term self-renewing liver organoid formation capacity was found in only a subset of epithelial cells defined as CD45-/CD31-/DHIC5-4D9+. Organoid formation efficiency occurred between 6-12% in these cells among the 5 patients samples studied. Single cells formed organoid structures comprising thousands of cells that could be passaged and reinitiate daughter organoid structures in a 3-dimensional matrigel culture system. Organoid formation was specific to the DHIC5-4D9+ population; hepatocytes, endothelium, fibroblasts, and stellate cells were unable to form organoid structures. The more abundant CD45-/CD31-/DHIC5-4D9- /DHIC2-4A10+ duct cells did not initiate organoids and likely represent a more mature duct cell.
Immunohistochemical analysis confirmed that DHIC5-4D9 and DHIC2-4A10 antibodies localize to distinct cell populations within the biliary system, which co-express duct marker CK19. To assess gene expression, RNA-sequencing libraries were successfully made from as few as 15,000 fresh-sorted cells. The RNA-sequencing profiles of the DHIC5-4D9+ and DHIC2-4A10+ epithelial subsets, and the results of cell transplantation studies into immunodeficient mice will be useful in further characterizing the stem-potential of these cells.

3-Iodothyronamine Undergoes Clathrin-mediated Endocytosis in Cells

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3-Iodothyronamine (T1AM) is an endogenous derivative of thyroid hormone. Administration of T1AM to animals induces profound physiological effects such as hypothermia, hyperglycemia, reduction in cardiac drive, and behavioral anergia. The exact mechanisms for these effects exerted by T1AM are largely unknown.

T1AM is rapidly taken up by various mammalian cell lines suggesting that intracellular uptake may be an important component of T1AM action. Cellular uptake of T1AM has been characterized as sodium and chloride independent, pH dependent, thyronamine specific, and not to involve the likely candidate transporters of other monoamines, organic cations, or thyroid hormones. In the present study we propose that T1AM uptake occurs via a clathrin-mediated endocytic process in HeLa cells. Inhibitors of clathrin-mediated endocytosis dramatically reduced internalization of 125I-T1AM and a fluorescent analogue, T1AM-Rhodamine, whereas pharmacological blockade of caveolae-related endocytosis exerted little or no effect on the uptake of 125I-T1AM and T1AM-Rhodamine. In summary, our studies provide direct evidence of the involvement of endocytosis machinery in the intracellular uptake of T1AM. Our data suggest that T1AM undergoes a classical clathrin-mediated endocytosis pathway.

Dynamic Structural Rearrangement of Translocon Proteins Sec61, TRAP, and OST Coincides with Nascent Chain Entry into the Lumen

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The Sec61 translocon is a large ER membrane protein complex that together with active ribosomes co-translationally directs nascent secretory and membrane protein movement into the ER lumen, cytosol, and lipid bilayer. Although structural studies to date have largely focused on the core protein conducting channel Sec61abg to explain functional complexities of this remarkable machine, the composition and structural organization of functionally engaged translocons remains a critical and largely unexplored question. Here we show that solubilized Sec61 heterotrimers released from active ribosomes trapped in mid-translation form multiple, stable macromolecular complexes that contain stoichiometric quantities of oligosaccharyltransferase (Sec61-OST), and translocation associated protein complex (Sec61-OST-TRAP). These complexes are derived from functional translocons and remain associated with translocation substrates after premature ribosome release. Surprisingly, translocon complexes undergo distinct conformational transitions that depend on the precise stage of translation. During early stages of membrane targeting and ER docking of
secretory protein preprolactin (≤ 105aa in length), Sec61 associates with nascent polypeptides primarily in its heterotrimeric form. However, affinity for OST and TRAP increase markedly as the chain elongates (137 to 163 aa in length). Interestingly, stabilization of OST-containing complexes was unrelated to either N-linked glycosylation, the presence of glycosylation consensus sites or cleavage by signal peptidase. Instead, stable Sec61-OST-TRAP complexes were established coincident with initial movement of the nascent chain passenger domain through the translocon pore into the ER lumen. This event was tightly dependent on chain length and appears to be driven by signal sequence-mediated translocation through the Sec61 pore. We propose that global translocon structure is dynamic and modulated by substrate in such a manner as to assemble non-pore proteins into a stable larger functional unit during the translocation process.

**Unraveling the Long Distance Electron Transfer Mechanism: A Substrate Mediated Approach**

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Many bioactive peptides require amidation of their carboxy terminus to exhibit full biological activity. Peptidylglycine α-hydroxylation monooxygenase (PHM; EC 1.14.17.3) is the enzyme that catalyzes the first of the two steps of this reaction. PHM is composed of two domains, each of which binds one copper atom (CuH and CuM). These copper atoms are situated 11Å apart and PHM reaction requires electron transfer between these two sites. Long distance electron transfer in PHM has always been an intensely debated topic. We used stopped-flow to record the reduction kinetics spectrum and performed non-linear regression on the data to determine the key parameters. Reduction kinetics data showed that the electron transfer process is a biphasic process in absence of substrate. This becomes monophasic in presence of the substrate (Ac-YVG). Different PHM substrates (Hippuric acid, 4-Nitrohippuric acid and Ac-tyrosyl threonine) were also used with the same results. Kinetic data on CuH site ligands (CuH107A, CuH108A and CuH172A) showed loss of coupling between the copper centers. Hence, the reduction remained biphasic even in presence of the substrate. Both studies indicate that the substrate and the ligands of CuH center play important roles in coupling of the two copper centers and may provide an electron transfer pathway during the PHM catalytic cycle.

**The green pathway toward purple assembly of Thermus thermophilus CuA**

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Metalation of the Tt Cu₄ center is of great interest within the context of the assembly of the mature cytochrome c oxidase. In mammalian systems, this process is thought to occur via interactions with Sco1 and Sco2, redox-active components of the inner mitochondrial membrane, but in prokaryotes, other candidates such as Tt PCu₄C and its homologues have been implicated in the transfer chemistry. To gain a better understanding of the fundamental mechanisms underlying the metalation of Tt Cu₄, we have studied the mechanism of copper incorporation by simple inorganic Cu (II) and Cu (I) species, using a recently developed technique of selenium labeling of the Met 160 ligand. Here we present evidence of a novel Cu (II) mononuclear intermediate via stopped-flow, electron paramagnetic resonance (EPR) and x-ray absorption spectroscopy (XAS) at the Cu and Se edges. XAS and EPR spectroscopy of the Tt
CuA center incubated with less than stoichiometric amounts of Cu(II) show a type-2 mononuclear copper center ligated by two Cys and one His residue. However, Se edge EXAFS of the selenomethionine-labeled protein indicates that the Met 160 ligand is not coordinated in the intermediate. Stopped-flow data agree with the previously defined mechanism in which an early thiol-centered capture complex is rapidly converted into a mononuclear T2 intermediate, which is subsequently converted cleanly into the mixed-valence species. We also present the first evidence of a Cu(I) mononuclear species using Se and Cu XAS. The structural and electronic findings on these mononuclear species may aid in understanding which copper accessory proteins are involved in the in vivo maturation of the *Tt* CuA center.

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**Old drugs, new chemistry: unbridged, aryl-4-quinolone esters as novel antimalarials**

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For centuries, the treatment of malaria has been hampered by the inability of drugs to permanently and safely eliminate *Plasmodium* parasites. Although the *Plasmodium* life cycle is complex and presents a constantly-shifting pool of drug targets, most antimalarial drugs are effective only against late-emerging, blood stage parasites. In order to remedy this problem, recent drug development has focused on inhibitors of the parasitic electron transport chain, which have the dual benefit of reducing available energy and blocking pyrimidine biosynthesis in *Plasmodium*. One of the most promising families of parasitic mitochondrial inhibitors is that of the 4-quinolone esters. These drugs are well-established as anticoccidial agents in veterinary medicine, and have recently been characterized as potent inhibitors of blood, liver, sexual, and latent stage *Plasmodium* parasites. Unfortunately, the use existing 4-quinolone esters as antimalarials is limited by their poor solubility, low bioavailability, and high potential for drug resistance. This project demonstrates that these problems can be offset by replacing the 4-quinolone esters’ original, long hydrocarbon side chains with less planar, aromatic components. The resulting compounds demonstrate impressive activity against a range of *P. falciparum* strains in vitro, and the associated chemical synthesis is ideal for high-yield, iterative modification.

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**Peptides, Proteins and Networks: Removing obstacles and getting on the road to systems integration.**

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Data integration is a key component in systems biology [1]. Successfully unraveling complex disease etiology requires multiple data types, from a range of omic sources [2]. With our current methods and technology, genomics and proteomics are often seen as “worlds apart”. Integrative methods attempt to shrink that distance. Here we discuss a de-novo inference approach applied to proteomics data that not only assists in biomarker discovery, but also improves data QA/QC, discrepancy resolution and peptide-protein mapping that can improve data integration and subsequent downstream analyses.

In our recent work, we demonstrate the utility of de novo peptide and protein networks in the analysis of LC-MS shotgun proteomics. Like other biological networks, peptide and protein networks have an approximately scale-free topology [3]. In-silico validation of the Modules is done via permutation testing. With regard to biological and clinical relevance, we show that the module summaries, eigenvectors, correlate with biological phenotypes. Modules are
enriched in protein-protein interactions and functional annotation (gene ontology). By comparing the connectivity between module eigenvectors and peptide or protein abundance profiles, we can rank candidates by their role in the network. Peptides derived from the same protein have both a statistically higher topological overlap and concordance in abundance when considering connected sub-graphs, important for inferring protein abundance. These networks can aid in discovering novel biomarkers and aggregate signatures of disease.

In order to integrate de novo peptide networks with other sources of information, we face the problem of protein inference [4]. This problem concerns estimating the set of proteins present in the original biological mixture given the observed set of data. This problem is made difficult by degenerate peptides matching multiple proteins, their true source unknown. Our approach is in three parts. First, using a bipartite graph mapping observed peptides to potential parent proteins, we partition the full graph into four classes of “annotation sub-graph”. These annotation sub-graphs clearly illustrate individual types of problems found in protein inference. Second, abundance trends, topological overlap and co-expression module assignments provide rich sources of information in determining potential source proteins. Lastly, making several clear assumptions, we treat peptide signals in a manner analogous to RNA-Seq exon reads, and use mixture models to apportion peptide signal to high confidence protein parents. This allows us to discriminate between similar protein family members, improve protein identification and provide higher confidence data for downstream analysis and data integration.

To validate the method, simulated LC-MS datasets were produced using MSSimulator, part of the OpenMS / TOPP mass spectrometry software platform [5]. Protein abundance profiles (across samples) are sampled from distributions generated from the original LC-MS datasets. This produces multiple datasets containing random permutations of proteins with known abundances. The simulated data sets reflect real-world networks, and are another tool to aid in validating the protein inferences.

References:


Phosphoric acid released dentin matrix components affect pulp cell behavior

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Acids, such as those used in adhesive dentistry, have been shown to solubilize bioactive molecules from dentin. These dentin matrix components (DMC) may promote dentin remineralization.

**Objective:** The objective was to evaluate the potential for varying concentrations of DMC extracted from human dentin by phosphoric acid of varying pH to stimulate proliferation and mineralization of cultured pulp cells.

**Methods:** DMC were solubilized from ground human dentin (7 days-4°C) by phosphoric acid of pH 1, 3, 5 and EDTA (all with protease inhibitors). Extracts were dialyzed for 7 days in distilled water and lyophilized. Mouse dental pulp cells (MDPC-23) were exposed to DMC daily for 7 days (cell proliferation; 35 mm dishes; 100,000 cells/seeded) or 12 days (mineralization; 96 well plates; 5,000 cells/seeded) at concentrations (µg/ml) of 0.01, 0.1, 1.0 and 10.0 (mineralization only). Cell proliferation was measured by cell counting (Trypan blue; n=5) and WST-1 assay. Mineralization was assessed by Alizarin red assay (n=5). Controls were media (DMEM) and dexamethasone (DEX; mineralization). Results were analyzed by ANOVA/Tukey’s (p≤0.05).

**Results:** There was a dose-dependent trend for enhanced cell proliferation with DMP exposure (Figure-left), especially at lower concentrations (0.01 and 0.1) from the phosphoric acid extraction, however differences compared with DMEM did not reach significance. DEX exposure resulted in less cell growth compared with several of the phosphoric acid extracts (pH1-0.01; pH3-0.01;0.1,1.0; pH5-0.01,0.1). Cell counts correlated with WST-1 results (R²=0.59). Mineralization was significantly higher with DEX exposure (Figure-right). DMC exposure demonstrated significantly greater mineralization than DMEM for pH1-10.0 and 1.0, pH3-10.0 and 0.01, and pH5-10.0.

**Conclusion:** Human dentin matrix components solubilized by acids at pH levels relevant to those used in commercial dentin adhesives enhanced mineralization of mouse dental pulp cells when presented in certain concentrations, but did not show significant enhancement of cell growth.

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**Novel Use of One Dried Blood Spot for Tacrolimus and creatinine Determination in kidney transplant recipients**

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Monitoring therapeutic blood levels of immunosuppression drugs as well as kidney function are essential for kidney transplant recipients. Clinical success requires many blood draws early in therapy and then subsequent monthly clinical visits are required to monitor drug adherence and to ensure renal function. In young children, blood draws are difficult to obtain add the required clinic visits can result in significant absences from work and school and this can often lead to noncompliance of drug therapy and/or a failure to recognize inadequate dosing that can cause kidney damage. Dried blood spots (DBS) are being used more extensively to measure drug levels. The preparation of DBS is less invasive than whole blood draws and can be performed at home by patients. The aim of this study was to develop and validate analytical methods using a single dried blood spot on filter paper to determine both tacrolimus (TAC) for immunosuppression monitoring and creatinine (Cr) to assess kidney function and to then compare these values to the standard blood tests performed in the clinical laboratory.
**Methods:** Twenty one patient (ages 2 to 20) with kidney transplants participated in the study during routine clinic visits. DBS were prepared on Whatman FTA DMPK-A cards from the index finger following a finger prick with assistance from clinic staff at the same time a venous blood sample was taken for the determination of TAC by radioimmunoassay and Cr in the clinical laboratory using the automated Jaffe picric acid colorimetric method. A 6 mm punch was made from the center of the spot and TAC and Cr were determined using liquid chromatography tandem mass spectrometry (LC-MS/MS) from a single spot after extraction with 250 µl of methanol/acetonitrile (80:20) containing 1 ng of the internal standard, ascomycin, for TAC and creatinine (methyl-d₃) for Cr. A 15 µl aliquot was removed for Cr determination and both were measured using LC-MS/MS with selected reaction monitoring.

**Results:** For TAC the intra-day assay variation was 3.1% at 3 ng/ml and 1.33% at 30 ng/ml (N=6) and the inter-day variation was 5.85% and 4.43% (N=12) at the low and high concentrations, respectively. The lower limit of quantification was 1.0 ng/ml with a RSD of 11.1% (N=6). Since Cr is present in all blood samples, intra- and inter-day variability was determined on pooled samples spiked with an additional 0.5 and 5.0 mg/dL of Cr. The bias was 3.75% at 0.5 mg/dL and 2.45% at 5.0 mg/dL (N=6) and intra-day variability was 4.11% (N=6) and inter-day variability of 3.79% (N=18) for a sample containing 0.82 mg/dL Cr. Using these assays we then compared the values of TAC and Cr with the clinical values from 21 patients seen in the OHSU clinic after kidney transplantation. The results demonstrated excellent comparisons. For Cr the correlation coefficient (R²) was 0.890, P<0.001 with a slope of 1.002 and an intercept of 0.170. For TAC the correlation coefficient (R²) was 0.742, P<0.001 with a slope of 0.969 and an intercept of 0.272. Only 18 samples were compared for TAC since two patients refused the finger prick and one had a clinical value reported as < 2 ng/ml the lower limit of quantification for the RIA used in the clinical lab. For that particular patient the DBS had no detectable levels of TAC. There is an excellent correlation between the values obtained with the newly developed DBS method and the clinical laboratory for both Cr and TAC. As a result, with this assay developed we are prepared to determine if a similar correlation can be obtained when patients prepare their own blood spots in the clinic and at home.

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**Examination of βB2/βB3 crystallin heterodimer conformation using hydrogen/deuterium exchange**

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**Purpose:** Ordered structures in crystallins, the major proteins of the lens, are required to maintain vision. During normal aging and cataracts, aberrant interactions in crystallins can lead to protein aggregation and light scatter. In order to identify these aberrant interactions, the normal structure of lens crystallins must first be defined. βB1- and βB2-crystallin homodimers are two lens proteins whose x-ray crystallographic structures are known. βB1-crystallin has closed conformation with a bent linker connecting its N- and C-terminal domains, while βB2-crystallin has an open conformation with an extended linker. The purpose of this study was to examine the structures of βA3 homodimer and βB2/βA3 heterodimer, both of which have not been crystallized, and have unknown structures.

**Methods:** Complexes were formed after incubating recombinant human βA3 and βB2 crystallins at 37 degrees C for 18 hours. Heterodimers were isolated by size exclusion chromatography and confirmed by blue-native electrophoresis.
Solvent accessibility changes were detected by analysis with hydrogen/deuterium exchange coupled to high-resolution mass spectrometry.

**Results:** The average deuterium uptake per exchangeable residue was greatest for the N- and C-terminal extensions of \( \beta \)A3 and the N-terminal extension of \( \beta \)B2, with no change upon complex formation, suggesting the extensions were not involved in heterodimer formation. Heterodimers also exhibited significant decreases in exchange in residues 70-84 and 121-163 of bB2. Regions of greatest decrease were localized at an interface in bB2 known to be important in \( \beta \)B2 homotetramer formation, suggesting this interface was involved in complex formation with \( \beta \)A3.

**Conclusion:** The data suggests that formation of \( \beta \)B2/\( \beta \)A3 crystallin heterodimer induces a closed conformation in \( \beta \)B2 leading to a more compact structure analogous to bB1-crystallin homodimer. The results demonstrate the utility of hydrogen/deuterium exchange to probe the structure of lens crystallins. Such data will provide a foundation to determine how changes in these structures lead to cataracts in aged lenses.
Anna Cedar | Validity of a web 2.0 instrument designed to assess needs of medical students and residents in diagnosing and managing pediatric respiratory emergencies

Megan Herting | Differences in brain activity during a verbal associative memory-encoding task in high and low-active adolescents

Cindy Mcevoy | Increased PRegravid BOdy Mass Index is Associated with Subsequent Bronchodilator Prescriptions in Early CHildhood.

Nichole Hildebrandt | Nurturing Healthy & Empowered Youth thru filmmaking

Validity of a web 2.0 instrument designed to assess needs of medical students and residents in diagnosing and managing pediatric respiratory emergencies

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Background: Assessment of learners’ knowledge is critical in curriculum development though there are few validated assessment tools and little is published on educational needs of trainees.

Objectives: We developed a free online video-based instrument to identify knowledge and clinical reasoning deficits of medical students and residents for pediatric respiratory emergencies. We hypothesized that it would be a feasible and valid method of differentiating educational needs of different levels of learners.

Methods: This was an observational study of a free, web-based needs assessment instrument that was tested on 44 third and fourth year medical students (MS3-4) and 29 pediatric and emergency medicine residents (R1-3). The instrument uses YouTube video triggers of children in respiratory distress. A series of case-based questions then prompts learners to distinguish between upper and lower airway obstruction, classify disease severity, and manage uncomplicated croup and bronchiolitis. Face validity of the instrument was established by piloting and revision among a group of experienced educators and small groups of targeted learners. Final scores were compared across groups using t-tests to determine the ability of the instrument to differentiate between different levels of learners (concurrent validity). Cronbach’s alpha was calculated as a measure of internal consistency.

Results: Response rates were 19% among medical students and 43% among residents. The instrument was able to differentiate between junior (MS3, MS4, and R1) and senior (R2, R3). learners for both overall mean score (61% vs.78%, p<0.01) and mean video portion score (74% vs. 84%, p=0.02). Table 1 compares results of several management questions between junior and senior learners. Cronbach’s alpha for the test questions was 0.47.
**Conclusions:** This free online video based needs assessment instrument is feasible to implement and able to identify knowledge gaps in trainees’ recognition and management of pediatric respiratory emergencies. It demonstrates a significant performance difference between the junior and senior learners, preliminary evidence of concurrent validity and identifies target groups of trainees for educational interventions. Future revisions will aim to improve internal consistency.

Table 1. Needs Assessment Results

<table>
<thead>
<tr>
<th></th>
<th>Juniors n=57</th>
<th>Seniors n=16</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordered CXR for bronchiolitis</td>
<td>91%</td>
<td>88%</td>
<td>0.7</td>
</tr>
<tr>
<td>Ordered steroids for croup %</td>
<td>56%</td>
<td>88%</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ordered albuterol for croup %</td>
<td>26%</td>
<td>13%</td>
<td>0.2</td>
</tr>
<tr>
<td>Identified normal respiratory rate in 1 month old</td>
<td>47%</td>
<td>63%</td>
<td>0.3</td>
</tr>
<tr>
<td>Identified normal respiratory rate in 1 year old</td>
<td>40%</td>
<td>63%</td>
<td>0.1</td>
</tr>
</tbody>
</table>

**Differences in brain activity during a verbal associative memory-encoding task in high and low-active adolescents**

Megan M. Herting* and Bonnie J. Nagel

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Aerobic fitness is associated with better memory performance, as well as larger volumes in memory-related brain regions, in children, adults, and elderly. It is unclear if aerobic exercise also influences learning and memory-related functional neural circuitry. In the current study, we examined brain activity in 17 high-active (HA) and 17 low-active (LA) adolescents during a subsequent memory encoding paradigm using functional magnetic resonance imaging (fMRI). Participants were presented with a series of novel word-pairs to learn in the scanner, and then performed a post-scan recognition task. Despite similar memory performance, LA youth displayed a number of differences in memory-related and default mode brain regions compared to their HA peers during encoding of word-pairs later remembered versus those later forgotten. While HA youth displayed robust deactivation in default mode brain areas during successful encoding of later remembered word-pairs, including the ventral medial prefrontal cortex and posterior cingulate cortex, LA youth did not show this pattern (p < .01). Furthermore, LA youth showed greater bilateral hippocampal (p < .05) and right superior frontal gyrus (p < .01) activation during encoding of later remembered versus forgotten word-pairs; presumably reflecting compensatory processes to allow LA to accomplish similar memory performance to their HA peers. The present study is the first to examine the impact of aerobic fitness on hippocampal function and memory-related neural circuitry using fMRI. Taken together with previous research, these findings suggest aerobic activity can influence brain function in youth and provide support for memory-related benefits of aerobic exercise across the lifespan.

**Increased Pregravid Body Mass Index is Associated with Subsequent Bronchodilator Prescriptions in Early Childhood**

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**Rationale:** Obesity and asthma are two concurrent worldwide health care crises that may be linked. Previous studies have utilized parental recall and questionnaires to evaluate the impact of pregravid body mass index (BMI) on subsequent childhood respiratory health. We hypothesized that the prescription rate for inhaled beta agonists bronchodilators (BD), as a surrogate marker for reactive airway disease, would be greater in children born to obese pregravid BMI women than that of children born to normal pregravid BMI women.

**Methods:** A retrospective cohort study was performed using a validated electronic medical record (EMR) in Kaiser Permanente Northwest (KPNW), a group practice health maintenance organization. Singleton gestations resulting in a live birth occurring between 1/1/2000 and 12/31/2005 were identified. The primary outcome was the prescriptions for bronchodilators in the children through 3.99 years of age as documented through the KPNW pharmacy dispensing records. Pregnancies were identified using a previously published and validated algorithm (Obstet Gynecol 2009; 114:1069-75). Pregravid BMIs (documented at -6 to +3 months of pregnancy onset) were categorized as underweight, normal, overweight, obese, and extremely obese using the 2009 Institutes of Medicine criteria, outlined in Table 1. Exclusion criteria included: births < 24 weeks of gestation, major chromosomal anomalies, < 1 year of infant enrollment in KPNW, mother baby pairs missing either maternal BMI or infant birth weight. Logistic regression was used to examine the associations between pregravid BMI and BD prescriptions, adjusting for the confounders of Medicaid insurance, maternal smoking, and maternal history of asthma. We also examined maternal comorbidities, gestational age at delivery, delivery mode, infant age and weight at time of first BD, and duration of KPNW enrollment of infant as confounding factors.

**Results:** 8,237 singleton pregnancies were identified. Maternal characteristics included: median age 28 yrs; median parity 1; 68% Caucasian; 5.6% public insurance; 12.2% smoked during pregnancy; 16.2% maternal asthma; 4.4% diabetes; 10% gestational hypertension. Infant characteristics included: 50% female; median gestational age 39.6 wks; 25.1% delivered by Cesarean section; 13.4 % large for gestational age; 6.4% small for gestational age. Pregravid obesity/extreme obesity occurred in 23.7% of this population and there was a association between increased pregravid BMI and BD prescriptions in their children (Table). At BD initiation, there were no differences in the median age (range 422-461 days) or median weight (20.9-21.8 kg) between the children according to maternal pregravid BMIs.

**Table. Association of Maternal Pregravid BMI and Bronchodilator Prescription in Early Childhood**

<table>
<thead>
<tr>
<th>Categories</th>
<th>Pregravid BMI (kg/m²)</th>
<th>Total N</th>
<th>BD Tx (%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Underweight</td>
<td>&lt;18.5</td>
<td>178</td>
<td>17.4%</td>
<td>0.63 (0.42-0.93)</td>
<td>0.64 (0.43-0.95)</td>
</tr>
<tr>
<td>Normal</td>
<td>18.5-24.9</td>
<td>3934</td>
<td>25.2%</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Overweight</td>
<td>25-29.9</td>
<td>2165</td>
<td>28.1%</td>
<td>1.16 (1.03-1.31)</td>
<td>1.14 (1.01-1.29)</td>
</tr>
<tr>
<td>Obese</td>
<td>30-39.9</td>
<td>1577</td>
<td>30.2%</td>
<td>1.28 (1.13-1.46)</td>
<td>1.19 (1.04-1.36)</td>
</tr>
<tr>
<td>Extremely Obese</td>
<td>≥40</td>
<td>383</td>
<td>31.1%</td>
<td>1.34 (1.06-1.68)</td>
<td>1.16 (0.92-1.46)</td>
</tr>
</tbody>
</table>

*Adjusted for maternal smoking, medicaid insurance and maternal history of asthma

**Conclusions:** Increased maternal pregravid BMI was associated with increased bronchodilator prescriptions in their children. In this cohort, pregravid underweight was associated with significantly decreased bronchodilator prescriptions in their children and may reflect demographic factors in this subgroup. Our findings support the hypothesis that the chronic inflammatory state associated with obesity programs fetal lungs to airway reactivity in early childhood.

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**The Healthy & Empowered Youth Project (HEY)**
American Indian youth are more likely to be sexually active, and demonstrate a higher prevalence of STDs and HIV/AIDS in both rural and urban settings. Further, the use of illicit substances is high in Native teens, putting them at additional risk for STDs, HIV, teen pregnancy and sexual violence. “Native STAND”, an adaptation of the STAND curriculum previously demonstrated to be effective in rural Georgia, has been implemented for 66 Native teens in a Jr/Sr high school in a Northwest reservation community. We have evaluated the curriculum to determine its effectiveness in the domains of academic enrichment, life skills, personal development and enrichment. An additional innovation is skill development in film-making, with the intent to empower youth to create risk messages for peers that are relevant to the perspectives of Native teens and result in diffusion to other tribal communities. Several short films have been produced to date and students have participated in a regional film festival. This prevention education intervention is in its second year, and we will present measures of shifts in knowledge, attitudes and behaviors, as well as examples of student film projects. The combination of film-making and media literacy with conventional classroom sexual health education shows potential for ensuring engagement and retention of youth, and provides a means for dissemination of risk messages to peers. This project was funded by the Youth Empowerment Program of the Office of Minority Health, US Department of Health and Human Services (1MP090037).
Stephen Hyter | ET-1 is a transcriptional target of p53 in epidermal keratinocytes and in a non-cell autonomous manner controls UV radiation-induced melanocyte homeostasis in vivo

Zhixing Wang | Selective ablation of keratinocyic Ctip2/Bcl11b triggers AD-like skin inflammatory responses in adult mice

Renato Goreshi | Double-blind, randomized, bilateral comparison of skin irritancy following application of the combination acne products clindamycin/tretinoin and benzoyl peroxide/adapalene

Amala Soumyanath | Piperine and melanoma: a crucial issue for future clinical trials in vitiligo

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**ET-1 is a transcriptional target of p53 in epidermal keratinocytes and in a non-cell autonomous manner controls UV radiation-induced melanocyte homeostasis in vivo**

Stephen Hyter\(^1,2\), Daniel Coleman\(^1,2\), Steven Ma\(^3\), Masashi Yanagisawa\(^4\), Gitali Ganguli Indra\(^1,2\) and Arup K. Indra\(^1,2,5,6\)

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Melanoma, the deadliest form of skin cancer, arises due to transformation of pigment producing melanocytes within the skin. Proliferation and differentiation of melanocytes can be orchestrated by various keratinocyte derived growth factors and signaling pathways. Alteration in melanocyte homeostasis is the source of many pathological conditions, including melanoma. Previous *in vitro* studies have shown that ultraviolet B (UVB) exposure increases secretion of keratinocyte derived endothelin-1 (ET-1), a 21 amino acid signaling peptide, which regulates proliferation, melanogenesis, migration and dendricity of human melanocytes via a receptor-mediated pathway. However, the *in vivo* role of ET-1 in skin homeostasis and in melanogenesis is unknown.

We recently characterized the *in vivo* function of keratinocytic endothelin-1 (ET-1) in modulating keratinocyte and melanocyte homeostasis. To that end, the gene encoding ET-1 was selectively ablated from epidermal keratinocytes using the Cre-LoxP strategy to generate the ET-1\(^{ep-/}\) mouse line. We investigated melanocyte activation, proliferation and migration post-UV irradiation in ET-1\(^{12/+}\) (control) and ET-1\(^{ep-/}\) mice. Interestingly, a significant decrease in both epidermal and dermal melanocyte population was observed in the mutant skin, 72 and 96 hours post-UVR. Results indicated a non-cell autonomous role of ET-1 in regulating melanocyte homeostasis *in vivo*. Interestingly, expression of other paracrine factors such as SCF, aMSH, HGF and FGF2 could not compensate for the loss of ET-1 in the epidermis.

Selective inhibition of ET-1 receptor (Ednrb) in cultured melanocytes abrogated downstream signaling by inhibiting PKC activation and MAPK pathway. Furthermore, topical treatment of neonatal wild type mice with Ednrb inhibitor
abrogated melanocyte activation induced by UVB irradiation and recapitulated the effects on melanocyte homeostasis observed in the ET-1$^{pp/-}$ transgenic mice. We discovered that transcription factor p53 directly and positively regulate ET-1 expression in epidermal keratinocytes post UVB irradiation. Altogether, results indicate that ET-1 in epidermal keratinocytes is essential in mediating UV induced melanocyte homeostasis and provide photo-protection in a murine model.

Selective ablation of keratinocytic Ctip2/Bcl11b triggers AD-like skin inflammatory responses in adult mice

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$^{2}$Environmental Health Science Center, OSU, Corvallis, OR
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Atopic dermatitis (AD) is a long-term (chronic) skin inflammatory disorder that is characterized by pruritic and eczematoid skin, epidermal barrier defects and systemic immunological abnormalities. AD is associated with defective epidermal barrier. In the early development of AD, defective barrier allows allergens to penetrate through skin and interact with immune cells. Chicken ovalbumin upstream promoter transcription factor (COUP-TF)-interacting proteins 2 (Ctip2) is crucial in epidermal homeostasis and permeability barrier formation. Ablation of Ctip2 in murine epidermal keratinocytes leads to AD-like inflammatory responses in a Th2-dependent manner, which is initiated by TSLP overexpression in epidermis. Barrier defects in Ctip2$^{pp/-}$ mice can possibly increase the susceptibility to allergens, and trigger secondary inflammatory reactions in Ctip2$^{pp/-}$ adult mice.

Double-blind, randomized, bilateral comparison of skin irritancy following application of the combination acne products clindamycin/tretinoin and benzoyl peroxide/adapalene

Renato Goreshi; Aman Samrao; Benjamin D. Ehst

Department of Dermatology, Oregon Health & Science University, Portland, OR

We sought to compare the tolerability of two combination topical acne products, clindamycin 1.2%-tretinoin 0.025% (CLIN/RA) gel and benzoyl peroxide 2.5%-adapalene 0.1% (BPO/RA) gel. The use of topical medications for acne vulgaris is often limited by their irritant properties. Newer combination preparations are available and offer convenience but irritant potential may still be a hindrance, perhaps more so with the addition of two agents. Few studies have compared these formulations directly for tolerability. CLIN/RA and BPO/RA were applied daily to opposite sides of a subject’s face for 21 days in a double-blinded fashion. Investigator and study subject self assessments of burning/stinging, itching, erythema, and dryness/scaling were collected. Transepidermal water loss (TEWL) was also measured as an objective measure of skin irritation. A mixed model analysis and repeated measures ANOVA were used to compare outcomes for both acne formulations. CLIN/RA produced significantly less burning/stinging than BPO/RA (p<0.001) as well as significantly less pruritus than BPO/RA (p<0.001). BPO/RA caused significantly more TEWL than CLIN/RA (p=0.005). There was no significant difference in the amount of erythema or the amount of dryness/scaling caused by either formulation. In conclusion, CLIN/RA produced significantly less skin irritancy and TEWL than BPO/RA.
Piperine and melanoma: a crucial issue for future clinical trials in vitiligo [OCTRI]

Philippe Thuillier¹, Julia Sonka¹, Kevin Phillips²,³, Ravikant Samatham³, Aznegashe Yelma¹, Neha Reddy¹, Meenakshi Vanka⁴, Satya Tatapudi¹, Justin Williams¹, Vikram Sankar¹, Steven Jacques²,³ and Amala Soumyanath⁴*

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²Dept. of Dermatology, Oregon Health & Science University, Portland, OR
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*Presenting author: soumyana@ohsu.edu

Piperine (PIP), a compound found in black pepper, is a novel, potential treatment for the depigmentary skin disease vitiligo. We have shown that PIP, stimulates the proliferation of murine and human melanocyte cell lines as well as human primary melanocytes in reconstructed epidermis. Applied in vivo, PIP increases melanocyte density and pigmentation of sparsely pigmented mice, an effect enhanced by concomitant administration of UV radiation. This suggests that PIP could be used to stimulate repigmentation in vitiligo, potentially in conjunction with UVB therapy. However, PIP’s stimulatory effect on melanocyte proliferation gives rise to concerns about potential effects on melanoma development, particularly if used with UVB radiation. Clearly, this concern must be addressed prior to initiating clinical investigations of PIP in human vitiligo. In vitro, PIP inhibits the growth of murine B16 F10 melanoma cells in monoculture, and human melanoma cells in reconstructed dermis. We are currently performing studies in the HGF/SF BL6 mouse – a murine model susceptible to melanoma development. We will evaluate the effects of long term application of PIP with and without UVB on melanoma development. We are optimistic that data from this project will support PIP’s lack of melanomagenic effect, and accelerate the translation of our preclinical studies into clinical trials of PIP in humans with vitiligo.

Studies in reconstructed skin were funded by AdPharma Inc. The HGF/SF BL6 mouse study is funded by a T1 translational grant from OCTRI, and an equipment grant from AdPharma.
Matthew McCarroll | Graded levels of Pax2a and Pax8 regulate cell fate during sensory placode formation

Molly Harding | FGF-Ras-MAPK signaling drives apical constriction via apical positioning of Rho-kinase during mechanosensory organ formation.

Kateryna Kyrylkova | Integration of BCL11B into the FGF Signaling Network in the Mouse Incisor

Jenna Ramaker | Interactions between Amyloid Precursor Proteins and the heterotrimeric G protein Goα may regulate neuronal migration.

Katie Kindt | Kinocilia mediate mechanosensitivity in developing zebrafish hair cells

Biliana Veleva | A Role for Pseudokinases During Development of the Cerebral Cortex

Graded levels of Pax2a and Pax8 regulate cell fate during sensory placode formation

Matthew N. McCarroll*, Zachary R. Lewis, Maya Deza Culbertson, Benjamin L. Martin, David Kimelman, Alex V. Nechiporuk

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*Corresponding author mccarrom@ohsu.edu

Haploinsufficiency of pax genes cause a variety of congenital defects including renal-coloboma syndrome, which results from mutations in pax2 and is characterized by kidney hypoplasia, optic nerve malformation, and hearing loss. While this underscores the importance of pax gene dosage in normal development, how differential levels of these transcriptional regulators control cellular fate decisions and subsequent tissue morphogenesis is still poorly understood. In this study, we show that differential levels of zebrafish Pax2a and Pax8 direct fate decisions in the multipotent cells that eventually contribute to the otic vesicle and the epibranchial placodes. Initially, a subset of epibranchial placode precursors is comingled with the otic precursors within a single Pax2a/8-positive domain; these cells exhibit cellular movements as they segregate into distinct placodes. Using lineage tracing and ablation analyses, we show that cells in the Pax2a/8+ domain become biased towards certain fates at the beginning of somitogenesis. Experiments involving either Pax2a overexpression or partial loss-of-function of both Pax2a and Pax8 reveal that high levels of Pax expression bias cells towards an otic fate while low levels of Pax expression bias cells toward an epibranchial fate. In addition, Pax2a levels are controlled by FGF and Wnt signaling pathways: FGF is necessary to induce Pax2a, whereas Wnt activity is required to induce the high levels of Pax2a that bias cells toward an otic fate. Our studies reveal the importance of Pax levels during sensory placode formation and provide a mechanism by which these levels are controlled.

FGF-Ras-MAPK signaling drives apical constriction via apical positioning of Rho-kinase during mechanosensory organ formation

Molly Harding and Alex Nechiporuk

Department of Cell and Developmental Biology, Oregon Health & Science University, Portland OR
Cell shape changes driven by apical constriction, a narrowing of the apical side of the cell, are important for diverse developmental processes, ranging from gastrulation to neural tube closure. In polarized epithelial cells, apical constriction is required for activation of an apically localized acto-myosin network; however, how this process is controlled in the context of organ formation is not well understood. We asked how extracellular signals regulate formation of polarized epithelial rosettes in the zebrafish posterior lateral line primordium (pLL). The pLL is a patterned group of ~100 cells that migrates along the trunk of the zebrafish during embryonic development. The migrating pLL is organized into polarized rosettes. Within rosettes, cells are polarized: they are apically constricted, with nuclei basally displaced. During the course of migration, the pLp deposits mature rosettes from the trailing edge. Concurrently, new cells are generated, polarized and incorporated into nascent rosettes in the leading edge. We have previously demonstrated that rosette renewal in the pLL is dependent on FGF signaling. However, how extracellular FGF ligands are transduced intracellularly to control cell shape is not known. Here, we show that FGF signaling activates intracellular Ras-MAPK to drive these cell shape changes. Using pharmacological and genetic approaches, we demonstrate that loss of FGF-Ras-MAPK signaling causes a loss of apical constriction. This failure was correlated with a loss of apically localized Rho-kinase 2a (Rock) in the non-constricted cells. Additionally, loss of apically localized Rock2a resulted in failed activation of the acto-myosin cytoskeleton. Importantly, other cytoskeletal and polarity components, including f-actin, myosin-II and polarity molecules, remained undisturbed, demonstrating selectivity of this FGF dependent phenotype. Using mosaic analyses, we also show that apical constriction and Rock localization are driven by a cell autonomous Ras-MAPK signal. Based on these data, we propose a model where extracellular FGF signals through Ras-MAPK to induce apical localization of Rock in cell-autonomous manner, which in turn activates acto-myosin network to drive apical constriction and rosette formation.

Integration of BCL11B into the Fibroblast Growth Factor Signaling Network in the Mouse Incisor
Kateryna Kyrylkova*, Sergiy Kyryachenko, Ophir Klein, Chrissa Kioussi, Mark Leid
*Corresponding author email: kryylkok@onid.orst.edu

Development of the mammalian tooth has been intensively studied as a model system for epithelial-mesenchymal interactions during organogenesis. Furthermore, the mouse incisor regenerates constantly and thus is an excellent model to analyze regulatory pathways that affect dental stem cells. In addition, enamel on the mouse incisor is deposited asymmetrically, which allows the study of differential patterning of the tooth. We have previously shown that mice lacking the transcription factor BCL11B/CTIP2 (BCL11B hereafter) exhibit severely disrupted formation of enamel-secreting cells, ameloblast, in the developing incisor. We now report that BCL11B is a key factor controlling epithelial proliferation and overall developmental asymmetry of the mouse incisor: BCL11B is necessary for proliferation of the labial (facing the lip) epithelium and development of the epithelial stem cell niche, which gives rise to ameloblasts; conversely, BCL11B suppresses epithelial proliferation, and development of stem cells and ameloblasts on the lingual (facing the tongue) side of the incisor. This bidirectional action of BCL11B in the incisor epithelia appears responsible for the asymmetry of ameloblast localization in developing incisor. Underlying these spatio-specific functions of BCL11B in incisor development is the regulation of a large gene network comprised of genes encoding several members of the FGF, Sprouty proteins that encode FGF antagonists, and Sonic hedgehog. Our data integrate BCL11B into these pathways during incisor development and reveal the molecular mechanisms that underlie phenotypes of both Bcl11b<sup>−/−</sup> and Sprouty mutant mice. In conclusion, we demonstrated that BCL11B functions as a critical regulator of proper incisor development, and this study may contribute to the generation of the bioengineered teeth.
Interactions between Amyloid Precursor Proteins and the heterotrimeric G protein Goα may regulate neuronal migration

JM Ramaker, TL Swanson, PF Copenhaver

Amyloid Precursor Protein (APP) is a transmembrane protein that is cleaved to produce the amyloid peptides that accumulate in Alzheimer’s disease, but APP is also strongly expressed by neurons during development and following injury. Both full-length APP and its cleaved fragments may affect neuronal motility and remodeling, although attempts to characterize the physiological role of APP in the mammalian nervous system have produced conflicting results, in part due to molecular redundancy with two closely related proteins (APLP₁ and APLP₂). To address this issue, we have exploited Manduca sexta (hawmoth) as a novel preparation for investigating how APP family proteins control neuronal motility in vivo. Unlike vertebrates, insect neurons express only one APP ortholog (APPL, for APP-Like), greatly simplifying an analysis of its normal function. During the formation of the enteric nervous system (ENS) in Manduca, APPL and Goα are co-expressed by developing neurons during active phases of their migration. Knocking down APPL produced a distinctive pattern of ectopic migration and outgrowth, similar to the effects of altering Goα activity. These observations support the hypothesis that APP and Goα function in a convergent pathway to regulate neuronal motility. To test whether APPL functions as a Goα-associated receptor, we first showed that APPL co-immunoprecipitates with endogenous Goα in Manduca embryonic lysates, and that stimulating Goα activity decreased binding between Goα and APPL. Likewise, inhibiting Goα activity in lysates increased APPL-Goα interactions, supporting the model that APPL binds inactive, but not active Goα. We then used Bimolecular Fluorescence Complementation (BiFC) assays to show that APPL can directly bind Goα, but not other related G proteins, in exogenous cell lines. We have also generated transgenic Drosophila lines to show that APPL and Goα directly interact in vivo when co-expressed in photoreceptors. We are currently using embryo culture assays to define the precise role of APPL in regulating specific aspects of neuronal motility, and to determine whether these effects require Goα activation.

Kinocilia mediate mechanosensitivity in developing zebrafish hair cells

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Mechanosensitive cilia are vital to signaling and development across many species. In sensory hair cells, sound and movement is transduced by bundles of apical cilia. Each bundle is comprised of a single primary cilium (kinocilium) flanked by multiple rows of actin-filled projections (stereocilia). Extracellular tip links that interconnect stereocilia are thought to gate mechanosensitive channels. In contrast to stereocilia, kinocilia are not critical for hair-cell mechanotransduction. However, by sequentially analyzing the structure and activity of individual hair bundles in vivo, we have uncovered a novel role for kinocilia in mechanosensation during development. Our data demonstrate that nascent hair cells require kinocilia and kinocilial links for mechanosensitivity. Although nascent hair bundles have correct planar polarity, the polarity of their responses to mechanical stimuli is initially reversed. Later in development, a switch to correctly polarized mechanosensitivity coincides with the formation of tip links and the onset of tip link-dependent mechanotransduction.

A role for pseudokinases during development of the cerebral cortex
Approximately 10% of the mammalian kinome encodes proteins that lack critical catalytic residues, but it remains unclear how these functionally inactive kinases contribute to cellular physiology. Ste20-related adapter protein α and β (STRADα and STRADβ) are two of these pseudokinases. To date, the only known functions of STRADα and STRADβ involve the allosteric activation and translocation of the protein kinase LKB1, but a recent report has identified a human pediatric syndrome, Polyhydramnios, Megalencephaly, and Symptomatic Epilepsy (PMSE) resulting from a partial deletion of the STRADα gene. PMSE patients exhibit several CNS symptoms including cell migration defects, severe cognitive delay, and infantile-onset epilepsy. To better understand the STRAD proteins’ functions during development, we have characterized their expression in the mouse cortex. STRADα is observed in both progenitors and post-mitotic neurons in the developing cortex while STRADβ is expressed primarily in post-mitotic neurons. We have previously demonstrated a requisite role for LKB1 in the establishment of neuronal polarity during cortical development, but the mechanisms by which STRADα loss leads to PMSE Syndrome remain unclear. We are exploring a loss of function model for STRADα in order to better understand the etiology of PMSE, and our results suggest a critical role for this pseudokinase during brain development.

Funding for this work: Developmental Biology Training Grant and The Doernbecher Foundation
**Opioid Sensitivity in Mice Selectively Bred to Consume or Not Consume Methamphetamine**

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Genetic factors likely influence individual susceptibility to escalating MA use. Replicate mouse lines were produced that consume high (MAHDR) or low (MALDR) amounts of MA in a two-bottle choice MA drinking (MADR) procedure. Selective breeding was used to aggregate risk alleles in one line and protective alleles in the other. Quantitative trait locus (QTL) analysis identified a QTL on mouse chromosome 10 in both sets of lines, mapping near the mu-opioid receptor (MOP-r) gene, Oprm1. MALDR mice have greater expression of Oprm1 than MAHDR mice in the medial prefrontal cortex. To examine differences between the lines in opioid system sensitivity, the magnesium sulfate abdominal writhing test was used. The MADR lines did not differ in sensitivity to the analgesic effects of the MOP-r agonist, fentanyl, consistent with results from other nociception tests. To assess differences in aversity for opioids, morphine drinking was measured. MALDR mice consumed more morphine than MAHDR mice in a two-bottle choice morphine drinking study, using a saccharin fading procedure. These data are consistent with previous results for morphine versus quinine drinking. Finally, because MALDR mice exhibit higher Oprm1 expression, naltrexone, a MOP-r antagonist, was hypothesized to enhance drinking; however, this result was not obtained. We speculate that extreme avoidance of MA by the low line may provide an explanation. These results support a negative genetic correlation between the consumption of MA and opioids and support additional consideration of Oprm1 as a candidate gene that influences differences in MA consumption between the MADR lines.

Funding Support: NIDA T32 DA07262, Department of Veterans Affairs and NIDA P50 DA018165.

**Effects of Sodium Butyrate on Methamphetamine Sensitized Locomotor Activity**

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Neuroadaptations associated with behavioral sensitization induced by repeated exposure to methamphetamine (MA) appear to be involved in compulsive drug pursuit and use. Increased histone acetylation, an epigenetic effect resulting in altered gene expression, may promote sensitized responses to psychostimulants. The role of histone acetylation in MA-induced locomotor sensitization was examined by measuring the effect of inhibiting histone deacetylase with sodium butyrate (NaB) on expression and acquisition of sensitization. For the effect on expression, vehicle or NaB (630 mg/kg, ip.) was administered 30 min prior to MA challenge in mice treated repeatedly with MA (10 days of 2 mg/kg MA) or saline (10 days) and then locomotor response to MA challenge was measured. NaB treatment increased the locomotor response to MA in both acutely MA treated and sensitized animals. For acquisition, NaB was administered 30 min prior to each MA exposure (10 days of 1 or 2 mg/kg), but not prior to the MA challenge test. Treatment with NaB during the sensitization acquisition period significantly increased locomotor activation in sensitized mice only. NaB alone did not significantly alter locomotor activity. Acute NaB or MA, but not the combination, appeared to increase striatal acetylation at histone H4. Repeated treatment with MA, but not NaB or MA plus NaB, increased striatal acetylation at histone H3. Although increased histone acetylation may alter the expression of genes involved in acute locomotor response to MA and in the acquisition of MA-induced sensitization, results for acetylation at H3 and H4 showed little correspondence with behavior.

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**Impulsivity Related to Subjective Nicotine Withdrawal**

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**Rationale.** Previous research indicated that relapse during smoking cessation attempts is more likely for individuals who reported that they were more impulsive, where impulsivity is characterized as a relatively increased sensitivity to immediate events and decreased sensitivity to delayed events. Increased sensitivity to short-term withdrawal (i.e., a heightened sensitivity to an immediate aversive event) may be one mechanism that prevents highly impulsive smokers from stopping smoking.

**Objectives.** The objective of this study was to assess the relationship between baseline levels of impulsivity and subjective reports of nicotine withdrawal during an abstinence period in smokers not engaged in a quit attempt.

**Methods.** Twenty-nine healthy smokers who reported smoking an average of 20.5 cigarettes per day participated. Participants served as their own controls as we examined alterations in impulsivity and nicotine-withdrawal/smoking-urges across two deprivation conditions (abstinence duration: 0 and 48 hours, order counterbalanced). Impulsive decision-making was assessed using 3 questionnaires (Barratt Impulsivity Scale version 11 [BIS-11]; Sensation-Seeking Scale Form V [SSS-V]; and Tridimensional Personality Questionnaire [TPQ]) and 1 behavioral computer task (Delay Discounting). Nicotine-withdrawal/smoking-urges were assessed using the Minnesota Withdrawal Scale [MNWS] and the Questionnaire on Smoking Urges [QSU] at several time points during each condition. Abstinence from smoking was confirmed by breath and cotinine measures.

**Results.** Participants reported typical subjective effects of nicotine withdrawal as measured by the MNWS and QSU. However, the measures of impulsivity were not related to measures of nicotine-withdrawal/smoking-urges.
Conclusions. While nicotine abstinence caused withdrawal scores to increase systematically, this increase was unrelated to baseline levels of impulsivity. Thus the relationship between high levels of impulsivity and smoking cessation success does not appear to be attributable to differences in withdrawal severity.

Does yoga improve smoking cessation outcomes? A systematic review of the literature

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Purpose: To evaluate the effectiveness of a yoga intervention for smoking cessation.

Methods: A systematic search, review and synthesis of existing literature on yoga interventions for smoking cessation was conducted. Online literature searches through MEDLINE, PsycINFO, EBM, PubMed, clinicaltrials.gov and NIH RePORTER were carried out using an array of search terms and combinations. Manual search of reference lists and specific authors was also performed. Studies were selected that had: (1) smoking-related primary outcomes and (2) an intervention consisting of yoga or a component of yoga (e.g. pranayama).

Results: A small number of studies met our inclusion criteria. The variation between studies was substantial in terms of study population, study design, sample size, control condition, type of yoga intervention, implementation of the intervention, adherence rates, length of follow-up and number of outcomes. However, despite the variability and limited number of reports available, all selected studies found changes in smoking behavior or attitude towards smoking after the intervention.

Conclusions: Our review of the literature suggests that yoga can aid smoking cessation by influencing the desire and motivation to quit smoking, reducing smoking urges, and increasing pulmonary health awareness. However, the variety of study designs, the non-standardized nature of the interventions, the short follow-ups, and the differences in study population and sample size, limit our capacity to draw definitive conclusions. Therefore, assessing yoga as an effective component of smoking cessation treatments, needs randomized controlled clinical trials with larger sample sizes, clearly defined yoga interventions, longer follow ups, and efficient measures of compliance and adherence to treatment.
Aisling Fernandez | NAMCS/NHAMCS: National Ambulatory Health Care Data, 2009

Sadee Saithong | Caregiver preparedness over a 10-year period: Parkinson's disease Spouses

Mellisa Pensa | Using Vital Statistics to Estimate the Frequency of Elective Early-Term Deliveries: Implications for Hospital Improvement

Wael Sabbah | Oral Health as a Risk Factor for Mortality in Middle-Aged Men: the Role of Socioeconomic Position and Health Behaviours

Benjamin Sun | Randomized Evaluation of an Emergency Department Observation Syncope Protocol (EDOSP)

Karen Lyons | Predicting Covarying Depressive Symptoms in Lung Cancer Dyads over Time

Andrea Cedfeldt | A Comparison Between Physicians and Demographically Similar Peers in Accessing Personal Healthcare

Rates of Acute Injury Visits across United States Ambulatory Care Settings in 2009

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Background: In the United States (US), injuries are the leading cause of death among individuals aged 1-44 years and are the fifth leading cause of death among those older than 44 years (National Vital Statistics Reports, 2010). Less is known about nonfatal injuries. Understanding population rates and frequencies of nonfatal injury, and differences by demographic and geographic characteristics, is essential for the prioritization of injury prevention interventions, particularly when resources are slim. The purpose of this study was to generate current estimates of the rates and frequencies of acute injuries that are treated in ambulatory care settings across the US.

Methods: We combined data from the 2009 National Ambulatory Medical Care Survey (NAMCS) and the 2009 National Hospital Ambulatory Medical Care Survey (NHAMCS) for these analyses. Both the NAMCS and the NHAMCS are national probability sample surveys that estimate the frequency of US healthcare encounters in three settings: office-based physicians, emergency departments (ED), and hospital outpatient departments (OPD). We defined injury visits as those that were coded in one of several ways as injury-related and were initial visits for an acute (rather than chronic) problem. We used imputed values for missing race and ethnicity data as provided in the datasets. For each setting, we calculated frequencies of injury visits by patient demographics and geographic characteristics. Population rates of injury visits were calculated using 2000 US census data. Results were weighted to account for the complex sampling strategy and represent all injury-related ambulatory care visits in the US.
Results: There were an estimated 106 million visits to physicians’ offices, EDs and OPDs during which acute injuries were treated. The overall rate of acute injury visits in 2009 was 35.3 per 100 in the US population (95% CI: 30.9-39.7). Rates were nearly equivalent for males and females (36.5 per 100 versus 34.0 per 100 persons, respectively). Rates of injury visits for people under 75 years old were highest for 15 to 24 year olds with a rate of 35.2 per 100 persons (29.4-40.9), although only 2.6% points higher than other young adults and children. The highest injury rate, at 53.3 per 100 person s (44.5-62.2) was among those ages 75 and older. By race and ethnicity, non-Hispanic whites and non-Hispanic black persons had similar injury visit rates, at 39.0 per 100 person s (95% CI: 34.3-43.8) and 40.3 per 100 persons (95% CI: 31.0-49.6), respectively. The confidence interval for the Hispanic injury visit rate, 25.2 per 100 Hispanics (95% CI: 17.8-32.6), overlapped with that of non-Hispanic blacks, but was significantly lower than the non-Hispanic white population.

Most injuries were treated in private physicians' offices (60 million; 56%), followed by EDs (39 million; 37%) and hospital OPDs (7 million; 7%). Physicians’ office visits were made by females slightly more often than males (51% versus 49%, respectively) while ED (47% versus 53%) and OPD (49% versus 51%) visits was made more often by males than females. Non-Hispanic whites tended to seek care for acute injuries in physicians’ offices while non-Hispanic blacks, Hispanics and non-Hispanics of other races more often sought care in EDs. In all three settings, the majority of injury-related visits were paid with private insurance. However, private insurance payment was more frequent in physicians’ offices (57%) than in EDs (35%) or OPDs (44%).

Conclusion: This study provides the most current estimate of acute injuries treated in ambulatory care settings in the US. The greatest proportions of injuries were treated in physicians’ offices and tended to involve non-Hispanics, older adults, Non-Hispanic whites, and males. By US population, the highest rates were among non-Hispanic blacks, older adults, and males. These findings suggest that interventions targeted to these higher risk groups may be the most effective in preventing large numbers of injuries. Ongoing assessment of the population burden of nonfatal injury will help inform intervention approaches and the most appropriate uses of public health resources.

Caregiver preparedness over a 10-year period: Parkinson’s disease Spouses

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Introduction: Parkinson’s disease (PD) impacts more than 1.5 million Americans, the majority of whom are physically impaired older adults. Spouses provide most of the support and care. Providing care to a relative with PD has been associated with depression and poor quality of life, and these have lasting effects on the patient, caregiver, and care provided. Research shows the important link between high levels of preparedness and low levels of role strain. The goal of this study was to describe feelings of being prepared, termed caregiver preparedness, over a 10-year period in spouse caregivers of a person with PD and to identify early-warning risk factors for low caregiver preparedness over time, using transition theory.

Design and Methods: A secondary analysis was used to examine 95 spouse caregivers over a 10-year period (baseline, Year 2, and Year 10) using repeated measures ANOVA.
**Results:** Repeated-measures ANOVA revealed that there was no time effect on caregiver preparedness. The findings suggested that on average, spouse caregivers of person with PD felt somewhat well-prepared for their spouse caregiver role ($M = 2.36$, $SD = .09$), and their feeling of being prepared remains stable over a 10-year period. After controlling for spouse age, results suggested that baseline spouse depression, mutuality, and predictability played significant roles in predicting stability in caregiver preparedness over time.

**Implications:** The study focused on spousal caregivers in early stage of PD. Caregiver preparedness remains stable over time. Findings suggested that spouses who have high depression, low levels of mutuality, and low predictability of the PD caregiving situation at baseline are at risk for decrease in caregiver preparedness over time. Clinicians could target intervention early in the caregiving trajectory based on inter- and intrapersonal conditions of spousal caregiving transition as well as the nature of caregiving situation.

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**Using Vital Statistics to Estimate the Frequency of Elective Early-Term Deliveries: Implications for Hospital Improvement**

Mellisa Pensa, Kendra Bunker, Sarah Hargand, Valerie King

**Statement of Purpose:** To estimate the proportion of non-medically indicated (NMI) early term deliveries in Oregon hospitals using birth certificate data, and to compare to hospital-based estimates.

Statement of Methods Used: Birth certificate data from 2008-2010 were used to estimate the percentage of NMI births occurring between 37 0/7 and 38 6/7 weeks gestational age in Oregon hospitals. NMI births were composed of two populations: (1) Cesarean births without demonstrated trial of labor and (2) Induction of labor, both without indication, as defined by the Joint Commission. Estimates were compared to those reported by hospitals via the Leapfrog Group. Hospitals were interviewed regarding their reporting methods.

**Summary of Results:** From 2008-2010, Oregon’s overall proportion of NMI early term deliveries was 39.7%, with individual hospital estimates ranging from 19.4% to 67.4%. Seven hospitals demonstrated a significant downward trend over the three year period. Six Oregon hospitals participated in voluntary public reporting of early-term NMI deliveries. Birth-record estimates were higher than hospital-reported estimates, and differences ranged between 4% and 38%. Direct estimate comparison is not possible because hospitals did not utilize a shared set of methods.

**Statement of Conclusions Reached:** Vital statistics provide an efficient way to estimate and compare uniformly across hospitals the proportion of NMI early term deliveries. Adoption of this method may allow low-cost tracking for rapid-cycle improvement processes to reduce NMI early-term births. A focused chart review is needed to determine validity of our methods prior to state-wide utilization.

Public Health Implication: Early term NMI deliveries increase neonatal morbidity and costs. The Joint Commission and the National Quality Forum have identified elective early term deliveries as a key quality indicator for hospitals. Birth certificate records may provide an accessible estimate of hospital performance and improvement in reducing the proportion of NMI early term deliveries.


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**Oral Health as a Risk Factor for Mortality in Middle-Aged Men: the Role of Socioeconomic Position and Health Behaviors**
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Objectives: To assess the association between oral health and all-causes mortality among middle aged men, and whether it could be explained by socioeconomic position and behaviors.

Methods: Data were from the Vietnam Experience Study, a prospective cohort study of Vietnam War-era (1965-1971), American male army personnel. Risk of mortality from all-causes at an average age of 53 years was assessed using Cox regression.

Results: Persons with poor oral health experienced a higher risk of cause-specific and all-causes mortality. Hazard ratios for all-causes mortality were 2.02 (95%CI: 1.56, 2.64) among individuals with poor oral health, and 3.01 (95%CI: 1.91, 4.91) among edentates, adjusting for ethnicity and age. The association attenuated but remained significant after adjusting for systemic conditions, socioeconomic position and behaviors. Socioeconomic and behavioral factors explained 52% and 44% of mortality risks attributed to poor oral health and being edentates, respectively.

Conclusion: The findings imply that oral health is a marker of socioeconomic and behavioral risk factors related to all-cause mortality.

Predicting Covarying Depressive Symptoms in Lung Cancer Dyads over Time

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Lung cancer will contribute 15% of all new cancer cases and 29% of all cancer deaths in the United States. Approximately 58% of lung cancer patients die within 1 year of diagnosis with a 5-year survival rate of 16%. This rapid trajectory, and the difficult decisions families are often faced with, heightens the need for early intervention in this end-of-life population. The goal of the study was to examine covarying trajectories of depressive symptoms within lung cancer dyads over time, and identify early-warning risk factors. Lung cancer dyads were recruited through a cancer registry using rapid case ascertainment 1-6 months post-diagnosis (M=4.15; SD=2.8). Baseline data indicated a moderate level of depressive symptoms for patients and family members, with 25% and 28% above clinical cut-offs respectively. Longitudinal data from 114 dyads were analyzed using multilevel modeling. Results revealed that, on average, patient and family member depressive symptoms remained relatively stable over the first six months, although there was significant variability around the average (p < .001). Controlling for stage of disease, patient age, and family member role, level 2 models found patient concealment, family member role overload, communication problems, and a negative family decision making process played significant roles in predicting baseline depressive symptoms and change over time. As expected, depressive symptoms within dyads was moderately correlated. Findings will be discussed in regard to identifying those families and patients most at risk, the role of patient symptoms over time, and need for communication-based interventions for such vulnerable end-of-life populations.
Randomized Evaluation of an Emergency Department Observation Syncope Protocol (EDOSP)

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Background: Syncope, defined as a transient loss of consciousness, accounts for over 700,000 emergency department (ED) visits per year. Because of uncertainty about potentially dangerous causes, patients are frequently hospitalized after an episode of unexplained syncope. Current admission patterns account for over $2.4 billion annual hospital costs with little evidence of benefit. We evaluated an Emergency Department Observation Syncope Protocol (EDOSP) as an alternative to routine admission.

Methods: This was a randomized trial at five emergency departments (EDs). Eligible patients had a chief complaint of syncope or near syncope, were aged 50 years or greater, and were classified as intermediate risk for subsequent serious outcomes. EDOSP arm patients received serial troponin testing, 12-24 hours of cardiac monitoring, and selective echocardiogram testing in an ED observation unit. Control arm patients received routine and unstructured care by an inpatient medical team. Primary outcomes included hospitalization rate and length-of-stay. Secondary outcomes included 30-day serious outcomes, 30-day hospital costs, and quality-of-life scores. All analyses were intent-to-treat.

Results: Of 2,724 screened patients, 202 met all eligibility criteria and 123 were randomized. Compared to routine admission, EDOSP resulted in a lower admission rate (11% vs 78%, p<0.001) and shorter hospital length-of-stay (29 vs 46 hours, p=0.001). The overall 30-day serious outcome rate was 6% (EDOSP 8% vs control 3%, p=0.2). EDOSP resulted in lower 30-day hospital costs ($2,100 vs $3,600, p=0.02). There were no differences in general health utility (p=0.5) or symptom-specific quality of life (p=0.3).

Conclusions: EDOSP can be replicated in multiple ED settings, and it represents a cost-effective alternative to routine admission for intermediate-risk patients with syncope. Clinical outcome rates were not significantly different between the two arms; however, this study was not sufficiently powered to demonstrate non-inferiority. Future research is needed to definitively demonstrate the safety of EDOSP.
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**Background:** Data suggest that physical or emotional stress in physicians leads to poor patient outcomes and that physicians in general do not pursue their healthcare reliably. Resident physicians are a unique subpopulation, training in a complex work and educational environment which puts them at risk for not obtaining preventive and illness related healthcare.

**Objectives:** This study was designed to compare resident physician utilization of personal healthcare services to that of demographically similar peers.

**Methods:** All 675 residents at Oregon Health & Science University were invited to participate in a confidential and web-based cross-sectional survey in January 2008. Survey responses to questions addressing personal healthcare were compared to those of a demographically similar group using the 2008 survey from the Behavioral Risk Factor Surveillance System (BRFSS). The final weights in BRFSS were used for a post-hoc stratified adjustment in analysis. Logistic regression was employed to compare subgroups.

**Results/Outcomes/Improvements:** Resident physicians are significantly less likely than demographically similar peers to have a primary care provider or dentist, or to participate in routine health maintenance. In subgroup analyses, gender was the most significant contributor to differences in accessing healthcare. Female residents were more likely to have healthcare providers and to have seen their providers for routine healthcare within the last year. Number of years in training was not related to likelihood of seeking routine healthcare, but was associated with ability to obtain non-routine healthcare. Residents in primary care training programs were almost twice as likely to have a physical healthcare need for which they had not scheduled an appointment. Training specialty was not associated with other differences in odds of accessing health care resources.

**Significance/Implications/Relevance:** Our study documents that physicians in training do not pursue their healthcare reliably. If one accepts the premise that preventive healthcare is important to overall wellness, these findings are of concern. Since the literature also suggests that physical or emotional stress in physicians contributes to poorer patient outcomes, we propose that intervention early in the education of physicians is necessary to promote physician wellness and to improve patient care. Further research into both the barriers that prevent residents from accessing healthcare, and opportunities to address them is needed.
Lourdes Quintanilla-Dieck | Expression of Inflammatory Cytokines and Ion Homeostasis Genes in the Cochlea Is Up-regulated by Lipopolysaccharide-induced Sepsis

Sripriya Ramamoorthy | Potentiometric measurement of the transmembrane potential distribution along isolated outer hair cells in an external electrical field

Lina Reiss | Plasticity in pitch perception with cochlear implant experience

Expression of Inflammatory Cytokines and Ion Homeostasis Genes in the Cochlea
Is Up-regulated by Lipopolysaccharide-induced Sepsis (RDRC)

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Objective: The mechanism by which sepsis-induced inflammation enhances aminoglycoside trafficking into the cochlea remains poorly understood. This study sought to determine sepsis and/or ototoxic drug-induced changes in gene and protein expression of inflammatory-mediated cytokines and ion homeostasis proteins in the cochlea.

Methods: Three groups of mice received one of the following treatments: gentamicin, lipopolysaccharide (LPS) for sepsis induction, or both gentamicin and LPS. A fourth group served as controls. After sacrifice, cochleae were collected and underwent either RNA extraction for PCR, or protein extraction for enzyme-linked immunosorbent assay. A total of 32 cytokine and ion homeostasis genes/proteins were analyzed.

Results: The cochlear gene expression analysis revealed that gentamicin alone did not induce significant cytokine up-regulation, compared to bacteria-derived LPS. The most up-regulated cytokine genes with LPS were interleukin(IL)-6, macrophage inflammatory protein(MIP)-2α, and neutrophil activating protein(CXCL)-1. LPS also up-regulated ion homeostasis genes, including gap junction-α1, claudin-4, and claudin-14. Cochlear protein quantification revealed that LPS greatly elevated IL-6, keratinocyte-derived cytokine (KC), and MIP-2α.

Conclusions: Sepsis induces a systemic pro-inflammatory state that enhances aminoglycoside uptake by cochlear tissues. During sepsis, there is statistically significant up-regulation of cytokine gene and protein expression and of specific ion homeostasis genes in the cochlea, regardless of aminoglycoside exposure. Given that cytokines increase permeability across blood-endothelial barriers, this phenomenon could enhance aminoglycoside trafficking into cochlear tissues. LPS-induced up-regulation of ion homeostasis gene expression may provide additional clues to the mechanisms by which enhanced aminoglycoside trafficking into the cochlea during systemic infection occurs. Thus, systemic infection may synergistically enhance the ototoxicity of aminoglycosides.

Potentiometric measurement of the transmembrane potential distribution along isolated outer hair cells in an external electrical field
The sensory outer hair cells (OHC) in the inner ear play an important role in active amplification of sound-induced vibrations by virtue of their somatic motility. The OHC forms a crucial part of the path of flow of transduction-current generated by deflection of the hair bundles at their apex. They are also electrically excited during current injection experiments in animals as well as in cochlear implants. The distribution of transmembrane potential along the OHC is therefore important for auditory research. We measure the transmembrane potential induced along the perimeter of isolated OHCs from guinea pigs in response to electrical field at various orientations relative to the OHC axis. The electrical field is set up using a wire-electrode pair. Percentage changes in the fluorescence intensity of the potentiometric dye ANNINE-6plus loading the cell membrane is used to determine the changes in transmembrane potential. The potentiometric sensitivity of the dye is calibrated by applying known voltage-steps (hyper-polarizing as well as de-polarizing) to cells under simultaneous whole cell patch-clamp. The effect of the distribution of conductance along the OHC perimeter, such as the larger conductance at the OHC base vs. apex, on the induced transmembrane potential is investigated theoretically and experimentally. The implications of this study for cochlear mechanics are discussed. Research supported by NIH grant DC000141.

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**Plasticity in pitch perception with cochlear implants**

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Recent experiments show that pitch perceived through a Hybrid short-electrode cochlear implant (CI) can shift over several months of implant experience, by as much as 2 octaves (Reiss et al., JARO 2007). Here we describe updated results in short- and long-electrode CI users recruited to have pitch perception measured longitudinally at several time points from hookup to up to 5 years of CI experience; long-term CI users were also recruited to measure electrode pitch adaptation patterns. Pitch perception was measured by having the subject compare the pitch elicited by stimulation of a single electrode with acoustic tones presented to the non-implanted ear. Acoustic tone frequency was varied across trials in pseudorandom, counterbalanced sequence, and the pitch match was estimated as the 25-75% range of the psychometric function.

As in short-electrode CI users, electric pitch changes were observed in long-electrode CI users over time. For both groups, pitch changes often aligned with the CI frequency-to-electrode allocations, consistent with the hypothesis that the brain adapts pitch perception to minimize perceived mismatches between electrically and acoustically stimulated cochlear place frequencies. However, not all subjects exhibited changes consistent with this trend; a subset of long-electrode CI users exhibited downward pitch shifts over time for all electrodes independent of frequency allocation. The reasons for this variability are not clear, but may include differences in hearing loss patterns, hearing aids, and environmental listening experience. These findings have implications for the role of brain plasticity in auditory prosthesis perception, and suggest that experience-dependent changes may need to be measured and factored into device evaluation and design.
Work supported by NIH-NIDCD grants R01DC000377, P50DC00242, and P30DC010755, the Iowa Cochlear Implant Clinical Research Center, and the OHSU Cochlear Implant Program.
Using SVV infection of aged RM to understand VZV reactivation

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Reactivation of latent varicella zoster virus (VZV) results in herpes zoster (HZ, shingles), which causes significant morbidity and occasionally mortality in the elderly and immune compromised. Clinical studies show that the current FDA-approved vaccine offers only 51% protection. With 17% of the US population estimated to be over the age of 65 by 2020, the incidence of HZ and its associated complications will certainly increase. Thus, continued investigations to develop more effective treatments against VZV, particularly in the elderly, are needed. In order to improve vaccine efficacy, we need a more thorough understanding of the anti-VZV immune response. We aimed to accomplish this by using our novel animal model wherein rhesus macaques (RM) inoculated with simian varicella virus (SVV), a homolog of VZV, recapitulate the disease hallmarks of VZV infection in humans. Since VZV is strictly a human pathogen, and VZV reactivation is associated with renewed viral replication, our model of acute VZV infection provides a unique opportunity to uncover immunological mechanisms that may contribute to poor control of VZV reactivation in the elderly. Data from our studies show that in contrast to young RM, aged RM experience increased peak SVV viral loads and more severe disease. Interestingly, aged RM generate IgM and IgG responses comparable in kinetics and magnitude to that of young RM. In contrast, CD4 and especially CD8 T cell responses are delayed and reduced in aged RM. One potential mechanism for this delayed T cell response is a reduced frequency of plasmacytoid DCs (pDCs) in the aged. Collectively, these data suggest that similar to clinical observations of VZV infection in humans, resolution of acute SVV infection is dependent on cellular rather than humoral immunity. Additionally, our data suggest reduced frequencies of pDCs, may play an extrinsic role in the age-associated global defect in T cell responses during acute SVV infection. These results provide the framework for future studies aimed at improving current vaccines against herpes zoster.
Rational design of novel adeno-associated virus vectors by a next generation sequencing-based approach (Barcode-Seq) to study virus biology

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Discoveries of a number of naturally occurring new AAV serotypes showing altered tropism or other distinctive biological features have significantly increased usefulness of rAAV vectors. Although AAV capsid protein primarily determines the distinctive biological properties of each AAV serotype, it has yet to be elucidated which amino acids in the capsid protein are responsible for manifesting each property. To comprehensively understand the AAV capsid amino acid sequence-phenotype relationships, we have recently established a new systematic approach, named “AAV Barcode-Seq”. The AAV Barcode-Seq takes advantage of DNA barcoding and Illumina sequencing technologies and makes it possible to investigate multiple phenotypic changes caused by mutations of each of all the amino acids in the AAV capsid protein with minimum time and efforts. Here we show that the AAV Barcode-Seq successfully identifies a motif in the AAV9 capsid that binds to its primary cell surface receptor (i.e., terminal galactose) and demonstrate that AAV2 vectors exhibiting AAV9-like biological properties can be designed and created based on the knowledge obtained by the AAV Barcode-Seq. In this study, we produced a total of 191 AAV9 capsid mutants to scan the entire region of the carboxy-terminal half of the AAV9 capsid by double alanine mutagenesis. To identify AAV9 capsid amino acids required for binding to terminal galactose in the cell surface glycans, CHO Lec2 cells were incubated with the AAV9 mutant libraries at 4 °C to allow viral particles to bind to terminal galactose expressed on the cells. Then viral genomes in the surface-bound viral particles were recovered and subjected to the AAV Barcode-Seq analysis. More than ten mutants showed significant impairment in binding to Lec2 cells. By taking into consideration the evolutionary conservation of the identified amino acids and their topological locations, we narrowed down the amino acids that potentially constitute the galactose binding motif in the AAV9 capsid. Based on the knowledge we obtained in the above study, we designed and created AAV2R585E.9, an AAV2-based variant that carried the galactose binding motif in addition to R585E, a mutation that ablates heparin binding. The AAV2R585E.9 showed a dramatic increase in transduction in Lec2 cells compared with the parental AAV2R585E, while the transduction efficiencies of both AAV2R585E.9 and AAV2R585E were equally low in Pro5 cells devoid of expression of terminal galactose. Thus, AAV Barcode-Seq significantly accelerates the elucidation of the capsid amino acid sequence-phenotype relationships, and hence significantly promotes rational design approaches to develop new rAAV vectors with the most desirable biological properties.

NF-κB-inducing kinase interferes with regulatory T cell function and phenotypic stability

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NF-κB is a conserved intracellular signaling pathway that in the mammalian immune system responds to infection, damage, and other danger signals. Activation of NF-κB can occur via two related, but distinct, pathways—canonical and non-canonical (also referred to as alternative). NF-κB-inducing kinase (NIK) is required only for activation of non-canonical NF-κB, and as such, mice deficient in NIK have been instrumental in elucidating the receptors that utilize this pathway. In fact, only a handful of TNFR family members are known to activate the non-canonical NF-κB pathway. We
recently discovered that the T cell costimulatory receptor, OX40, activates non-canonical NF-κB, and that its in vivo costimulatory function absolutely requires NIK. In addition to activating conventional T cells, OX40 can also inhibit differentiation and function of regulatory T cells (Treg), a cell subset critical to dampen immune responses and prevent autoimmunity. Here, we investigate the role of NIK in Treg. We show that the ability of OX40 to antagonize Treg differentiation and function depends on NIK. Using a mouse model of conditional NIK overexpression in T cells, we found that modest upregulation of NIK is sufficient to inhibit Treg differentiation and function and that this dysfunction contributes to lethal autoimmunity. The poor suppressive function of NIK-overexpressing Treg is associated with decreased expression of Treg “signature genes”, including the Treg master transcription factor, Foxp3. In addition, NIK destabilizes Treg phenotype, propelling Treg conversion to a T helper type 1 phenotype. These data position NIK as a key player that integrates the response to TNFR costimulation by both activating conventional T cells and inhibiting Treg-mediated suppression. This allows for a robust immune response, but also necessitates tight regulation of NIK in T cells to avoid immunopathology and autoimmunity.

NF-κB inducing kinase negatively regulates invariant natural killer T cell development

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Invariant natural killer T (iNKT) cells are a rare subset of αβ T cells that recognize both self glycolipid and microbial antigens presented on the MHC class I-like molecule, CD1d. iNKT cells are an attractive innate immune cell therapeutic target for the following reasons: i) a general iNKT cell population can be effectively activated by well-characterized glycolipid antigens, ii) iNKT cell activation not only promotes tolerance in the mouse models of autoimmune disease but also enhances immunity against tumor and infection, and iii) iNKT cells are involved in pathogenesis of chronic inflammation including asthma and atherosclerosis. However, the number of iNKT cells varies widely among humans for unknown reasons. Moreover, a loss of iNKT cells in patients with autoimmune diseases, cancers, and severe infections provides a barrier to effective use of iNKT cell-targeted therapy. Therefore, it is important to understand their development in the thymus.

The NF-κB inducing kinase (NIK)-dependent non-canonical NF-κB pathway governs development of lymphoid organs and promotes survival of B lymphocytes. We recently showed that stimulation via OX40, a member of TNF receptor (TNFR) family, causes non-canonical NF-κB signaling in T cells, resulting in acquisition of effector functions [Murray et al (2011) J Clin Invest 121:4775]. In contrast, low level overexpression of NIK in iNKT precursors (NIK-tg) results in a massive loss of iNKT cells. Moreover, we have observed that NIK-deficient (NIK-KO) iNKT cells have a developmental advantage over control cells in mixed bone marrow chimeras. Normal proliferation and increased apoptosis in iNKT cells have been detected in NIK-tg mice, and the levels of survival factors such as PLZF and IL-7Ra are strongly reduced in NIK-tg iNKT cells. Taken together our data suggest that NIK negatively regulates iNKT cell development through downregulation of critical survival factors. Our study suggests that inhibition of NIK and the non-canonical NF-κB pathway might improve efficacy of iNKT cell-targeted immunotherapy by increasing number of iNKT cells.

Binding of partial MHC class II constructs to monocytes reduces CD74 expression and induces both specific and bystander T-cell tolerance

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Treatment with partial (p)MHC class II-β1α1 constructs linked to antigenic peptides (pMHC/peptide) can induce T-cell tolerance, inhibit recruitment of inflammatory cells and reverse autoimmune diseases. Here we demonstrate a requirement for pMHC/peptide binding to CD11b+ mononuclear cells through a receptor comprised of MHC class II invariant chain (CD74), cell-surface histones and MHC class II itself for treatment of experimental autoimmune encephalomyelitis (EAE). Binding of pMHC/peptide constructs with CD74 involved a previously unrecognized MHC class II-α1/CD74 interaction that inhibited CD74 expression, blocked activity of its ligand, macrophage migration inhibitory factor, and reduced EAE severity. These findings implicate binding of pMHC/peptide constructs to CD74 as a key step in both antigen-driven and bystander T-cell tolerance important in treatment of inflammatory diseases.

The big ball of wax: novel insights into the mycobacterial cell wall

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The goal of the Purdy Lab is to further define the dynamic interface between the human pathogen Mycobacterium tuberculosis and the host macrophage. We use bacterial genetics and biochemistry to explore the pathogenic mechanisms of TB in combination with cell biology and novel genetic approaches to study the mycobactericidal properties of macrophages. M. tuberculosis is exposed to a number of host-associated environmental stresses during infection, and the pathogen possesses mechanisms to resist these stresses when imposed by the host immune system. These stress response pathways are necessary for the bacterium to establish and maintain infections. The bacterial response and resistance mechanisms to promote survival in the host mycobactericidal conditions of oxidative and/or nitrosative stress and host antimicrobial peptides are our highest priority.

The bacterial cell wall provides significant intrinsic resistance to both the host immune response and antibiotics. We are characterizing a number of bacterial mutants with altered susceptibility to antimicrobial ubiquitin peptides. These mutants lack proteins that are involved in a variety of cell wall biosynthetic pathways including: peptidoglycan biosynthesis, mycobacterial lipid synthesis and lipid transport to the outer membrane. As such, they are excellent candidates for novel TB drug design. Of particular interest are the MmpL transporters. We have shown that MmpL3 is essential for bacterial viability. Both MmpL3 and MmpL11 both contribute to cell wall biogenesis and MmpL11 is required for full virulence of M. tuberculosis using the mouse model of infection.
Virk: An Active Learning System for Optimally Identifying Biomedical Community Database Contributions

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The frequency and volume of newly-published scientific literature is quickly making the maintenance of publicly available scientific databases unrealistic and costly. The amount of time database curators must spend identifying and processing documents containing information relevant to their databases is prohibitive to their continued development. Although supervised document classification is useful for developing models for identifying database-relevant publications, developing such models necessitates manually annotating an unrealistic number of documents. Active learning is a machine learning approach to building such classification models that proceeds by iteratively identifying documents that will provide the most information to the classifier. Although this approach has been shown to be effective for related problems, in the context of optimally curating scientific databases, it falls short in two key ways. First, the process of developing traditional active learning models does not immediately yield curatable information for the database—an expert annotator must spend time reading an approximately equal number of relevant and irrelevant documents. Second, traditional active learning models are evaluated by assessing its performance against a separate evaluation document collection, rather than by examining its ability to accurately identify documents containing relevant information. Virk is a system that embodies a modified active learning approach that addresses both of these shortcomings. Here, we describe the method and the experiments conducted to validate this approach and identify documents containing information relevant for the Neuron Registry, a Neuroinformatics community database.

Clinician Perspectives on the Quality of Patient Data Used for Clinical Decision Support: A Qualitative Study

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Objective: Clinical decision support (CDS), defined broadly to encompass patient-specific information and knowledge provided at the point of care, depends on a foundation of high quality electronic patient data. Little is known about how clinicians perceive the quality and value of data used to support CDS within an electronic health record (EHR) environment.

Methods: During a three-year research study, we collected ethnographic data from ten diverse organizations, including community hospitals, academic medical centers and ambulatory clinics.

Results: An in-depth analysis of the theme “data as a foundation for CDS” yielded five subthemes related to data quality: completeness; accessibility; context specificity; accuracy; and reliability.

Conclusion: Based on our results, we propose a new framework for describing the factors impacting clinicians’ perceptions of data quality related to CDS in the organizations we studied.

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Is Perceived Quality in Primary Care Associated with Practice Size and/or Use of Health Information Technology?

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Background: Patient safety initiatives have focused largely on inpatient settings, but the majority of medical encounters occur in ambulatory practice, and less is known about the determinants of quality and safety in these settings.

Methods: We surveyed 6534 clinicians and staff in 306 medical practices from 11 practice based research networks in 16 states. We collected background data on office size, ownership, and use of health information technologies (HIT), and assessed patient safety and quality of care using the Medical Office Survey of Patient Safety. After adjusting for the role of respondents, we examined the relationship between perceived safety and quality of care with office size, ownership, and implementation of HIT.

Results: The highest proportion of positive responses about quality and safety occurred in small practices (3-15 personnel), and the lowest proportion of positive responses occurred in large (41-70 personnel) and very large practices (over 70 personnel). After controlling for office size, we found no relationship between perceived quality and safety and practice ownership. The relationship of perceived quality and safety to HIT implementation was not clear. We found the highest proportion of positive response in practices with the least HIT and those with the most HIT, and the lowest proportion was found in practices with intermediate levels of HIT implementation.

Conclusion: Clinicians and staff in small practices reported the highest overall quality and safety of care, and perceived quality and safety declined with increasing office size. No clear relationship was found between perceived quality and safety and implementation of HIT.
Identifying Patients for Clinical Studies from Electronic Health Records: The TREC Medical Records Track

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The growing investment in electronic health record (EHR) systems provides an opportunity to augment clinical research by allowing the “secondary use” of clinical data. However, there are a number of challenges to using EHR data, the most significant of which is it being inaccessible and difficult to structure due to it being in mostly free-text form. A related challenge has been a lack of robust collections of such data to facilitate research in this area. This has been addressed by the Text Retrieval Conference (TREC, trec.nist.gov), an annual challenge evaluation for information retrieval (IR) researchers. TREC is usually organized into 5-6 tracks focused on different IR problems. In 2011, TREC instituted a Medical Records Track, focused on the retrieval of medical records that could enable the selection of patients for possible participation in clinical studies.

The data for the task came from the University of Pittsburgh NLP Repository, a repository of 95,702 de-identified clinical reports available for NLP research purposes. The reports were generated from multiple hospitals during 2007, and were grouped into “visits” consisting of one or more reports from the patient’s hospital stay. Each document was formatted in XML, with a cross-walk table that matched one or more documents to visits. There are a total of 17,199 visits.

A total of 35 “topics” for retrieval were developed based on the Institute of Medicine priorities for comparative effectiveness research. Retrieval performance was measured by recall, precision, and related measures (e.g., mean average precision [MAP] and binary preference [B-Pref]). Retrieved documents were assessed by relevance judges who were physicians and students in the OHSU biomedical informatics graduate program.

A total of 29 research groups from around the world participated in the track. Results showed that while many systems achieved a good level of performance, there is still substantial room for improvement. In addition, techniques known to improve general IR (i.e., queries using conventional search engines) were counterproductive for this task.

CTSAconnect: A Linked Open Data approach to represent clinical and research expertise, activities, and resources

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Teams of scientists are now much more common than single scientists in the production of biologically meaningful and clinically consequential breakthroughs. There are a myriad of data sources about investigators, physicians, research resources, clinical encounters, and expertise to promote team interaction. However, much of this valuable information is locked in silos and is not easily connected. The CTSAconnect project, a collaboration between the Ontology Development Group in the OHSU Library (http://bit.ly/ohsuontdevgroup) and OCTRI (http://www.ohsu.edu/xd/research/intstitutes/octri/index.cfm) aims to facilitate information aggregation about investigators, physicians, biomedical research resources, services, and clinical activities. The goal is to provide a more
well-rounded representation of a person’s expertise for the purposes of promoting collaboration and data and resource sharing, as well as understanding funding outcomes and targets.

Researchers can be characterized by their organizational affiliations, grant and project participation, research resources (such as reagents, biospecimens, animal models) they have generated, and publications they have (co-)authored. Clinician profiles can be characterized by training and credentials, by clinical research topic, and by the kinds of procedures and specialization that can be inferred from encounter and billing data. We believe that integrating this diversity of information sources and platforms to fully represent a person’s expertise requires addressing the overlap between research resources and the attributes and activities of researchers and clinicians.

Linked Open Data (LOD) refers to a collection of structured data, for the purpose of sharing and linking data and information on the Semantic Web. Notwithstanding the large amount of data published as LOD, there remains a significant gap in the representation of research resources and clinical expertise. Where it does exist, there is insufficient consistency across sources. CTSAnnect is creating an Integrated Semantic Framework (ISF) that enables projects that represent information about resources and people on the Semantic Web (eagle-i, VIVO, ShareCenter) to work together to facilitate biomedical research. Our dissemination website (ctsanconnect.org) will illustrate repeatable methods and examples of how to extract, consume, and utilize this valuable new LOD using freely available tools like VIVO, eagle-i, and Google APIs.

**Semantic Linking of Biospecimen Resources**

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Biomedical researchers studying human disease have an opportunity to take advantage of the large amount of clinical, laboratory, and genetic information and resources being accumulated during clinical care. One important aspect of this research is being able to obtain biospecimens, and related clinical metadata, that meet specific criteria to address a specific research question. To address these research needs, OHSU has recently developed the Biolibrary data repository and a search engine that allows researchers to identify relevant biospecimens. However, the benefits of the shared Biolibrary database and its search application is somewhat limited by the disparate data representations from the various data sources being incorporated into the Biolibrary system. Also, a large portion of specimen-related information is in the form of unstructured natural-language pathology reports. These various data sources have overlapping domain knowledge and domain terminology however it is difficult to exploit this similarity due to incompatibilities in information modeling, data encoding, and the use of natural language instead of a shared controlled vocabulary and a common information model.

This project is a pilot study that aims to develop an ontology (a controlled vocabulary with additional logic-based semantics) that represents the various entities and relationships in the domain of biomedical specimens. The ontology will then be mapped to existing pathology reports to enable better search capabilities. The Biolibrary search system currently relies on text-based search capabilities to retrieve relevant biospecimens from existing pathology reports. These search techniques do not take into considerations issues such as synonymy, anatomical parts and relationships, anatomical entities and their possible pathologies, etc. These issues, when not taken into account, could negatively impact search results. We believe that an ontology-based search system will enhance the Biolibrary search capabilities by taking advantage of the logical relations represented in the ontology together with a concept-based mapping of the
ontology to existing pathology reports. The ontology will also provide a logical layer that could simplify future integration of new biospecimen repositories into the Biolibrary system.
Estrogen regulation of the M-current in GnRH neurons

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Throughout most of the estrous cycle, gonadotropin-releasing hormone (GnRH) neurons are quiescent and except for short periodic pulses of activity. Prior to ovulation, estrogen and timing cues prime GnRH neurons for a transient period of burst firing to propel the GnRH surge. A possible mediator of the GnRH quiescent state is the M-current (I_m), which is generated by KCNQ 1-5 channels. I_m is a non-inactivating, voltage-dependent potassium current that is activated at subthreshold membrane potentials and has relatively slow kinetics. Previous studies in our lab have demonstrated that male GnRH neurons exhibit I_m, which is activated by the GnRH peptide and contributes to an autoregulatory negative feedback loop. Although I_m acts to suppress neuronal activity, the downregulation of I_m in GnRH neurons is a possible excitatory mechanism. As there have been no studies examining the role of I_m in modulating the excitability of GnRH during the preovulatory surge, we sought to characterize I_m in female animals using molecular and electrophysiological techniques. Ovariectomized animals were treated with an established 17β-estradiol (E2) paradigm to mimic the day of proestrus, and control animals were oil-treated. Using single-cell harvesting, pools of 5 cells were collected from E2- and oil-treated animals in the morning (negative E2 feedback) and afternoon (positive E2 feedback) of induced proestrus.
After reverse transcription, the pools were evaluated for mRNA quantities of KCNQ channel subtypes in GnRH neurons. This revealed that KCNQ 2 and 3 transcripts were equally expressed, whereas KCNQ5 was found in much lower quantities. In initial experiments we evaluated E2-induced changes in KCNQ3 and -5. We found that KCNQ3 mRNA was significantly downregulated by E2 compared to oil in the morning and afternoon of proestrus (n=5, 2-3 pools/animal). Whole-cell voltage-clamp recording from eGFP-GnRH neurons revealed a robust, voltage-sensitive $I_m$ which reversed around -80 mV. The robust $I_m$ current was maximal at -30 mV (112 ±50, n=5) and was inhibited by TEA (10mM, n=7). $I_m$ fully recovered from the TEA inhibition after prolonged recordings (60 min) from GnRH neurons, indicating very little rundown of this potassium current. Therefore, the E2 regulation of KCNQ channel mRNA expression in GnRH neurons may attenuate the $I_m$ contribution to neuronal excitability in GnRH neurons.

Opposing respiratory effects of two analgesic drugs in the brainstem revealed using a novel method of plethysmography

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The analgesic effects of systemic opioids depend on the rostral ventromedial medulla (RVM), a brainstem region that modulates pain through descending projections to the spinal cord. Ablation of the RVM blocks the analgesic effects of systemic opioids, and opioid microinjection into the RVM produces analgesia comparable to systemic administration. However, opioid action in the RVM also leads to respiratory depression. Here our goal was to compare the respiratory effects of the novel analgesic improgan applied directly in the RVM with those of the µ-opioid receptor agonist DAMGO.

To make this comparison in lightly anesthetized rats, we validated two methods of measuring respiration against simultaneous measurements using whole body plethysmography. Validation was done using 10% carbon dioxide and systemic morphine (4 mg/kg) challenges in isoflurane-anesthetized animals. First, an accelerometry-based approach (Devonshire et al., Lab Anim, 2009;43:382) adequately represented ventilatory rate ($r^2 = 0.93 ± 0.04$), but was less accurate in correctly measuring tidal volume ($r^2 = 0.84 ± 0.03$ vs whole-body plethysmography). Second, we developed a novel method of measuring respiration, ventilatory pressure-transduced (VPT) plethysmography. VPT plethysmography detects subtle changes in air pressure around the animal’s nose during each breath. VPT plethysmography measurements of rate were highly correlated with those obtained using whole-body plethysmography ($r^2 = 0.99 ± 0.001$), and instantaneous measurements of rate differed by less than 1% from those of the whole-body approach. VPT underestimated changes in tidal volume (73.8 ± 16.6% of whole-body measure), but was still well correlated ($r^2 = 0.90 ± 0.03$) with the whole-body approach. VPT therefore provides an accurate measure of respiratory rate, and a useful relative measure of respiratory volume.

Using these methods, we found that an analgesic dose of the µ-opioid receptor agonist DAMGO microinjected into the RVM produced a sustained depression of both respiratory rate and tidal volume (-5.9% ± 2.6% and -9.4% ± 5.1%, respectively). By contrast, analgesic doses of improgan in the RVM produced a sustained increase in both rate (14.5% ± 3.2%) and volume (19.7% ± 6.6%).
VPT plethysmography provides an accurate measure of respiratory rate and relative tidal volume without the use of whole body plethysmography. We also found that the potent analgesic drug imopran strongly drives respiration at a brainstem site where opioid analgesics produce respiratory depression. These results open up the possibility for development of potent new pain-killing drugs without the respiratory depressant effects of opioids.

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The neurodegenerative *Drosophila melanogaster* AMPK mutant loe interferes with the RHO pathway and actin dynamics

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The *Drosophila* mutant *loe* lacks a neuronal isoform of the AMPK (AMP-activated protein kinase) γ subunit and shows neuronal cell death of the adult nervous system, a lower cholesterol ester level and a reduced APPL (amyloid precursor protein-like, a key player in Alzheimer’s disease) processing (1). A mutation in PRKAG2, the γ2 isoform of the human AMPK, can lead to Wolff-Parkinson-White syndrome, which causes arrhythmia (irregular heartbeat) (2). AMPK negatively regulates the isoprenoid pathway by inhibiting the HMGR (hydroxymethylglutaryl-CoA Reductase). Isoprenylation is an important mechanism allowing intracellular proteins, like small G proteins (e.g. RHO) to associate with the membrane, which is then followed by activation of the protein. This step is critical for signal transduction of cellular hormones, growth factors, and survival of the cell. Besides AMP, AMPK can also be activated by LKB1. LKB1 is a tumor suppressor that is mutated in Peutz-Jegher syndrome, a rare genetic disease that causes harmatomas (polypes in the intestine) and increased incidence of epithelial cancers (3). In vertebrates, AMPK inhibits the isoprenoid/mevalonate pathway, which regulates protein preylation and cholesterol synthesis. In *loe*, due to the not functioning γ subunit, its not able to inhibit HMGR, leading to increased farnesylation (adding hydrophobic anchor) of small G-Proteins (e.g. Rho). In order to determine the correlation between the *loe* mutation, isoprenylation and the RHO1 pathway, we generated and analyzed flies with mutations in RHO1 and its downstream targets. We were able to show that the *loe* mutation interferes with the prenylation of RHO1 and the regulation of the downstream LIM-Kinase pathway, which plays an important role in actin turnover and axonal remodeling. In addition, we used western blotting to show the concentration of coflin, which regulates actin turn over. Interestingly, all these defects including a behavior phenotype, can be detected before a severe neurodegeneration is histologically visible.


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Using fruit flies to model the role of TDP-43 in Amyotrophic Lateral Sclerosis

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The human Tar-DNA binding protein TDP-43 is closely associated with ALS and other neurodegenerative disorders. TDP-43 contains two highly conserved RNA-binding motifs and possesses a variety of documented roles in RNA metabolism, including pre-RNA splicing and repression of transcription. In post-mortem samples of patients with ALS, TDP-43 is found in cytoplasmic aggregates and is reduced or missing from its normal location in the nucleus. It is unclear whether the deleterious effects are due to loss-of-function from the nucleus or novel toxic effects due to the cytoplasmic aggregates. We are using the fruit fly, Drosophila melanogaster to model this by assessing the effects of knockout and over expression of the fly ortholog of this protein. Here I will summarize the molecular, physiological and behavioral consequences of these manipulations, focusing on future goals to understand the molecular basis for TDP-43 proteinopathies.

STAT3 activation by NGF and gp130 cytokines stimulates sympathetic axon regeneration

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Nerve growth factor (NGF) stimulates sympathetic axon outgrowth through activation of TrkA, but the mechanisms are not completely understood. Studies in PC12 cells indicate that TrkA stimulates neurite outgrowth through phosphorylation of S727 on STAT3 (S727-P) (Ng et al., 2006). Cytokines like ciliary neurotrophic factor (CNTF) simulate phosphorylation of Y705 on STAT3 (Y705-P), and nerve injury studies suggest that Y705 phosphorylation may also be important for regeneration. We investigated the role of STAT3 in sympathetic axon regeneration, and found that STAT3 phosphorylation on both sites is required for NGF-stimulated outgrowth. NGF stimulated phosphorylation of S727 in sympathetic neurons via ERK1/2, and S727-P STAT3 was detected throughout the axon. CNTF stimulated phosphorylation of Y705 and translocation to the nucleus. Inhibiting all phosphorylation with Stattic (20μM) completely blocked sympathetic axon outgrowth, while disrupting STAT3 DNA binding partially inhibited outgrowth. To test the role of Y705 and S727 selectively, neurons lacking STAT3 were co-transfected with GFP and wild-type STAT3 (Stat3-WT), Y705 mutated (Stat3-Y705F, or S727 mutated (Stat3-S727A). Neurons were treated with NGF and CNTF, and green axons measured. Stat3-WT significantly increased axon outgrowth compared to vector alone, but neither of the mutant constructs restored growth when added alone, indicating that both S727 and Y705 phosphorylation are required for maximal axon outgrowth. We asked if gp130 signaling and STAT3 was involved in sympathetic axon regeneration in the heart following myocardial infarction, using mice whose sympathetic neurons lack gp130 (gp130 KO) and have low STAT3. We found that peri-infarct sympathetic nerve regeneration was impaired in the gp130 KO mice. This confirms that STAT3 is required for sympathetic regeneration, and is consistent with the notion that cytokine signaling is required for NGF-induced axon regeneration.

Neuroligin Involvement in Synapse Formation During Adult Neurogenesis

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Constitutive neurogenesis continues throughout the life of adult mammals in at least two brain regions, the hippocampal dentate gyrus and the subventricular zone. Unlike neurons generated during embryonic development, newly born neurons in adults must navigate through the relatively hostile environment of mature tissue to extend neurites. As neuroligins are known to drive synapse formation during embryonic development, we examined whether neuroligins have a specific role in the formation of synapses by new adult-born neurons in the dentate gyrus. We used replication-deficient retroviruses to specifically infect dividing neuroblasts and manipulate neuroligin levels as these newborn granule cells differentiated in adult mice. Co-expression of GFP via an IRES sequence labeled infected neurons. Retrovirally expressed HA-tagged neuroligin proteins trafficked to punctate structures on granule cell dendrites in the dentate molecular layer. Over-expression of neuroligin-1 accelerated the development of dendritic spines in newborn granule cells, increasing the number of spines in neurons as they matured. These results are consistent with a role for neuroligin in the formation of excitatory synapses onto adult-generated granule cells. Whole cell recordings from infected cells demonstrated an increase in excitatory synaptic function, as well as a change in the ratio of synaptic AMPA-type to NMDA-type glutamate receptors. These experiments suggest that manipulations of neuroligin levels in other populations of newly differentiating adult neurons, such as those formed after brain injury or after stem cell transplantation, could modulate functional integration of these cells.

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**Measuring vesicular release of dopamine from single periglomerular neurons**

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The olfactory bulb contains the largest population of dopaminergic neurons in the brain. These periglomerular cells have several special characteristics: they are dual transmitter neurons also containing GABA; they lack axons and therefore neurotransmitter release must occur in the dendrites or soma; and they are replenished from newborn neurons migrating from the subventricular zone. Little is known about where dopamine and GABA are stored in these cells and whether dopamine is released from vesicles.

We used amperometry, electrophysiology, electron microscopy and real time PCR to elucidate how dopamine is stored and released from individual dopamine periglomerular neurons. Amperometry allows detection of oxidizable neurotransmitters on the time scale of microseconds, and can detect single vesicular release events. We demonstrate that periglomerular neurons secrete oxidizable quanta, likely indicating that dopamine is packed into vesicles in those cells. Moreover we were able to simultaneously monitor GABA and dopamine release from the same neuron. The two neurotransmitters showed a very different pattern of release. Although GABA release was tightly coupled to the stimulus, secretion of dopamine was asynchronous. Taken together, our data suggest that dopamine and GABA are likely to serve temporally distinct roles in the function of this neural circuit.

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**Regulated Expression of MicroRNAs by Activation of the Group I Metabotropic Glutamate Receptors in Mouse Brain**
MicroRNAs are small non-coding RNAs that regulate post-transcriptional gene expression. In the short time since the discovery of microRNAs, the literature has burgeoned with studies focused on the biosynthesis of microRNAs, target prediction and binding, and mechanisms of translational repression by microRNAs. Given the prominent role of microRNAs in all areas of cell biology, it is not surprising that microRNAs are also linked to human diseases, including those of the nervous system. One of the least-studied areas of microRNA research is how their expression is regulated outside of development and cancer. Thus, we examined a role for regulation of microRNAs by neurotransmitter receptor activation in mouse brain. We focused on the group I metabotropic glutamate receptors by using intracerebroventricular injection of the selective agonist, (5)-3,5-dihydroxyphenylglycine (DHPG) in mouse brain. We then examined the expression of microRNAs in the cerebral cortex by Ambion and Invitrogen microarrays, and the expression of mature microRNA sequences by SABiosciences qPCR arrays, at 4, 8 and 24h after DHPG injection. These studies revealed that the largest number of significantly regulated microRNAs was detected 8h after DHPG injection in the microarrays and qPCR arrays. We then used RNA blots to quantify microRNA expression, and in situ hybridization to examine cellular distribution of the microRNAs regulated by DHPG. Bioinformatic analysis of the microRNAs regulated 8h after DHPG in all three arrays revealed KEGG pathways that are known to correlate with group I mGluR effects, as well as recently described and novel pathways. These studies are the first to show that DHPG regulates the expression of microRNAs in mouse cerebral cortex, and support the hypothesis that group I mGluRs may regulate microRNA expression in mouse brain.

Dysfunctional Neuronal M<sub>2</sub> Muscarinic Receptor Causes Airway Hyperresponsiveness in Diet-induced Obese Rats

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Obesity is associated with asthma but the molecular mechanisms are unclear. Asthma is a chronic disease that is characterized by airway narrowing due to increased contraction of airway smooth muscle and airway hyperresponsiveness. Airway hyperresponsiveness can occur through dysregulation of parasympathetic nerves, which provide dominant autonomic control of airway smooth muscles. Parasympathetic nerves release acetylcholine (ACh), which binds to M<sub>2</sub> muscarinic receptors on smooth muscle, causing contraction and bronchoconstriction. ACh also activates inhibitory M<sub>2</sub> muscarinic receptors on the nerves themselves, inhibiting further ACh release and limiting bronchoconstriction. Dysfunctional M<sub>2</sub> receptors have been reported in patients with asthma and in animal models of asthma. Thus, we investigated the mechanism of obesity induced increase in airway responsiveness in diet-induced obese rats. Out-bred obesity-prone and obesity-resistant rats were fed either a high fat or a low fat diet for 5 weeks. Food intake and body weight were recorded every three days. Body fat was measured by nuclear magnetic resonance. After 5 weeks, obese-prone rats had higher body weight and percentage of body fat than obese-resistant rats, regardless of diet. Fasting glucose was not significantly different among groups, but fasting insulin was significantly higher in the high fat diet fed obese-prone rats compared to all other groups. Airway responsiveness to parasympathetic nerve stimulation, measured in anesthetized, paralyzed and ventilated rats, was not different between obese-prone and obese-resistant rats on a low fat diet. However, when rats were fed a high fat diet, bronchoconstriction was significantly increased in obese-prone rats. In contrast, bronchoconstriction in response to i.v. ACh was not significantly different among groups of rats vagotomized to remove any reflex. These data demonstrate that smooth muscle is not changed by obesity, but parasympathetic nerves release increased ACh in obese animals. Neuronal M<sub>2</sub> muscarinic receptor function was measured using a muscarinic agonist, pilocarpine, to inhibit parasympathetic nerve induced bronchoconstriction. M<sub>2</sub>
Receptor function was significantly decreased in obese-prone rats compared to obese-resistant rats on a high fat diet, indicating that M2 receptors are no longer inhibiting ACh release in diet-induced obese rats. Thus, the airway hyperresponsiveness in high fat diet induced obese rats is mediated by dysfunctional M2 muscarinic receptors on parasympathetic nerves, leading to increased ACh and subsequently to increased bronchoconstriction as seen in asthma.

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**Adenosine A1 receptor activation in rat brain induces a hypothermic and hypometabolic state**

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Hibernating animals can reduce their energy expenditure by suspending both shivering and non-shivering thermogenesis, even in the face of marked falls in skin and core temperatures. In hibernating species, icv injection of the adenosine A1-receptor agonist, N6-cyclohexyladenosine (CHA), can induce a hypothermic and hypometabolic state characteristic of hibernation. In the current studies we investigated: 1) the potential for an adenosine receptor agonist to induce hypothermia in the rat (a non-hibernating species), 2) the precise thermo-effector mechanisms contributing to the fall in body temperature and 3) the brain circuits involved in the adenosine agonist-induced hypothermia. In inactin-anesthetized, Wistar rats, icv administration of CHA (10μl, 1mM) decreased core, skin and BAT temperatures, reduced expired CO2, heart rate (HR), brown adipose tissue (BAT) sympathetic nerve activity (SNA) and shivering EMG activity. In awake rats in a 15°C ambient, a similar icv injection of CHA decreased BAT temperature and induced a sleep-like state, in contrast to icv injection of saline vehicle which had no effect on BAT temperature or observed behavioral state. In urethane/chloralose anaesthetized rats direct nanoinjection of CHA (60nl, 1mM) in the nucleus of the solitary tract (NTS) produced a reduction in cold-evoked BAT SNA, BAT temperature, expired CO2 and HR concomitant with an increase of mean arterial pressure. These effects were reversed by direct nanoinjection of muscimol (60nl, 1mM) and prevented by nanoinjection of an adenosine-A1 antagonist in the NTS. We conclude that adenosine A1-receptor stimulation in the NTS can produce hypothermia in the rat and that inhibition of BAT and shivering thermogenesis contributes to this marked reduction in core temperature. This pharmacological approach to inducing a hypothermic and hypometabolic state could provide the basis for a therapeutic intervention to reduce ischemic tissue damage in pathologies such as stroke, traumatic brain injury and cardiac arrest.

Supported by NIH grant NS40987.
Behavioral Activation as an Alternative Treatment for PTSD among Returning Veterans

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Although empirically supported treatments exist for PTSD, many veterans do not seek or remain in these treatments. Perceived stigma and difficulties accessing care are two factors that have been implicated in low treatment adherence among returning veterans. Behavioral Activation is a well-established treatment for depression with accumulating support for the treatment of PTSD. Importantly, the emphasis in BA on engaging in goal-directed activity to prevent chronic patterns of avoidance may be more consistent with the preferences of many veterans and therefore aid treatment engagement and adherence. We will overview the core components of our adaptation of BA for PTSD and present data on a randomized controlled trial of BA compared to treatment as usual (specialty PTSD care) among a cohort of veterans returning from the wars in Iraq and Afghanistan (N=60). Preliminary analyses indicate that BA is an efficacious treatment for PTSD that is well-received by veterans.

SKF 81297, a dopamine D1 receptor agonist, enhances extinction of contextual fear

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Previous research has demonstrated that dopaminergic signaling is important for learning and memory, as well as a critical mediator of motivation and salience. Fear extinction, the process by which a conditioned fear response is suppressed, involves the formation of new inhibitory memories and dopamine D1 receptors are distributed through regions of the brain that are important to fear extinction, such as the hippocampus, prefrontal cortex, and amygdala. Previous studies with dopamine D1 receptor antagonists have demonstrated fear extinction impairments with D1 receptor blockade. Here, we show that SKF 81297, a full D1 agonist, administered prior to or following a fear extinction session will enhance extinction retention in C57BL/6 mice. Mice received contextual fear conditioning on Day 1 (four
0.35 mA foot shocks in a 12-min session). On Day 2, mice were returned to the context for a 12-min exposure with no foot-shock and given doses of SKF 81297 ranging from 1-10 mg/kg or vehicle (saline) prior to or immediately following the session. On Days 3-5, mice were returned to the context each day for 12-min non-reinforced tests. Animals who received SKF 81297 prior to or following extinction (Day 2) showed decreased freezing on Days 3-5, suggesting enhanced extinction consolidation and retention. These studies are consistent with previous research indicating improvements in working memory following administration of D1 agonists, and suggest that D1 dopamine receptors could be a potential pharmacological target for PTSD patients undergoing exposure therapy. Future studies will extend these findings by examining the cellular mechanisms that guide the SKF 81297-mediated fear extinction enhancements.

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Maternal high-fat diet consumption suppresses serotonergic system signaling in juvenile nonhuman primate offspring resulting in increased anxiety, decrease social interactions and perturbations in energy balance regulation

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Maternal nutrition and energy status have long term effects on offspring behavior and energy balance regulation. Given the current prevalence of obesity in developed nations, it is critical to examine the consequences of maternal over nutrition and obesity on offspring behavior. We previously determined that maternal high-fat diet consumption caused perturbations in the central serotonin system of fetal offspring and increased anxiety-like behavior in infant offspring. As the serotonergic system is a critical regulator of both energy balance and behavior, the goal of this study was to use a non-human primate model of diet-induced obesity to examine the consequences of maternal obesity and high-fat diet (HFD) consumption on the behavioral determinants of energy balance (feeding behavior, food preference and physical activity), anxiety like behavior and social behavior in juvenile offspring. Offspring from female Japanese macaques consuming either a low fat control diet (13% of calories from fat) or a HFD (36% calories from fat) were examined. The Human Intruder test and novel object tests were used to assess stress and anxiety responses to a social threat or novel item when offspring were 11 months of age. These tests were adapted from tests commonly used in children and have been shown to reliably assess individual differences in primate stress response and anxiety. Behavior in social housing was assessed by frame by frame analysis of videography. Preference for diets of differing fat and sugar content were examined at 8 and 10 months of age. Physical activity was measured by accelerometry from 6-12 months of age. Offspring from mothers fed a HFD exhibited increased latency to explore novel objects indicating increased anxiety. In social housing, offspring from HFD mothers spend more time alone and less time engaged in play with other animals. After weaning, offspring from HFD mothers spend more time eating and display differences in preference for diets of varying compositions. For example, offspring from HFD mothers placed on the CTR diet at weaning display increased preference for diets high in sugar and fat. Also, maternal-diet dependent changes in physical activity level were observed such that HFD offspring that continued to consume the HFD diet post-weaning were less active than CTR offspring placed on the HFD at weaning. Moreover, we observe a suppression in the serotonin system in juvenile offspring as indicated by a reduction in tryptophan hydroxylase 2 (THP2; the rate limiting enzyme in serotonin synthesis) in the dorsal raphe and a reduction in CSF serotonin in juvenile offspring from HFD fed mothers suggesting that perturbations in the serotonin system persist into the post-weaning period. Together these data suggests that maternal over nutrition causes decreased serotonergic tone which results in long term changes in energy balance regulation and heightened anxiety in juvenile nonhuman primates.
Support: This work was supported by grants from the US National Institutes of Health (RO1DK079194, DK60685-S2, DK7919481, DK079194-S1, R00163 and 5 UL1 RR24140-05).

**Functional connectivity patterns of the reward system inform distinct communities in youth with and without ADHD**

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**Introduction and Objectives:** An obstacle likely limiting our ability to clarify ADHD etiology is heterogeneity in the population. Including etiologically distinct subgroups as a unitary sample in any study is likely to produce muted effects (Hyman, 2007, *Nature Reviews.Neuroscience, 8*(9), 725-732). Graph theory and community detection techniques, combined with brain functional connectivity, may assist to overcome this obstacle (Newman, 2006, *Proceedings of the National Academy of Sciences of the United States of America, 103*(23), 8577-8582). We aimed to evaluate whether distinct subgroups could be determined based on functional connectivity patterns of the reward network, which has been hypothesized as one of the core systems involved in ADHD.

**Methods:** The region most selectively associated with reward in the functional neuroimaging literature was determined by an automated brain-mapping meta-analysis platform (Yarkoni, Poldrack, Nichols, Van Essen, & Wager, 2011, *Nature Methods, 8*(8), 665-670) (consistent with left nucleus accumbens). We then applied a community detection procedure to the functional connectivity maps (using the reward region as a seed) of 117 children with and without ADHD (aged 7-12), to identify groups based on connectivity patterns.

**Results:** Subjects were found to be divided into 2 distinct communities: group A (30 controls, 20 ADHD), group B (27 controls, 26 ADHD), differentiated by the strength of the decoupling between the seed region and the thalamus (group A: weaker negative connectivity between the reward seed region and the thalamus). Within group A, compared to ADHD, controls had stronger functional connectivity between the reward region and regions of the default network. Within group B, controls displayed stronger negative connectivity to the dorsal anterior cingulate cortex.

**Discussion:** Our findings show that children with ADHD and typically developing children can be classified in distinct subgroups according to functional connectivity; and also suggest that variation within ADHD follow the same trends of normal variation. Combining neuroimaging data and community detection techniques might be an important tool to elucidate heterogeneity in ADHD neurobiology.
Television and the social idealization and disillusionment of medicine

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Medical drama on television often depicts doctors and modern medicine as infallible and capable of unrealistic feats of curing the sick. The public tends to believe these misrepresentations as medical facts which leads the public to idealize medicine. This paper explores the idealization of medicine as a defense to protect individuals emotionally from considering their own mortality. But having unrealistic faith in medicine can lead to disillusionment in patients when the medical system cannot provide adequate treatment, information, or results. In these circumstances, patients are more likely to discontinue medical care or seek alternatives such as complementary and alternative medicine. It becomes the role of the physician to educate patients where television drama fails. Talking with patients about their expectations regarding their medical care gives physicians a chance to dispel medical myths and provide more accurate health information while assisting patients in making more informed choices in their own health care.

The Patient Centered Outcomes Research Institute (PCORI) Expert Interviews Project

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Patient involvement in research is emerging as a priority in the US. To promote patient-centered outcomes research, Congress established an independent organization, the Patient Centered Outcomes Research Institute (PCORI). In November 2011, the Center for Evidence-based Policy (CEBP) was selected to conduct interviews with citizens and a comprehensive group of experts in the field of patient and public engagement to help identify best practices and effective methods for incorporating the patient voice in research.
Working with a representative advisory panel, CEBP conducted 87 in-depth interviews with national and international experts and 12 facilitated discussions with 123 patients and carers across six regions of the US. We will discuss the findings from the expert interviews and facilitated discussions, including key principles and effective methods of successful patient and public involvement. These findings formed the basis of a subset of research standards for PCORI’s Methods Guide and indicated areas of future research needs. Research standards cover the impact of research for patients, planning for patient involvement, selection of patients for involvement activities, dissemination of research results to patients and reporting of involvement methods and results in research reports.

Disclaimer: This work was funded by PCORI. The views expressed in this submission are those of the research team at the Center for Evidence-based Policy and do not necessarily reflect those of PCORI.

Oregon State Guidelines: Development of a Statewide, Multi-stakeholder Guidelines Program

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Background: Oregon’s legislature passed comprehensive health reform legislation in 2009 that directed the state to “set standards for safe and effective care,” including development of “best practice guidelines and standards that can be uniformly applied across public and private health care.”

Objective: Develop guideline methodology and guidelines for Oregon clinicians and payers.

Methods: Employed ADAPTE framework for guideline development. Initial guidelines selected for development included three low back pain (LBP) topics: general evaluation and management of LBP, advanced imaging for LBP, and percutaneous interventions for LBP. (Description below for first guideline only.) Seed guidelines identified by searching 17 databases. Quality evaluated using modified AGREE II instrument. Multidisciplinary guideline development group (GDG) selected and adapted seed guidelines. Stakeholder peer review and public comments were solicited.

Results: 13 seed guidelines identified and 10 met minimal inclusion criteria for LBP evaluation and management topic. Dual quality rating found five of good or fair quality. Final seed guideline selected based on quality and attention to acute and chronic back pain. A consumer booklet was developed and distributed electronically and in hard copy to consumer, provider, and payer groups. Over 400 booklets were distributed with 25,000 page views (as of 1/12). Professional organizations were largely supportive of guideline. Clinical and resource utilization outcomes are pending. Initial guideline process took over one year to complete at cost of ~$200,000.

Discussion and implications: Starting a new multi-stakeholder guideline development program requires substantial investments of methodological expertise, staff time, funds and political capital.

Effectiveness of public reporting of health care quality information as a quality improvement strategy

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Objectives: The goal of this review was to evaluate the effectiveness of public reporting of health care quality information as a quality improvement strategy. We sought to determine if public reporting results in improvements in health care delivery and patient outcomes. We also considered whether public reporting affects the behavior of patients or of health care providers. Finally we assessed whether the characteristics of the public reports and the context affect the impact of public reports.

Data Sources: Articles were identified through searches of the following bibliographical databases: MEDLINE, EMBASE, EconLit, PsychINFO, Business Source Premier, CINAHL, PAIS, Cochrane Database of Systematic Reviews, EPOC Register of Studies, DARE, NHS EED, HEED, NYAM Grey Literature Report database, and other sources (experts, reference lists, and grey literature).

Review Methods: We screened citations based on inclusion and exclusion criteria developed based on our definition of public reporting. We initially did not exclude any studies based on study design. Of the 11,809 citations identified through title and abstract triage, we screened and reviewed 1,632 articles. A total of 97 quantitative and 102 qualitative were included, abstracted, and entered into tables and evaluated. The heterogeneity of outcomes as well as methods prohibited formal quantitative synthesis. Systematic reviews were used to identify studies, but their conclusions were not incorporated into this review.

Results: For most of the outcomes the strength of the evidence available to assess the impact of public reporting was moderate. This was due in part to the methodological challenges researchers face in designing and conducting research on the impact of population-level interventions. Public reporting is associated with improvement in health care performance measures such as those included in Nursing Home Compare. Almost all identified studies found no evidence or only weak evidence that public reporting affects the selection of health care providers by patients or their representatives. Studies of health care providers’ response to public reports suggest they do engage in activities to improve quality when performance data are made public. Characteristics of public reports and the context, which are likely to be important when considering the diffusion of quality improvement activities, were rarely studied or even described.

Conclusions: The heterogeneity of the outcomes and the moderate strength of evidence for most outcomes make it difficult to draw definitive conclusions; however some observations were supported by research. Public reporting is more likely to be associated with changes in health care provider behaviors than with selection by patients or families of health services providers. Quality measures that are publicly reported do improve over time. Although the potential for harms are frequently cited by commentators and critics of public reporting, the amount of research on harms is limited and most studies do not confirm the potential harm.

Fatigue: Take Control: A VA Multi-Center Randomized Controlled Trial for Treatment of MS-Related Fatigue

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Background: Multiple sclerosis (MS) is a common and often disabling disease of the central nervous system affecting approximately 400,000 Americans and 2.5 million people worldwide. Fatigue occurs in 75-95% of people with multiple sclerosis and is often reported as the most disabling symptom. In 1998 the MS Council for Clinical Practice Guidelines published a clinical practice guideline Fatigue and MS recommending comprehensive treatment for fatigue. The widely
distributed guideline does not include an implementation program. A formal group program *Fatigue: Take Control* was created in partnership with the National MS Society to implement the recommendations. Hundreds of people with MS have taken the program but its effectiveness is undetermined. Our randomized, controlled pilot trial suggested that it may reduce fatigue and increase self-efficacy in ambulatory people with MS but a larger, multicenter trial is needed to clearly prove effectiveness.

**Objective:** To determine if *Fatigue: Take Control* improves fatigue in MS subjects more than a control general education program on MS-related topics.

**Method:** This will be a single blind randomized controlled two-arm trial comparing *Fatigue: Take Control* with a general MS group education program called *MS: Take Control* with MS participants at Portland, Seattle, Baltimore and North Florida/South Georgia VA Medical Centers. We will enroll 50 subjects at each site for a total enrollment of 200 subjects. Subjects will receive the intervention for 6 weeks and be followed for an additional 26 weeks. The primary outcome will be changes in scores on the Modified Fatigue Impact Scale (MFIS). Secondary outcome measures include self-efficacy, activity and quality of life.

**Results:** The VA RR&D Research Service recently funded this study and we will commence enrollment in the fall of 2012.

**Conclusions:** Currently there are few symptomatic therapies for people with MS. Demonstrating that any new treatment for fatigue in MS is effective would be a significant innovation that would improve fatigue and its associated limitations. *Fatigue: Take Control* is available at all VA Medical Centers and all National MS Society chapters so results could be rapidly applied to clinical and community practice. If shown to be effective and with the program’s ready availability, it will immediately be possible to deliver cost-effective proven fatigue management information to people with MS throughout the US and other English speaking countries.

The project described is supported by project no. F7777-R from the Rehabilitation Research & Development Service of the VA Office of Research and Development.
Xuehong Liu | Thermal Instability of ΔF508 CFTR Channel Function: Protection by Single Suppressor Mutations and Inhibiting Channel Activity

Yohei Norimatsu | Molecular Modeling of the CFTR Chloride Channel

David Koeller | Evidence for an association between infant mortality and homozygosity for a carnitine palmitoyltransferase 1a genetic variant

Thermal instability of ΔF508 CFTR channel function: Protection by Single Suppressor mutations and inhibiting channel activity

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Deletion of Phe508 from CFTR results in a temperature-sensitive folding defect that impairs protein maturation and chloride channel function. Both of these adverse effects, however, can be mitigated to varying extents by second-site, suppressor mutations. To better understand the impact of second-site mutations on channel function, we compared the thermal sensitivity of CFTR channels in Xenopus oocytes. CFTR-mediated conductance of oocytes expressing wt or ΔF508 CFTR was stable at 22°C and increased at 28°C; a temperature permissive for ΔF508 CFTR expression in mammalian cells. At 37°C, however, CFTR-mediated conductance was further enhanced, whereas that due to ΔF508 CFTR channels decreased rapidly towards background, a phenomenon referred to here as “thermal inactivation”. Thermal inactivation of ΔF508 was mitigated by each of five suppressor mutations, I539T, R553M, G550E, R555K and R1070W; but each exerted unique effects on the severity of, and recovery from, thermal inactivation. Another, K1250A, known to increase open probability (P0) in ΔF508 CFTR channels, exacerbated thermal inactivation. Application of potentiaters known to increase P0 of ΔF508 CFTR channels at room temperature failed to protect channels from inactivation at 37°C and one, PG-01, actually exacerbated thermal inactivation. Unstimulated ΔF508CFTR channels or those inhibited by CFTRinh-172, were partially protected from thermal inactivation; suggesting a possible inverse relationship between thermal stability and gating transitions of ΔF508 CFTR channels. Thermal stability of channel function and maturation of the mutant protein appear to reflect distinct, but related facets of the ΔF508 CFTR conformational defect, both of which must be addressed by effective therapeutic modalities.

Molecular Modeling of the CFTR Chloride Channel

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We developed molecular models for the CFTR chloride channel based on the prokaryotic ABC transporter, Sav1866. Here we analyze predicted pore geometry and side-chain orientations for TM S 3, 6, 9 and 12; with particular attention to the location of the rate-limiting barrier for anion conduction. Side-chain orientations assayed by cysteine scanning were found to be from 77% to 90% in accord with model predictions. The predicted geometry of the anion conduction path was defined by a space-filling model of the pore and confirmed by visualizing the distribution of water molecules from a molecular dynamics (MD) simulation. Pore shape is that of an asymmetric hour glass, comprising a shallow outward-facing vestibule that tapers rapidly toward a narrow “bottleneck” linking the outer vestibule to a large inner cavity extending toward the cytoplasmic extent of the lipid bilayer. The junction between the outer vestibule and the bottleneck features an outward-facing rim marked by T338 in TM6 and I1131 in TM12, consistent with the observation that cysteines at both of these locations reacted with both channel-permeant and channel-impermeant, thiol-directed reagents. Conversely, cysteines substituted for S341 in TM6 or T1134 in TM12, predicted by the model to lie below the rim of the bottleneck, were found to react exclusively with channel-permeant reagents applied from the extracellular side. The predicted dimensions of the bottleneck are consistent with the demonstrated permeation of Cl−, pseudohalide anions, water and urea.

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**Evidence for an Association Between Infant Mortality and Homozygosity for a Carnitine Palmitoyltransferase 1A Genetic Variant**

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**Background:** In 2003 Alaska initiated newborn screening by tandem mass spectrometry, which identified an unexpectedly high incidence of carnitine palmitoyltransferase 1A (CPT1A) deficiency, one of a group of disorders of fatty acid oxidation that has been associated with sudden infant death. Affected infants are all of Alaska Native heritage, and homozygous for a single variant in the CPT1A gene (c.1436C→T; p.P479L). Statewide, 26% of Alaska Native infants are homozygous for this variant, and in Western and Northern Alaska 51% of Alaska Native infants are homozygous, and 37% heterozygous (allele frequency, 0.7). The infant mortality rate in these regions of Alaska (Western and Northern) is almost twice as high (11.4/1,000 vs. ~6/1,000) as that in Anchorage and the rest of the state.

**Methods:** We performed an unmatched case-control study to test the hypothesis that homozygosity for the variant is a risk factor for infant death. Based on the availability of newborn screening cards, cases were 110 Alaska Native infant deaths that occurred from 2006 through 2010; controls were 395 Alaska Native births from the same time period. Because the CPT1A variant in Alaska is highly concentrated among Yupik/Inupiat populations, and Alaska Native sub-populations have substantial differences in risk factors for infant mortality, we conducted two analyses to increase the likelihood of having both cases and controls come from the Yupik/Inupiat populations. The first used all genotypes but was limited to the Western and Northern regions, which contain primarily Yupik/Inupiat people. The second included all regions but was limited to infants homozygous or heterozygous for the variant. Genotyping was by an allelic discrimination assay.
**Results:** Among Western/Northern residents, 66% of cases and 51% of controls were homozygous for the variant (crude odds ratio [OR] 1.8; 95% CI: 1.0-3.3). In a multivariate model used to adjust for possible confounding factors (maternal education, prenatal substance use, and a composite variable of marital status and the presence of a father’s name on the birth certificate), infant mortality and homozygosity remained associated (OR, 2.5; 95% CI, 1.3-5.0). Results for the all-Alaska analysis were similar (crude OR, 1.6; 95% CI 0.92-2.6) (adjusted OR, 2.0; 95% CI, 1.1-3.6). Cause of death analysis is ongoing.

**Discussion:** Homozygosity for the c.1436C→T sequence variant of CPT1A is associated with an increased risk of infant mortality. The associated risk accounts for the majority of the increased risk of infant mortality observed in Western and Northern Alaska. These results suggest that early identification of homozygous infants, in combination with education of parents and local health providers about prevention of symptoms of CPT1A deficiency could significantly reduce infant mortality among affected Alaska Native infants.
**Dabigatran Etxilate: Management in Acute Ischemic Stroke**

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**Background:** After years of using warfarin for long-term oral anticoagulation to prevent cardiac thromboembolism in patients with non-valvular atrial fibrillation (AF), new drugs are emerging. Without the need for ongoing monitoring, the twice daily dosing of dabigatran makes it a potential drug of choice for both patients and practitioners. Thus, practitioners must familiarize themselves with clinical challenges posed by dabigatran.

**Case Report:** A 54-year-old man presented 69 minutes after experiencing an ischemic stroke. His past medical history included medically controlled hypertension on atenolol 25mg daily and AF status post ablation and on dabigatran 150mg twice daily. Neurological findings included: inability to follow commands with the right arm while able to follow commands on the left side, right-sided pronator drift, right-sided facial droop, and aphasia. Motor strength was: 3/5 proximal and distal right upper extremity, 5/5 proximal and distal left upper extremity, and 5/5 right and left lower extremities. Recombinant tissue plasminogen activator (rtPA) administration was not recommended secondary to dabigatran therapy.

The patient was transferred to our institution where a National Institutes of Health Stroke Scale (NIHSS) was 9. Angiography demonstrated occlusion of the left middle cerebral artery (MCA), and suction thrombectomy achieved flow through the inferior division of the left MCA. CT showed possible intracranial hemorrhage (ICH), and dabigatran reversal was attempted with prothrombin complex concentrate (PCC) and recombinant factor VIIa (rFVIIa). Coagulation studies prior to the administration of the reversal blood products demonstrated a partial thromboplastin time (PTT) 30.3 seconds and 1 hour after administration PTT was 28.5 seconds.

The patient did have an episode of AF with rapid ventricular response requiring metoprolol and diltiazem. There was no evidence of ICH on repeated CT scans of the brain. He was discharged on hospital day 7 to rehabilitation on aspirin and warfarin with an NIHSS score of 8.
**Conclusion:** There is a lack of clinically sensitive assays to examine the anticoagulant effect of dabigatran. The thrombin time, Hemoclot® thrombin inhibitor assay, ecarin clotting time (ECT), and ecarin chromogenic assay are emerging as sensitive laboratory tests, but PTT insensitive. Only 3 case reports on the use of IV rtPA with concurrent dabigatran use exist in the literature. Conservative recommendations suggest waiting >48 hours since the last dabigatran dose with a normal PTT or waiting >24 hours since the last dabigatran intake with a normal ECT before administering rtPA. Although the use 3-factor PCC and 1mg/kg rFVIIa has been reported as a potential reversal agent, a true reversal agent has not yet been developed for dabigatran and clear guidelines do not exist for administration of available agents. This report is based on the limited available literature. Further studies are necessary as dabigatran use becomes more widespread in clinical practice.

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**LPS preconditioning redirects TLR signaling following stroke: TRIF-IRF3 plays a seminal role in mediating tolerance to ischemic injury**

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Background: Toll-like receptor 4 (TLR4) is activated in response to cerebral ischemia leading to substantial brain damage. In contrast, mild activation of TLR4 by preconditioning with low dose exposure to lipopolysaccharide (LPS) prior to cerebral ischemia dramatically improves outcome by reprogramming the signaling response to injury. This suggests that TLR4 signaling can be altered to induce an endogenously neuroprotective phenotype. However, the TLR4 signaling events involved in this neuroprotective response are poorly understood. Here we define several molecular mediators of the primary signaling cascades induced by LPS preconditioning that give rise to the reprogrammed response to cerebral ischemia and confer the neuroprotective phenotype. Methods: C57BL6 mice were preconditioned with low dose LPS prior to transient middle cerebral artery occlusion (MCAO). Cortical tissue and blood were collected following MCAO. Microarray and qRT-PCR were performed to analyze gene expression associated with TLR4 signaling. EMSA and DNA binding ELISA were used to evaluate NF B and IRF3 activity. Protein expression was determined using Western blot or ELISA. MyD88/- and TRIF/- mice were utilized to evaluate signaling in LPS preconditioning-induced neuroprotection.

Results: Gene expression analyses revealed that LPS preconditioning resulted in a marked upregulation of anti-inflammatory/type I IFN-associated genes following ischemia while pro-inflammatory genes induced following ischemia were present but not differentially modulated by LPS. Interestingly, although expression of pro-inflammatory genes was observed, there was decreased activity of NF B p65 and increased presence of NF B inhibitors, including Ship1, Tollip, and p105, in LPS-preconditioned mice following stroke. In contrast, IRF3 activity was enhanced in LPS-preconditioned mice following stroke. TRIF and MyD88 deficient mice revealed that neuroprotection induced by LPS depends on TLR4 signaling via TRIF, which activates IRF3, but does not depend on MyD88 signaling. Conclusion: Our results characterize several critical mediators of the TLR4 signaling events associated with neuroprotection. LPS preconditioning redirects TLR4 signaling in response to stroke through suppression of NF B activity, enhanced IRF3 activity, and increased anti-inflammatory/type I IFN gene expression. Interestingly, this protective phenotype does not require the suppression of pro-inflammatory mediators. Furthermore, our results highlight a critical role for TRIF-IRF3 signaling as the governing mechanism in the neuroprotective response to stroke.
Preconditioning is the phenomenon in which pre-exposure to a low dose of an otherwise damaging stimulus induces protection against a higher dose of a damaging stimulus (e.g. stroke). Preconditioning stimuli include ligands to the Toll-like receptors (TLRs), a family of evolutionarily conserved innate immune receptors that recognize pathogen or damage associated molecules. Stimulation of TLRs with their cognate ligands before injury induces a state of tolerance to a subsequent ischemic challenge resulting in neuroprotection against stroke. Experimental models of preconditioning focus on the central nervous system as the cellular target of cerebral protection while little attention has been paid to the cerebrovascular compartment whose role in the pathogenesis of ischemic brain injury is crucial. Preconditioning has been shown to attenuate blood-brain barrier (BBB) disruption and brain edema; however, the signaling pathways involved in the preconditioning-induced BBB protection are not known. We have previously shown that preconditioning with a stabilized form of polynosinic polycytidylic acid (poly-ICLC), a TLR3 and melanoma differentiation associated gene-5 (MDA-5) agonist, protects against cerebral ischemic damage. However, the site of action for poly-ICLC-induced neuroprotection is unclear. Here we asked the fundamental question of whether poly-ICLC induces tolerance to ischemia by preserving the function of the BBB. The BBB was modeled in vitro by co-culturing primary murine brain microvessel endothelial (BMEC) and astroglial cells in transwell plates. We found that poly-ICLC (2μg/ml) treatment of the BBB prior to 5h of oxygen glucose deprivation (OGD), which models stroke in vitro, maintained transendothelial electric resistance (TER) and endothelial paracellular and transcellular transport compared to controls. We also found that poly-ICLC treatment induced IFNβ mRNA expression in glial cells and that type I IFN signaling in BMECs was required for poly-ICLC-induced protection of the in vitro BBB. This last finding is supported by in vivo data showing that poly-ICLC requires type I IFN signaling to induce neuroprotection against ischemic injury.

In conclusion, we are the first to show that preconditioning with poly-ICLC attenuates ischemia-induced BBB dysfunction. Our data suggests that poly-ICLC preconditioning protects the BBB by inducing glial cells to produce IFNβ. The IFNβ signals via IFNARs on the endothelium to increase resistance against ischemic injury. Strategies that target these endothelial signaling pathways may serve as new therapeutic tool for neurovascular unit protection in stroke patients.
Hemiparesis is commonly observed following stroke, although a wide-range of motor deficits can be seen in both humans and experimental animals. Quantification of the extent of motor deficit following stroke can therefore be problematic and has been limited historically to the use of subjective neurological scales. While these sophisticated neurological scoring systems have great value, they also have obvious limitations, in that they provide a discontinuous view of the status of motor function of a subject and require significant training and expertise to implement. In the search for more objective measurements of physical activity, lightweight miniaturized accelerometer devices offer promise. The major advantage of accelerometer activity monitoring is that it enables the continuous objective evaluation of motor activity using a non-invasive, safe, and convenient method. The goal of our study was to assess the applicability of accelerometer-based measurements in experimental animals undergoing surgically-induced cerebral ischemia (stroke). We have developed a nonhuman primate (NHP) model of stroke that uses a minimally invasive transient vascular occlusion to elicit brain damage primarily localized to the cortex and as such, represents a model with substantial clinical relevance. Using this NHP cortical stroke model, we demonstrate for the first time that monitoring locomotor activity prior to and following cerebrovascular ischemic injury using an accelerometer is feasible in adult male rhesus macaques and that the measured activity outcomes significantly correlate with severity of brain injury. We further demonstrated the applicability of this technology in primate pre-clinical studies looking at the protective potential of the neuroprotective candidate D192935. Primates treated with D192935 showed more locomotor activity following stroke than the control group. In addition D192935 treatment also resulted in a 2-fold lower infarct volume and 2-fold improvement in cumulative 7-day neurological score compared to controls. Importantly, the accelerometry data significantly correlated with the extent of brain injury measured by infarct volume and cumulative neurological findings. We show that data derived from accelerometers can be useful in determining the preclinical efficacy of novel agents being tested in our NHP model of ischemic brain injury. The use of accelerometry as an unobtrusive, objective preclinical efficacy determinant complements our standard practices involving subjective neurological scoring and magnetic resonance imaging in NHPs, providing a continuous account of locomotor activity using this less biased approach to the assessment of neurological deficits following brain injury.
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Shreya Bhattacharya | Role of transcriptional regulator COUP-TF-interacting protein 2 (Ctip2) in hair follicle morphogenesis and postnatal hair cycling

Danielle Williamson | Intramolecular chaperones are sufficient to regulate organelle-specific pH-dependent activation of Furin and Proprotein Convertase 1/3

Jessica Martin | A Role for Adenine Nucleotides in the Sensing Mechanism to Purine Starvation in Leishmania donovani

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Daniel Austin | A State-Space Model for Finger Tapping with Applications to Cognitive Inference

Role of transcriptional regulator COUP-TF-interacting protein 2 (Ctip2) in hair follicle morphogenesis and postnatal hair cycling

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COUP-TF-interacting protein 2 (Ctip2), also known as Bcl11b, is a transcriptional regulatory protein that is highly expressed in mouse epidermis during embryogenesis and in adulthood (1). It is also expressed in bulge stem cells of adult hair follicles (1). Ctip2 null mice (Ctip2−/−), with a germline deletion of Ctip2 gene, exhibit defects in epidermal terminal differentiation and a compromised epidermal permeability barrier formation (2). Furthermore, Ctip2ep−/− mice, with an epidermal specific deletion of Ctip2, display aberrant expressions of hair follicle stem cell markers during wound healing (3). Hair follicles reconstitute themselves through hair cycling by proliferation of intrinsic stem cells. Therefore, our present studies focus on the role of Ctip2 in hair follicle morphogenesis and in postnatal hair cycling. We recently discovered that neonatal Ctip2−/− mice exhibit reduced hair follicle density. Since Ctip2 null mice die at birth, Ctip2ep−/− mice were utilized for the postnatal hair cycling studies. We have observed deregulated hair cycling and altered hair follicle morphology in the Ctip2ep−/− mice. The expressions of a subset of stem cell markers, involved in hair cycling were altered in the mutant epidermis. Interestingly, Ctip2 expression was found to be regulated in a synchronized manner during depilation induced adult murine hair cycling. To elucidate the role of Ctip2 in induced hair cycling, ligand inducible Ctip212/12-K14Cre-ER72 mouse line was used. In those mice, intraperitoneal injections of ligand (tamoxifen) lead to the selective loss of Ctip2 in epidermal keratinocytes thereby generating the Ctip2ep−/− mice. The Ctip2ep−/− mice exhibited extended anagen and reduced apoptosis during depilation induced hair cycling. Altogether, our results suggest that Ctip2 plays an important role in hair follicle morphogenesis, besides normal and in depilation induced hair cycling.


Intramolecular Chaperones are Sufficient to Regulate Organelle-Specific pH-Dependent Activation of Furin and Proprotein Convertase 1/3

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3These authors contributed equally to this work

Proprotein convertases (PCs), furin and proprotein convertase 1/3 (PC1), cleave substrates at dibasic residues along the eukaryotic secretory/endocytic pathway. PCs are evolutionarily related to bacterial subtilisin and are synthesized as zymogens, with N-terminal propeptides that function as dedicated intramolecular chaperones (IMCs). These IMCs facilitate folding and regulate activation of cognate proteases through multiple-ordered cleavages. Previous studies identified a histidine residue (His69) that functions as a pH sensor in the IMC-domain of furin (IMCfurin), which regulates furin-activation at pH~6.5 within the trans Golgi network. Although this residue is conserved in the PC1 IMC-domain (IMCPC1), PC1 nonetheless activates at pH~5.5 within the dense core secretory granules. Here we analyze the mechanism by which IMCfurin regulates furin activation and examine why IMCfurin and IMCPC1 differ in their pH-dependent activation. Spectroscopy and molecular dynamics establish that histidine-protonation significantly unfolds IMCfurin when compared to IMCPC1 to enhance autoproteolysis. Sequence analyses establish that while both IMCfurin and IMCPC1 are enriched in histidines when compared with cognate catalytic-domains and prokaryotic orthologs, histidine content in IMCfurin is ~two-fold greater than IMCPC1, which augments its pH sensitivity. We further demonstrate that IMCfurin and IMCPC1 are sufficient to confer organelle-sensing on folding and activation of cognate proteases. Swapping IMCs between furin and PC1 transfers pH-dependent protease activation in an IMC-dictated manner in vitro and in cells. Since prokaryotes lack organelles and eukaryotic PCs evolved from IMC-dependent, not IMC-independent prokaryotic subtilases, our results suggest that histidine- enrichment may have enabled IMCs evolve to exploit pH-gradients to activate within specific organelles.

A Role for Adenine Nucleotides in the Sensing Mechanism to Purine Starvation in Leishmania donovani

Jessica L. Martin¹, Philip A. Yates, Jan M. Boitz, Audrey L. Fulweiler, Buddy Ullman, Nicola S. Carter
Leishmania are kinetoplastid protozoan parasites with a complex digenetic life cycle during which they encounter dramatic physiological alterations in their host milieu as they transition from extracellular promastigotes in the insect vector to intracellular amastigotes that reside in the phagolysosome of mammalian macrophages. Acclimatization to these divergent environments is essential for parasite survival. Despite their significance, little is known about the molecular mechanisms used by these parasites to sense and respond to environmental changes, such as nutrient availability. Furthermore, the leishmanial genome, although rich in kinase-like sequences, lacks both G protein-coupled receptors and receptor tyrosine kinases, and the canonical signaling pathways present in higher eukarya are either absent or substantially different.

The salvage of purines in Leishmania is an obligatory process that impacts both cell viability and growth. Our previous studies have shown that purine starvation is easily induced in vitro and that the adaptive response to purine stress is both robust and readily tractable, provoking significant morphological and metabolic changes. Thus, the induction of purine starvation provides an ideal paradigm for the elucidation of nutrient stress response mechanisms in this parasite.

Leishmania are able to transport and readily convert any single purine nucleobase or nucleoside to fulfill their adenylate and guanylate nucleotide requirement needed for their viability and growth. We have assessed whether the adaptive response to purine starvation is triggered by perturbation of the intracellular pools of adenylate or guanylate nucleotides through the use of purine pathway mutants that allow the synthesis of adenosine monophosphate (AMP) or guanosine monophosphate (GMP) to be selectively and inducibly disrupted. In addition, we have used these purine pathway mutants to investigate whether adaptation to purine starvation arises from a direct sensing of available purine in the extracellular milieu by restricting the amount and type of extracellular purine available in the growth medium.

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**Role of Melanocytic RXRα/β in solar UV induced tissue homeostasis**

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Retinoid-X-Receptors (RXRs) α, β, and γ are members of the nuclear hormone receptor (NR) superfamily, and act as central coordinators of cell signal transduction through heterodimerization with several other NRs. In malignant human melanoma samples, loss of RXRα expression has been previously observed both in the melanoma cells themselves (Chakravarti et al, 2007) and in adjacent epidermal keratinocytes (Hyter et al, 2010). Previously, epidermal-specific ablation of RXRα in a mouse model has been shown to promote increased melanocyte proliferation after UV radiation (Wang et al, 2010) and increased susceptibility to malignant melanomas (Hyter et al, 2010). We find that ablation of RXRα and RXRβ specifically in the melanocytes of the skin results in an increase in apoptosis in non-melanocytic cells in the dermal layer following UV radiation. Ablation of RXRs and RXRβ results in decreased infiltration of F4/80+ immune cells and downregulated expression of interferon-γ (IFNγ) in the dermal layer following UV radiation, suggesting a defect in secretion of inflammatory chemokines. RT-PCR analysis of irradiated RXRα/β shRNA knockdown melanocytes in vitro revealed significant changes in expression of chemoattractive and chemorepulsive ligands implicated in chemotaxis of IFNG-secreting immune cells. Of these, Cxcl12 has also been previously reported to be upregulated in
metastatic melanoma (Vianello et al, 2006), and several other genes implicated in melanoma were found to be differentially regulated in the irradiated shRNA knockdown cells. Additionally, melanocytic RXRa/ RXRβ ablation resulted in a decreased percentage of apoptotic melanocytes following UV radiation in vivo, suggesting an increased survival of melanocytes themselves following DNA damage. This reduced apoptosis could result in increased incorporation of mutation into the melanocytes and increased susceptibility of tumor formation in the long-term.

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**A State-Space Model for Finger Tapping with Applications to Cognitive Inference**

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Sensory-motor functions have been repeatedly linked to both cognitive and physical functions. One common test of sensory-motor performance frequently used for neuropsychological evaluation is the Halstead-Reitan finger tapping test (FTT). While this test has been normed and used extensively, the underlying sensory, motor and cognitive processes mediating tapping behavior during the test are not well understood. As a first step towards investigating the behavioral aspects manifested by these processes, we describe a state-space model for finger tapping during the FTT. This state-space model exploits quasiperiodicity to decompose tapping into a set of time-varying states corresponding to the instantaneous amplitude of the finger oscillation, the instantaneous frequency (or speed) of tapping, and a phase that keeps track of the current finger position during the cycle. We evaluate the model by showing a good fit between estimated and actual measurements, and outline an experiment that will relate features from the model to cognitive function.
Selective involvement of the neuropeptide urocortin-1 in long-term alcohol consumption in mice

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Prior work from our laboratory revealed that ethanol (EtOH) drinking resulted in robust activation of neurons within the rodent centrally-projecting Edinger-Westphal nucleus (EWcp) containing the CRF-related neuropeptide urocortin-1 (Ucn1). In addition, expression of Ucn1 in the EWcp was genetically associated with sensitivity to several EtOH-related phenotypes, and electrolytic lesion of the EWcp attenuated EtOH intake and preference.

We also recently reported that genetic deletion of Ucn1 in mice on a C57BL/6J background decreased EtOH consumption in a long-term, continuous access two-bottle choice procedure using 3-10% EtOH, but not in a short-term, intermittent access single-bottle procedure using 20% EtOH. Therefore, we set out to determine the relative importance of each of these differentiating variables in mediating Ucn1’s effects on EtOH intake and preference.

Experiment A modified the short-term intermittent access study by offering mice concurrent access to water during the EtOH access session, thereby unmasking a minor and sex-specific role for Ucn1 in EtOH preference in this paradigm. Experiment B modified the long-term continuous access procedure by increasing the concentrations of EtOH from 3-10% to 10-40%, thereby confirming a concentration-independent role for Ucn1 in EtOH intake and preference in this paradigm. Experiment C used a long-term intermittent access procedure (3-20% EtOH) to demonstrate that Ucn1’s involvement in EtOH consumption is dependent on the extent of prior history with EtOH self-administration, rather than whether or not the access is intermittent or continuous.

Together, these studies are consistent with earlier place conditioning studies showing that mice deficient in Ucn1 are insensitive to EtOH-induced reward, and suggest that Ucn1 plays a critical role in long-term EtOH consumption. These findings lend further support for the importance of Ucn1 in the development of alcohol use disorders resulting from prolonged alcohol self-administration.

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Mild stress increases sensitivity to pain through the dorsal medial hypothalamus and rostral ventromedial medulla

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Stress produces a myriad of physiological and psychological effects, some beneficial and some maladaptive. Mild, prolonged stress leads to increased sensitivity to pain (hyperalgesia) through yet unknown neural circuits; mild stress also produces physiological changes, including elevated heart rate and blood pressure, which are driven by the dorsal medial nucleus of the hypothalamus (DMH). The DMH has anatomical connections to the rostral ventromedial medulla (RVM), a region of the brainstem that modulates pain via projections to the spinal cord. I hypothesize that mild stress increases sensitivity to pain via a functional connection between the DMH and RVM.

A model of restraint and air puff to the face was used to induce a mild stress in awake Sprague-Dawley rats. This mild stress has been previously shown to produce the physiological changes described above, and in this case it also produced hypersensitivity to pain and increased responsiveness to non-painful stimuli. In order to assess the role of the DMH and RVM in this model of stress-induced hyperalgesia (SIH) a guide cannula was stereotaxically implanted into one of these brain areas. An intraparenchymal injection was made through the guide cannula to inactivate one area or the other prior to the stress. Neurons in the RVM were inactivated with an injection of the sodium channel blocker lidocaine, which attenuated SIH, while a control injection of artificial cerebrospinal fluid prior did not change the effect of stress on pain thresholds. Similarly, neurons in the DMH were inhibited with an injection of the GABA agonist muscimol. Inactivation of the DMH also attenuated SIH compared to a control aCSF injection. In the absence of stress, activation of the DMH with an injection of the GABA antagonist bicuculline led to pain hypersensitivity.

A functional connection between the DMH and RVM is responsible for the increased sensitivity to pain resulting from mild, prolonged stress; inactivation of either region blocks SIH. Additionally, activation of the DMH alone is sufficient to induce pain hypersensitivity. These data give additional insight into pain modulation and how stress influences pain perception. Understanding the influence of stress on pain has important implications for pain treatment, and highlights the importance of effectively managing chronic stress when treating pain conditions. Further research is needed to better understand these complex neural pathways, and will hopefully lead to more effective pain treatments for patients.

Salivary alpha amylase (sAA) as a stress biomarker in middle-aged adults

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**Background:** Salivary alpha amylase (sAA) is an emerging stress biomarker with a stress response profile distinctive from that of salivary cortisol. Previous studies describing sAA stress-related changes reported some inconsistent findings about sAA stress reactivity possibly due to using different stressor tasks, and few studies looked at sAA in adults 50 or older. This study aimed at evaluating changes in sAA after three laboratory stressors of different types in adults over 50 and relating them to subjective stress ratings and several physiologic measures.
Methods: sAA level was assessed using a portable biosensor (Nipro, Osaka, Japan) prior, during, and after three laboratory stressors: physical stressor that involved completing the Cold Pressor Task, an emotional stressor that included viewing unpleasant pictures from International Affective Picture System, and a mental stressor that involved completing a computerized Montreal Imaging Stressor Task (MIST). sAA and subjective stress ratings were collected at baseline, after each stressor (5-10 min after each stressor onset), and 30 min after the final stressor onset. Additionally, simultaneous recordings of several physiologic markers (blood pressure, heart rate variability, and respiration rate) were obtained.

Results: Preliminary results show that, while participants rated perceived stress level after all three stressors significantly greater than their baseline stress level (all p’s < .001), sAA levels were significantly elevated only after the mental stressor, MIST (p < .05). The baseline and post-stressor sAA levels were similar (p > .10). Relationships among sAA levels, physiologic markers, and subjective stress ratings will be also addressed.

Conclusions: sAA might be useful as a stress biomarker in a population of adults over the age of 50, and it might be sensitive primarily to the task requiring mental effort with psychosocial judgment component.

SVM to Detect the Presence of Visitors in a Smart Home Environment

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With the rising age of the population, there is increased need to help elderly maintain their independence. Smart homes, employing passive sensor networks and pervasive computing techniques, enable the unobtrusive assessment of activities and behaviors of the elderly which can be useful for health state assessment and intervention. Due to the multiple health benefits associated with socializing, accurately tracking whether an individual has visitors to their home is one of the more important aspects of elders’ behaviors that could be assessed with smart home technology. With this goal, we have developed an SVM model to identify periods where untagged visitors are present in the home. Using the dwell time, number of sensor firings, and number of transitions between major living spaces (living room, dining room, kitchen and bathroom) as features in the model, and self report from two subjects as ground truth, we were able to accurately detect the presence of visitors in the home with a sensitivity and specificity of 0.90 and 0.89 for subject 1, and of 0.67 and 0.78 for subject 2, respectively. This performance is sufficient for estimating intensity of socialization and detecting potentially important changes.
**Soft tissue shadow on lateral cervical spine radiograph does not predict development or severity of chronic dysphagia.**

Khaki F, Zusman NL, Nemecek AN, Ching AC, Hart RA, Yoo JU.

**Introduction** Dysphagia is commonly reported in the early postoperative period following anterior cervical spine surgery. Although prevertebral soft tissue swelling (STS) has been hypothesized as a potential risk factor for development of chronic dysphagia, this has not been previously studied. This study is a longitudinal radiographic evaluation of the STS and its relationship to the problem of long term dysphagia in patients undergoing anterior cervical surgery.

**Methods** We retrospectively reviewed the medical records and radiographs of patient who underwent elective anterior cervical surgery from our institution during the period of 2008-2011. Patients with preoperative dysphagia were excluded. To be included in the study, the follow up of greater than 6 months and lateral cervical radiographs at preoperative, immediate postoperative, 6 week and 3 month were required. Soft tissue shadow was measured at the lower endplates of C2 and C6. Presence and severity of dysphagia was evaluated prospectively using previously published Bazaz-Yoo Scale.
**Results**  Sixty-seven patients met the inclusion criteria. Soft tissue shadow was greatest at immediate postoperative x-ray measuring 10.9 ± 4.7 mm at C2 and 18.9 ± 5.5 mm at C6 from preoperative measurements of 4.5 ± 1.7 mm and 14.5 ± 3.7 mm, respectively. By 6 weeks, these measurements returned to baseline levels. The prevalence of dysphagia was 73% (21% mild, 39% moderate, and 13% severe). There were no statistically significant differences in the measurements between patients with and without dysphagia. Also there were no significant differences in soft tissue shadow between mild, moderate and severe dysphagia patients.

**Conclusion**  Although marked increase in the STS in the immediate postoperative period may be responsible for dysphagia in the acute stage of recovery, soft tissue shadow at immediate postoperative period, 6 weeks or 3 months does not predict either the presence or severity of chronic dysphagia.

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**Correction of lumbar hypolordosis with Smith-Petersen osteotomy and transforaminal interbody fusion**

Farbod Khaki BS, Robert A. Hart MD

**Introduction:**
The Smith-Petersen osteotomy (SPO) and pedicle subtraction osteotomy (PSO) represent polar alternatives in the correction of lumbar hypolordosis. SPO is a simple technique that yields less potential correction, whereas PSO provides substantial correction, but with greater technical difficulty and operative risk. The purpose of this study was to evaluate the radiographic results of coupling a Smith-Petersen osteotomy with a transforaminal lumbar interbody fusion (SPO + TLIF) for the correction of lumbar hypolordosis.

**Methods:**
We retrospectively reviewed the medical records and radiographs of patients who underwent SPO + TLIF to correct lumbar hypolordosis. Operative and perioperative data was collected. The Cobb angle was used to measure the overall lumbar lordosis and focal lordosis at the osteotomy level on lateral lumbar radiographs. Radiographic measurements were made on preoperative, postoperative, one year and two year films.

**Results:**
Fourteen patients underwent SPO + TLIF with an average age of 64 years (47–77). Eleven patients had both one and two year follow-up. The average focal correction at the osteotomy level at one and two years was 13.6 ± 7.7 degrees and 13.4 ± 6.1 degrees. The average correction in overall lumbar lordosis at one and two years was 17.6 ± 11.9 degrees and 15.1 ± 10.6 degrees. Blood loss averaged 2132 ml, operating time averaged 452 minutes, and hospital stay averaged 9.7 days. Five patients experienced complications, which included excessive blood loss, unplanned termination of procedure, wound infection, epidural hematoma and cardiac arrhythmia.

**Discussion and Conclusion:**
We achieved an average increase in focal lordosis of 13.4 degrees at two years using SPO + TLIF. Although five patients experienced complications, all underwent more extensive procedures at the time the osteotomy was performed. Our results indicate that SPO + TLIF may represent an intermediate option in the correction of lumbar hypolordosis.

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**Comparative Effectiveness of Robotic vs Conventional Total Laparoscopic Hysterectomy for Benign Indications**

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**Objective:** To compare surgical outcomes, hospital charges, and patient satisfaction amongst women undergoing total laparoscopic hysterectomy for non-malignant indications with and without robotic assistance.

**Methods:** Retrospective chart review was conducted for patients who underwent total laparoscopic hysterectomy for non-malignant indications at two community hospitals and one academic medical center from 2008 - 2010. Subjects were invited to complete follow-up questionnaires.

**Results:** Hospital records were searched using an ICD9-based search protocol, yielding 411 cases which were screened for inclusion. 299 cases met study criteria. Data were extracted by manual chart review of electronic records. 134 cases (44.8%) involved robotic assistance. Robotic assistance was associated with increased likelihood of blood loss >100mL (adjusted OR: 2.133, 95% CI: 1.088, 4.183), major complications (OR: 2.101, 95% CI: 0.921, 4.799), and total hospital charges (median difference: $3487 USD). Robotic assistance was not associated with significant differences in operative time (adjusted Ratio 1.035, 95% CI: 0.947, 1.131), conversion to open laparotomy (OR: 0.854, 95% CI: 0.316, 2.231) or likelihood of prolonged hospital stay (OR: 1.278, 95% CI: 0.761, 2.150). Ninety-nine subjects (33%) completed questionnaires. Robotic assistance did not demonstrate superior patient satisfaction or recovery experience.

**Conclusion:** In women undergoing total laparoscopic hysterectomy for non-malignant indications, robotic assistance is associated with increased likelihood of blood loss >100mL, major complications, and greater total hospital charges. These data do not demonstrate significant differences in operative time, length of stay, or patient satisfaction among patients whose operations involved robotic assistance.

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**Non-Visual CT Data is useful in the Differentiation of Jaw Lesions.**

Philipp Kupfer DMD, Jia Ooi BA, Mark Engelstad DMD, MD

In the management of intraosseous jaw lesions, computed tomography (CT) is commonly used to help define a pre-treatment differential diagnosis. Most clinicians use CT data visually by viewing a holistic image made of shades from black to white. Unfortunately, the value of visually-interpreted CT information is highly dependent upon a clinician’s personal experience and other human factors. However, CT images are also full of objective, raw computable data. But in most cases, clinicians rely solely on the visual component of CT data and ignore the raw data.

The Hounsfield Unit (HU) is the objective digital tissue density unit of measure used in all modern medical grade CT machines. HU are calibrated and standardized among machines; with Air = -1024 HU, water = 0 HU, and bone =700 to 3000 HU. HU data exist for each voxel in all scanned tissues, including those within jaw lesions. In related studies, Hounsfield units have been proven useful to determining the nature of soft tissue neoplasms (1) as well as bone density around dental implants (2).

This study retrospectively analysed two data sets associated with jaw lesions evaluated and treated at our institution: 1) jaw lesion CT HU digital data and, 2) jaw lesion final histopathological diagnoses. These two data sets were examined for correlations or patterns that could answer the preliminary research question, “Can Non-visual CT data be analysed to create pre-treatment diagnosis probabilities for jaw lesions?” Institutional IRB approval for this study was obtained.
We obtained and studied a total of twenty CT records from patients with unilocular soft tissue jaw lesions from one two lesion types: keratinizing odontogenic tumors (KOT), n=10 or solid ameloblastomas (AMB), n=10.

CT data were analysed using NIH-funded open-source medical imaging software (3D slicer, Boston MA). In each patient CT, 3D Slicer was used to isolate a representative sample of the jaw lesion, creating a set of HU data associated specific to each individual jaw lesion. The HU data from each lesion was analyzed using one of two methods: 1) visual analysis of a density histogram or 2) creation of descriptive statistics.

Preliminary lesion data shows: average HU for four representative KOT 0.39 and two AMB 34.94. Statistical significant difference calculation pending.

Non-visual CT data in the form of Hounsfield units can probably be used in computerized analysis to pre-operatively differentiate between differing types of jaw lesions. The specific Hounsfield unit value of jaw lesions can aid clinicians in preclinical decision-making.

References:

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**Routine Completion Axillary Lymph Node Dissection for Positive Sentinel Node in Mastectomy Patients is not Associated with Improved Local Control**


The current practice of completion axillary node dissection (ALND) for patients found to have a positive sentinel node (+SLN) is being questioned. Recently published results from the Z-11 trial suggest that in patients undergoing lumpectomy and adjuvant radiation there is no difference in outcome with ALND. This led us to examine the outcomes of SLN+ mastectomy patients with and without ALND. Review of cancer registry data from two community hospital systems identified 575 women with breast cancer with +SLN that underwent mastectomy. Of these SLN+ women 438 underwent formal ALND and 137 were managed expectantly. The patients were seen between 2000-2010. Disease-free survival was defined by no local, regional or distal recurrence assessed at 24 months and 120 months.

The two groups, ALND and No ALND are compared in Table 1. The disease-free survival of patients at 24 months follow-up undergoing ALND was 92.2% (89.8-94.8%) compared to 97.1% (94.3%-99.9%) in the No ALND. Survival rates at 120 months: ALND 84.4% (83.8%-96.8%), No ALND 90.0% (80.3%-88.6%). Figure 1 shows the survival curves of the two groups. In our experience there was no significant difference in disease-free survival in mastectomy patients with +SLN when completion ALND was not performed. This is in concert with recent data suggesting that a closer look at the indications for completion dissection in early breast cancer should be further explored. Given the known risk of lymphedema and neuralgia associated with ALND this study suggests that morbidity may be obviated with expectant management without compromising disease burden or oncologic outcome.

<table>
<thead>
<tr>
<th>Table 1: Demographics</th>
<th>ALND (n=438)</th>
<th>No ALND (n=137)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at presentation</td>
<td>55.7 (12.6)</td>
<td>58.6 (13.5)</td>
<td>0.029</td>
</tr>
<tr>
<td>Stage</td>
<td>2.3(0.5)</td>
<td>2.1(0.4)</td>
<td>&lt;0.001</td>
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<tr>
<td>ER+</td>
<td>87%</td>
<td>90%</td>
<td>0.458</td>
</tr>
</tbody>
</table>

Figure 1: Disease-free survival in SLN+ patients undergoing mastectomy

**INR Fails to Accurately Portray Coagulation Status Following Liver Resection**

Jeffrey Barton, Gordon Riha, Jerome Differding, Samantha Underwood, Igor Kremenevskiy, Brett Sheppard, Rodney Pommier, Susan Orloff, Thomas Deloughery, Martin Schreiber, and Kevin Billingsley

Department of Surgery, Oregon Health & Science University, Portland, OR

**Introduction:** Following hepatectomy, regulation of the coagulation system remains poorly understood. Prior attempts to characterize post-hepatectomy coagulation have focused on either coagulation assays or factor studies, without synthesizing the data into a complete picture of coagulation status. The aim of this study was to prospectively evaluate coagulation status in liver resection patients using standard coagulation tests, thrombelastography (TEG) and coagulation factor activity assays.

**Methods:** Forty patients were enrolled prior to undergoing elective hepatectomy. Standard coagulation tests, TEG and plasma for coagulation assays were performed prior to incision, post-operatively and on post-operative days 1, 3 and 5.

**Results:** Post-operative INR increased significantly when compared to pre-operative values at all time points ($p<0.01$). The time to onset of clot (R-value) decreased significantly immediately following hepatectomy ($p=0.04$), consistent with relative hypercoagulability, then normalized for the remainder of the study. Remaining TEG values did not change significantly. Plasma activity of hepatically synthesized pro- and anti-coagulant factors decreased significantly ($p<0.01$). Factor VIII activity increased significantly at all post-operative time-points ($p<0.01$). aPTT remained within normal limits throughout the study, and fibrinogen transiently decreased significantly, but remained within normal limits.
**Conclusions:** Decreases in pro-coagulant factors account for elevation in INR following hepatectomy. However, activity of these factors remains adequate for robust clot formation. Decreases in proteins C and S, and increased factor VIII activity likely contributed to the normal coagulation observed by TEG and aPTT. Due to its sensitivity to vitamin K dependent factors, INR does not depict coagulation status following hepatectomy.

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**Use of Technology for Communication after Laryngectomy: Three Patterns of Utilization**

JM Childes*, AD Palmer, M Fried-Oken, DJ Graville

*Presenting Author: Jana Childes (childesj@ohsu.edu)

The majority of patients who undergo treatment for head and neck cancer experience some change in their ability to communicate, which may range from mild difficulties to profound alterations in their ability to produce voice and speech. The communication needs and challenges of these patients vary considerably by the site of the primary lesion, their treatment method (i.e., surgery or radiation), reconstruction, co-existing medical problems, or disease recurrence. Many patients note ongoing difficulty communicating, which may persist even after they are considered to be rehabilitated or cured of their disease. Communication impairments are isolating and affect participation in activities of daily living.

The head and neck cancer population has been traditionally viewed as technology-adverse and have not been previously thought of as successful users of communication technology. However, the population is changing and demonstrating increased facility with computer technology. Literature review reports numerous efforts to improve communication and information dissemination to this patient population through the use of e-mail and telehealth applications. Our recent international study of 250 head and neck cancer survivors demonstrates that members of this population communicate frequently, with 90% engaging in face to face communication daily. Among study participants, 91% communicate by telephone and the majority (56%) of those who use the telephone engage in telecommunication on a daily basis. Scores on the Communicative Effectiveness Scale (CES) ranged from 8-32 with a mean of 22.25 (±5.18). It appeared that individuals tended to become better communicators over time as there was a significant positive correlation between number of years since surgery and CES score (r = .29, p < .001). Interestingly, however, there was no association between CES score and age. A minority of individuals (22%) used a single method of communication, with 64% of respondents using 2 or 3 methods. Individuals who used more communication methods had a significantly lower score on the CES (r = -.26, p < .001). Not all speaking situations were rated as being equally challenging, however, and communicating in a noisy environment and at a distance were notably more difficult for our respondents. In terms of technology use, survey results show that 76% of participants describe themselves as being “very comfortable” with computer use and demonstrate increasing use of computer technology, including personal and laptop computers, smartphones, and video conferencing (e.g., Skype). The most common use of the computer by respondents was for email (98%).

Within the group of 250 study participants, 26 persons identified that they currently use speech generating technology for communication. These participants were invited to participate in a second survey that was designed to gather qualitative information regarding their experiences with speech generating technology. Eighteen persons were enrolled in this phase of the study. The 18 participants included 12 males and 6 females who range in age between 48-82 years. Speech generating technology was used as either a primary or secondary method of communication, and included both dedicated communication devices and commercially available technology (e.g., smartphones, laptop computers, and multimedia devices). Nine participants used a speech generating device several times per day, while the remainder of
the participants used their devices weekly to rarely. Three themes of use emerged from the survey responses and include: 1) use for communication in the post-operative period as a temporary communication method while undergoing speech rehabilitation, 2) use of speech generating technology as a secondary method or “back-up” used in association with an alaryngeal method of communication, and 3) as a primary communication method when functional verbal communication was not possible. This presentation will include qualitative information related to the three themes of communication technology use in the head and neck cancer population, including: device selection methods, operations and aesthetics, as well as both positive and negative experiences related to communication with a speech generating device.

Compliance with Long-term Surveillance Recommendations following Endovascular Aneurysm Repair or Type B Aortic Dissection


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Objectives: Lifelong surveillance is recommended for both endovascular aneurysm repair (EVAR) and acute, uncomplicated type B thoracic aortic dissection, though compliance remains a significant challenge. We sought to determine factors associated with failure to obtain recommended surveillance.

Methods: Patients surviving to discharge who received EVAR for thoracic or abdominal aortic aneurysms or medical management for type B dissections from 2004-2011 were reviewed. Primary endpoints were compliance with follow-up and need for re-intervention. Co-morbidities included coronary artery disease, CHF, hypertension, COPD, diabetes, and chronic kidney disease. Socioeconomic factors examined were age, gender, distance from hospital, discharge destination (i.e. home with or without home-health/family assistance, or skilled nursing facility), and insurance type. Complications included endoleak, sac expansion, endograft migration, infection or thrombosis, and aneurysm degeneration.

Results: 157 patients, median age 72.5 years, were identified; 127 had EVAR and 30 had type B dissection. Median follow-up was 34 months. Overall, 48% were lost to follow-up, while 9% never returned for surveillance after initial hospitalization. Follow-up was compared for each of the co-morbidities and socioeconomic factors; none were found to significantly affect follow-up. The known complication rate was 31% (n=49), with re-intervention performed in 21% of EVAR patients, and crossover to intervention in 33% with dissection. All-cause mortality was 20% as determined by the Social Security Death Index.

Conclusions: Despite a significant rate of re-intervention in patients with EVAR and type B dissection, long-term compliance with surveillance is limited. In addition, predicting who is at risk of being lost to follow-up remains difficult. Coordinated protocols to capture EVAR and type B dissection patients for surveillance studies are needed to ensure optimal follow-up for these patients.

Surgical intervention for radial artery catheter-associated ischemic complications
Phong T Dargon, Timothy K Liem, Margaret C Gorman, Amir F Azarbal, Erica L Mitchell, Gregory J Landry, Gregory Moneta

Oregon Health & Science University, Portland, OR, United States.

Objectives: Radial artery catheterization may be complicated by arterial thrombosis and hand ischemia. We sought to identify risk factors for radial catheter-associated ischemic hand complications and need for operative intervention.

Methods: All patients with radial artery catheter-associated ischemic hand complications at a single hospital between 2006 and 2011 were identified. Clinical risk factors (vascular comorbidities, anticoagulation or antiplatelet therapy, shock, sepsis, and APACHE score) in patients whose complications lead to surgical consultation, were compared with an age- and gender-matched control patient cohort with uncomplicated radial artery catheters. Nominal variables were compared using McNemar test, chi-square, and fisher’s exact test (P<.05). Paired t-test and one-way ANOVA were used for continuous variables.

Results: There were 23 consultations performed for hand ischemia related to radial artery catheters. Compared to nonischemic controls consulted patients were more likely to have shock (P=.002), sepsis (P=.01), and be receiving anticoagulation (P=.04). Twelve patients (52%) required surgical revascularization; thrombectomy (91.7%), intraoperative thrombolysis (58.3%), vein patch angioplasty (33.3%), and intraoperative angiography (50%). In patients who underwent revascularization, 2 required digital amputation, 1 required major upper extremity amputation, and 2 patients died. In patients who underwent nonoperative management, none required amputation. Overall mortality was 22% in patients requiring vascular consultation and 17% in those requiring operation for radial artery catheter ischemic complications.

Conclusions: Fifty-two percent of patients with ischemic complications of radial artery catheters require operation and mortality is high. Patients with shock and sepsis are more likely to develop radial catheter-associated upper extremity ischemia. Those requiring revascularization have a high rate of digital or major upper extremity amputation.

Contribution of Factor XI Activation to the Pathomechanism of Polymicrobial Abdominal Sepsis

András Gruber1,2, David Gailani3, Owen JT McCarty1, Erik I Tucker1,2

1Department of Biomedical Engineering, Oregon Health and Science University, Portland, OR, 2Aronora, LLC, Portland OR, and 3Department of Pathology, Vanderbilt University, Nashville, TN.

Certain severe infections are accompanied by intravascular coagulation and fibrinolysis, resulting in a coagulopathy with thrombotic and hemorrhagic components (disseminated intravascular coagulation – DIC). We have shown that the plasma coagulation protease factor XI (FXI) contributes substantially to experimental thrombus formation in baboons and mice. In human populations, FXI deficiency confers a decreased risk of thrombotic ischemic stroke and deep venous thrombosis, while the associated bleeding diathesis is often mild. Factor XI deficiency reduces DIC and improves survival of abdominal sepsis in mice. However, the role of factor XIIa (FXIIa) in the activation of FXI, in vivo, and in the pathogenesis of infections remains unclear. We tested the hypothesis that selective inhibition of FXI activation by FXIIa would improve the outcome of fecal peritoneal sepsis in mice, and would be significantly safer in this setting than infusion of activated protein C (APC, Xigris), which could exacerbate hemorrhage. We developed an antibody, 14E11, that binds to the A2 domain of FXI and inhibits the activation of FXI by factor XIIa without affecting FXIIa activity or FXI
activation by thrombin. Injection of 14E11 (4mg/kg, SC) increased the APTT of mice 3-fold for >48 hrs. Following cecal ligation and puncture (CLP), mice were treated with vehicle (PBS, SC), APC (6 mg/kg, SC), or 14E11 (4 mg/kg, SC) (n=20 for each group). Overall survival was 45% for vehicle, 15% for APC, and 80% for 14E11 treated mice (P < 0.001 for 14E11 vs. both APC and vehicle). 24 hrs after CLP, platelet count in vehicle, APC, and 14E11 treated mice (n=8 each) decreased 24±7%, 25±5%, and 12±6%, and leukocyte counts decreased by 51±15%, 42±14%, and 43±13% respectively. Thrombin/antithrombin complex levels were higher (5.0±1.2 μg/L) in the vehicle treated group 24 hrs after CLP compared with 2.2±0.2 μg/L in normal healthy mice (P<0.05), while APC and 14E11 treated groups showed only moderately elevated TAT levels (2.6±0.2 and 2.7±0.4 μg/L, respectively). In a separate cohort (n=12 each), tail-clip bleeding times were 12.8±1.0, 17.9±1.8, and 12.1±1.7 min for vehicle, APC, and 14E11 treated animals respectively (P<0.05 for APC vs. vehicle and 14E11) 30min after injection. In summary, the outcome was better for 14E11 treated mice in CLP-induced sepsis compared to vehicle or APC treatment, and the data indicated that consumptive coagulopathy was less severe following inhibition of FXI activation. Furthermore, mice treated with 14E11 showed no increase in bleeding compared with vehicle treatment, while APC prolonged the tail bleeding time. The results suggest that molecules in bacteria that activate and exploit the contact system may be part of their virulence factors that support their ability to invade the host. We thus also propose that early therapeutic inhibition of FXI activity could be safe and effective in the prevention or initial adjuvant treatment of certain infections.
Aaron Cohen | Studying the Potential Impact of Automated Document Classification on Scheduling a Systematic Review Update

Aaron Jacobs | Inhibitors of Base Excision Repair Glycosylases

Alex Johnson | High-Throughput Screen for Small Molecular Inhibitors of Parasite Glucose Transporters

Amy Cantor | Body mass index and breast cancer risk of women in their 40s: Systematic review and meta-analysis

Barent DuBois | Maternal pre-gravid BMI and placental cytochrome P4501A1 activity

Bernadette Zakher | Reproductive Risk Factors for Breast Cancer for Women in their Forties: Systematic Review and Meta-analysis

Brandon Dyer | Hypoxic & Reperfusive Responses are Selectively Altered During Non-Small Cell Lung Cancer Progression

Britta Torgrimson-Ojerio | The Effect of Strength Training on Body Composition in Breast Cancer Survivors with Premature Menopause: Preliminary Findings from a One-Year Randomized, Controlled Trial

Chelsea Jenkins | Evidence for a complex containing FANCM and Rif1

Christine Ernst | Type II Diabetes and Depression in Adults from One Primary Care Practice

Cristina Butterfield | Purifying the active marine Bacillus sp. PL-12 Mn(II) oxidase from E. coli

Derek Zachman | Endothelial Cells Mitigate Radiation-Induced Hematopoietic Stem Cell Injury

Dian Chase | An in silico model of insulin/glucose transport

Eric Brown | Deconstructing the Fanconi Anemia Pathway


James Keller | Accumulated Dose Reduction of Optically Stimulated Luminescent Dosimeters through Fluorescent Light Annealing
Jennifer DeVoe | Variability in Care Quality: Do Federally-Qualified Health Center Patient Demographics Correlate with Quality of Diabetic Care?

Jenny Kan | A "Rare Disease" Approach to Cancer Therapeutics

Jessica Dobek | The Effect of Strength Training on Body Composition in Prostate Cancer Survivors on ADT: Preliminary Findings from a One-Year Randomized, Controlled Trial

John Bissonnette | Depressed serotonin (5-HT) contributes to suppressed CO2 chemosensitivity in MeCP2 deficient mice

Kati Geszvain | Identification of the Mn(II) oxidases and accessory proteins involved in Mn(II) oxidation in Pseudomonas putida GB-1

Kim Jones | A Randomized Controlled Trial of 8-form Tai Chi Improves Symptom and Functional Mobility in Fibromyalgia Patients

Kinrin Yamanaka | The Discovery of Small Molecule Inhibitors of DNA Polymerase Kappa

Mark Engelstad | Clinical Documentation Quality- Can Our Notes Be Used For Outcomes Research?

Michael Wallisch | High-Throughput Platform for Screening Small Molecule Inhibitors for the Fanconi Anemia Pathway using Bioluminescence Resonance Energy Transfer (BRET) in a Novel Cell-Free Assay

Mike Danilchik | Ligand-sensing in a large embryonic extracellular space by membrane nanotubes

Ravikant Samatham | Image Contrast Based on Nano-Architecture of Tissues and Biomaterials.

Richard Davis | Community Composition and Carbon Fixation of Neutrophilic Iron-Oxidizing Chemoautotrophs at Hydrothermal Vents

Sara Kelley | Vancomycin Area Under the Curve (AUC) in Patients with Methicillin-resistant Staphylococcus aureus Bacteremia

Scott Sherry | One if by Land and Two if by Air: Evaluation of Internet Mapping Technology and the Decision to Transport Trauma Patients by Helicopter Emergency Medical Services (HEMS).

Seth Lewin | Cisplatin Treatment Enhancement with Acetaminophen in a Model of Human Medulloblastoma in Nude Rats

Skye Barendt | The Identification of Genes Regulated by Paralogous Spx Proteins in Bacillus anthracis

Sung-Woo Lee | Effects of common groundwater constituents on coupled Mn(II)/U(IV) oxidation by Bacillus sp. SG-1

Tracy Edinger | Barriers to Retrieving Patient Information from Electronic Health Record Data: Failure Analysis from the TREC Medical Records Track
Travis Lovejoy | Chronic Pain Treatment and Health Service Utilization of Veterans with Hepatitis C Virus Infection

Vishnu Mohan | Interdisciplinary collaborative research by the Physician Order Entry Team (POET) of the Department of Medical informatics and Clinical Epidemiology (DMICE) at Oregon Health & Science University.

Wendy Smythe | Iron & Manganese Depositing Cold-Seeps: Mineral Formation Along A Freshwater To Marine Ecosystem At Soda Bay, Alaska

Yasuhiko Kawano | A potential functional domain rich in basic amino acids within the AAV2 Assembly-Activating Protein (AAP2) constrains the structural diversity of the AAV2 capsid

Ying-Chih Lin | Mutagenesis of Aflatoxin B1-DNA adducts in mammalian cells

Zhi Hu | Validating Therapeutic siRNA against HER2 in Breast Cancer Cell

Poster Session 2 | 5 – 7 PM Tuesday, May 8

Abby Rynko | Etanercept Blocks Parainfluenza Downregulation of M2 Muscarinic Receptor mRNA in Parasympathetic Nerves In Vivo

Alexandre Colville | c-Fos Induction Associated With Ethanol Withdrawal In Chromosome 1 Congenic and GIRK3 Knockout Mice.

Angela Senders | Mindfulness in Multiple Sclerosis: A Cross-sectional Survey Study

Annika Giesbrecht | Behavioral Risk Factors and Recent Colorectal Cancer Screening Among American Indian and Alaska Native People in the Pacific Northwest: Tribe A BRFSS Project 2009-2010

Ashley Franklin | Implications of Process-Focused Learning Activities for Care of Multiple Patients

Ben Kong | Valganciclovir Pharmacokinetics and Area-Under-the-Curve Profiling in Pediatric Kidney Transplant

Binglin Li | Physical and biological controls on Mesodinium rubrum blooms in the lower Columbia River estuary

Chiemi Tanaka and Katie Loera | Residual Hearing in a Guinea Pig Model of Hybrid Cochlear Implants

Christine Nelson | Facilitators of FQHCs' Participation in Practice Based Research: A Qualitative Study

Cong-Qiu Chu | NLRP3 Gene Analysis for Patients with Schnitzler's Syndrome

Damian Zuloaga | The effects of neonatal methamphetamine exposure on expression of hypothalamic pituitary adrenal axis-associated proteins in adulthood
Measuring and Promoting Social Connectedness in Older Adults: Development of the Social Footprint Model

Evaluating Communication Skills of Children with Low-Incidence Disabilities

Effects of Ulnar Shortening on Wrist Pressure and Dynamic Work of Rotation: A Cadaveric Biomechanical Model

Rural-Urban Differences in Access, Utilization and Time Spent

Epithelial Sensory Hyperinnervation And Increased Vagally-Mediated Airway Resistance In A Mouse Model Of Eosinophilic Asthma

Efficacy and Safety of Daptomycin in the Treatment of Gram-Positive Infections in Patients with Renal Impairment

Piperacillin-Tazobactam Extended Infusion Therapy to Treat Pulmonary Exacerbations in Patients with Cystic Fibrosis

Relationship between heart failure self-care and provider consultation

Risk Factors for Complication during Outpatient Parenteral Antibiotic Therapy (OPAT) for Orthopedic and Neurosurgery Infections

Validation of Limited Sampling Equations to Predict Midazolam AUC For CYP3A4 Phenotyping in Obese Women

COMPARING DIFFERENT MEASURES OF IMPULSIVITY BETWEEN REGULAR SMOKERS, CHIPPERS AND NON-SMOKERS

MPDZ Knockout and Bacterial Artificial Chromosome (BAC) Transgenic Overexpression Models Support its Involvement in Ethanol Withdrawal and Consumption

Challenges in Detecting and Preventing Pressure Ulcers in Older Adults with Dark Skin

Integrating High Fidelity Simulation into a Nurse-Midwifery Curriculum

Systemic Blockade of Dopamine D1 but not D2 Receptors Impairs the Development of Ethanol-Conditioned Place Preference

Severe Postnatal Growth Retardation, Immunodeficiency, Pulmonary Disease: Insights from Clinical and Functional Evaluations of Two Rare STATuesday, 5 PMB Missense Mutations

SIGNIFICANTLY IMPROVED PREDICTIVE VALUE PROVIDED BY PROEXCTM STAINING OF PAP SMEARS DIAGNOSED AS HSIL OR ASC-H WARRANTS LEAP TO LEEP
Michelle Maier | Impact of chytrid parasites on diatoms in the lower Columbia River

Miriam Elman | Application of a novel method for age-period-cohort analysis to Oregon viral hepatitis mortality, 1995-2010

Mojgan Rostaminia | Impact of Sea Level Rise on Habitat Opportunity of Columbia River Juvenile Chinook Salmon

Monique Rennie | Extracellular Matrix Remodeling of the Embryonic Chicken Outflow Tract in Response to Altered Hemodynamics

Nancy Findholt | Barriers to Treatment and Prevention of Childhood Obesity in Rural Primary Care

Natalie Vuylsteke | Assessing the Impact of a Pilot Antimicrobial Stewardship Program in Pediatrics at an Academic Medical Center

Natasha Chattergoon | Fetal Myocardial Thyroid Hormone Deiodinases are Regulated by Fetal T3

Perrie O'Tierney | Placenta #11035: Risks and benefits of de-identifying data in birth cohort repositories

Peter Kahn | Ecology and genetic characterization of Katablepharis CRE, a heterotrophic flagellate that 'blooms' in the Columbia River estuary during the spring

Rhonda Vandersluis | Balancing Survival & Resistance: Faculty of Color in Euro-American Schools of Nursing

Rowena Vilches-Tran | Understanding Empiric Antibiotic Prescribing Among Primary Care Residents

Samantha Louey | IGF-1 Does Not Restore Cell Cycle Activity In Fetal Sheep Cardiomyocytes Following Placental Insufficiency

Shelley Selph | Conflict of interest among clinical practice guidelines

Sonnet Jonker | Sexual Dimorphism Of Cardiomyocyte Size In Adult But Not Fetal Sheep

Steven Mansberger | Compliance with Annual Diabetic Eye Exams Survey (CADEES): Preliminary Results

Steven Mansberger | Cost-Effectiveness of Telemedicine Screening for Diabetic Retinopathy

Takatoshi Karasawa | CLIMP-63 is a gentamicin-binding protein involved in drug-induced cytotoxicity

Tracie Nettleton | Mapping neural response to alcohol using optical imaging techniques

William Martin | Effectiveness of a multi-component, community-based noise-induced hearing loss and tinnitus prevention intervention in an Oregon American Indian community

Yingxin Chen | The effect of moderate and excessive alcohol exposure on male experimental stroke outcomes in mice is androgen-independent
Yosef Berlow | Increased striatal iron accumulation in methamphetamine users

**Poster Session 3 | 2 - 3 PM Wednesday, May 9**

Alison Ting | Optimization of rhesus macaque ovarian tissue vitrification in a closed system

Allison Lindauer | Managing Behavior Symptoms of Dementia: What Can We Learn from Black Family Caregivers?

Andrew Terker | Adrenergic regulation of the renal sodium chloride cotransporter

Anke Vermehren-Schmaedick | Quantum Dots to study live, molecular dynamics of ligand-receptor complexes in neurons

Anna Wilson | Adolescents at risk for chronic pain

Anna Wilson | Overview of research activities in the Child Development and Rehabilitation Center's Division of Psychology

Becky Proskocil | Airway hyperreactivity is potentiated in ovalbumin sensitized guinea pigs treated with chlorpyrifos, but not diazinon or permethrin, and is IL-5 dependent.

Brett Dufour | Assessing a global therapeutic strategy for Huntington's Disease: Systemic RNAi therapeutics for central and peripheral symptoms

David Stein | Inhibition of dengue virus infections in vitro and in vivo by an siRNA targeting highly conserved sequence.

Doria Thiele | Maternal vitamin D transfer to infants via breast milk: An evidence-based literature review.

Elizabeth Brass | Maternal Obesity Suppresses Male Dominance of Placental Fatty Acid Uptake

Eric Earl | Novel Quantitative Ischemic Lesion Analysis Method in a Non-Human Primate Stroke Model

Henryk Urbanski | The Secrets of Healthy Aging: Gaining Insights from Nonhuman Primate Research

Hongzhe Li | Gentamicin uptake in acoustically traumatized cochleae

Jeremy Woods | Development and application of a polymicrobial, in vitro, biofilm wound model.

Jesse Lorton | New eosinophils recruited to the lungs of sensitized guinea pigs after ozone exposure persist in the airway tissues

Jessica Hebert | Promoter DNA Hypomethylation in the Fetal Kidneys of Intrauterine Growth Restricted Mice
Jessica Hebert | Fetal Programming of White Coat Hypertension in Growth Restricted Male Mice

Jessica Stanley | Medical Termination of Pregnancy in Cynomolgus Macaques

Katie Schenning | Ischemic Injury to Glomerular Endothelium: Proposed Mechanism of Hyperglycemic Protection

Kentaro Yomogida | Cell penetrating recombinant Foxp3 protein enhances Treg function, suppresses Th17 cells and ameliorate arthritis

Lance Johnson | Diet-induced Insulin Resistance, ApoE, and Cognitive Function

Laurie King | Comparison of individual, group and home agility boot camp (ABC) exercise program for people with Parkinson's disease?

Maria Manczak | Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage

P. Hemachandra Reddy | Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: implications for selective neuronal damage

P. Hemachandra Reddy | Mitochondria-targeted catalase reduces abnormal APP processing, amyloid beta production, and BACE1 in a mouse model of Alzheimer's disease: Implications for neuroprotection and lifespan extension

P. Hemachandra Reddy | Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease

P. Hemachandra Reddy | Gene Expression Profiles of Mitochondrial Structure/Function and Amyloid Beta Production in Brain Tissues from Alzheimer's Disease Patients: Implications to Neuronal Damage and Cognitive Decline

Rebecca Tempel | Tularemia Vaccine Trials in Nonhuman Primates

Sarah Wicher | Testing whether sustained or bolus delivery of BrdU labels dividing inflammatory cells

Sheetal Bodhankar | Indispensable role of B cells in mediating protective effects of estrogen against EAE

Sudeshna Dutta | The role of Swiss Cheese, the Drosophila homologue of Neuropathy target esterases, in glia.

Tao Wu | Optogenetic control of mouse outer hair cells

Tessa Steel | Environmental Food Balance: The Neighborhood Context of BMI and Diet Among Landless Northwest American Indians

Tetsuhiro Fujiyoshi | Memory dysfunction following cardiac arrest and cardiopulmonary resuscitation in mice is associated with hippocampal inflammation

Tosha Zaback | Summative Evaluation of a Tribal Telemedicine Project
Tracy Zitzelberger | Intelligent Systems for Detection of Aging Changes

Valerie Conrad | TLR4- and TLR9-induced neuroprotection against stroke is mediated by IRF signaling

Yingxin Chen | Intrastratal B cell administration limits infarct size after stroke in B cell deficient mice

Zachary Urdang | Intravital microscopy imaging of cochlear lateral wall in live mice through a thinned otic capsule

**Poster Session 4 | 5 – 7 PM Wednesday, May 9**

Aaron Grossberg | Inflammation-induced lethargy is mediated by suppression of orexin signaling.

Abby Floeter | Adherence to guidelines for heart failure with preserved ejection fraction (HFPEF) at the Portland Veterans Affairs Medical Center (PVAMC)

Aimee Mooney | A Brain Computer Interface using the RSVP keyboard for users who are locked-in

Amy Trevor | Children with Severe Early Childhood Caries: Identification of Cariogenic Mutans Streptococci Genetic Strains Harbored Within Carious and White Spot Lesions

Anders Goranson | Evaluation of a Home Based Telemental Health (HBTMH)

Andy Barnett | Curative versus palliative therapy for patients with colorectal cancer presenting to the emergency department

Anita Csverenka | Gender differences in the neural substrates of emotional conflict during adolescence.

Beth Darnall | Medication taking attitude/behavior is influenced by relationships: Results from the CARE Scale online survey

Carmem Pfeifer | Conversion-dependent shrinkage in (meth)acrylates as a function of irradiance

Carolina Glogowski | Physiology of unipolar brush cells in the dorsal cochlear nucleus

Carrie Farrar | CTRC Study Coordinator Unit

Cheng Fang | Inhibition of axonal transport is an early event following exposure to reactive oxygen species

Clive Woffendin | OCTRI Core Laboratory

Corinne Stevens | Functional brain connectivity in infants with prenatal risk for ADHD

Daniel Kriz | Navigating the Unstandardized Approaches to Cortical Mapping
David Grayson | Altered Structural Connectivity in Youth with ADHD

David Grayson | Resting-state Functional Connectivity MRI Reveals Large-Scale Differences Between Monkeys Raised on High vs Low Omega-3 Fatty Acid Diets

Diana Parrish | Proneurotrophins cause peri-infarct sympathetic denervation

Eli Schwarz | Dental Care Needs Among Underserved Oregonians - the Oregon Mission of Mercy

Ian Tagge | Population-Generalized vs. Individual-Specific AIF in Human Prostate DCE-MRI Pharmacokinetic Analysis

Izabela Chamot | Variability in the diagnostic criteria to evaluate heart failure with preserved ejection fraction (HFPEF) in a veteran affairs (VA) patient population

Jacob Pearson | The Effect of Maternal Low Protein Diet on Indoxyl Sulfate and Oat1/3 Expression

Joannah Vaughan | How Effective is the PediaVision SO9 in Detecting Amblyopic Risk Factors in Children, Ages 3-5 Years?

John Mitchell | Flexural strength and modulus of bioactive glass-containing, anti-microbial dental composites

John Muschler | Loss of cell-surface laminin anchoring promotes tumor growth and is associated with poor clinical outcomes.

Joseph Haynes | Dental Implants Placed from 2002-2009 in an Advanced Specialty Education Program in Periodontics: A Radiographic and Clinical Review

Julia Jordan | Bionutrition Resources for Clinical Research

Junan Zhang | A method of improving the effective spatial resolution for small field IMRT QA with 2D-array device

Kristen Mackiewicz Seghete | White matter microstructure and cognitive control in a developmental sample

Lani Doser | Perinatal Mortality of Planned Out of Hospital Births Transferred to an Oregon Hospital, 2004-2008

Lillian Nail | Describing the Experience of Hair Loss with Cancer Chemotherapy

Lyndsey Miller | Conflict, Decision-Making, and Social Support during Lung Cancer: The Roles of Age and Spousal Relationships

Manoj Sammi | Decreased Cellular Energetics in Multiple Sclerosis Gray Matter: a 7T Phosphorus Spectroscopy Study

Mary Samuels | The Clinical and Translational Research Center

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**Studying the Potential Impact of Automated Document Classification on Scheduling a Systematic Review Update**

Aaron M. Cohen\(^{1\#}\), Kyle Ambert \(^1\), Marian McDonagh \(^1\)

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**Background:** Systematic Reviews (SRs) are an essential part of evidence-based medicine, providing support for clinical practice and policy on a wide range of medical topics. However, producing SRs is resource-intensive, and progress in the research they review leads to SRs becoming outdated, requiring updates. Although the question of how and when to update SRs has been studied, the best method for determining when to update is still unclear, necessitating further research.

**Methods:** In this work we study the potential impact of a machine learning-based automated system for providing alerts when new publications become available within an SR topic. Some of these new publications are especially important, as they report findings that are more likely to initiate a review update. To this end, we have designed a classification algorithm to identify articles that are likely to be included in an SR update, along with an annotation scheme designed to identify the most important publications in a topic area. Using an SR database containing over 70,000 articles, we annotated articles from 9 topics that had received an update during the study period. The algorithm was then evaluated in terms of the overall correct and incorrect alert rate for publications meeting the topic inclusion criteria, as well as in terms of its ability to identify important, update-motivating publications in a topic area.

**Results:** Our initial approach, based on our previous work in topic-specific SR publication classification, identifies over 70% of the most important new publications, while maintaining a low overall alert rate.

**Conclusions:** We performed an initial analysis of the opportunities and challenges in aiding the SR update planning process with an informatics-based machine learning approach. Alerts could be a useful tool in the planning, scheduling, and allocation of resources for SR updates, providing an improvement in timeliness and coverage for the large number of medical topics needing SRs. While the performance of this initial method is not perfect, it could be a useful supplement to current approaches to scheduling an SR update. Approaches specifically targeting the types of important publications identified by this work are likely to improve results.

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**Inhibitors of Base Excision Repair Glycosylases**

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Center for Research on Occupational and Environmental Toxicology, Oregon Health & Science University, Portland, OR
DNA base excision repair (BER) is the primary pathway for the recognition and removal of nucleobase damage following exposures from alkylating agents, oxidative stressors and various forms of radiation. Defects in DNA glycosylases that initiate the BER cascade can result in a variety of disease manifestations such as cancer, metabolic disorders and altered inflammatory responses. Following the introduction of oxidative DNA damage, several glycosylases are required to span the spectrum of lesions formed including NEIL1, the enzyme primarily responsible for removal of ring-fragmented purines. In order to develop specific inhibitors to NEIL1, we have developed a fluorescent-based assay that utilizes incision of site-specifically modified oligodeoxynucleotides to detect enzymatic activity. This assay was miniaturized to a 1536 well format and used to screen small molecule libraries for inhibitors of the combined glycosylase/AP lyase activities. The top hits of these studies have been further characterized for their ability to specifically inhibit individual glycosylases. Ongoing studies are focusing on a series of purine analogs and the development of cell- and animal-based assays for biological readouts of efficacy.

High-Throughput Screen for Small Molecule Inhibitors of Parasite Glucose Transporters [OCTRI]

Diana Ortiz¹, Alex Johnson¹, Armand Guigemde², Caroline Elya¹, Johanna Hayenga¹, Kip Guy², and Scott M. Landfear¹

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Several parasitic protozoa that cause widespread human disease, including Leishmania, African trypanosomes, and malaria, are dependent upon uptake of the nutrient glucose for survival inside the human host. Hence small molecules that selectively inhibit the parasite glucose transporters, but not their human counterparts, could serve as leads for development of desperately needed anti-parasitic drugs. We have developed a live cell assay that allows us to screen for such selective inhibitors of glucose transporters from the 3 parasites mentioned above, and we have initiated high-throughput screens (HTS) at the St. Jude high-throughput screening facility.

The HTS assay is based upon expression of glucose transporters from Leishmania mexicana (LmxGT2), Plasmodium falciparum (malaria, PfHT), and Trypanosoma brucei (TbHT1), or humans (GLUT1) in a glucose transporter null mutant (gene knockout) of L. mexicana. The assay monitors growth of each transgenic L. mexicana line, employing glucose as an essential nutrient for growth. Cell growth is quantified using the fluorescent signal from the dye SYBR Green that fluoresces upon binding to cellular DNA and RNA. Compounds that inhibit the relevant glucose transporter will prevent uptake of glucose and inhibit growth of the transgenic parasite. Hits from the primary screen (e.g., growth of the transgenic line expressing the malaria glucose transporter PfHT) of a large chemical library are then rescreened in a second step to select for compounds that do not inhibit growth of a transgenic line expressing the human glucose transporter GLUT1. This dual screen identifies candidate compounds that may be selective inhibitors of the parasite but not the human glucose transporter. The most potent and selective hits will be further screened for those that inhibit uptake of [³H]D-glucose by the parasite transporter but not by GLUT1.

The St. Jude library (~600,000 drug-like compounds) has been screened against the L. mexicana LmxGT2 and the human GLUT1 and identified 14 compounds that appear to be high affinity (IC₅₀ <1 µM) selective inhibitors of transgenic lines expressing the Leishmania glucose transporter LmxGT2. Of interest, 6 of these hit compounds also preferentially inhibited growth of transgenic parasites expressing the malaria glucose transporter PfHT. The best such compound had an IC₅₀ for PfHT of 0.4 µM and a 13-fold lower IC₅₀ for PfHT compared to GLUT1, suggesting that it may be the most promising compound yet identified.
We have also screened a library from Glaxo-Smith-Kline of ~13,500 compounds that have previously been shown to inhibit growth of malaria parasites with an IC₅₀ of <1 μM, i.e. a ‘focused’ library of anti-malarial compounds. Some 400 of these compounds exhibited the ability to strongly inhibit growth of the PfHT-transgenic parasites, and these compounds are being tested for efficacy and selectivity for PfHT versus GLUT.

**Funding Support:** Support for this research has been provided by NIH (AI079092), Department of Defense (W81XWH-09-1-0429), and OCTR.

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**Body mass index and breast cancer risk of women in their 40s: Systematic review and meta-analysis**

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Background: Body mass index (BMI) has a paradoxical relationship with breast cancer risk in epidemiologic studies. Higher BMI has been associated with lower risk for breast cancer in premenopausal women, but higher risk after menopause. Underlying mechanisms for this paradox are unknown.

Objectives: To determine the strength of the evidence and quantify the effect of BMI on breast cancer risk of women in their 40s through a systematic review and meta-analysis.

Methods: A systematic evidence review was conducted using methods of the AHRQ Evidence-based Practice Centers. Electronic literature searches used MEDLINE (1995 through June 2011), Cochrane, and Scopus. English-language studies were included if they provided data about BMI measured within two years of the study’s baseline, and subsequent invasive breast cancer incidence for women aged 40–49 years in populations relevant to screening in the U.S. BMI categories corresponded to World Health Organization definitions of underweight, normal weight, overweight, and obese. A random effects model was used to determine summary estimates of associations using normal BMI as the reference group.

Results: Eighteen observational studies enrolling over 335,000 participants met criteria for meta-analysis. Most individual studies did not demonstrate statistically significant relationships between BMI and breast cancer incidence, although point estimates were consistent across studies. Meta-analysis indicated breast cancer risks that were significantly decreased for overweight (RR = 0.86 [95% CI 0.82, 0.90]) and obese (RR = 0.74 [95% CI 0.68, 0.81]) women compared to normal weight women.

Conclusions: For women in their 40s, breast cancer risk was reduced by 14% for overweight and 26% for obese women compared to normal weight women. While this analysis demonstrates an association between BMI and breast cancer incidence, it does not prove causation.

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**Maternal pre-gravid BMI and placental cytochrome P4501A1 activity**

Barent DuBois*, Perrie O’tierney, Jacob Pearson, Kent Thornburg, Ganesh Cherala
Purpose: To compare in vitro cytochrome P4501A1 (CYP1A1) activity of cytosol and microsomes from human term placentas, and to examine the effect of maternal pre-gravid BMI on CYP1A1 in these subcellular fractions.

Methods: Thirty-eight women who were 18 years of age or older, at term (≥37 weeks gestation) and had uncomplicated pregnancies (no gestational diabetes, preeclampsia or hypertension) were consented upon admission for scheduled caesarean section. The placental villous tissue was collected within 20 min of delivery, snap frozen in liquid nitrogen, and stored at -80°C until further analysis. Placental cytosol and microsomes were collected using differential centrifugation. Microsomes or cytosol protein was incubated in potassium phosphate buffer with an NADPH regenerating system and 7-Ethoxyresorufin. Fluorescence was measured at excitation / emission wavelength of 530nm / 590nm in a kinetic mode. The CYP1A1 specific activity was measured as pmoles of resorufin formed /min/mg of protein. Cytosolic protein was pre-incubated with anti-CYP1A1 antibody and α-naphthoflavone, and CYP1A1 activity was measured as described above. Gel electrophoresis was carried out to confirm the presence of CYP1A1 in cytosol of placenta.

Results: A 144% greater CYP1A1 specific activity in the cytosolic fraction compared to the microsomal fraction in placenta was observed. Both chemical and antibody inhibition studies abolished 50% of CYP1A1 activity. Western blots confirm the presence of CYP1A1 in cytosol. Obese mothers (BMI > 30) have significantly reduced placental microsomal CYP1A1 activity compared to lean mothers (BMI < 30) (0.082 vs. 0.046 pmoles/min/mg). Multiple linear regression analysis of placental efficiency (newborn’s weight/placenta weight) indicates cytosolic CYP1A1 specific activity, age, BMI, and gender of the fetus as strong main effects.

Conclusion: Placentas of obese mothers have reduced microsomal CYP1A1 specific activity. We have also observed significant EROD specific activity in placental cytosol that is attributable to CYP1A1. Regression analysis demonstrates that cytosolic CYP1A1 is an important determinant of placental efficiency. Together, these data suggest that maternal lifestyle could have a significant impact on CYP1A1 activity, and hints at a possible role for CYP1A1 in placental growth and thereby well-being of fetus.

Reproductive Risk Factors for Breast Cancer for Women in their Forties: Systematic Review and Meta-analysis

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Background: Identifying risk factors for breast cancer specific to women in their forties could inform screening decisions.
Objective: As part of a larger review, this analysis determined which reproductive risk factors are associated with breast cancer for women aged 40 to 49 years. Reproductive risk factors included oral contraceptive use, age at menarche, parity, age at first birth, and breastfeeding.

Methods: Studies were identified using MEDLINE (January 1996 to November 2011), Cochrane Central Register of Controlled Trials and Cochrane Database of Systematic Reviews (fourth quarter of 2011), Scopus, and hand searching reference lists of published studies. English-language studies reporting breast cancer outcomes for women aged 40 to 49 years in populations relevant to screening in the U.S. were selected. Included studies of oral contraceptive use investigated any oral formulation, used any definition of “ever” and “never” use, and measured current use within 1 year of breast cancer diagnosis. Studies defined parity as birth of a full-term infant, live birth, or pregnancy continuing 6 months or more regardless of outcome. Studies of breastfeeding used non-breastfeeding parous women as the reference category and determined breastfeeding activity (ever/never) and total duration. Data on population characteristics, study design, analysis, follow-up, and outcomes were abstracted, and studies were rated for quality using U.S. Preventive Services Task Force criteria. Meta-analyses were performed for studies rated good or fair quality that adjusted for at least one variable in their analysis. Sensitivity analysis was performed to determine the impact of outliers and the differences in the number of adjusted variables, measures, and reference categories.

Results: Thirty-four observational studies met inclusion criteria for the meta-analysis. Age at menarche ≥14 years versus ≤12 years (relative risk [RR] 0.81, 95% confidence interval [CI] 0.75-0.86; 10 studies), parity versus nulliparity (RR 0.84, 95% CI 0.74-0.96; 17 studies), ≥3 births versus none (RR 0.73, 95% CI 0.61-0.87; 13 studies), ever breastfeeding (RR 0.87, 95% CI 0.77-0.98; 14 studies), and breastfeeding for ≥12 months (RR 0.85, 95% CI 0.73-0.99; 11 studies) versus never breastfeeding were significantly associated with reduced risk of breast cancer. Age at first birth of ≥30 years versus <22 years was significantly associated with increased risk (RR 1.47, 95% CI 1.25-1.73; 15 studies), and oral contraceptive use within the previous 5 years was not significantly associated with risk (RR 1.10, 95% CI 0.93-1.29; 8 studies).

Limitations: Studies were observational and varied by measures, reference groups, and adjustment for confounders; effects of multiple risk factors were not considered; non-English-language studies were excluded.

Conclusions: Older age at menarche, parity, 3 or more births, and breastfeeding were associated with significantly reduced risk of breast cancer, while older age at first birth was associated with increased risk, although the magnitudes of effect were small for all reproductive risk factors. While the biological mechanisms underlying these associations are poorly understood, clinicians may find this information useful when determining breast cancer risk for women in their forties.

Support: National Cancer Institute

HYPOXIC & REPERFUSIVE RESPONSES ARE SELECTIVELY ALTERED DURING NON-SMALL CELL LUNG CANCER PROGRESSION.

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Purpose/Objectives Solid tumors undergo repeated cycles of hypoxia & reperfusion (h/r) of variable duration & magnitude. H/r episodes, referred to as acute hypoxia, occur hundreds to thousands of times during tumor initiation &
progression, providing selective pressure which influences genomic & epigenetic alterations favoring tumor survival & expansion, & leading to epithelial-to-mesenchymal transition (EMT), local invasiveness, distant metastasis & therapeutic resistance. Studies have indicated that such selective pressure is exerted on specific oncogenic signaling pathways (i.e. p53 & PTEN); however, no systematic studies have examined acute hypoxia in specific pathway activation, alteration or termination. Our hypothesis is that acute hypoxia results in the specific selection of kinase-regulated pathways allowing tumor growth in a harsh micro-environment. This study is designed as a pilot examination to determine if the phospho-, ubiquityl- & acetyl-proteome of normal bronchial epithelia (NBE) differs from that of non-small cell lung cancer (NSCLC) at 1% hypoxia (60 minutes) & 1% hypoxia (60 minutes) followed by reperfusion. Protein lysates from NBE & NSCLC lines were examined for altered global tyrosine phosphorylation, phosphorylation of specific serine or threonine motifs & global protein acetylation & ubiquitylation.

**Materials/Methods**  
NBE cells & associated small airway epithelial cell growth kit, A549 (CCL-185) cells, SW1573 (CRL-2170) cells, H1650 (CRL-5883) cells & complete growth media for each cell line, respectively, were purchased from ATCC Life Science Research (Manassas, VA). Cells were grown at 37°C, 5% CO₂, 15% water vapor under atmospheric O₂ to 70-80% confluency in 75cm² sterile flasks. Primary cultures were split into 35cm² sterile culture dishes & allowed reach confluency prior to experiments. Cells were subsequently grown under either: 1% O₂, 5% CO₂, 84% N₂ in a Hypoxen glove box (Frederick, MD) for 60 minutes followed by 60 minutes of reperfusion under the previously mentioned atmospheric conditions, or were allowed to continue growing under atmospheric conditions for 120 minutes.

After the growth period, cells were lysed in culture using ice cold NP-40 Cell Lysis Buffer with Thermo Scientific (Rockford, IL) protease & phosphatase inhibitor cocktail. Lysate was transferred to sterile 1.5mL Eppendorf tubes & incubated on ice for 30 minutes with occasional mixing. Tubes were spun for 10 minutes at 10,000g at 4°C using a Thermo Scientific Sorvall Legend X1R centrifuge (Rockford, IL). Supernatant was aspirated to sterile 1.5mL Eppendorf tubes & immediately stored at -80°C. Cellular extracts were shipped on dry ice to Cell Signal Technologies (Danvers, MA) & were sonicated & centrifuged to remove insoluble material. Protein concentrations were measured by Bradford Assay & 15μg of total protein were run by Western blot using proprietary antibodies. Western blots were developed using a LI-COR Odyssey NIR imaging system (Lincoln, NE).

**Results**  
Antibodies (Abs) directed phospho-motifs, ubiquitin-motifs, & acetyl-motifs indicate numerous PTMs responsive to changes in incident oxygen growth conditions in both the NBE & NSCLC lineages. This study’s initial novel findings are the hypoxic &/or h/r activation of specific kinases not previously associated with h/r. Further, results indicate that multiple PTMs occur in NSCLC that differ from those in NBE suggesting that such alterations could convey a selective advantage to the tumor cells. Of note, the two NSCLC selected for their notably different known oncogenic alterations also displayed limited common PTMs in response to h/r. Whether this is due to such oncogenic differences will be a central focus in future studies.

Common PTM responses between NBE & NSCLC are hypothesized to be critical regulatory steps necessary for cell survival regardless of oncogenic state, while those PTMs lost are hypothesized to be inhibitory growth regulatory steps selected against during rounds of acute hypoxia during tumorigenesis; PTMs gained in NSCLC but absent in NBE may confer a growth advantage that has been acquired during acute hypoxia-selected oncogenesis. While other explanations may underlie these alterations, future mutagenesis studies related to acquired oncogenic phenotype will begin the validation of these hypotheses.

H/r induced changes in PTMs are indicative of alterations in signal transduction pathway activation status, differential complex formation (phosphorylation), protein degradation, localization or complexation (ubiquitylation) & protein confirmation/activity status (acetylation). Identifying the targets of these PTMs is the first step in defining h/r-induced
PTM-phenotype relationships. Future studies will verify that these alterations convey a selective growth &/or survival capability & expand upon this pilot study by determining if cycles of acute hypoxia in NBE eventually lead to the specific patterns of PTM seen in the NSCLC cells.

**Conclusions** Recent proteomic advances have facilitated global mapping of changes in protein PTMs including phosphorylation - particularly detailed elucidation of kinase-specific phosphorylations, as well as ubiquitylation & acetylation. This study is intended as a pilot study to determine if differential PTM responses exist between NBE & NSCLC under micro-environmental acute hypoxic challenge. This has proven to be the case, justifying a larger study comparing h/r induced PTM profiles in a wider sampling of NSCLC to determine if specific PTM-regulated pathways are selected for during tumorigenesis & if specific gene mutations common to NSCLC influence specific PTM profiles. Future studies will evaluate if repeated cycles of h/r in NBE & NBE cells transformed with oncogenic Kras(G12D) or a dominant negative form of p53 [p53(R175H)] or combined Kras(G12D)+p53(R175H) lead to h/r-induced PTM profiles seen in mature cancers.

By comparing the effects of acute h/r on the phosphoproteomic spectrum in NBE to NSCLC of varying genotype/stage/grade, PTMs are indeed altered & specific h/r kinase-regulated pathways are selected for during oncogenesis. These PTM profiles are potentially instrumental in both diagnosis, as well as determining prognosis - particularly in relation to radiation & genotoxic therapy resistance. As current clinical methodologies poorly determine not only the hypoxic state of the tumor, but perhaps more importantly, the acute hypoxia-induced aggressiveness & resistance of the tumor, this research will provide key insights into determining these factors & thus influencing treatment options. Additionally, verification that PTM alterations convey a selective growth &/or survival capability may help suggest novel, non-redundant nodes suitable for therapeutic targeting thus adding another layer to the systemic biology underlying h/r-modulated tumor development & therapeutic resistance.

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**The Effect of Strength Training on Body Composition in Breast Cancer Survivors with Premature Menopause:**
**Preliminary Findings from a One-Year Randomized, Controlled Trial**

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Premature menopause is a risk for young breast cancer survivors (BCS) treated with chemotherapy. Cancer treatment-induced menopause can cause unhealthy body composition changes that may compromise physical function and increase the risk of comorbid conditions and breast cancer recurrence.

**Purpose:** To present preliminary findings from a one-year RCT of strength training (STR) versus a control program of flexibility training (FLEX) in prematurely menopausal BCS.

**Methods:** 48 early-stage BCS with treatment-induced menopause who completed baseline and 12 month measurements and who were randomized to either STR (N=23) or FLEX (N=25). Select measures include: Total body fat mass, total body lean mass, percent body fat (% BF) and bone mineral density (BMD) at the lumbar spine (L1-L4), total hip, femoral neck, and greater trochanter. Separate 2 (group) x 2 (time) RM-ANOVAs for each outcome measure were run to examine significant group x time interactions (p<.05).
Results: There were no significant group x time differences for any measure, however there was trend for FLEX to increase % BF compared to STR (p=0.06) (Table 1).

Table 1. % Changes in body composition over 12 months for STR and FLEX groups.

<table>
<thead>
<tr>
<th></th>
<th>Total % Body Fat</th>
<th>Total Lean Mass</th>
<th>Total Spine BMD</th>
<th>Total Hip BMD</th>
<th>Fem Neck BMD</th>
<th>G. Troch BMD</th>
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<tbody>
<tr>
<td>STR N=23</td>
<td>0.2±1.32</td>
<td>1.7±1.27</td>
<td>-1.2±0.64</td>
<td>-1.1±0.59</td>
<td>-0.6±0.63</td>
<td>-0.8±0.71</td>
</tr>
<tr>
<td>FLEX</td>
<td>3.6±0.53</td>
<td>0.9±0.17</td>
<td>-1.9±0.48</td>
<td>-0.6±0.36</td>
<td>-1.1±0.67</td>
<td>-0.6±0.45</td>
</tr>
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Means ± SEM.

Conclusions: These preliminary data show that one year of strength training may prevent increases in body fat for breast cancer survivors with premature menopause, but may not have an appreciable effect on the skeleton. Definitive conclusions are precluded until complete analyses are performed considering moderating effects of hormone manipulation therapy and participant adherence.

Supported by the American Cancer Society

Evidence for a complex containing FANCM and Rif1

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Fanconi anemia (FA) is a genetic disease leading to genomic instability and cancer. Our long-term goal is to understand the functional relationships among the proteins in FA and its collateral pathways to develop novel strategies for anticancer treatments. The current model for the FA pathway consists of three parts. First, a multiprotein “core complex,” including the highly conserved protein FANCM, is required for the monoubiquitylation of the second tier of the FA pathway, the FANCD2/FANCI protein complex and for other functions that are not well understood. The third tier of the FA pathway consists of breast cancer susceptibility components FANCD1/BRCA2, the partner of BRCA2 (Palb2/FANCN), and a helicase associated with BRCA1, FANCJ. FANCM is a component of a large complex of at least twelve proteins that responds to replication stress and specific types of DNA damage such as DNA interstrand crosslinks. Defects in the function of FANCM result in accumulation of errors during genomic replication and following treatment with specific types of DNA damaging agents.

We established Xenopus cell-free assays to identify proteins that associate with FANCM and to evaluate protein function in context with full replication. Xenopus extracts are a concentrated source of cell-cycle synchronized proteins that have been used for biochemical isolation and characterization of novel protein complexes. Because egg extracts contain stockpiles of nuclear proteins and are precisely synchronized in S or M phase, we reasoned that FANCM-containing complexes isolated from egg extracts might be enriched for FANCM-associated proteins that would be difficult to detect in human cell extracts.

We found FANCM sub-complexes in egg extracts that contained the DNA damage response protein Rif1. Rif1 was recently identified as a component of the BLM helicase complex, and shown to promote recovery of stalled replication
forks in vertebrate cells. Rif1 contains C-terminal binding domain that preferentially binds fork or HJ DNA and is required for BLM/Rif1 to prevent accumulation of stalled replication forks. We found that Rif1 was undetectable in cells lacking FANCM but readily in wild-type cells or in cells defective for the downstream helicase FANCJ. Rif1 co-precipitates with FANCM in wild-type cells, but is not detected in immune complexes in cell lines lacking FANCM, although Rif1 is expressed. These data suggest that FANCM and Rif1 are together in a complex and that the complex may be unstable when FANCM is absent or defective. Cells depleted of Rif1 are not MMC sensitive and the absence of xRif1 had no detectable effect on the monoubiquitylation of xFANCD2 suggesting that xRif1 is not required for xFANCD2 monoubiquitylation and thus is not part of the linear FA pathway. Rif1 forms DNA damage induced nuclear foci that are reduced in cells depleted of FANCM, suggesting that FANCM is required for localization of Rif1 in subnuclear foci, perhaps at sites of DNA repair. Identification of new proteins and protein complexes in the FA pathway could lead to a new understanding of FA protein function and targets for drug design for treatment of FA patients. In conclusion, we identified xRif1 as an interactor of xFANCM using a proteomics approach. Taken together, our data suggest that xFANCM is a component of a non-FA core complex containing Rif1. Rif1 may function with xFANCM during DNA damage response to maintain genomic stability.

Type II Diabetes and Depression in Adults from One Primary Care Practice

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Purpose: (1) To determine the prevalence of depression among adult type II diabetics and (2) assess if a relationship exists between glycemic control and depressive symptoms.

Background: Type II diabetes mellitus is a serious chronic illness that, when poorly controlled, can have significant lifelong effects. Depression often occurs as a comorbid condition. Despite uncertain causality, studies have shown that screening and detecting depression among diabetics may lead to improved health outcomes.

Methods: A convenience sample of 96 adult type II diabetics from one primary care clinic were screened for depression using the patient health questionnaire-9 (PHQ-9). Scores greater than nine were considered positive screenings to determine prevalence. A partial correlation was used to assess a relationship between hemoglobin A1c (Hb A1c) scores in the last 6 months with PHQ-9 scores, controlling for demographic and patient data.

Outcomes: Prevalence of depression in adult type II diabetics was 24%. Controlling for age, gender, education, race/ethnicity, and body mass index, Hb A1c and depression did not have a significant correlation (r=.091, p=.392). In post-hoc analysis, when using only participants with current Hb A1c values (within the last month, n=43), results showed a stronger correlation, but were not statistically significant (r=.31, p=.06).

Conclusion: In this sample, approximately 1 in 4 adults with T2DM had at least moderate depressive symptoms. There is some evidence of a relationship between glycemic control and severity of depressive symptoms. This pilot study has limited power and generalizability outside the clinic because of its small, non-random sample from one site. This study demonstrates that routine depression screening may be beneficial to detect current depression in type II diabetics, and may be correlated with poor glycemic control.
Purifying the active marine Bacillus sp. PL-12 Mn(II) oxidase from E. coli

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Manganese, like iron and copper, is a transition metal vital to life on Earth, yet much of the world’s metals are trapped in the Earth’s crust as minerals. These metals must cycle to their soluble form in order to be used by cells, but too much of any one metal is toxic. Understanding metal cycling is a central component to understanding how organisms function and how they interact with their environment. Manganese is solubilized to Mn(II) from Mn(IV) minerals and can oxidize back by cycling through a Mn(III) intermediate. Some bacteria and fungi can enzymatically oxidize Mn(II) several orders of magnitude faster than abiotic. Bacillus sp. PL-12 is a marine Firmicute that oxidizes Mn(II) to Mn(IV) on its exosporium. The Mn(II) oxidase has been directly identified as MnxG, a multicopper oxidase (MCO), that lies in a polycistronic operon made up of mostly proteins of unknown function. In order to elucidate the mechanism of this MCO we have successfully produced an active Mn(II) oxidase from an E. coli expression system. We have discovered that unlike any MCO studied, this enzyme requires the co-expression of at least two of its operon neighbors in order to be active. We have also observed similarities to the Bacillus spore coat MCO, CotA, in that it is heat resistant, binds copper, and runs at a higher apparent molecular weight in an SDS PAGE gel when fully denatured. Characterizing this enzyme complex will describe novel MCO/accessory protein binding and the elusive mechanism of biological Mn(II) oxidation.

Endothelial Cells Mitigate Radiation-Induced Hematopoietic Stem Cell Injury

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The hematopoietic system is exquisitely sensitive to damage by ionizing radiation. Unsuspected radiation exposure, as has occurred in tragedies such as the Chernobyl disaster and more recently in Japan, places large populations at risk for developing bone marrow failure. Currently, transplantation is the only curative therapy for these individuals, and non-transplant maintenance therapies are often wrought with complication. As such, the development of effective treatments that can be administered to irradiated victims post-hoc and still restore normal hematopoiesis is essential to limit the huge health burdens these disasters can exact. We have previously shown that endothelial cells (ECs) are capable of restoring endogenous hematopoietic stem cell (HSC) function and promoting long-term survival when injected into lethally irradiated mice at the time of radiation exposure. Herein, we extend our findings by providing evidence that this EC-mediated phenomenon remains effective up to 48 hours following irradiation. Commencement of EC-bone marrow cell (BMC) co-culture 24 and 48 hours after radiation exposure partially prevented loss of BMC colony-forming capacity and increased HSC numbers in the BMC population as evidenced by flow cytometry, when compared with irradiated BMCs cultured in the absence of ECs. Our results suggest that ECs are capable of rescuing HSCs up to 48 hours after irradiation in vitro. Coupled with ongoing in vivo experiments, these data warrant our continued search for the molecular mediators of this phenomenon as putative therapies for mitigating radiation-induced hematopoietic disease.
In-silico customization of insulin dosing regimens
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Background: Titration of insulin dosing is an ongoing process for type 2 diabetics. Insulin sensitivity changes with weight, stress, and other medications. Variations in diet and exercise change glucose supply and usage. This project is a proof of concept for a simple tool to model individual insulin/glucose transport characteristics. This model can then be used to evaluate alternative insulin dosing regimens.

Methodology: A simplified model of the insulin/glucose transport system was developed using pharmacokinetic principles. This model, developed using the VENSIM systems dynamics program, is based on a series of ordinary differential equations that define the absorption, distribution, metabolism, and elimination of both insulin and glucose in diabetic patients.

Results: With the model, we were able to model reference behaviors for both healthy and diabetic patients. Sensitivity analysis showed that insulin production and sensitivity, carbohydrate intake, and exercise were the key variables in glucose homeostasis. Based on these modeled behaviors, the balance between carbohydrate intake and insulin dosing could be evaluated, and optimal insulin dosages for different types of insulin calculated.

Conclusion: The use of a similar model could improve the ability of diabetic patients and their providers to better manage their conditions.

Deconstructing the Fanconi Anemia pathway

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Our laboratory is interested in understanding the molecular function of the Fanconi anemia (FA) protein network in context with other proteins that regulate or influence genomic stability. Fanconi anemia (FA) is a genetic disease with characteristic developmental abnormalities, bone marrow failure, genomic instability and predisposition to cancer. The FA pathway consists of a multiprotein “core complex,” required for the monoubiquitylation of the FANCD2/FANCI protein complex and for other functions that are not well understood. Components downstream of the FANCD2/FANCI complex include breast cancer susceptibility components FANCD1/BRCA2, the partner of BRCA2 (Palb2/FANCN), and a helicase associated with BRCA1 (FANCJ/BACH1), and several newly identified proteins including FAN1, FANCO/RAD51C, FANCP/SLX4. Together, the FA/BRCA pathway responds to replication stress and specific types of DNA damage such as DNA interstrand crosslinks. There are components in the pathway yet to be discovered, and the function of most of the FA proteins is unclear. Our lab uses assays in human cells and Xenopus cell-free assays to identify proteins that participate in the function of the FA pathway, and to analyze the function of the FA proteins in context with DNA replication. We recently identified Rif1 as an interactor of the FA core complex protein FANCM, using a proteomics approach. Rif1 was recently identified as a component of the BLM helicase complex that promotes recovery of stalled replication forks, raising the possibility that Rif1 may function with xFANCM during DNA damage response to maintain genomic stability. In a second research focus, we designed a cell-free assay to screen for small molecules that modulate the FA/BRCA pathway. We are using this assay to identify potential therapeutic targets and to dissect the activity of the FA proteins within the network of proteins that guard genomic stability. Ultimately, insights into the mechanism of the FA/BRCA network of proteins will lead to an understanding of the underlying molecular defect in FA and may lead to more effective avenues of treatment for this devastating pediatric disease and for certain types of cancer.

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\(^3\)The Ohio State University

**Background:** Recent policy changes have affected access to health insurance for families in the United States (US): private health insurance premiums have increased, state Medicaid programs have cut coverage for adults, and the Children’s Health Insurance Program has made public insurance available to more children. Parental coverage impacts their children’s coverage and having health insurance is associated with improved health outcomes, thus it is important to understand how family coverage patterns have changed as a result of these policies.

**Methods:** We analyzed data from the nationally-representative Medical Expenditure Panel Survey, comparing cohorts from 2003 to 2008. We evaluated cross-sectional and full-year coverage patterns for child/parent pairs, stratified by income. We conducted chi-square tests to assess significant differences in coverage over time.

**Results:** Middle-income (200-<400% Federal Poverty Level) child/parent pairs had the most significant changes in their coverage patterns when comparing 2003 to 2008. Specifically, the cross-sectional percentage of insured middle-income child/parent pairs significantly decreased from 85.4% to 80.6% (p=0.01), child/parent pairs with private coverage dropped from 75.7% in 2003 to 70.2% in 2008 (p=0.01), and there was a significant increase in the percentage of uninsured middle-income child/parent pairs (from 5.6% in 2003 to 8.3% in 2008; p=0.05). In addition, the percentage of middle-income child/parent pairs who were either uninsured all year or had a gap in insurance at some point during the year significantly increased from 9.7% in 2003 to 13.0% in 2008 (p=0.04).

**Implications/Discussion:** The percentage of middle-income child/parent pairs who were lacking insurance, for all or part of the year has risen suggesting that these families may be caught between affording private coverage and being eligible for public coverage. Unless private coverage becomes more affordable, insurance instability among middle-income families may persist.

Accumulated Dose Reduction of Optically Stimulated Luminescent Dosimeters through Fluorescent Light Annealing

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Optically stimulated luminescent dosimeters, OSLDs, are a new type of therapy approved dosimeter which have a light tight case encapsulating an active crystal region of aluminum oxide doped with carbon (Al\(_2\)O\(_3\):C). The active region of the OSLDs stores energy upon exposure to ionizing radiation, which is released when the OSLD is stimulated with optical light (540nm). As the OSLD is used for various treatments, the amount of accumulated dose on the device reaches a
saturation point. Optically annealing the OSLD’s provides a method for removing accumulated dose allowing further use of the device.

We present results illustrating the reduction of the accumulated dose stored on OSLDs through annealing. Our method for annealing uses a common light box found in most clinics as the light source, which contains 14W fluorescent bulbs. The experiment exposes the OSLDs with a known amount of accumulated dose for various amounts of annealing time. Our results show the remaining dose over time follows a second ordered exponential relationship, where after 20 minutes less than 5% of the original dose remains on the OSLD and after an hour, approximately 1.5% of the initial dose remains.

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**Variability in Care Quality: Do Federally-Qualified Health Center Patient Demographics Correlate with Quality of Diabetic Care?**

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**Research Objectives:**

1. To describe differences in the demographics of Oregon patients with diabetes in the OCHIN network of federally qualified health centers (FQHCS);
2. To assess variability in rates of recommended diabetes preventive care services in the OCHIN network between 2005-2007;
3. To evaluate whether the demographic patient profile of an FQHC correlates with its rates of recommended diabetes services.

**Study Design:** We conducted a retrospective study of patient data from FQHCS in the OCHIN community health information network that provided care to adult patients with diabetes mellitus in 2005-2007. We used individual-level linkages to create a combined dataset using electronic health record (EHR) and Medicaid administrative data, then conducted a series of descriptive and univariate analyses in this dataset.

**Population Studied:** We included 21 Oregon FQHC members of the OCHIN network that had provided services to >100 established diabetic patients between 2005-2007. We assessed patients’ receipt of the following diabetes services: influenza immunization, low density lipoprotein (LDL) cholesterol screening, urine microalbumin, and/or glycosolated hemoglobin (HbA1c) screening. Patient characteristics included: number of languages spoken, services received for other conditions, variability in insurance coverage, and income as a percent of the federal poverty level (FPL).

**Principal Findings:** The percentage of patients who received ≥1 service between 2005-2007 varied by clinic, ranging from 19% to 98% for LDL screening, 8% to 87% for influenza immunization, 14% to 96% for urine microalbumin, and 56% to 100% for HbA1c screening. Patient demographics also differed among these clinics. There was either no correlation or a positive correlation between the percentage of diabetic patients receiving services for morbidities other than diabetes, and receipt of diabetic services. There were significant positive Spearman correlations between percent of clinic patients with continuous insurance coverage and clinic rates for LDL screening (0.57), influenza immunizations (0.53) and HbA1c screening (0.52) (p<0.05). Microalbumin screening rates were not significantly correlated with clinic-level insurance coverage rates. Median income as percent of FPL at visit, and percentage of patients with average income <50% of FPL, were not significantly associated with percentage of patients receiving diabetic services.
Conclusions: In a network of FQHCs that routinely provide care to diabetic patients, there was wide variability in rates of diabetes preventive service administration. Positive correlations between percent of patients with co-morbid conditions and higher clinic rates of diabetic services suggests that the burden of delivering services for other comorbidities did not negatively impact delivery of diabetes services. The positive correlation between the percent of patients with continuous insurance coverage and higher clinic rates of LDL, influenza immunization and HbA1c screenings suggests that clinic-level insurance coverage rates might influence whether or not certain diabetic tests are performed. The lack of association between income and patient care might be due to the restricted range of income, as most FQHC patients are low income.

Implications for Policy, Delivery or Practice: Patient demographics correlate with quality of care. In this study, FQHCs with a higher percentage of continuously insured patients had better rates of diabetes care delivery. Quality of care comparisons between clinics must take into account the patient demographic profile of the clinic.

A “Rare Disease” Approach to Cancer Therapeutics

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Abstract

Current approaches in cancer treatment are typically not selective, affecting both cancer cells and normal cells. However, inactivation of DNA repair pathways, an event that occurs frequently during tumor development (Rai, 2007), can make cancer cells over-dependent on a reduced set of DNA repair pathways for survival. Targeting the remaining functional pathways may be useful for single-agent and combination therapies in such tumors. The most successful example of this approach so far is specific targeting of BRCA-deficient tumors with PARP (Poly [ADP-Ribose] Polymerase) inhibitors (Fong, 2009). This targeted chemotherapeutic approach could improve cancer therapy with more specificity for tumor cells and with less toxicity for normal cells (Iglehart, 2009). The challenge in this approach is that patients must be stratified for specific treatments through biomarkers and surrogate indicators to find the (sometimes rare) subpopulation that is likely to respond.

Treating BRCA-deficient tumors with PARP inhibitors appears to block repair of single strand breaks. Unrepaired single strand breaks can lead to DNA double strand breaks (DSBs) that must be repaired for the cell to survive. In normal cells, DSBs can be repaired by homologous recombination (HR), a process that is defective in certain tumors. The ability of a cell to perform HR could be evaluated indirectly by identifying mutations in BRCA genes or by determining if cells are capable of forming RAD51 foci, a surrogate marker of HR function. The ability of a cell to form RAD51 foci formation may indicate “BRCA-ness,” and sensitivity to PARP inhibitors (Lord & Ashworth, 2012).

PARP inhibitors may also have broader indications. A deficiency in the tumor suppressor gene PTEN results in HR defects and reduced RAD51 foci formation (Mendes-Pereira, 2009). Reduced RAD51 foci formation due to certain PTEN mutations could indicate defective HR, and sensitivity to PARP inhibitors. However, only a subset of PTEN mutations are likely to result in reduced RAD51 foci and HR deficiency.

We are interested in:
1. Developing a screening assay for RAD51 foci as a predictor of PARP inhibitor sensitivity.
2. PTEN sequencing and expression analysis to identify PTEN mutations associated with reduced RAD51 foci and PARP inhibitor sensitivity
3. Analysis of primary tumor samples with a PTEN mutations for potential HR defect by screening for PTEN expression, RAD51 foci formation (as biomarker for HR) and PARP inhibitor sensitivity.

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**The Effect of Strength Training on Body Composition in Prostate Cancer Survivors on ADT: Preliminary Findings from a One-Year Randomized, Controlled Trial**

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Androgen deprivation therapy (ADT) is a common treatment for prostate cancer survivors with advanced disease. Side effects of ADT are rapid bone and muscle loss and fat gain that can compromise physical function and increase the risk of comorbid conditions.

**Purpose:** To present preliminary data from a recently completed randomized controlled trial of one-year of strength training (STR) versus a control program of flexibility training (FLEX) in prostate cancer survivors on ADT.

**Methods:** To date, 37 men completed baseline and 12 month testing and were randomized to one year of STR (N=26) or FLEX (N=11). Select measures include: Total body fat mass, total body lean mass, % body fat (%BF) and bone mineral density (BMD) at the lumbar L1-L4 spine, total hip, femoral neck, and greater trochanter. Separate 2 (group) x 2 (time) RM-ANOVAs for each outcome measure were run to examine significant group x time interactions (p<.05).

**Results:** After one year, there was a significant group x time interaction for total fat mass (p=.03; Table 1). There were no other significant differences over time between groups for remaining variables (Table 1.).

<table>
<thead>
<tr>
<th></th>
<th>%Body Fat</th>
<th>Total Fat Mass(kg)</th>
<th>Total Lean Mass(kg)</th>
<th>Total Spine BMD</th>
<th>Total Hip BMD</th>
<th>Fem Neck BMD</th>
<th>G.Troch BMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>STR</td>
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<td>-.05±.04</td>
<td>-.04±.04</td>
<td>0.1±0.7</td>
<td>-0.9±0.2</td>
<td>-2.6±0.6</td>
<td>-0.3±0.4</td>
</tr>
<tr>
<td>FLEX</td>
<td>0.8±0.5</td>
<td>1.5±1.0</td>
<td>.07±1.3</td>
<td>-1.6±0.8</td>
<td>-0.8±1.3</td>
<td>-1.1±1.6</td>
<td>-0.4±1.3</td>
</tr>
</tbody>
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Means ± SEM.

**Conclusion:** These preliminary data suggest that one year of strength training can prevent an increase in fat mass in men on ADT for prostate cancer. Definitive conclusions are precluded until the final analyses are complete.

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Depressed serotonin (5-HT) contributes to suppressed CO2 chemosensitivity in MeCP2 deficient mice

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Loss of function mutations in the transcription factor Methyl-CpG-binding protein 2 (MeCP2) causes the X-linked disorder Rett syndrome. Frequent apnea is a common and disturbing feature of this autism spectrum disorder. Mouse models faithfully mimic the respiratory phenotype. CO2 chemosensitivity is depressed in MeCP2 deficient mice especially at physiological levels of CO2 (Am J Physiol 301:C729, 2011; FASEB J 26:894.6, 2012). This deficiency may contribute to their respiratory disorder by raising the level of CO2 at which breathing ceases. Studies were preformed in B6.129P2(C)-Mecp2<sup>tm1.1Bird</sup> mice. Using an in situ preparation allowed CO2 to be held at any desired level in the arterial perfusate while recording from the phrenic nerve. At 3% CO2 phrenic amplitude in wild type mice was ~60% of the control value. In contrast the phrenic nerve stopped firing in 6 of 8 MeCP2 deficient mice at this level of hypopapnia. Brain levels of 5-HT are decreased in Rett subjects and MeCP2 deficient mice (PNAS 106:21966, 2009). We hypothesized that increasing central 5-HT would restore CO2 chemosensitivity. CO2 chemosensitivity was determined using body plethysmography and exposing the mouse to sequential 1, 3 and 5% CO2. At baseline the increase in minute ventilation was 30 to 50% in MeCP2 deficient mice compared that in wild type. Pre-treatment with citalopram, a selective 5-HT reuptake inhibitor (2.5 mg/Kg ip) in MeCP2 deficient mice resulted in a significant increase in their response to CO2 such that it equaled that of wild type animals. These results suggest that decreased serotonin in MeCP2 deficient mice dis-facilitates CO2 chemosensitivity.

Support: International Rett Syndrome Foundation; Rett Syndrome Research Trust; NewLife Foundation (UK)

Identification of the Mn(II) Oxidases and Accessory Proteins Involved in Mn(II) Oxidation in Pseudomonas putida GB-1

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Bacterially mediated Mn(II) oxidation profoundly affects the bioavailability of Mn, which in turn affects the availability of trace metals and other compounds that associate with the insoluble Mn(III,IV) oxides. While bacterial Mn(II) oxidation has been studied for years, it is relatively recent that the enzymes responsible have begun to come to light. In the alpha-proteobacteria Erythrobacter sp. SD-21 and Aurantimonas maganoxydans SI85-9A1, a homolog of calcium-binding heme peroxidase, MopA, was identified biochemically as a Mn(II) oxidase. In Bacillus sp. SG-1 and related organisms, a multicopper oxidase (MCO), MnxG, was identified as the Mn(II) oxidase both genetically and biochemically. Despite repeated, extensive genetic screens in the Mn(II) oxidizing strain Pseudomonas putida GB-1, the nature of its Mn(II) oxidase had remained unknown. However, recent work has identified two genes (mcoA and mnxG), both encoding MCOs, as Mn(II) oxidases. Complete loss of Mn(II) oxidase activity is only achieved when both of these genes are deleted from the chromosome. The mnxG gene encodes a protein with homology to the Bacillus MnxG (e = 1 x 10^-22). Through analysis of the P. putida GB-1 genome and site-directed mutagenesis, as well as biochemical purification, additional factors involved in Mn(II) oxidation have been identified. The P. putida GB-1 genome encodes a homolog to MopA. However, deletion of this gene only decreases oxidation in a strain also lacking mnxG, suggesting that, unlike in the alpha-proteobacteria,
MopA plays only an accessory role in Mn(II) oxidation. Both \textit{mnxG} and \textit{mcoA} are located in putative operons with downstream genes encoding SCO proteins. This class of protein has been implicated in loading Cu into cytochrome c oxidase. Our results show that deletions of each of these genes result in decreased Mn(II) oxidation, supporting a role for these Cu chaperones in MCO function. Mass spectrometric analysis of partially purified active fractions identified two hypothetical proteins that co-purify with McoA. The genes encoding these proteins are the first two genes of a putative operon of four genes total, with the other two genes encoding proteins with possible curlin domains. Expression of the first gene in the putative operon is regulated by the Mn(II) oxidase regulator MnxR and this operon is conserved among Mn(II) oxidizing pseudomonads but not among non-oxidizers. Furthermore, deletion of either of the two hypothetical protein genes results in decreased Mn(II) oxidation. Therefore, Mn(II) oxidation in \textit{P. putida} GB-1 involves two separate MCO enzymes, multiple SCO Cu chaperones, a possible contribution from the heme-peroxidase MopA under some conditions and two novel hypothetical proteins. From these preliminary results we have proposed a model for a Mn(II) oxidase complex in \textit{P. putida} GB-1 upon which to base future investigations.

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**A Randomized Controlled Trial of 8-form Tai Chi Improves Symptom and Functional Mobility in Fibromyalgia Patients**

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**Background:** Previous researchers have found that 10-form Tai chi yields symptomatic benefit in patients with fibromyalgia (FM). The purpose of this study was to further investigate these findings and focus on functional mobility.

**Methods:** We conducted a parallel-group randomized controlled trial FM-modified 8-form Yang-style Tai chi program compared to an education control. Participants met in small groups twice weekly for 90 minutes over 12 weeks. The primary endpoint was symptom reduction and improvement in self-report physical function, as measured by the Fibromyalgia Impact Questionnaire (FIQ), from baseline to 12 weeks. Secondary endpoints included pain severity and interference (Brief Pain Inventory (BPI), sleep (Pittsburg sleep Inventory), self-efficacy and functional mobility.

**Results:** Of the 98 randomly assigned subjects (mean age 54 years, 93% female), those in the Tai chi condition compared to the education condition demonstrated clinically and statistically significant improvements in FIQ scores (16.5 vs. 3.1, \(p<0.0002\)), BPI pain severity (1.2 vs. 0.4, \(p<0.0008\)), BPI pain interference (2.1 vs. 0.6, \(p<0.0000\)), sleep (-2.0 vs. -0.03, \(p<0.0003\)) and self-efficacy for pain control (9.2 vs. -1.5, \(p<0.0001\)). Functional mobility variables including Timed get-up and go (-0.92 vs. -0.25, \(p<0.0001\)), static balance (7.5 vs. -0.3, \(p<0.0001\)) and dynamic balance (1.6 vs. -0.5, \(p<0.0001\)) were significantly improved with Tai chi compared to education control. No adverse events.

**Conclusions:** Tai chi appears to be a safe and effective mind/body exercise treatment that could be used as an adjunctive modality in FM patients for both symptom reduction and functional mobility improvement. (ClinicalTrials.gov Identifier, NCT01311427

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**The Discovery of Small Molecule Inhibitors of DNA Polymerase Kappa**

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Human DNA polymerase kappa (pol κ) is a Y-family translesion synthesis (TLS) polymerase that has been demonstrated to catalyze efficient TLS past various minor groove linked lesions including acrolein-mediated DNA-peptide cross-link lesions and DNA–DNA interstrand cross-link lesions (ICLs), and polycyclic aromatic hydrocarbon-derived lesions. It also plays a role in the processing of chemotherapeutic agent mitomycin C and ultraviolet (UV) light-induced DNA lesions. Since the TLS activity of pol κ could result in cellular resistance to chemotherapy and since gliomas overexpress pol κ, small molecule library screens targeting pol κ were carried out in order to develop a new cancer therapeutic that can lead to the improvement of chemotherapeutic efficacy. A high throughput, fluorescence-based DNA strand displacement assay using non-damaged DNA has been utilized to screen for diverse bioactive compound libraries. The hit compounds have been identified and authenticated by radioactive gel-based TLS assays with non-damaged DNA. The lead compounds were characterized for their specificity toward pol κ in radioactive gel-based TLS assays with DNAs containing site-specific acrolein-mediated ring-opened form of γ-HOPdG adduct. Furthermore, the lead compound has been shown to enhance toxicity of UV irradiation in xeroderma pigmentosum variant cells, suggesting the intracellular activity of the compound against pol κ. Collectively, these studies highlight the ability to target pol κ, as well as the discovery of compounds that could serve as prototypes for future design of pol κ inhibitors.

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Clinical Documentation Quality- Can Our Notes Be Used For Outcomes Research?

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Oregon Health and Sciences University: Dept of Oral and Maxillofacial Surgery⁴, Dept Medical Informatics and Clinical Epidemiology⁵

The Problem

Clinical notes are usually written in unstructured narrative formats. Researchers rely on this unstructured documentation for surgical outcomes research. We assessed the documentation quality of clinic encounters following treatment of mandibular fracture - a common injury with known functional and clinical outcomes. We asked the question, “Will current documentation practices support future outcomes research?”

Materials and Methods

Data Source: 54 paper-based medical records from patients with primary diagnosis = mandible fracture and at least one postoperative encounter.

Site: Dental School-based OMS residency training program.

Abstraction: Paper charts were de-identified, scanned to PDF, then abstracted and rated by two authors (AR/ME). A standardized rating method was developed because none existed. 10 separate records were used for initial reviewer calibration and establishment of inter-rater reliability.

Scoring: The quality of documentation of basic clinical outcomes were scored in the categories of wound healing, bone union, sensory nerve, motor nerve, facial morphology, occlusion, scar, range of motion, pain, and function. For each record in each category, outcome documentation was given a quality rating (0 – 4). 0= undocumented, 1= subjective report only, 2= ambiguous documentation, 3= explicit documentation, 4= documented by validated method. When unsure, a higher score was assigned.
The quality of all post-op documentation was rated as a whole. For instance, if sensory nerve function was documented at only one of three postoperative encounters, that record would still receive a score of 3 for sensory.

Statistics: Descriptive statistics were generated, including Cohen’s Kappa (κ) for inter-rater reliability.

Conclusions

- Significant variability exists in the consistency and quality of documentation of mandible fracture outcomes.
- Few clinical categories are regularly documented in a structured format that is useful for outcomes based research.
- Future surgical outcomes research will require development of structured terminologies and documentation standards.

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**High-Throughput Platform for Screening Small Molecule Inhibitors for the Fanconi Anemia Pathway using Bioluminescence Resonance Energy Transfer (BRET) in a Novel Cell-Free Assay**

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The Fanconi anemia (FA) pathway is a DNA damage response network involved in the maintenance of genomic stability. The FA pathway is synthetic lethal with several DNA repair genes such as ATM, NBS1, NEIL1 and PARP1, that are linked to a wide range of inherited and sporadic hematological malignancies. FA pathway inhibitors may therefore selectively kill malignant cells bearing defects in other DNA repair pathways.

We recently developed a new platform to identify and evaluate inhibitors of the FA pathway by monitoring the monoubiquitylation status of FANCD2 in Xenopus cell-free extracts. As a pilot study, various curcumin analogs and small molecule libraries were screened and the most active compounds were further characterized using densitometry of immunoblots.

Our preliminary data suggests that our established cell-free FA inhibition assay can be converted to a Bioluminescence Resonance Energy transfer (BRET)-based high-throughput screen (HTS) that will be needed to identify compounds that inhibit the FA pathway more effectively than those we already identified. Our goal is to identify small molecules from established compound libraries that inhibit formation of monoubiquitylated FANCD2 as a marker for down-regulation of the FA pathway.

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**Ligand-sensing in a large embryonic extracellular space by membrane nanotubes**

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In early, cleavage-stage *Xenopus* embryos, an unusual class of extremely long, stable filopodia forms complex arrays that span the blastocoel and interconnect blastomeres that are separated by hundreds of microns. The time of appearance
and distribution of these structures suggest a direct role in some aspect of early, pre-midblastula transition embryonic patterning. In support of this possibility, we present recent findings, including that the long filopodia are particularly active in endocytosis of blastocoelar contents via clathrin-coated vesicles and caveolae, in contrast to the comparatively quiescent nearby blastocoel-facing cellular surfaces. Because endocytosis is known to play a key role in both positive and negative regulation of Wnt signaling, filopodia may function as Wnt-selective probes of the complex 3-dimensional extracellular environment of the *Xenopus* blastocoel.

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**Image Contrast Based on Nano-Architecture of Tissues and Biomaterials**

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The status of a cell or tissue is encoded in its nano-scale architecture, i.e., nuclear organization, mitochondria and organelles, cytoskeleton, extra-cellular matrix. Optical scattering is able to sense the size distribution of this nano-architecture. We have developed a novel approach toward characterizing the nano-architecture using reflectance-mode confocal laser scanning microscopy (rCLSM). The axial position (zf) of the focus of an rCLSM system is moved down into the first ~100 μm of a tissue. The reflectance signal (R) falls exponentially versus depth zf: $R = \rho \times \exp(-\mu \cdot z)$. The parameter $\rho$ [dimensionless] is the local reflectivity of the tissue. The parameter $\mu$ [cm$^{-1}$] is the attenuation coefficient. Experimental data is fit by this equation. We have used computer simulations to specify the relationship between the experimentally observed $(\rho, \mu)$ and the tissue optical properties $(\mu_s, g)$. The property $\mu_s$ [cm$^{-1}$] is the optical scattering coefficient. The property $g$ [dimensionless] is the anisotropy of scattering which describes the angle of photon deflection by each scattering event. The $g$ value is particular sensitive to the size distribution of the nano-architecture of a tissue. We have used this $(\rho, \mu) \rightarrow (\mu_s, g)$ characterization in 3 examples: (1) detect the effect of a single gene mutation (*Osteogenesis Imperfecta*) on dermal structure in mice. The mutation affects the nano-scale collagen structure where the ability of the collagen fibrils to organize into collagen fibers is hindered; (2) detect the remodeling of a collagen gel by smooth muscle cells, where the remodeling involved the degradation of collagen fiber bundles in the bundles into smaller fibrils by matrix-metalloproteinases (MMPs); (3) study the mechanism of optical clearing of dermis treated with glycerol, where we show that the dermis becomes clear not because of reduced scattering but rather because the angle of scattering becomes very forward directed. The rCLSM method can scan a tissue and create values of $\mu_s$ and $g$ for each pixel in a field of view, yielding an image whose contrast mechanism is the nano-architecture of the tissue.

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**Community Composition and Carbon Fixation of Neutrophilic Iron-Oxidizing Chemoautotrophs at Hydrothermal Vents**

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Loihi Seamount is an active submarine volcano that marks the southernmost extent of the Hawaiian hotspot. Numerous diffuse hydrothermal vents have been found near the summit with fluids containing millimolar concentrations of Fe(II) and almost completely devoid of reduced sulfur species. Microbial mats growing around these vents are dominated by
neutrophilic iron-oxidizing chemoautotrophic bacteria. T-RFLP community fingerprinting coupled with clone library analysis has shown many of these communities are dominated by phylotypes belonging to the newly described *Zetaproteobacteria* class of bacteria. Quantitative PCR analysis shows the populations of *Zetaproteobacteria* is variable and can range from 10 to 70% of the bacterial community. Clone library analysis of the Calvin Cycle carbon fixation genes *cbbL* and *cbbM* from both DNA and mRNA templates indicate the Rubisco form II *cbbM* gene is expressed at a greater level than *cbbL*, and *cbbM* phylotypes primarily group with *Mariprofundus ferrooxydans*. Reductive TCA cycle genes are also present in some microbial mats. A metagenomic library constructed from a microbial mat sample using Illumina sequencing supports the PCR based analysis, and also identifies potential carbon fixation genes utilized in the reductive acetyl-CoA pathway and the 3-hydroxypropionate/4-hydroxybutyrate cycle. This suggests that the chemoautotrophic iron-oxidizing communities at Loihi Seamount are either microaerophilic or anaerobic and utilize diverse carbon fixation pathways.

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**Vancomycin Area Under the Curve (AUC) in Patients with Methicillin-resistant Staphylococcus aureus Bacteremia (MRSAB)**

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**Introduction:** MRSA infections have been on the rise over the past decade. Vancomycin is the primary treatment for invasive MRSA infections, particularly bacteremia. The vancomycin AUC relative to minimum inhibitory concentration (MIC) is a major determinant of efficacy. Since vancomycin has time dependent bactericidal activity, further investigation of determining AUC as a therapeutic target is needed.

**Objectives:** To calculate the AUC and determine if vancomycin doses and trough concentrations pre- and post-publication of the Vancomycin Therapeutic Guidelines attained target AUC/MIC ratios > 400 mg*hr/L; to assess vancomycin response pre- and post-publication of the Vancomycin Therapeutic Guidelines.

**Methods:** Retrospective cohort of adult patients who received intravenous vancomycin for MRSAB from May 2008-present. Data collected: demographics, labs, antibiotics, microbiology, medications and comorbidities. Outcomes: Resolution of fever and clinical signs of infection and negative cultures for microbiology.

**Results:** Data collected in n = 28 patients (27 Caucasian, 1 Black, 10 women, 18 men, TBW 69.4 +/- 15.1 kg, BMI range 15.3 – 31.8 kg/M², and eGFR range 6.38 - >150 mL/min/1.73M²). Of 8 patients with vancomycin AUC/MIC < 400 mg*hr/L, 4 patients had MRSAB for > 3 days, and 3 patients febrile > 5 days during vancomycin therapy. Sixteen of the 20 patients with AUC/MIC > 400 mg*hr/L had MRSAB for < 3 days, and 1 patient was febrile > 5 days during vancomycin therapy.

**Conclusions:** Preliminary results in a larger cohort will be presented. These results will provide valuable information regarding the utilization of the target AUC/MIC ratio to optimize vancomycin therapy in patients with MRSAB.

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**One if by Land and Two if by Air: Evaluation of Internet Mapping Technology and the Decision to Transport Trauma Patients by Helicopter Emergency Medical Services (HEMS)**
Scott Sherry, Zeki Karahan, Karen Dooley, Apostolos Alexandridis, Kyle Lamb, Samantha Underwood, Jennifer Watters

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Introduction: In the past 10 years there have been a number of HEMS crashes that have resulted in the deaths of patients and air crews with the peak coming in 2008 when there were 9 fatal crashes causing 29 deaths. This has lead to increased scrutiny of the use of these resources and their application. Guidelines suggest that HEMS may only be beneficial if it reduces transport time to a trauma center by more than 10 minutes. With emerging computer software mapping technology, dispatch centers could provide EMS personnel additional information to make decisions for mode of transportation.

Hypothesis: Estimations of time to trauma center from the scene using internet mapping technology are similar to that of time from notification to arrival at the trauma center for HEMS.

Methods: A trauma registry at a level 1 trauma center was reviewed for all patients admitted via HEMS from a trauma scene from 2007 to 2009. There were 329 HEMS transportations and of those 210 had complete information available. HEMS notification time to arrival at hospital time was calculated in each case (NAT). Using www.google.com/maps, estimated time by ground (GT) was obtained for each transport using the scene location and the trauma center location. Injury severity score (ISS) and mortality were also recorded.

Results: The GT and the NAT times were compared at straight distances of <15 and ≥15 miles, <20 and ≥20 miles, and <25 and ≥25 miles. ISS and mortality were compared at those same distances. GT was significantly shorter than NAT in the <15, <20, and <25 miles (p<0.001), but not at ≥15, ≥20, and ≥25 miles. There were no differences in ISS and mortality at each distance.

Conclusions: Utilization of internet mapping technology can aid in the correct application of HEMS. Use of ground EMS to transport trauma patients should be the first consideration especially within a 25 mile radius from a trauma center. Prospective studies and application of additional mapping technology needs to be investigated.

Limitations of this study include variables such as reasons for HEMS activation (limited EMS units, advanced procedures, prolonged extrication, and traffic conditions). Further research should explore decision making in utilization in HEMS.

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Cisplatin Treatment Enhancement with Acetaminophen in a Model of Human Medulloblastoma in Nude Rats

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Purpose: To determine the efficacy of cisplatin treatment when enhanced by high dose acetaminophen (APAP) and followed by N-acetylcysteine (NAC) rescue in a nude rat model of medulloblastoma.

Methods: Human medulloblastoma (DAOY) cells were inoculated into the cerebellum of female athymic nude rats (nu/nu). On day 10, rats were randomized to treatment groups (n = 6 per group): (1) saline controls; (2) IP cisplatin 2 mg/kg; (3) PO APAP 600 mg/kg + IP cisplatin; and (4) APAP + cisplatin + NAC 1 g/kg. All treatments were given twice a
week for two weeks. Cisplatin treatment was given one hour after APAP, and NAC was given 4 hours following cisplatin. T2 and contrast-enhanced T1 magnetic resonance (MR) imaging sequences were performed prior to treatment and just prior to sacrifice at 14 days following initial treatment. Cerebrospinal fluid (CSF) was collected for polymerase chain reaction (PCR) assay of human DNA. In vitro cell sensitivity to a dose escalation of APAP and cisplatin was assessed in medulloblastoma cell lines DAOY and D238-MED.

**Results:** In vitro viability assays showed that combination APAP plus cisplatin induced synergistic toxicity in human medulloblastoma cells. In saline control rats, the tumor xenograft localized within the cerebellum with spread to the meningeal surfaces and brainstem, and had a volume of 27.1 ±6.7mm³ (mean±SEM). All cisplatin therapy treatment groups showed a significant reduction in final tumor volume when compared to the control group (P < 0.0004). A trend for the cisplatin single therapy group to be less efficacious when compared to the combined cisplatin treatment groups was observed however, the difference was not significant. No reduction in efficacy was seen in the NAC rescue group, with a tumor volume of 4.42±1.36 mm³, compared to 3.30±0.99 mm³ in the APAP plus cisplatin group. Tumor response was not measurable by MR, however the presence of human DNA in the CSF, was correlated with tumor size.

**Conclusions:** This treatment model for medulloblastoma may provide a mechanism to improve chemotherapy efficacy without increasing chemotherapy dose. In vivo and in vitro data correlate to show that the pretreatment with APAP sensitizes cells to cisplatin allowing efficacy at a lower doses, meanwhile delayed treatment with NAC staves off the systemic toxicity normally associated with cisplatin treatments. APAP enhancement with NAC rescue may provide a less toxic option to patients who might otherwise experience systemic toxicity from high doses of cisplatin.

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**The Identification of Genes Regulated by Paralogous Spx Proteins in Bacillus anthracis**

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Bacteria must quickly adapt to environmental conditions that lead to changes in the internal redox state of the cell. Thiol-based, redox-sensitive proteins provide one mechanism that has evolved in bacteria to monitor and maintain the intracellular redox environment. Spx of *Bacillus subtilis* is a redox-sensitive protein which, under disulfide stress, interacts with the C-terminal domain of the RNA polymerase α subunit to activate genes required for maintaining thiol homeostasis. Spx activity is controlled by a thiol/disulfide switch, and its concentration is regulated proteolytically by the ATP-dependent protease, ClpXP. Spx orthologs are highly conserved among low %GC Gram-positive bacteria, and often exist in multiple paralogous forms within species. It remains unclear how multiple Spx proteins participate in transcriptional regulation. One hypothesis is that paralogous Spx proteins confer greater promoter specificity when they simultaneously interact with RNA polymerase to exert transcriptional control. In this study, we used *B. anthracis* Sterne, which harbors two paralogous *spx* genes, *spxA1* and *spxA2*, to study Spx-dependent promoter specificity. *B. anthracis* strains expressing *spxA1*<sup>00</sup> or *spxA2*<sup>00</sup> alleles encoding stable, protease resistant products were constructed. RNA was harvested from wild-type and *SpxA1*<sup>00</sup>- and *SpxA2*<sup>00</sup>-producing strains for use in microarray and RT-PCR analyses in order to uncover genes under *SpxA1*, *SpxA2*, or *SpxA1/SpxA2* control. Comparison of transcriptomes identified many genes that were upregulated when either *SpxA1*<sup>00</sup> or *SpxA2*<sup>00</sup> were produced, but several genes were uncovered whose transcript levels increased in only one of the two *SpxA*<sup>00</sup>-expression strains, suggesting that each Spx paralog governs a unique regulon. Among genes that were upregulated were those encoding orthologs of proteins that are specifically involved in maintaining intracellular thiol homeostasis or alleviating oxidative stress. Some of these genes have important roles in *B. anthracis* pathogenesis, and a large number of upregulated hypothetical genes have no homology.
outside of the *B. cereus/thuringiensis* group. These and future results will be important in uncovering activator-mediated transcriptional control during the stress response in bacteria that harbor Spx paralogs.

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**Effects of common groundwater constituents on coupled Mn(II)/U(IV) oxidation by Bacillus sp. SG-1**

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Bioreduction of U(VI) to U(IV) is being considered an attractive approach in remediation of U in the subsurface as it transforms U into a less mobile form. The product of U(VI) bioreduction was considered to be crystalline uraninite (UO2). But recent studies have shown that other non-UO2 phases such as monomeric U(IV), which is presumed to be more labile compared to UO2, can also be formed depending on the groundwater conditions. Manganese oxides (MnO2) can be produced by bacteria even under low oxygen concentrations and jeopardize the stability of U(IV) as MnO2 are known to be strong oxidants. Once U(VI) is formed due to oxidation, it can be adsorbed to the surface of MnO2 such that its transport in the subsurface will be retarded. As the presence of MnO2 can greatly impact the fate and transport of U in the subsurface, it is imperative to understand the effects a variety of constituents commonly found in the groundwater may have on the interaction between biogeochemical cycling of Mn and U.

In this study, the effects of common groundwater constituents, O2 (0-5 %), Ca2+ (5 mM), and HCO3− (1 mM), on the coupled Mn/U oxidation processes were investigated. A model Mn(II)-oxidizing microorganism, Bacillus sp. SG-1 spores, was incubated with Mn(II) in the presence and absence of the above solutes and U(IV) as either biogenic UO2 or monomeric U(IV). As expected, increasing levels of O2 resulted in stimulation of Mn(II) and U(IV) oxidation. Although addition of Ca2+ stimulated Mn(II) oxidation, UO2 was less oxidized. Addition of HCO3− had no apparent effect of Mn(II) oxidation but resulted in higher release of U(VI) from UO2. Interestingly, oxidation of monomeric U(IV) appeared to be dictated by the level of O2 at higher levels (1 and 5 %) while at lower levels (0.1 and 0.5 %) impact of Mn(II) oxidation on U(IV) oxidation became significant. Also, effects of Ca2+ and HCO3− observed in the presence of UO2 was not present for monomeric U(IV).

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**Barriers to Retrieving Patient Information from Electronic Health Record Data: Failure Analysis from the TREC Medical Records Track**

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**Objective:** Secondary use of electronic health record (EHR) data relies on the ability to retrieve accurate and complete information about desired patient populations. The Text Retrieval Conference (TREC) 2011 MedicalRecords Track was a challenge evaluation allowing comparison of systems and algorithms to retrieve patients eligible for clinical studies from a corpus of de-identified medical records, grouped by patient visit (or encounter). Participants retrieved cohorts of
patients relevant to 35 different clinical topics, and visits were judged for relevance to each topic. This study identified the most common barriers to identifying specific clinic populations in the test collection.

**Methods:** Using the runs from track participants and judged visits, we analyzed the five nonrelevant visits most often retrieved by submitted systems and the five relevant visits most often overlooked. Categories were developed iteratively to group the reasons for incorrect retrieval for each of the 35 topics.

**Results:** Reasons fell into nine categories for non-relevant visits and five categories for relevant visits. Non-relevant visits were most often retrieved because they contained a non-relevant reference to the topic terms. Relevant visits were most often infrequently retrieved because they used a synonym for a topic term. There were also visits judged by the relevance assessors that our review believed incorrect.

**Conclusions:** This failure analysis provides insight into areas for future improvement in EHR-based retrieval with techniques such as more widespread and complete use of standardized terminology in retrieval and data entry systems.

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**Chronic Pain Treatment and Health Service Utilization of Veterans with Hepatitis C Virus Infection**

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**Objectives:** Hepatitis C virus (HCV) infection is estimated to affect 2% of the general U.S. population and chronic pain is a common comorbidity among persons with HCV. The primary purpose of this study was to compare health service utilization of U.S. military veterans with HCV with and without the presence of comorbid chronic pain.

**Design:** Cross-sectional study with retrospective review of patient medical records.

**Patients:** One hundred seventy-one U.S. military veterans with confirmed HCV, recruited through a U.S. Veterans Administration hospital.

**Outcome Measures:** Medical service utilization data from the past five years were extracted from participants’ electronic medical records.

**Results:** Sixty-four percent of veterans with HCV (n = 110) had chronic pain. Veterans with HCV and chronic pain utilized more health services including inpatient general medicine (OR = 2.41 [1.36, 4.27]) and psychiatric services (OR = 4.63 [2.98, 7.21]), compared to participants with HCV but no chronic pain, after statistically adjusting for demographic, psychiatric, and substance use covariates. In addition, those with HCV and chronic pain had more than twice as many total outpatient visits with primary care providers (OR = 2.10 [1.46, 3.03]). Both pharmacological and non-pharmacological treatments for pain were very acceptable to patients with comorbid HCV and chronic pain.

**Conclusions:** Patients with HCV and chronic pain utilize medical services to a greater extent than patients with HCV but no chronic pain. Future studies that examine the efficacy of both pharmacological and nonpharmacological pain treatment for patients with comorbid HCV and chronic pain appear warranted.
Interdisciplinary collaborative research by the Physician Order Entry Team (POET) of the Department of Medical informatics and Clinical Epidemiology (DMICE) at Oregon Health & Science University

POET Members:
Dean. F. Sittig, Ph.D., (University of Texas)
Adam Wright, Ph.D., (Harvard Medical School)
Carmit McMullen, Ph.D., (Kaiser Center for Health Research)

POET Consultants:
Jim Carpenter, R.Ph., M.S., Kenneth Guappone, M.D., Ph.D., (Providence Health Systems)

This presentation describes the research performed by the Physician Order Entry Team (POET) of the Department of Medical informatics and Clinical Epidemiology (DMICE) at Oregon Health & Science University.

Funded by a grant from the National Library of Medicine, POET first studied factors that contributed to the successful implementation of computerized physician order entry (CPOE), and examined unintended consequences associated with CPOE implementation. POET went on to define best practices for clinical decision support, and studied barriers and facilitators towards successful CDS implementation in community inpatient and outpatient settings.

POET members also play a key role in clinical decision support research as co-investigators of the Clinical Decision Support Consortium project funded by the Agency for Healthcare Research and Quality (AHRQ) led by researchers at Brigham and Women’s Hospital, Harvard Medical School, and Partners HealthCare Information Systems.

POET has more recently turned its attention to studying health information technology (HIT) safety.

POET authored a white paper commissioned by the Institute of Medicine as background for its November, 2011 report Health IT and Patient Safety. Currently, POET members are focusing on improving the safety of electronic health records as participants in a project sponsored by the Office of National Coordinator for Health IT (ONC) - the Safety Assurance Factors for EHR Resilience (SAFER) project, in collaboration with the University of Texas and Baylor University.

POET developed an innovative research methodology in the form of a Rapid Assessment Process (RAP) for clinical informatics interventions, which utilizes multiple qualitative and quantitative techniques to gather data expeditiously. Current POET members represent a truly collaborative spectrum of disciplines, including informaticians, social scientists, clinical anthropologists, a physician, a nurse, and a pharmacist.

POET has won the prestigious Diana Forsythe Award sponsored by the American Medical Informatics Association for their research, as well as several best paper awards at national and international conferences.

Iron & Manganese Depositing Cold-Seeps: Mineral Formation Along A Freshwater To Marine Ecosystem At Soda Bay, Alaska

Wendy F. Smythe¹², Melanie Kadake¹, Sean McAllister³, Richard Davis¹, Craig Moyer³ & Bradley Tebo².
Soda Bay is a pristine low-temperature iron and manganese-depositing ecosystem in Southeast Alaska. Groundwater fluids, supersaturated with dissolved minerals, are transported through fissures of a massive limestone deposit. These fluids are discharged from cold-seeps at the surface forming large mounds. X-ray absorption near edge structure (XANES) mapping shows that seeps are dynamic carbonate rich environments where both reduced and oxidized forms of iron and manganese minerals co-exist. Inductively coupled plasma (ICP) analysis indicates that seep fluids are enriched in both CO₂ and high concentrations of dissolved Fe (> 1mM) and Mn (> 100µM). These reduced metals may provide fuel for metal-oxidizing chemolithoautotrophic microbes. As fluids flow from their sources CO₂ is out gassed, pH rises and metal oxidation becomes more thermodynamically favorable, increasing the potential for abiotic oxidation, making it more difficult for microbes to compete with chemical reactions for chemical energy. Preliminary evidence from molecular analyses indicates an abundance of metal-oxidizing microbes and the potential for chemolithoautotrophy within microbial communities. Ultra high-resolution scanning electron microscopy (UHR-SEM) shows close microbe-mineral associations, and suggests microbial dissolution of carbonate minerals. Our goal is to better understand the diversity of metal oxidizers, their contribution to overall mineral formation, geochemical controls dictating why Fe-oxides dominate in the high flow system while Mn-oxides dominate in the low flow system.

A Basic Amino Acid-Rich Nucleolar Localization Signal Within the AAV2 Assembly-Activating Protein Constrains the Structural Diversity of the AAV2 Capsid

Yasuhiro Kawano¹²*, Kei Adachi¹, Shane Neeley¹, Mushui Dai¹, Xiao-Xin Sun¹ and Hiroyuki Nakai¹

Abstract withheld by author’s request.

Mutagenesis of Aflatoxin B₁-DNA adducts in mammalian cells

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Aflatoxin B₁ (AFB₁) exposure via ingestion of food contaminated by the fungus Aspergillus gives rise to hepatocellular carcinoma (HCC) in humans. Upon metabolic activation, the intermediate, AFB₁-8,9-epoxide, can damage DNA by covalent conjugation to the N7 atom of deoxyguanosine via nucleophilic attack. DNA damage has been linked to many human diseases including cancer. Replication of unrepaired DNA lesions may lead to mutations, and mutagenesis plays a critical role in cancer development. Here, we tested the mutagenic potential of two major DNA adducts of AFB₁, 8,9-dihydro-8-(N7-guanyl)-9-hydroxyaflatoxin B₁ (AFB₁-N7-Gua) and AFB₁-formamidopyrimidine (AFB₁-FAPY) using a single-stranded shuttle vector DNA containing a site-specific adduct. Both
AFB$_1$-N7-Gua and AFB$_1$-FAPY adducts were highly mutagenic when vectors were replicated in green monkey kidney COS-7 cells and the mutation activity of AFB1-FAPY was twice as much as that of AFB$_1$- N7-Gua. The predominant mutations were G->T transversions, which is consistent with observations of hepatocellular carcinoma patients with AFB$_1$ exposure. Moreover, we demonstrated that the translesion synthesis (TLS) polymerases κ could bypass these AFB$_1$ DNA adducts in vitro, with a preference for insertion of an A opposite the lesions. Overall, these findings suggested a molecular mechanism of AFB$_1$ mutagenicity that may contribute to its carcinogenesis.

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**Validating Therapeutic siRNA against HER2 in Breast Cancer Cell**

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Over-expression of oncogene HER2 is found in 20-30% primary breast cancers, and is involved in tumor initiation and progression. Breast cancer patients with over-expressed HER2(HER2 positive) show poor clinical prognosis. Increasing evidence has indicated that the majority of HER2 positive patients do not respond well to anti-HER2 drugs Trastuzumab (Herceptin) and Lapatinib. Even those who show initial response ultimately become resistant to the treatment. The mechanism of resistance includes the mutation of HER2 gene and the masking binding epitope of Her2 protein. The discovery of small interfering RNA (siRNA) raises the possibility to explore new approaches for cancer therapy. To develop a high efficacy siRNA against HER2 that could be used with nanoparticle delivery into human breast cancer cells , we designed and synthesized seventy-six siRNA sequences targeting the coding region of human HER2 gene . We evaluated the potency of these siHER2 and scramble control siRNAs in three HER2+ breast cancer cells (BT474, SkBr3 and HCC1954). Ten siRNA sequences with greater than 50% knockdown efficiency plus 40% inhibition of cell viability were selected for further evaluation. The growth inhibitory effect of the top 5 siHER2 sequences was measured in 20 HER2+ cell lines. The dose response data showed that most of cell lines resistant to Trastuzumab were still sensitive to siRNA mediated HER2 inhibition. Moreover, nanoparticle delivery of the siRNA resulted in successful HER2 silencing. Therefore, our data indicates that RNA interference of HER2 can potentially be used to overcome resistance of HER2 drugs, making it an alternative cancer therapeutic to HER2 positive patients. A mouse xenograft study of the in vivo delivery of the optimized siHER2 using nanoparticles is under evaluation.
Abby Rynko | Etanercept Blocks Parainfluenza Downregulation of M2 Muscarinic Receptor mRNA in Parasympathetic Nerves In Vivo

Alexandre Colville | c-Fos Induction Associated With Ethanol Withdrawal In Chromosome 1 Congenic and GIRK3 Knockout Mice.

Angela Senders | Mindfulness in Multiple Sclerosis: A Cross-sectional Survey Study

Annika Giesbrecht | Behavioral Risk Factors and Recent Colorectal Cancer Screening Among American Indian and Alaska Native People in the Pacific Northwest: Tribe A BRFSS Project 2009-2010

Ashley Franklin | Implications of Process-Focused Learning Activities for Care of Multiple Patients

Ben Kong | Valganciclovir Pharmacokinetics and Area-Under-the-Curve Profiling in Pediatric Kidney Transplant

Binglin Li | Physical and biological controls on Mesodinium rubrum blooms in the lower Columbia River estuary

Chiemi Tanaka and Katie Loera | Residual Hearing in a Guinea Pig Model of Hybrid Cochlear Implants

Christine Nelson | Facilitators of FQHCs' Participation in Practice Based Research: A Qualitative Study

Cong-Qiu Chu | NLRP3 Gene Analysis for Patients with Schnitzler's Syndrome

Damian Zuloaga | The effects of neonatal methamphetamine exposure on expression of hypothalamic pituitary adrenal axis-associated proteins in adulthood

Daniel Mick | Measuring and Promoting Social Connectedness in Older Adults: Development of the Social Footprint Model

Emily Quinn | Evaluating Communication Skills of Children with Low- Incidence Disabilities

Farees Tavangari | Effects of Ulnar Shortening on Wrist Pressure and Dynamic Work of Rotation: A Cadaveric Biomechanical Model

Glenn Kautz | Rural-Urban Differences in Access, Utilization and Time Spent

Gregory Scott | Epithelial Sensory Hyperinnervation And Increased Vagally-Mediated Airway Resistance In A Mouse Model Of Eosinophilic Asthma

Ian McClellan | Efficacy and Safety of Daptomycin in the Treatment of Gram-Positive Infections in Patients with Renal Impairment
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Natalie Vuylsteke | Assessing the Impact of a Pilot Antimicrobial Stewardship Program in Pediatrics at an Academic Medical Center

Natasha Chattergoon | Fetal Myocardial Thyroid Hormone Deiodinases are Regulated by Fetal T3
Etanercept Blocks Parainfluenza Downregulation of M2 Muscarinic Receptor mRNA in Parasympathetic Nerves In Vivo

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Rationale: The majority of asthma exacerbations in children and adults are associated with viral infection. Virus-induced airway hyperreactivity is mediated by loss of neuronal M2 muscarinic receptors that normally function to limit acetylcholine release and inhibit vagally mediated reflex bronchoconstriction. In vivo and in isolated nerve cells, blocking tumor necrosis factor alpha (TNF-α) with Etanercept (a TNF-α receptor IgG fusion protein) protects M2 receptor function and prevents virus-induced hyperreactivity. In cell culture, M2 receptor gene expression is decreased by direct viral...
infection of airway nerves or with addition of TNF-α alone. Here we test whether viral infection of airway nerves, or TNF-α is the in vivo mechanism of virus-induced decrease in M2 receptor expression and resulting airway hyperreactivity.

**Methods:** Guinea pigs were infected with parainfluenza (Sendai) virus. Controls received uninfected media. In some animals, TNF-α was blocked in vivo with Etanercept 24 hours before virus infection. Four days after infection lungs were harvested for viral titers. In addition, guinea pig parasympathetic ganglia were harvested. The tracheas were removed, opened longitudinally and pinned lumen side down. A 0.05% neutral red solution was applied for 15 minutes to stain the ganglia. Using a dissecting microscope, smooth muscle was pulled away from ganglia and each process of the ganglia was severed with tweezers. Ganglia were removed and cleaned in a separate dish to remove any additional non-neuronal tissue. Viral titers for lung and ganglia were determined using RNA from tissue homogenates and parasympathetic ganglia by quantitative real-time RT-PCR (qRT-PCR). M2 receptor gene expression levels were normalized to 18s and a pan-neuronal marker, PGP9.5, was used to verify nerve cell content.

**Results:** Guinea pig parasympathetic ganglia showed a 3.5 fold decrease in M2 receptor mRNA following virus infection. Pre-treating with Etanercept blocked this decrease in expression. Guinea pig lung viral titers were positive for Sendai virus demonstrating the lungs were infected, and the lung viral titers were not affected by Etanercept. No virus message could be detected in parasympathetic ganglia by qRT-PCR.

**Conclusion:** Parainfluenza (Sendai) downregulates M2 muscarinic mRNA in parasympathetic ganglia in vivo. This downregulation is induced by TNF-α, as it is blocked by Etanercept. There is no direct infection of airway parasympathetic nerves. These results show that TNF-α is a key mediator of M2 receptor dysfunction during infection in vivo.


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**c-Fos Induction Associated With Ethanol Withdrawal In Chromosome 1 Congenic and GIRK3 Knockout Mice**

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Our goal is to dissect the neural and molecular substrates by which quantitative trait loci (QTLs) influence ethanol dependence and associated withdrawal. Using mapping populations derived from DBA/2J (severe EtOH withdrawal phenotype) and C57BL/6J (mild EtOH withdrawal phenotype) mice, we previously identified QTLs on distal Chromosome 1 with large effects on chronic and acute ethanol withdrawal. We created a congenic strain with this segment of Chr 1 from the B6 strain superimposed on a genetic background that is >98% from the D2 strain. Genetic noise from the remainder of the genome is nearly eliminated, which allows for comparisons of the neural circuitry between the congenic and background strains. Fine-mapping to a 0.44 Mb interval and detailed molecular analyses of the genes within this interval identified Kcnj9 (which encodes GIRK3, a subunit of G-protein-dependent inwardly-rectifying K+ channels) as a high-quality candidate gene for QTLs affecting withdrawal from ethanol, zolpidem and pentobarbital (J Neurosci 29:11662, 2009). c-Fos expression was used as a marker for neuronal activation to compare congenic and background strain mice at peak ethanol withdrawal and control animals administered saline. Our results revealed significant strain x treatment interactions (p<0.05)in the prelimbic and cingulate cortices, amygdala, nucleus accumbens shell, and substantia nigra pars reticulata. In addition, trends (p=~0.1) were seen in the nucleus accumbens core and
ventral pallidum. Preliminary data using GIRK3 knockout mice also indicate less ethanol withdrawal associated activation compared to wildtype mice in regions that include the prelimbic cortex and amygdala. We conclude that Chr 1 QTL effects on withdrawal may involve the amygdala and prelimbic cortex, where Kcnj9 mRNA and GIRK3 protein are abundant. Future studies will test the role of the neural and genetic targets identified in ethanol physiological dependence and associated withdrawal.

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**Mindfulness in Multiple Sclerosis: A Cross-Sectional Survey**

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**Objective:** To date there have been no studies evaluating stress and mindfulness in multiple sclerosis (MS). Our primary objective is to evaluate the association between mindfulness and perceived stress, coping, and resilience in MS and healthy controls. We will also analyze the correlation between mindfulness and depression and anxiety. Our secondary objective is to assess serum biomarkers of stress as an objective measure of mindfulness.

**Background:** MS patients report higher levels of perceived stress than healthy controls, independent of physical disability. Stress in MS is associated with increased relapse rate and lesion burden on MRI. Mindfulness-based interventions have been shown to reduce perceived stress and improve quality of life across a variety of patient populations. This is the first study to evaluate the relationship between mindfulness, perceived stress, psychosocial mediators of stress (e.g. coping, resilience, depression, anxiety), and objective serum biomarkers in MS. Data gathered in this study will help us to better understand how mindfulness may be applied as a therapy for MS in future trials.

**Design/Methods:** We are conducting a cross-sectional study. One hundred fifty people with MS and 100 healthy controls will be enrolled between December 2011 and October 2012. MS subjects meet the following: 18 – 90 years, definite diagnosis of MS (McDonald criteria), no relapse within 3 months prior to study visit. Healthy controls are matched to age (within 5 years), sex, and race. Subjects complete a battery of surveys and one blood draw during one study visit to OHSU. Primary outcome measures include: Perceived Stress Scale, Social Readjustment Rating Scale, Five-Facet Mindfulness Questionnaire, Brief Coping Orientation for Problem Experiences, and Connor-Davidson Resilience Scale. Secondary outcomes include: MS Quality of Life Inventory, Beck Depression Inventory, State Trait Anxiety Inventory, Patient Reported Outcomes Measurement System (PROMIS) surveys, and serum biomarkers of stress: Brain-Derived Neurotrophic Factor (BDNF), Tumor Necrosis Factor – alpha (TNF-α); Interleukins (IL) 1β, 6, 10, 17, and 22; C-X-C motif chemokine 10 (CXCL 10); Intercellular Adhesion Molecule 1 (ICAM-1), Vascular Cell Adhesion Molecule 1 (VCAM-1) and Neural Cell Adhesion Molecule (NCAM). Bivariate correlations will be used to examine the associations between mindfulness and outcomes. Significance of associations will include p< 0.05. Analysis will be adjusted for age, gender, type of MS, stressful life-events, self-reported disease severity, and MS disease modifying medication use (yes or no). Resilience, coping strategies, and the association between stressful life events and perceived stress for MS and controls will be compared using a t-test.

**Preliminary results:** This study is still enrolling subjects. Preliminary results (n=37 MS, n=0 controls) indicate that mindfulness is negatively associated with perceived stress (r= -0.54, p=.001), depression (r= -0.53, p<.001), and trait anxiety (r= -0.69, p<.001), and positively associated with resilience (r= 0.63, p<.001) in people with MS. There is a significant negative correlation between mindfulness and avoidant coping strategies (r= -.50, p=.002), but no
relationship between level of mindfulness and emotion-based or problem-focused coping strategies ($r=0.21$, $p=.208$; $r=0.21$, $p=.207$, respectively).

After accounting for age, gender, disability status, and disease modifying therapy use (yes or no), mindfulness is significantly associated with perceived stress ($t_{35} = -3.78$, $p = 0.001$) in MS and accounts for 54% of the variation in perceived stress scores.

**Conclusions:** Preliminary evidence suggests that people with MS who report higher levels of mindfulness report lower levels of perceived stress. Higher reported levels of mindfulness are also associated with lower depression and anxiety scores, and lower levels of mindfulness are associated with less constructive coping strategies. In addition, people who are more mindful in their daily lives are more resilient to stressful circumstances. Causal relationships cannot be inferred from this cross-sectional study, yet the data suggest that mindfulness training may be an appropriate way to shift people with MS from avoidant to more constructive coping strategies, enhance psychosocial wellbeing and strengthen resilience to adverse circumstances in MS.

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**Behavioral Risk Factors and Recent Colorectal Cancer Screening Among American Indian and Alaska Native People in the Pacific Northwest: Tribe ‘A’ BRFSS Project 2009-2010**

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American Indian and Alaska Native (AI/AN) people living in the Pacific Northwest (PNW) have a higher incidence of colorectal cancer (CRC) and higher 1- and 5-year mortality after colorectal cancer-related diagnosis compared to the Non-Hispanic White (NHW) population. Colorectal cancer screening is a cost effective service that has been proven to reduce incidence and mortality. National data indicate low prevalence of colorectal cancer screening in the AI/AN population. We know of no study that has been conducted to determine the prevalence of recent colorectal cancer screening and prevalence of behavioral risk factors associated with colorectal cancer among AI/AN people in the PNW. Using data collected from a Northwest Tribe Behavioral Risk Factor Surveillance System (BRFSS) survey project, 2009-2010, this study will (1) quantify the prevalence of behavioral risk factors associated with colorectal cancer among Tribe A’s members age 18 and older compared to the NHW population in Washington State, (2) quantify the prevalence of recent colorectal cancer screening among Tribe A members age 50 and older compared to the NHW population in Washington State, and (3) identify key variables associated with recent colorectal cancer screening using logistic regression modeling. We hope this study will elucidate factors contributing to increased colorectal cancer incidence and mortality in AI/AN people living in the PNW. Founded upon community-based participatory research principles, the information gathered in this study will be used to guide colorectal cancer education initiatives and cancer prevention programs tailored to the needs of the Tribe A population.

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**Implications of Process-Focused Learning Activities for Care of Multiple Patients**

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**Background.** Process-focused learning activity intervention developed Summer 2011 for six senior pre-licensure students who needed additional clinical elective. Clinical group included two students who missed med-surg clinical experience for high-acuity patients during previous semester, two students with past clinical failures, and two International students who requested clinical elective course to provide additional opportunities for hands-on patient care.

**Methods.** Clinical objectives centered on care processes such as taking/giving shift report, implementing safety checks, performing focused physical assessments, and medication administration. Intervention implemented during 10 week clinical course helped prepare students for capstone clinical experience and providing care to multiple med-surg patients during 12 hours shifts.

**Findings.** Interventions increased learner confidence in providing care to multiple patients. Intervention provided more opportunities for learners to administer medications and practice psychomotor skills. At the end of the course, learners demonstrated ability to transfer knowledge and provide care for multiple simulated patients.

**Conclusions/Implications.** Use of process-focused learning activities increased learners’ clinical judgment as measured by self-report and Lasater Clinical Judgment Rubric. All of the students’ psychomotor and communication skills increased as a result of process-focused learning intervention. While small clinical group size may not be practical use of resources in large programs, the design used with this intervention provided measurable benefit to learners who would be considered high-risk for not being successful in capstone clinical course or on NCLEX-RN.

**Valganciclovir Pharmacokinetics and Area-Under-the-Curve Profiling in Pediatric Kidney Transplant Recipients**

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**Introduction** Valganciclovir (Valcyte™) is an antiviral medication FDA approved on August 31, 2009 for the prevention of CMV infection in pediatric transplant patients. Despite 20 years of extensive clinical use, there remains debate about the most effective means of dosage calculations for valganciclovir in pediatric patients.

**Objective(s)** To: (1) investigate oral doses of valganciclovir administered to pediatric transplant patients, (2) calculate pediatric valganciclovir dosages based on body weight and the manufacturer’s dosing equation, and compare calculated doses to prescribed doses, (3) relate valganciclovir doses to patient outcomes post-transplantation, (4) determine valganciclovir AUCs from three different dosing methods, and (5) relate AUCs to patient outcomes post-transplantation.

**Methods** An open-label, single-center, retrospective chart review at OHSU and Doernbecher Children's Hospital. Our inclusion criteria included pediatric kidney transplant patients who required CMV prophylaxis with oral valganciclovir between 2006 to 2010. There were no exclusion criteria.

**Results** Use of the manufacturer’s calculated daily doses would have resulted in doses much higher than the actual prescribed doses and weight-based doses in a majority of patients. Eight patients developed leukopenia during valganciclovir therapy. No patients developed nephrotoxicity, anemia or thrombocytopenia drug valganciclovir therapy.
Conclusions  When all three calculated methods were compared, the weight-based method was found to achieve prophylaxis against CMV in a majority of patients with minimal episodes of leukopenia. We recommend the weight-based method of 18 mg/kg/day with adjustment for creatinine clearance for determining oral valganciclovir doses.

Physical and biological controls on Mesodinium rubrum blooms in the lower Columbia River estuary

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Annually recurring blooms of the photosynthetic ciliate Mesodinium rubrum have been observed in the Columbia River estuary (CRE) for several decades. To date, the factors influencing the timing and magnitude of these blooms have not been identified. We examined the temporal relationship between coastal upwelling and M. rubrum blooms. Estuary blooms were preceded by strong upwelling in mid-late summer when river flow was reduced. Weekly spatial surveys conducted in 2011 indicated movement from retentive lateral bays out into the estuary main channels. The initiation of strong, sustained coastal upwelling coincided with the timing of bloom initiation within lateral bays during 2007-2011. Microscopic and 18S rRNA gene sequence analyses showed that free-living Teleaulax amphioxeia, the cryptophyte associated with M. rubrum, was only present prior to bloom formation within lateral bays. Our study suggests a potential interaction between upwelling and prey population dynamics in M. rubrum bloom phenology. Upwelling events, which supply nutrient rich water into the lower CRE, may therefore support M. rubrum/T. amphioxeia blooms. Strong upwelling events may therefore be used to forecast M. rubrum blooms in the CRE.

Residual Hearing in a Guinea Pig Model of Hybrid Cochlear Implants

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Background: A cochlear implant (CI) is a surgically implanted hearing device that provides hearing to people with hearing loss (HL) or deafness by directly stimulating auditory nerve with electrical pulses delivered to an implanted electrode array in the inner ear. A hybrid CI, also known as Electric Acoustic Stimulation, is a new type of CI that enables patients to use their residual low-frequency hearing in addition to electric hearing from the CI. However, ~30% of these CI patients develop >30 dB of mean low frequency hearing threshold shifts postoperatively (Gantz et al., 2010). Interestingly, low-frequency HL occurred at different time points ranging from between surgery and CI activation to within 3-36 months after CI activation. The cause of the residual hearing loss in hybrid CI patients is not well understood.

Rational: The delayed time course of the HL suggests that in addition to surgical trauma, electrical stimulation through the CIs may also contribute to the loss of residual hearing in these populations. As part of a preliminary study
investigating the causes of the residual HL, we measured changes in auditory function in a guinea pig model of the hybrid CIs with and without electrical stimulation in correlation with cochlear pathology.

Methods: Eight 2-month-old normal-hearing guinea pigs were implanted with an 8-ring animal electrode (Cochlear Limited, Australia) in the left cochlea via a cochleostomy. These animals were divided into three groups: 1) Non-implanted Chronic Acoustic Stimulation control (CAS, n=2); 2) Implanted Chronic Acoustic + Electric Stimulation (CAES, n=3); 3) Implanted No Stimulation (NS, n=3). Auditory brainstem responses (ABRs) at 1, 2, 6, and 16 kHz and electrically-evoked ABRs (EABRs) were recorded biweekly to monitor changes in acoustic and electric hearing, respectively. In the CAES animals, hybrid CIs were activated 5 weeks after surgery followed by sound stimulation 3 hours/day, 5 days/week for 10 weeks with modulated white noise delivered via a loudspeaker and a Freedom speech processor as applicable. The CAS group received the same acoustic sound stimulation during the same period of time. Histological analyses were performed at the end of the study to quantify hair cell loss, vascular density in stria vascularis (SV), spiral ganglion neuron density, degree of ossification at cochleostomy site, and amount of fibrosis in the scala tympani.

Results: Three animals developed HL at 6 and 16 kHz, closer to the electrode implantation sites, after the surgery. These postoperative ABR threshold shifts were associated with higher EABR thresholds, fibrosis, elevated impedance, ossification, and reduced vascular density. The CAES group showed statistically significant increases in the ABR threshold shifts at 1 kHz due to sound stimulation. However, no cochlear pathology specific to the CAES group was found to explain the cause of HL at 1 kHz. All implanted animals in the CAES and NS groups showed lower vascular density in the CI electrode region compared to non-implanted guinea pigs, suggesting possible changes in cochlear vasculature after implantation.

Conclusions: We successfully developed an animal model of hybrid cochlear implants, which can be utilized to continue investigating the mechanism of the low-frequency residual HL after hybrid cochlear implantation. Further investigation into SV vascular pathology and molecular analysis may be important to understand the mechanism of the residual HL.

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Facilitators of FQHCs’ Participation in Practice Based Research: A Qualitative Study

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Background: Since federally qualified health centers (FQHC) serve as major healthcare resources for underserved and vulnerable populations, incorporating FQHCs into practice-based research networks could enhance research with health disparity populations. However, it has proved challenging to include FQHCs in practice-based research.

Purpose: This qualitative exploratory study aimed to identify facilitators and barriers to research in FQHCs and factors most important to building successful research partnerships.

Methods: Individual, semi-structured interviews were conducted with senior leaders and managers (n=12) from five different FQHCs in the Western US. A follow-up focus group interview was conducted with direct service staff (n=5) from one of the participating FQHCs. Two investigators independently reviewed transcripts and categorized emergent
themes. Iterative analytic cycles and team discussion yielded consensus on factors to consider when partnering with FQHCs.

**Results:** Seven major themes were identified as important considerations when collaborating with FQHCs:

*Understand the Mission and Culture:* Research must be congruent with FQHCs’ core mission and with the specific culture of participating organizations;

*Collaborate:* Research of greatest value to FQHCs capitalizes on their unique models of care, their innovative interventions, and the practice adaptations they utilize with their populations. This requires extensive collaboration on ideas and strategies;

*Minimize Disruption:* Researchers should demonstrate flexibility in methods, adapt study procedures to accommodate FQHCs’ existing processes, and minimize disruption to clinic “flow;”

*Bring Value:* Researchers must recognize the revenue impact of FQHC participation and compensate accordingly, both financially and with non-monetary rewards;

*Invest in Champions:* Researchers need to identify, utilize, and fund champions within FQHCs to promote and manage the work;

*Communicate:* Ongoing communication and a spirit of true partnership throughout the research project are critical;

*Establish Trust:* Researchers should build and maintain trust relationships with FQHCs and assure that the study does not negatively affect trust between clinicians and patients.

**Implications:** The themes identified in this study provide guidance for developing and implementing effective approaches and collaborative strategies to facilitate FQHCs participation in research. They can be applied more broadly to all practice-based network research partners.

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**RDRC: NLRP3 Gene Analysis for Patients with Schnitzler's Syndrome**

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**Purpose:**
Schnitzler's syndrome is characterized by chronic urticaria, intermittent fever, arthralgia, bone pain, gammopathy and marked systemic inflammation. The striking response to IL-1 blockade suggests that Schnitzler's syndrome is an IL-1 mediated condition of the expanding spectrum of systemic autoinflammatory disorders. However, the mechanism leading to the increased IL-1 activity has remained elusive. We aim to identify genetic predisposition in patients with Schnitzler's syndrome.

**Methods:**
Genomic DNA was extracted from peripheral blood mononuclear cells from 4 patients with a clinical diagnosis of Schnitzler's syndrome. DNA sequencing was performed in both directions of NLRP3 gene regions, including exon 3 which contains the majority of mutations associated with the cryopyrinopathies. Genetic data of patients was compared with the reference sequence for mutations.
Results:
Of the 4 patients with Schnitzler's syndrome, 2 had classical findings of monoclonal IgM, kappa; one with polyclonal IgA; and one with polyclonal IgG and IgA. All of the 4 patients were refractory to non-biologic immunosuppressive treatment but achieved sustained clinical remission with daily use of anakinra. DNA sequence analysis of NLRP3 revealed a disease-associated mutation encoding the V198M substitution in the patient with polyclonal IgG and IgA. However, this mutation was not found in the other 3 patients. No other disease-associated mutations were identified in NLRP3 for this patient or in the other 3 patients.

Discussion:
Genetic studies of Schnitzler's syndrome have been scanty primarily due to the rarity of the condition and the results have been mixed. Our 4 patients consist of classical (2 cases) and atypical (2 cases) Schnitzler's syndrome. The V198M mutation was found in one with atypical Schnitzler's syndrome (polyclonal IgG and IgA). Interestingly, this mutation was previously reported in one patient with classical Schnitzler's syndrome with monoclonal IgM, kappa (1). However, another genetic study on a patient with classical Schnitzler's syndrome found no mutations in NLRP3 (2). The distinct feature of Schnitzler's syndrome is its gammopathy which is poorly responsive to IL-1 blockade suggesting that IL-1 may not mediate the gammopathy. Complete analysis of NLRP3 exons and their flanking regions in this cohort is underway as disease-associated mutations outside of exon 3 have been reported and there may be as yet unknown mutations involved in this phenotype. The presence of an NLRP3 mutation in another patient with Schnitzler's syndrome confirms the finding that this disorder is inflammasome-mediated.

References:

The effects of neonatal methamphetamine exposure on expression of hypothalamic pituitary adrenal axis-associated proteins in adulthood

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The psychostimulant methamphetamine is abused by approximately 1.2 million individuals over the age of 12 according to a recent survey. Many women that abuse methamphetamine are of childbearing age. As they often use methamphetamine to enhance their sex life, many pregnant women expose their offspring to methamphetamine in utero. Exposure to methamphetamine in utero can lead to long-term behavioral and cognitive deficits by altering the normal trajectory of brain development. One pathway through which methamphetamine can induce detrimental behavioral and cognitive effects is by increasing circulating levels of glucocorticoids. Exposure to high levels of glucocorticoids in early brain development can lead to long-term disruptions in the hypothalamic pituitary adrenal (HPA) axis which plays a critical role in the regulation of stress responses. Furthermore, alterations in proteins associated with the HPA axis, including glucocorticoid receptors and vasopressin, are correlated with disorders of anxiety and depression. Glucocorticoid receptors and vasopressin also play important roles in cognition. Recent studies in our laboratory indicate that methamphetamine exposure also increases glucocorticoid levels in mice during the stress
hypoactive period, a period in which stress hormone responses are naturally blunted possibly as a mechanism to protect developing brain regions from negative effects of glucocorticoid over-exposure. We hypothesize that methamphetamine exposure during the stress hypoactive period results in lasting alterations in the expression of HPA axis-associated proteins and that these effects may contribute to behavioral and cognitive alterations later in life. To test this hypothesis, male and female mice were treated with methamphetamine (1mg/kg daily) from postnatal day 11-20, a period of hippocampal development which occurs in utero in humans during the third trimester. At postnatal day 90, mice were perfused and brains processed for immunohistochemistry and confocal microscopy to detect glucocorticoid receptor and vasopressin levels in brain regions involved in HPA axis regulation (hippocampus and paraventricular nucleus of the hypothalamus). Results of these experiments will be presented and discussed.

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Measuring and Promoting Social Connectedness in Older Adults: Development of the Social Footprint Model

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Objectives. Social isolation is a complex topic with many correlates and has been linked to numerous negative physical and mental health outcomes, especially for older adults. The literature is a mix of definitions and correlates resulting in numerous piecemeal assessment models. My objective is to develop a participant-accessible multivariate data visualization model for assessing the objective social connectedness of social isolation, and which also identifies areas of opportunity for intervention.

Methods. The literature of the last three decades is reviewed to identify scales and models measuring social isolation/connectedness/network/support, then collate items correlated with objective social isolation, related in full/part to older adults, shared with at least one other model, and not impervious to therapeutic intervention. These are then grouped into subcategories to be included in a multivariate star plot.

Results. The “Social Footprint” model is created, a multivariate star plot that assesses social connectedness through 12 types of social interaction: Family, Romantic, Close friends, Neighbors, Organized groups, Business, Service positions, Spirituality, Education, Pets, Hobbies, and Athletic, grouped into four quadrants: Personal, Community, Contribution, and Activity.

Discussion. The Social Footprint model is a unique offering that both measures and promotes social connectedness in older adults with multiple variables. It is a visual model that clearly displays social network strengths and weaknesses and identifies areas for possible intervention.

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Evaluating Communication Skills of Children with Low- Incidence Disabilities

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An online version of the Communication Matrix (www.communicationmatrix.org), a communication skills assessment tool, is available in English and five other languages (Rowland, 2004). This instrument is designed for individuals functioning at the earliest stages of communication and for individuals who use any form of communication, including pre-symbolic and augmentative or alternative (non-speech) forms. Users enter information about an individual’s communication skills, as well as diagnosis and functional impairments. Interactive features make it possible to follow progress and review achievement in depth. The information that users provide is stored in a permanent database that collects extensive new information about the development of communication skills in children with specific disabilities. With approximately 400 new assessments being entered into the database each week, it presently includes over 30,000 assessments that have been entered from 117 countries. Many individuals have been assessed multiple times, providing information about communication skill development over time.

One of the hopes in developing the online version of the Matrix was that data could be collected on the communication skills of children with low-incidence disabilities on whom little data can be aggregated in any one geographical location. This expectation is being realized. For example, the database includes assessments on 270 children with Angelman syndrome (incidence = 1:15,000); 203 children with CHARGE syndrome (incidence = 1:11,000); 188 children with Rett syndrome (incidence = 1:16,000); 73 children with Cornelia de Lange syndrome (incidence: 1: 10,000-30,000); and 32 children with Wolf Hirschhorn syndrome (incidence = 1:50,000). Deafblindness is a low-incidence disability that is associated with many different etiologies. The database includes assessments on 1,270 children ages 0 to 21 years of age with a primary diagnosis of deafblindness who live in the U.S. This amounts to 14% of the 9,320 children ages 0 to 21 identified in the U.S. national 2010 child count (National Consortium on Deafblindness, 2011). The Communication Matrix database has the potential to provide a valuable knowledge base related to communication skill development in individuals with various rare disorders and in functionally defined low-incidence populations.

Effects of Ulnar Shortening on Wrist Pressure and Dynamic Work of Rotation: A Cadaveric Biomechanical Model

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Background: The ulnar shortening osteotomy procedure has been used to treat ulnar impaction syndrome. However, the biomechanical implications of the procedure are not completely understood. We sought to characterize the radiocarpal pressures and forearm work of rotation after ulnar shortening osteotomy.

Materials and Methods: Ten cadaver arms sectioned at the mid humerus were mounted to a rotatable jig. The scaphoid and lunate fossa pressures were measured during dynamic cycles of pronosupination. Additionally, a load cell was used to measure rotational torque in order to calculate the work of rotation. This was done for each arm before and after incrementally shortening the ulna by 3 and 5 millimeters. We analyzed the data for work of rotation for the entire cycle and the pressure data at neutral, 20, 40, and 60 degrees of pronation and supination.

Results: In general, the pressure increased in the lunate fossa at all degrees of rotation after shortening the ulna by 3 and 5 millimeters. In contrast, the pressure in the scaphoid fossa decreased as the ulna was shortened by 3 and 5 millimeters. The work of rotation decreased after shortening the ulna by 3 millimeters. The value then increased after the ulna was shortened 5 millimeters.

Conclusions: Ulnar shortening causes lunate fossa pressure to increase and scaphoid fossa pressure to decrease at all degrees of pronosupination. Too much ulnar shortening can potentially lead to radiolunate degeneration. The work of
rotation decreases at 3 millimeters of ulnar shortening before increasing at 5 millimeters of shortening. This supports the clinical improvement described in some reports.

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**Rural-Urban Differences in Access, Utilization and Time Spent**

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**Background:** Rural citizens face many barriers to accessing and utilizing both health care services and health insurance. Medicaid covers a large portion of the Oregon population, represents a high-risk group that is distinct from the general population and plays a particularly crucial role in rural health systems. For individuals without health insurance, accessing health care services can be difficult and can lead to accruing substantial medical debt. While comparisons among the general population indicate that rural citizens are at a higher risk of less access and utilization than their urban counterparts, few studies have examined these differences longitudinally among a Medicaid population. There is some evidence to suggest that urban and rural populations to have similar risk for less access and utilization when compared within a Medicaid population. The current study is framed in a time of significant policy changes for Oregon’s State Medicaid program, the Oregon Health Plan (OHP), when the State sought to increase enrollment by creating the OHP2 by shifting costs to a group of enrollees through premiums, copays and decreased benefit packages. In the ensuring months enrollment plummeted leaving many Oregonians without health insurance.

**Objective:** We sought to examine differences between urban and rural populations with reference to access, utilization, medical debt and time spent uninsured among beneficiaries of the OHP2 during a time of significant State Medicaid policy changes.

**Methods:** We utilized the Andersen Behavioral Model of Health Services Use as a conceptual model to frame our investigation. Our analyses utilized results from the Oregon Health Care Survey, a three-wave longitudinal panel study developed and implemented between 2003 and 2006 by the Providence Center for Outcomes Research and Education (CORE) and Portland State University. Our analyses included the 1535 adults who completed all three survey waves. We utilized univariate and multivariate logistic regression analyses to investigate the impact of living in a rural area on reported unmet medical need, unmet prescription need due to cost, unmet urgent care need, utilization of primary care services, utilization of emergency care services, medical debt and time spent uninsured while controlling for a common set of potential confounding variables.

**Results:** The results of the univariate analyses indicated that rural respondents were older, more educated and more likely to be Caucasian than urban respondents. Spending time without insurance was associated with increased risk for each of the outcomes except emergency department utilization. Further, the risk for these outcomes increased with increasing time spent uninsured. The multivariate models comparing rural versus urban revealed that the risk for unmet medical need, unmet prescription need due to cost, unmet urgent care need, going without primary care and emergency services and having greater medical debt were similar. Results also indicate that rural and urban respondents are equally likely to spend any amount of time uninsured.

**Conclusions:** Our findings support prior research, indicating that the longer individuals spend without health insurance the greater is their risk for less access and utilization of health care services, and greater medical debt. In addition, our results support the conclusions of prior research examining rural/urban differences within the Medicaid population, that rural and urban citizens experience similar levels of risk for less access and less utilization of health care services within
this distinct population. State officials should incorporate an understanding of urban/rural differences in both population compositions and health system structures into Medicaid policies in order to ensure that all Medicaid enrollees receive the same opportunity for good health, regardless of where they live.

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**Epithelial Sensory Hyperinnervation And Increased Vagally-Mediated Airway Resistance In A Mouse Model Of Eosinophilic Asthma**

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**Rationale:** Sensory hyperresponsiveness contributes to airway hyperreactivity but the underlying mechanism(s) remains understudied. In other organs (eg. skin, bowel), sensory hyperresponsiveness is associated with inflammation and branched outgrowth of peripheral sensory nerves. We had previously shown that eosinophils, an inflammatory cell associated with asthma, increase dorsal root ganglion sensory neuron branching in vitro. However, in lung tissue quantifying nerve branching and growth in airway tissue has been hindered by the complex three-dimensional structure of airway sensory nerves. We hypothesize that eosinophilic airway inflammation increases branched outgrowth of airway sensory neurons and increases reflex bronchoconstriction.

**Methods:** We overcame the hurdle of quantifying structural changes to airway sensory nerves by developing a computer modeling analysis of three-dimensional nerve images. We then used this method to quantify nerve length/branching in wildtype mice and transgenic mice with overabundant airway eosinophils (IL5 driven by the airway epithelial specific CC10 promoter). In order to isolate the effect of eosinophils from ILS, we also measure nerve length/branching in mice with overabundant IL5 but lacking eosinophils (dipetheria toxin driven by the eosinophil specific EPO promoter). We then measure airway resistance in the same mouse strains using mechanical ventilation before and after vagotomy.

**Results:** Using our new computer modeling analysis, we now show that epithelial sensory nerves in eosinophil transgenic mice contain double both the number of branchpoints (83.8 ± 7.0 vs 39.6 ± 10.4) and total neurite length (2126.4μM ± 415.4 vs. 1084.3μM ± 166.2). Mice lacking eosinophils but overexpressing IL5 contain equivalent nerve length and branching as wildtype. Airway resistance experiments show eosinophil transgenic mice exhibit augmented reflex responses to aerosolized serotonin, an effect inhibited by vagotomy. Mice lacking eosinophils but overexpressing airway IL5 did not exhibit augmented reflex bronchoconstriction.

**Conclusions:** We conclude that eosinophils promote dramatic airway sensory nerve growth, and that this may potentiate reflex bronchoconstriction.

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**Efficacy and Safety of Daptomycin in the Treatment of Gram-Positive Infections in Patients with Renal Impairment**

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Sharp Medical Center, San Diego, CA
**Introduction:** Multidrug-resistant Gram-positive infections are a growing concern. Vancomycin has been the primary treatment in patients with invasive Gram-positive infections; however, vancomycin-associated nephrotoxicity is a limitation. Daptomycin, a rapidly bactericidal lipopeptide, may serve as an alternative. Limited studies exist evaluating the clinical efficacy and safety in patients with renal impairment outside of the scope of dialysis.

**Objective:** To evaluate the efficacy and safety of daptomycin in patients with Gram-positive infections with renal impairment, defined as creatinine clearance ≤ 50 mL/min.

**Methods:** Multicenter retrospective analysis, including adult patients receiving daptomycin ≥ 3 days (2008-2011) for confirmed or suspected Gram-positive infections. Data collected: demographics, comorbidities, antimicrobial therapy, microbiologic cultures, outcomes, and adverse events.

Results: Results on 50 patients are presented, with median (IQR) or %. Age and APACHE-II score: 60 years (53-66) and 12 (9-15); Creatinine clearance: 27 ml/min (15.4-35.4); 94% prior hospitalization ≤ 1 year, 30% prior MRSA infection ≤ 1 year, 44% renal disease, 40% diabetes. Daptomycin dose: 6 mg/kg (5.8-7.8). 72% had at least one Gram-positive organism cultured, with MRSA most prevalent (47%). Concomitant sites of infection: 56% complicated bacteremia, 32% skin/wound, 22% bone/joint, 18% endocarditis, 14% intra-abdominal. Outcomes: 86% had clinical success; 83% had organism eradicated at end-of-therapy. Safety: 30% had peak creatine phosphokinase (CPK) values available, 53% of these had levels ≤ 200 IU/L (78-286); 60% had end-of-therapy CPK levels, 90% of these had levels ≤ 200 IU/L (21-72).

**Conclusion:** Daptomycin may be an efficacious and safe treatment option in patients with Gram-positive infections with renal impairment.

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**Piperacillin-Tazobactam Extended Infusion Therapy to Treat Pulmonary Exacerbations in Patients with Cystic Fibrosis**

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**Introduction:** Piperacillin-tazobactam, an effective agent for pulmonary exacerbations in patients with cystic fibrosis due to its bactericidal activity against *Pseudomonas aeruginosa*, exhibits time-dependent bactericidal activity. Extended infusions of piperacillin-tazobactam (e.g. over 3 to 4 hours) compared to traditional dosing (e.g. over 30 minutes) extends the time period that plasma concentrations remain above the MIC. Patients with cystic fibrosis have shorter piperacillin half-lives (t1/2) compared to patients without disease (median (range) 0.69h (0.34-1.19 h) vs 1.05 (0.49-7.52)). Therefore, T>MIC may be shorter in patients with cystic fibrosis, diminishing drug efficacy. Extended infusion regimens may be advantageous in cystic fibrosis patients to optimize efficacy, decrease the frequency of pulmonary exacerbations and hospitalizations, and slow or stop the progression of this disease.

**Objectives:** To determine C_{max,ss,free} / MIC and T>MIC in patients who received piperacillin-tazobactam for pulmonary exacerbations of cystic fibrosis. To utilize pharmacokinetic and pharmacodynamic data to design optimal piperacillin-tazobactam dosing regimens in patients with cystic fibrosis.

**Methods:** We conducted a retrospective analysis of adult patients who were admitted for pulmonary exacerbation of cystic fibrosis from January 2011 – June 2011. The study was approved by the OHSU IRB. Piperacillin pharmacokinetic
parameters were used to predict drug concentrations utilized for determining $C_{\text{max,ss,free}} / \text{MIC}$ and $T>MIC$ for prescribed piperacillin-tazobactam dosing regimens, with an MIC of 8 mg/L for susceptibility against *Pseudomonas aeruginosa*.

**Results:** Forty patients were admitted for 54 pulmonary exacerbations. Twenty-four patients (60%) received piperacillin-tazobactam therapy for two weeks. All dosing regimens achieved the target $C_{\text{max,ss,free}} / \text{MIC}$ (5x MIC). Sixteen of the 24 patients received piperacillin-tazobactam at doses recommended for nosocomial pneumonia (piperacillin-tazobactam 4.5 g IV over 30 minutes Q 6h); however, $T>MIC$ was $< 48\%$ and below the target $T>MIC$ of 90%. The remaining eight patients received lower doses (piperacillin-tazobactam 3.375 mg IV over 30 minutes – 4 hours Q6 – 8 h) even though creatinine clearance was greater than 40 mL/min, resulting in $T>MIC$ ranging from 43 – 69%. Six of 24 patients (25%) were readmitted for pulmonary exacerbation and received piperacillin-tazobactam.

**Conclusions:** Prescribed doses of piperacillin-tazobactam IV over a short infusion time of 0.5 h Q6 – 8 h, and use of lower doses (3.375 mg vs 4.5 mg) are suboptimal in this population. Suboptimal dosing regimens may result in greater episodes of pulmonary exacerbations and more frequent and longer hospitalizations. Higher doses and an extended infusion time of 3 – 4 hours instead of 0.5 hour would increase $T>MIC$, while achieving $C_{\text{max,ss,free}}$ that are more than 5 times above the MIC for efficacy.

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**Relationship between heart failure self-care and provider consultation**

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**Background:** Heart failure (HF) is a common and complex condition that requires optimal medical management and a number of maintenance and reactive self-care behaviors in response to changes in clinical stability. The relationships between different aspects of HF self-care behaviors are poorly understood.

**Objective:** To quantify the relationship between HF self-management and provider consulting behaviors.

**Methods:** We completed a secondary analysis of data collected during a cohort study on symptoms among adults with moderate to advanced HF. The Self-care of HF Index (SCHFIv6) was used to measure self-care maintenance (routine adherence behaviors) and management (recognition and self-response to HF symptoms), and the European HF Self-care Behavior scale (EHFScb-9) was used to measure consulting behaviors (whether a patient contacts a healthcare provider when symptoms occur). Linear correlations were quantified between indices of HF self-care correcting for multiple measures.

**Results:** This sample (n=167) was largely male (61.1%) and predominantly Caucasian (88%) with primarily class II/III HF (96.4%); mean age was 56.4±12.4 years. Consulting behaviors were moderately associated with self-care maintenance ($r=0.4102$, $p<0.0001$). There was a weak-to-moderate association between consulting behaviors and self-care management ($r=0.3758$, $p=0.0001$).

**Conclusion:** While patients who reported better self-care also reported being more likely to consult with providers when symptoms occur, there is considerable unexplained variability in HF self-care behaviors. As such, self-care and provider
consulting behaviors can neither be considered independent nor mutually explanatory. Future research is required to
determine if HF patients who are better at self-care are also those who reliably consult with providers when necessary.

Risk Factors for Complication during Outpatient Parenteral Antibiotic Therapy (OPAT) for Orthopedic and Neursurgery Infections
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Purpose: To determine factors significantly associated with major OPAT complication among patients treated with long-
term parenteral antibiotics for orthopedic and neurosurgery infections.

Subjects and Methods: We retrospectively reviewed the electronic medical records of 337 patients who received 365
courses of outpatient parenteral antibiotic therapy through the OHSU OPAT service during a 20 month period. Only
patients age 18 or older who had been recommended for 2 or more weeks of OPAT were included. Factors examined
included patient demographics, comorbidities, infection characteristics, lead-time factors, and OPAT treatment factors.
OPAT complication was considered to be an adverse antibiotic reaction (AAR), a vascular access problem (VAP), a failed
discharge plan (FDP) that required either a change in parenteral antibiotic, or replacement of a vascular access device, or
early discontinuation of OPAT.

Results: 46% of OPAT courses were marked by one or more OPAT complication. Two patients died during OPAT. By
univariable analysis, female sex, having insurance, having no primary care provider, being discharged less than 50 miles
away from OHSU, having a psychiatric disease, having a malignancy, and OPAT treatment with Vancomycin were
significantly associated with OPAT complications. OPAT complications were not associated with age, race, ethnicity,
cardiovascular disease, diabetes, renal disease, liver disease, chronic immunosuppression, recent alcohol or drug abuse,
length of hospital stay, having an ICU stay, total operating room time, infection organism, infection type, vascular access
lead time, initial antibiotic lead time, type of vascular access, or setting for OPAT delivery.

Conclusion: OPAT complications are common. Risk factors identified by this study can be used to develop a multivariable
model for assessing risk of OPAT complication. We plan to validate the model in another OHSU OPAT cohort and test
enhanced care interventions for high risk OPAT patients.

Validation of Limited Sampling Equations to Predict Midazolam AUC For CYP3A4 Phenotyping in Obese Women

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Introduction: Cytochrome P450 3A4 (CYP3A4) phenotyping using midazolam is resource intensive with multiple plasma
samplings. Lee L.S. et al. published four limited sampling strategies to simplify CYP3A4 phenotyping in normal body mass
index (BMI) women. In the current study, we tested the above four limited sampling strategies to predict area under the curve (AUC) of the concentration-time profile of midazolam in adult obese women.

Methods: Thirty-four obese women (BMI>30 kg/m²; Age=18-35 years) were consented for the study approved by institutional review board of Oregon Health & Science University. Serial blood samples were collected after a single, 2mg oral dose of midazolam, and plasma samples were quantified using liquid chromatography-tandem mass spectrometry. Pharmacokinetic parameters including AUC from time 0 to ∞ was computed using non-compartmental methods. The predicted AUC are compared to computed AUC using the coefficient of determination (r²). Bias and precision of the equations were evaluated by mean prediction error (MPE), mean absolute error (MAE), and root mean square error (RMSE).

Results:

<table>
<thead>
<tr>
<th>Equation</th>
<th>Sampling Time, h</th>
<th>Log (AUC predictive) pg*h/mL</th>
<th>r²</th>
<th>%MPE</th>
<th>%MAE</th>
<th>%RMSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equation-1</td>
<td>0.5, 2, 6</td>
<td>1.1+0.21(logC₀.₅)+0.43(logC₂)+0.30(logC₆)</td>
<td>0.0215 4</td>
<td>-1.033</td>
<td>1.033</td>
<td>1.0945</td>
</tr>
<tr>
<td>Equation-2</td>
<td>0.5, 2</td>
<td>0.73+0.24(logC₀.₅)+0.74(logC₂)</td>
<td>0.5282 1</td>
<td>-0.253</td>
<td>0.2735</td>
<td>0.2735</td>
</tr>
<tr>
<td>Equation-3</td>
<td>0.5, 6</td>
<td>1.67+0.21(logC₀.₅)+0.66(logC₆)</td>
<td>0.5064 8</td>
<td>-0.0174</td>
<td>0.0776</td>
<td>0.1105</td>
</tr>
<tr>
<td>Equation-4</td>
<td>6</td>
<td>2.24+0.76(logC₆)</td>
<td>0.5379 5</td>
<td>0.0838</td>
<td>0.1131</td>
<td>0.1319</td>
</tr>
</tbody>
</table>

Conclusions: Our analysis demonstrates that a single-variable model with time point at 6 hours and a 2-variable model with time points at 0.5 and 6 hours are acceptable and predictive of midazolam AUC in obese women, and the model using time points at 0.5 and 6 hours is the best-fitted equation in our population. These results are compared to two equations using the time points at 0.5 and 6 hours and 0.5,2, and 6 hours in normal BMI women.

Comparing Different Measures of Impulsivity Between Regular Smokers, Chippers and Non-Smokers

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Amongst smokers, the amount and frequency of smoking is highly variable: occasional smoking, regular light smoking, regular heavy smoking and all the in between. The majority of occasional smokers eventually become nicotine dependent. However, a fraction remains as non-dependent occasional smokers. These smokers, known as tobacco chippers, do not differ in nicotine absorption or metabolism from regular nicotine-dependent smokers. Studies comparing regular smokers and non-smokers have shown that specific traits are associated with nicotine dependence. However, few studies have analyzed the relationship between delay discounting, questionnaire measures of personality, and smoking levels by including chippers. This study aimed to address this gap.
Participants between 25 and 55 years of age were recruited from the general population. Based on the number of cigarettes smoked daily and the Fagerstrom Test for Nicotine Dependence, three study groups were defined: non-smokers with no history of regular smoking, chippers, and regular smokers. Impulsivity measures were obtained using a computer-based delay discounting task. Self-response questionnaires measuring personality traits included the NEO Five-Factor Inventory, the Sensation-Seeking Scale, the Kentucky Inventory of Mindfulness Skills, and the Perceived Stress Scale.

Nicotine dependent scores were significantly different between groups. As reported previously, smokers were significantly more impulsive than non-smokers in that they discounted delayed rewards more steeply. However, while exhibiting similar discounting rates, regular smokers exhibited significantly lower levels of impulsivity and risk-taking on the self-reported questionnaires than chippers.

**MPDZ Knockout and Bacterial Artificial Chromosome (BAC) Transgenic Overexpression Models Support its Involvement in Ethanol Withdrawal and Consumption**

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Using robust behavioral models, positional cloning, sequence and expression analyses, we have identified *Mpdz* as a quantitative trait gene (QTG) for ethanol withdrawal in mice (*Nat Neurosci* 7:699, 2004). *Mpdz* encodes the multi-PDZ domain protein (MPDZ/MUPP1). Currently, there is limited information about its function in the brain how it relates to withdrawal, and its role in other behaviors. Here, we address this using two novel genetic models. One is an MPDZ knockout (KO) developed from an embryonic stem cell line with an insertional mutation in *Mpdz* (XG734, Bay Genomics). MPDZ KO homozygotes are not viable, but KO heterozygotes are have significantly (*p*<0.05) more severe ethanol withdrawal convulsions than wildtype littermates (inbred C57BL/6 background). To assess its role in ethanol consumption, we compared KO and wildtype mice using a two-bottle choice paradigm. In two studies, mice had continuous access to ethanol (3% and 10%, 4 days per concentration). In a third study, mice had continuous access to ethanol (3%, 6%, 10% and 20%, 4 days each) and were then assessed for saccharin and quinine consumption. Across all studies, a main effect of genotype (*F*1,97=14.2, *p*=0.00027) is apparent (based on 3% and 10% tested in all 3 studies), with MPDZ KO mice consuming more ethanol than wildtype littermates. No difference was detected for water, saccharin or quinine consumption. We also report the completion of *Mpdz* overexpression mice. Microinjection of a BAC construct into embryos produced transgenic founders, from which we completed transgenic strains (inbred DBA/2 background). Transgenic mice, in which *Mpdz* expression is increased ~2-fold, showed significantly less severe ethanol withdrawal than non-transgenic littermates.

Thus, fine-mapping, molecular and in vivo analyses all point to *Mpdz* as a QTG for ethanol withdrawal. The strengths of the BAC transgenic approach complement the limitations of the KO approach, and vice versa, so the finding that both support a role for *Mpdz* in withdrawal is compelling. Our results also implicate MPDZ in ethanol consumption. Although limited to small populations thus far, two groups have found a potential role for *MPDZ* in alcoholism (*Alc Clin Exp Res* 33:712, 2009; *BMC Biol* 7:70, 2009). Our genetic models will be useful to elucidate the mechanism by which this translational target affects ethanol withdrawal and consumption.

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Challenges in Detecting and Preventing Pressure Ulcers in Older Adults with Dark Skin

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Purpose: This poster reviews the state of the science and best practices for identifying and staging pressure ulcers in older adults with dark skin, and describes factors associated with disparities in pressure ulcer recognition and treatment outcomes.

Background: Pressure ulcers are a preventable cause of morbidity, mortality, and impaired quality of life in older adults. Yet, in the United States, pressure ulcers are a cause of death among 60,000 persons annually. Nearly 80% of pressure ulcer-associated deaths occur in people over age 75. Costs associated with pressure ulcer management account for $18.5 billion dollars annually. The Centers for Medicare and Medicaid Services classified stage III and IV pressure ulcers as a preventable hospital-acquired condition and do not reimburse hospitals for care costs. State and national organizations have developed campaigns related to pressure ulcer prevention, early detection and management including the National Pressure Ulcer Advisory Panel, an independent non-profit professional organization. Despite increased attention on pressure ulcer prevention, detection and management, one poorly addressed area continues to be detection in people with dark skin. This may be related to difficulties recognizing non-blanchable erythema, an indicator of stage I ulcers, in dark skin. The National Pressure Ulcer Advisory Panel (2007) states that “in darker skin tones, the ulcer may appear with persistent red, blue, or purple hues.” Dark-skinned people are substantially less likely than whites to have a Stage I pressure ulcer documented, while higher-grade ulcers are more common in blacks than whites. As a result, pressure ulcer-associated mortality is higher among blacks than among whites. This problem will only grow in coming years with the expanding population of darker pigmented older adults. Further, anecdotal evidence suggests that nursing education programs are not teaching students how to assess for pressure ulcers in people with dark skin.

Best Practice: Only recently has there been increased awareness of this disparity. The 2010 Revised National Pressure Ulcer Advisory Panel Guidelines include specific recommendations for assessing and managing pressure ulcers in older adults with dark skin. Key components of these guidelines will be discussed and a photo-essay will illustrate their use in assessing older adults with dark skin.

Conclusions: There is a need for research and performance improvement projects to identify the dissemination and use of guidelines for recognizing pressure ulcers in older adults with dark skin, and their efficacy in reducing the incidence of higher stage pressure ulcers in this population. Recommendations will be made for future research regarding use of the 2010 guidelines, and implications will be identified for educating care providers and nursing students in early recognition of pressure ulcers in older adults with dark skin. Registered nurses have a key role in improving the health of these clients by identifying and preventing pressure ulcers through education, research, and in their professional practice. Therefore, nurses need to be familiar with the new guidelines for detecting pressure ulcers in older adults with dark skin, and able to use and teach these guidelines to others.

Integrating High Fidelity Simulation into a nurse-midwifery curriculum
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The Oregon Health and Science University Nurse-Midwifery education program has piloted the use of high fidelity simulation to teach team leadership and management of complex scenarios. In our School of Nursing, simulation has become an integral part of the pre-licensure program, with substantial expertise among simulation specialists and a state-of-the-art simulation laboratory. Evidence is accruing about the effectiveness of simulation for improving clinical judgment, providing opportunities for managing emergency situations, and development of team functioning skills. Faculty in the advanced practice nursing programs have begun drawing on this expertise to integrate simulation into their curricula.

Nurse-Midwifery Education has long used “skills lab” approaches to basic clinical teaching. In recent years some programs have integrated more complex forms of simulation such as the American Academy of Pediatrics Neonatal Resuscitation Program, which includes a mega-code. A few programs are exploring more robust simulation programs within their nurse-midwifery curricula. As part of a major transformation in educational approaches, nurse midwifery faculty at OHSU have introduced simulation as a means to improve students’ clinical judgment and to learn team roles, particularly in the management of emergent situations.

Methods: Over three years, a total of 30 nurse-midwifery students and 60 pre-licensure nursing students participated in simulation scenarios. The scenarios required that they manage: (1) a postpartum hemorrhage; (2) a complex delivery with a resuscitation of a depressed neonate; (3) a patient with bleeding in the second trimester.

Results: In the pilot test, the pre-licensure nursing students reported that they found having the provider be part of the scenario was much more realistic than in prior simulation experiences. Those who ultimately aspired to advanced practice roles were excited by the opportunity to interact with students in training for that role and with the midwifery faculty. The midwifery students were surprised at how difficult it was to communicate their orders to the nursing students. Some students did not like the unrealistic “patient”, but others wanted more opportunities to practice complex skills in emergency scenarios. After the pilot, we revised the scenarios and ran a second set of joint simulations. It became clear that the value of this exercise was less in mastering specific content, and more in trying on the roles they are preparing for and practicing complex processes of team leadership in a safe environment. Based on the evaluation data after the third year, a decision was made to continue the use of this educational innovation in the future. We hope to expand it to include inter-professional educational collaboration.

Systemic Blockade of Dopamine D1 But Not D2 Receptors Impairs the Development of Ethanol-Conditioned Place Preference

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Dopaminergic transmission has been implicated in ethanol reward and involvement of dopamine D1 and D2 receptors has been examined using various pharmacological and genetic manipulations. However, studies assessing the
consequences of systemic blockade of these receptors on the development of a place preference induced by ethanol remain largely absent from the literature. In the present experiments, we used systemic administration of selective dopamine receptor antagonists to examine the roles of D1 and D2 receptors in the acquisition of ethanol-induced conditioned place preference (CPP) in adult male DBA/2J mice. All experiments used an unbiased place conditioning procedure. Testing occurred 24 hrs after the first two conditioning sessions and again after another two sessions, so that mice received four conditioning sessions in total. In experiments 1 and 2, antagonists selective for D2 (raclopride, 0-1.2 mg/kg) and D1 (SCH-23390, 0-0.3 mg/kg) receptors were administered before conditioning sessions, where ethanol (2 g/kg) was paired with a distinctive tactile floor cue. While antagonism of D2 receptors produced no effect, D1 receptor blockade impaired ethanol-CPP acquisition. In experiment 3, we determined these impairments were not due to aversive properties of SCH-23390 (0.3 mg/kg) by demonstrating the inability of this drug to produce a conditioned place aversion (CPA). Experiment 4 examined the effect of SCH-23390 on the development of a CPA produced by post-trial ethanol (2 g/kg) injection. Ethanol-CPA was not impaired by SCH-23390, suggesting that reductions in ethanol-CPP acquisition were likely not due to general learning or memory impairments produced by the drug. Overall, these findings suggest that D1 receptor function is specific to the rewarding and not aversive motivational effects of ethanol.

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Severe Postnatal Growth Retardation, Immunodeficiency, Pulmonary Disease: Insights from Clinical and Functional Evaluations of Two Rare STAT5B Missense Mutations

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Background: Growth hormone (GH) promotes human growth primarily by regulating insulin-like growth factor (IGF)-I production through activation of GH receptor (GHR)-Janus kinase (JAK)-2 signaling cascades. Of the multiple pathways activated, the critical importance of the STAT (Signal Transducer and Activators of Transcription)-5b pathway in regulating human IGF-I production was confirmed by cases of rare STAT5b deficiency due to mutations in the STAT5B gene. We previously reported the first STAT5b deficient patient who carried the homozygous missense mutation, p.Ala630Pro, located within the highly conserved SH2 domain. This case presented with GH insensitivity (GHI), insulin-like growth factor-I deficiency (IGFD), but, unlike GHI and IGFD due to mutations in the GHR gene, also presented with severe immune dysregulation manifesting as progressive worsening of pulmonary function. We now describe a new case, a patient who carried a novel missense mutation also in the SH2 domain. Functional studies and clinical presentation of the novel mutation, compared to p.Ala630Pro, revealed some striking differences and provided valuable insights as to possible phenotype-genotype correlations.

Patient: The new patient, a girl adopted at four days of age, presented with severe cutaneous eczema, several infections associated with failure to thrive since the first year of life. Immunological evaluation revealed T lymphopenia but severe pulmonary symptoms were notably absent. The patient was concomitantly severely growth retarded, reaching an adult height of 124.7 cm (-5.9 SDS). Endocrine evaluations (normal provocative GH tests; low serum IGF-I, -3.7 SDS, and IGFBP-3, -4.5 SDS) were consistent with GHI and IGFD.
Results: Sequencing of the STAT5B gene revealed a novel homozygous missense mutation, p.Phe646Ser (exon 16), located in the SH2 domain, a highly structured domain essential for STAT5b to dock to activated receptors, to dimerize and to stably interact with DNA. Phe646 is positioned within the beta-D’ strand of the SH2 domain. Reconstitution studies demonstrated that the regenerated p.Phe646Ser variant was readily over-expressed in HEK293(GHRfl) cells, although expression was considerably lower than that of wild-type STAT5b. In contrast to our previously described SH2 missense mutation, p.Ala630Pro, p.Phe646Ser, could be phosphorylated in response to GH and to cytokines such as interferon-gamma. The phosphorylated p.Phe646Ser, however, could not drive transcription.

Conclusion: We describe the identification only the second homozygous STAT5B missense mutation, p.Phe646Ser, in a patient with profound postnatal growth failure due to IGF deficiency, but who lacked the severe chronic pulmonary disease and immunodeficiency observed in the patient carrying the first described STAT5B missense mutation, p.Ala630Pro. Both missense mutations are located within the critical SH2 domain. While the p.Ala630Pro substitution, mapping within the βC sheet, completely abrogated both the phosphopeptide binding and transcriptional functions, the p.Phe646Ser variant, located within the βD’ sheet, could still bind phosphopeptides, but was unable to drive transcription, thus demonstrating the importance of the SH2 βD’ strand for full transcriptional activity. This differential functional effect of p.Phe646Ser is unique amongst the STAT5B mutations and may be correlated to the absence of pulmonary symptoms displayed by the patient. Identification of additional STAT5B deficient patients should provide more thorough genotype-phenotype correlations.

Acknowledgement: This study was supported, in part, by a grant from the March of Dimes (RGR). EF is supported by a Fulbright Scholar Award.

Significantly Improved Predictive Value Provided by ProExCTM Staining of Pap Smears Diagnosed as HSIL or ASC-H Warrants Leap to LEEP

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Objectives: Management guidelines now allow for a “leap to LEEP” in women with a pap smear diagnosis of high grade dysplasia (HSIL). This change was due to an improved positive predictive value (PPV) of HSIL (70-80) provided by liquid-based paps sufficient to bypass inefficient colposcopy. In our experience, however, the consequence has been a reduction in HSIL and a doubling of ASC-H (atypical squamous cells, cannot exclude HSIL) diagnoses. Our clinicians are also hesitant to leap to LEEP because of significant false positive rates. HPV testing is not useful in these cases because the PPV is no better than cytology. Our objective was to test whether a specific marker of neoplastic transformation, such as ProExCTM, provides sufficient PPV to warrant “leap to LEEP.”

Methods: SurePathTM cervical pap smears diagnosed as either ASC-H (n=136) or HSIL (n=118) were immunostained for ProExC using a Ventana Benchmark XT. At least 5000 epithelial cells were required on each slide to be adequate for staining and outcome required either biopsy proven high grade dysplasia (CIN2+) or at least three years of negative followup. Biopsies were also stained for ProExC and a “Consensus Diagnosis” between gynecologic pathologists (TM and RK) provided the gold standard outcome. Stained pap smears were scored by a cytopathologist (TM) and resident (AS
and CR). Because of the difference in CIN2+ prevalence in ASC-H and HSIL, these groups were analyzed separately. Predictive values were calculated and associations tested for by Chi-square.

Conclusions: We observed excellent agreement between pathologists scoring ProExC (ASC-H kappa statistic 0.59; HSIL kappa 0.61). Discordant scores were primarily in paps with fewer than 10 positive cells (52% discordance compared to 3-5% in negative or abundant cases). Chi-square analysis revealed an association between ProExC staining in both ASC-H (p<0.0001) and HSIL (p<0.0001) compared to CIN2+ outcome. The prevalence of CIN 2+ in the ASC-H group was 37% with staining yielding a PPV of 73, NPV 62, and likelihood ratio of 1.8. The prevalence of CIN 2+ in HSIL was 81% with PPV of 95, NPV 78, and likelihood ratio of 4.5. We conclude ProExC staining may strengthen clinical confidence when leaping to LEEP and may warrant LEEP in ASC-H.

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**Impact of chytrid parasites on diatoms in the lower Columbia River**

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This study combined hourly biogeochemical data with weekly phytoplankton abundance and species composition analysis to examine the role of river discharge on chytrid-phytoplankton interactions in the lower Columbia River. Infected diatoms were enumerated using fluorescent stains and parasites were identified by sequencing the 18S rDNA. The dominant diatom, Asterionella formosa, was infected at high levels (40%) in the primary spring bloom prior to elevated river discharge events. Diatom abundances and infections decreased as river discharge and turbidity increased. High resolution data showed that river discharge controlled the timing, magnitude, and number of diatom bloom events that occurred each spring. River discharge may play an important role in modulating chytrid-diatom interactions and preventing epidemic events by influencing host growth and contact rates of host and parasites. The data indicate that during maximal infection, chytrid parasites can divert ~20% of the large diatom carbon pool away from export to the estuarine microbial food webs and into the river food web via zooplankton grazing of chytrid zoospores.

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**Application of a novel method for age-period-cohort analysis to Oregon viral hepatitis mortality, 1995-2010**

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**Background:** Evaluating mortality incidence over time using direct or indirect rates is a common public health tool for predicting disease trends, understanding etiology, and informing prevention and planning efforts. While rates are easily calculated, the factors which influence mortality risk over time are obscured in the averaging process. Age, period, and cohort (APC) analysis seeks to uncover these influences by partitioning trends into components associated with changes over time within a given age group (age effects), a given time period (period effects), and within a birth cohort (cohort effects). Because of challenges evaluating the extent of the population infected with chronic viral hepatitis and the ensuing burden of disease, an APC analysis of viral hepatitis mortality may contribute information about the factors
perpetuating the persistence of chronic infection, suggest whether current trends are likely to be sustained, and provide information to public health programs to guide planning efforts.

Objective: To evaluate Oregon viral hepatitis mortality applying a novel method of APC analysis$^{1,2}$

Methods: Deaths related to viral hepatitis (ICD-9: 070; ICD-10: B15-19, B94.2) were abstracted from multiple-cause mortality variables from Oregon death certificates for 1995-2010. These data were then evaluated for the presence of cohort effects using a three phase method: (1) data were assessed using graphical inspection; (2) log-additive components of age and period were removed using a median polish,$^{3,4}$ (3) remaining cohort effects were separated from error using a linear regression model and their relative magnitude calculated.

Results: Rates of viral hepatitis deaths were highest among individuals in the baby boomer generation, those born between 1946 and 1965. After removal of the log-additive effects of age and period, estimated rates remained significantly higher for individuals born between 1950 and 1965 than preceding birth cohorts for 1910-1949 (p=0.03) or subsequent birth cohorts for 1966-1985 (p=0.003).

Conclusion: We demonstrate a computationally-simple method for assessing temporal trends. By applying this method to mortality rates for viral hepatitis, a clear pattern of increase in deaths is discernible between 1950-1965 compared with the cohorts before and afterward. These findings may contribute to public health surveillance and planning efforts.

REFERENCES


**Impact of Sea Level Rise on Habitat Opportunity of Columbia River Juvenile Chinook Salmon**

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The Columbia River estuary and plume play a significant role in the life cycle of Columbia River salmon. Basin management requires anticipated knowledge of the changes in habitat for Endangered Species Act-listed species, which might occur as a result of climate change. To begin understanding these changes, we are conducting simulations of river-to-ocean circulation by the baroclinic circulation model SELFE, for scenarios of future sea level rise based on work by the Climate Impacts Group at University of Washington. Direct simulation outputs of SELFE include salinity, temperature, water depth, and velocity. These outputs are processed through filters to calculate (a) habitat opportunity for juvenile salmon, (b) salinity intrusion length. The impacts of sea level rise scenarios are compared, among themselves and with
contemporary conditions. Early results confirm the potential for substantive changes in the physics of estuary and the plume, resulting in changes in seasonal patterns of physical habitat opportunity.

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**Extracellular Matrix Remodeling of the Embryonic Chicken Outflow Tract in Response to Altered Hemodynamics**

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**Background:** Hemodynamic forces such as wall shear stress are critical environmental factors modulating the genetic program for heart development and cardiac remodeling. Alterations in blood flow at early embryonic stages can lead to detrimental remodeling and heart defects. However, the structural adaptations of the heart to altered hemodynamic conditions are not well understood. The early embryonic outflow tract (OFT), composed of collagens and proteoglycans, is known to be sensitive to hemodynamic conditions. We hypothesize that when shear stress is increased within the OFT the cardiac wall will become stiffer with increased collagen composition.

**Methods:** To test this hypothesis a suture was tightened around the OFT of stage HH18 (day 3) chick embryos to reduce the cross-sectional area of the OFT lumen. This banding procedure (OTB) increases blood velocities and thus shear stress at the wall. After 24 hours sham and OTB embryos were collected for histology or immunostained for collagen XIV prior to confocal microscopy imaging. Histological sections were stained with Picrosirius Red to assess collagen deposition and Alcian Blue to localize proteoglycans.

**Results:** Confocal microscopy results revealed that thin bands of collagen XIV are normally expressed along the inner lining of the OFT wall. Picrosirius red staining for collagen was most intense along this inner lining, directly underlying the endocardial cells. Confocal microscopy revealed that collagen XIV expression along the sub-endocardial lining was increased in the OTB group. Collagen XIV deposition was also observed in fibroblasts throughout the cardiac jelly of the OTB group, a finding also observed with picrosirius red staining. Alcian Blue staining revealed deposition of proteoglycans throughout the OFT’s cardiac jelly. Preliminary results suggest increased proteoglycan deposition in the OTB group, though numbers are too small to determine statistical reliability.

**Conclusions:** Preliminary results for collagen XIV suggest that OTB may increase collagen deposition. In future, these data will lead to a better understanding of early changes in cardiac wall adaptation to hemodynamic conditions and detrimental cardiac remodeling.

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**Barriers to Treatment and Prevention of Childhood Obesity in Rural Primary Care**

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Purpose: The purpose of this study was to explore the practices and perceived barriers, resources, and training needs of rural primary care providers as these pertain to prevention, assessment, and treatment of childhood obesity.

Background: Childhood obesity is a significant health concern that disproportionately affects rural populations. Visits to the health care provider offer a key opportunity for identification and treatment of obesity, and for education and counseling concerning nutrition and physical activity. Yet, despite the existence of clinical guidelines addressing childhood obesity, these have not been consistently translated into practice. While some barriers to prevention and management of childhood obesity within primary care have been identified, barriers encountered by rural clinicians have not been explored.

Methods: Semi-structured interviews were conducted with 13 clinicians (6 physicians, 5 nurse practitioners, and 2 physician assistants), from a total of 35 employed in a pediatric or family practice in a three-county region in rural Oregon. Interview transcripts were analyzed by two investigators using a modified version of focused coding and grounded theory methods.

Results: The majority of clinicians routinely assessed for obesity in pediatric patients. However, efforts to prevent or treat obesity were limited by time constraints, lack of reimbursement, inadequate patient education materials, lack of parent motivation, the sensitivity of the issue, and clinicians' self-perceived low proficiency in diet counseling and behavior management. Despite these barriers, clinicians viewed primary care as the “first and last stop” for addressing childhood obesity in these rural counties, where specialists and tertiary weight management centers were inaccessible to much of the population due to distance, low family income, and/or lack of health insurance.

Implications: Primary care providers have an essential role to play in preventing and treating childhood obesity within rural communities, where other resources for addressing obesity are often non-existent. Training; clinical aids, such as checklists for assessing diet and readiness to change; and high quality patient education materials are needed to assist clinicians with this responsibility. Internet or written formats for clinician training are preferred over conferences which usually require considerable travel. Additionally, alternative approaches, such as employing nurses with expertise in child nutrition and motivational interviewing to lead support groups and counsel families, could be a feasible and cost-effective way to improve the management of childhood obesity within rural primary care.

Acknowledgements: This research was funded by a grant from the Betty Gray Rural Health Development Award Program at Oregon Health & Science University School of Nursing.

Assessing the Impact of a Pilot Antimicrobial Stewardship Program in Pediatrics at an Academic Medical Center

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Introduction: Antimicrobial resistance continues to rise due to antibiotic overuse and inappropriate medication prescribing. The prevalence of adult antimicrobial stewardship programs continue to grow, however, the prevalence of pediatric programs is limited.
Objective: To assess the impact of a pilot antimicrobial stewardship program in pediatrics in regards to days of antimicrobial therapy, empiric therapy discontinuation, bug-drug mismatch, and intravenous (IV) to oral (PO) route conversions.

Methods: This study is a prospective chart review between January and March 2012 at Doernbecher Children’s Hospital. The clinical pediatric pharmacists use an integrated system to document their interventions; referred to as i-vents. These i-vents will be reviewed to assess the role of pharmacists in decreasing inappropriate antimicrobial use. Data collection includes the following interventions: IV to PO, discontinuation of empiric therapy, broadening or narrowing of antimicrobial therapy based on cultures, limiting double anaerobe coverage, appropriate use of restricted antimicrobials, and dose adjustments. Additionally, the principle investigator, a pharmacy resident, reviewed patients on prespecified antibiotics for the aforementioned criteria. Interventions were discussed with a pediatric infectious disease provider prior to implementation. Success of the program will be determined by comparing days of therapy to a similar time frame one-year prior.

Results and conclusions are pending.

Fetal Myocardial Thyroid Hormone Deiodinases are Regulated by Fetal T₃

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Objectives: Conversion of pro-hormone thyroxine (T₄) to active tri-iodo-L-thyronine (T₃) is accomplished under the catalytic action of deiodinases D1 and D2. T₃ is deactivated by D3 deiodinase. Current dogma holds that the near term cortisol surge regulates deiodinase expression in the fetal liver to facilitate the T₃ surge just prior to birth. However, regulation of expression of deiodinases in the heart by two powerful fetal cardiac growth regulating hormones, T₃ and insulin-like growth factor-1 (IGF-1), has not been studied. It is known that T₃ promotes maturation while IGF-1 promotes proliferation in the fetal heart.

Hypothesis: T₃ and IGF-1 would not influence the expression of the deiodinases in the heart because the control of T₃ levels is solely under the control of circulating fetal cortisol.

Methods: Fetal sheep were instrumented at 120 days gestational age (dGA, term ~145dGA). Fetuses were randomly assigned to 4 infusion groups (n=6 each group) and studied from 125-130dGA: 1) IGF-1 (715 g/d), 2) T₃ (54 g/d), 3) IGF-1 combined with T₃ (T₃+IGF-1), and 4) control (CON). At 130dGA, a section of the fetal heart was snap frozen for gene expression analysis of the three deiodinases by qPCR.

Results: T₃ stimulated expression of all three deiodinases compared to controls (p<0.05). IGF-1 alone did not influence expression of any of the deiodinases. The combination of T₃ and IGF-1 exposure did not alter D2 expression, but D1 and D3 levels decreased compared to T₃ alone (p<0.05 vs T₃).

Conclusions: Contrary to our hypothesis, T₃ increased expression of all three deiodinases but not in combination with IGF-1. Active T₃ production in the fetal heart appears to be a feed forward system that upregulates deiodinases and
leads to maturation of cardiomyocytes. This work is important because maternal hyperthyroidism can lead to an under-endowed fetal myocardium.

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**Placenta #11035: Risks and benefits of de-identifying data in birth cohort repositories**

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Banking of biological specimens for future unspecified research is a powerful resource and is becoming increasingly popular. However, these tissue repositories raise issues as how to protect participants’ rights while supporting scientific investigation. One important consideration a principle investigator must make is how to maintain confidentiality. We used responsible conduct of research methodology to investigate this issue in the context of birth cohort studies.

The current guidelines to maintain confidentiality present two options: 1) Maintaining identifiable or coded repositories within secure databases, limiting access to protected health information or 2) removal of all protected health information and any identifiers from the repository. This latter option is called “de-identification”.

We analyzed the risks and benefits of de-identification from the viewpoint of the researcher and the study subject in birth cohort studies. Though de-identification of data initially appears to be the simplest and safest method for maintaining confidentiality and flexibility for future scientific inquiry, it is not without risk. Researchers must consider the ethical implications of de-identification: obtaining truly informed consent; inability to contact subjects with research findings that may concern their health or that of their offspring; and the rights of the offspring to information concerning their tissue collected at birth, among other issues.

We developed a list of questions concerning potential ethical and scientific implications of data de-identification in birth cohort repositories for researchers to consider when creating a repository.

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**Ecology and genetic characterization of Katablepharis CRE, a heterotrophic flagellate that 'blooms' in the Columbia River estuary during the spring**

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The Columbia River estuary, which divides Oregon and Washington in the United States, is typically classified as a detritus-driven system due to light limitation of phytoplankton growth. Much of the microbial activity is therefore associated with particles. While the particle-associated bacterial community has been examined in the Columbia River estuary, major consumers of bacteria in the estuary are not well characterized. Molecular analysis of microbial eukaryotic (protist) assemblages in the Columbia River indicates that during April the heterotrophic flagellate, *Katablepharis*, dominates the estuary, comprising over 50% of 18S rDNA clone libraries in both 2007 and 2008. No other protist during the two-year time series was found at such high relative abundance. Other events occurring in the spring,
such as an upriver diatom bloom and spring runoff, deliver organic matter to the estuary that could fuel its presence. *Katablepharis* is a heterotrophic flagellate that is known to be both a primary consumer and bacterivore associated with particles in other freshwater and marine systems. To our knowledge, this is the first report of *Katablepharis* as the dominant member of an estuarine protist population. Sequence analysis of the rRNA gene of the *Katablepharis* strain found in the Columbia River estuary (referred to as *Katablepharis* CRE) reveals a 332 base pair variable region in the long subunit (LSU) region of the gene. This variable region appears to be unique to *Katablepharis* CRE, and does not align with any sequenced katablepharids or other eukaryotes in the sequence databases. To better resolve the spatial and temporal distribution of *Katablepharis* CRE in the Columbia River estuary, a specific molecular probe for *Katablepharis* CRE was developed based on this variable region of its LSU rRNA gene, and quantitative PCR (qPCR) was performed on water samples collected at different locations and depths throughout the estuary from 2007-2011. Due to its high abundance, *Katablepharis* could play an important, previously unseen role in the fate of spring phytoplankton organic matter.

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**Balancing Survival & Resistance: Faculty of Color in Euro-American Schools of Nursing**


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This poster represents findings from a grounded theory study of the experiences of 23 faculty of color (FOC) at predominately Euro-American Schools of Nursing. Findings indicated that a dominant group of individuals, sometimes referred to as the “Good Old Girls,” exerted “Patterns of Exclusion & Control” as a way of controlling the influence of FOC. FOC, in turn, learned to “Balance Survival & Resistance” by learning the rules of the game and engaging (and disengaging) strategically in ways that optimized their chances for success in the academic environment and beyond. The study also revealed the importance of mentoring and the social support of white allies in fostering an inclusive climate that promotes diversity.

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**Understanding Empiric Antibiotic Prescribing Among Primary Care Residents**

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**Background:** Despite widespread support for judicious antibiotic prescribing, inappropriate and excessive prescribing persists. Better understanding of clinicians’ decision-making process in empiric antibiotic prescribing would facilitate development of effective interventions.

**Objective:** To describe (1) medical residents’ familiarity with and application of hospital antibiograms and (2) information sources used during empiric antibiotic selection.

**Methods:** A survey has been developed for distribution to Family Medicine Pediatrics and General Internal Medicine residents. Survey questions explore residents’ familiarity with and use of hospital antibiograms. The survey also presents infectious disease scenarios and asks residents to report which information sources they would use to select
empiric antibiotic therapy as well as the targeted pathogens. Responses will be summarized using descriptive statistics and residency programs will be compared using the chi-square test.

**Results:** Survey data from the Internal Medicine program are pending. Among the 45 (96% response rate) respondents, the majority were residents (71%), female (71%), and had a primary affiliation with Pediatrics (54%). Fifty-six percent of respondents (25/45) reported being aware of the hospital antibiograms and feeling comfortable using them for empiric selection of therapy. Of those aware of the antibiograms, only 53% (18/34) knew how to access them yet 76% (26/34) reported having utilized them as resource for prescribing. In response to questions regarding relative antibiotic costs, 47% (21/45) of respondents reported that cost does not impact their empiric antibiotic choice for inpatients whereas only 7% (3/45) reported that it does not impact their choice for outpatients.

**Conclusion:** Our preliminary data indicate that antibiogram use by primary care residents is limited. Future research is needed to identify the generalizability of these findings to non-resident clinicians.

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**IGF-1 Does Not Restore Cell Cycle Activity In Fetal Sheep Cardiomyocytes Following Placental Insufficiency**

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**Aim:** Placental insufficiency induced by umbilicoplacental embolisation (UPE) in the fetal sheep suppresses cell cycle activity and maturation in cardiomyocytes (Louey et al., 2007), but the capabilities of the heart to recover from this insult is not known. The aim of this study was to determine the degree to which an undergrown heart can increase cell cycle activity (and hence cell number) in response to IGF-1, a known stimulant of cardiomyocyte proliferation.

**Methods:** Three groups of fetal sheep were studied between 115 and 130days gestational age (dGA, term ~145d GA): (1) controls, (2) 15d of UPE, (3) 10d of UPE followed by 5d of IGF-1 (UPE-IGF1). During UPE, non-soluble microspheres were injected into the umbilicoplacental circulation daily to induce fetal hypoxemia. We have previously shown 715µg/day Long R3 IGF-1 (~6.6µg/kg/hr) increases cardiomyocyte proliferation (Sundgren et al., 2003); given decreased body weight in UPE fetuses, a lower dose (560µg/day, 6.7±0.3µg/kg/hr i.v.) was used in the current study. Postmortem, fetal hearts were enzymatically dissociated. Isolated cardiomyocytes were analyzed for maturational state (% of binucleated myocytes) and cell cycle activity (Ki-67 staining). For clarity only left ventricular data are reported here. Data are expressed as mean±SEM.

**Results:** After 10d, all UPE fetuses were hypoxemic (PaO₂: 15±0.8mmHg) compared to controls (PaO₂ 20±1mmHg, p<0.01). UPE-IGF1 fetuses remained hypoxemic despite no UPE during the final 5d of study, and became hypoglycemic (0.3±0.04mmol/L) compared to control (0.8±0.03mmol/L, p<0.01) and UPE (0.6±0.03mmol/L, p<0.01) fetuses. Body weights of 15d UPE fetuses were 24% lighter (p<0.05) than control and UPE-IGF1 fetuses; heart weights (g/kg body) were not different between groups. As previous, 15d UPE suppressed cardiomyocyte cell cycle activity (3±1%) and binucleation (35±2%, p<0.05) compared to controls (8±1% Ki-67 positive; 50±4% binucleation). IGF-1 therapy failed to fully restore cell cycle activity (5±1%). Cardiomyocyte length was increased in UPE-IGF1 hearts, likely contributing to in the increased in heart weight in this group.
Conclusions: IGF-1 failed to ameliorate the negative effects of placental insufficiency on cardiomyocyte number. Perhaps of greater concern is the worsening hypoglycemia in response to IGF-1 despite the cessation of daily embolisation, which diminishes the appeal of IGF-1 alone as a viable therapy for correcting deficits in cell number in undergrown hearts.

Conflict of interest among clinical practice guidelines authors

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Background: Conflict of interest (COI) among clinical practice guideline (CPG) authors is an important potential source of bias for recommendations. A recent report of the Institute of Medicine (2009) suggests that the updated descriptive information is needed on these conflicts.

Objective: To describe the conflicts of interest of authors of CPGs in the National Guideline Clearinghouse (NGC) and to identify predictors of disclosures and conflicts among organizational and guideline characteristics.

Methods: A random sample of 250 CPGs was obtained from the NGC on 11/22/2010. For each CPG, data were abstracted on the funder, author disclosures, and characteristics of the CPG, organization, and publication venue. Regression techniques were used to predict the presence of COI among authors.

Results: In the final model, whether the CPG has a disclosure was significantly associated with whether the guideline was published in a journal and impact factor of the journal (p=0.001), whether the organization has a policy for COI (p=0.023) and the country of the organization (p=0.022). Compared to CPGs not published in a journal, CPGs published in a journal with an impact factor less than 5 are less likely to have a disclosure (OR 0.312; 95% CI 0.129, 0.752). CPGs published in a journal with an impact factor more than 5 tended to be more likely to have a disclosure, but the association was not significant. CPGs developed by an organization with a policy for COI are more likely to have a disclosure than those developed by an organization without a policy (OR 4.24; 95% CI 1.217, 14.772). In addition CPGs developed by a Canadian or European organization are more likely to have a disclosure compared to those developed by a US organization (p=0.033 and 0.018, respectively)

Conclusions: Funding of CPGs by private specialty organizations is common, and financial COI among CPG authors are increasingly widespread. A significant percentage of CPGs do not provide disclosures, especially those not published in a journal. In order to be trustworthy and transparent, efforts are needed to achieve accurate, relevant, and readily accessible disclosures in all CPGs.

Sexual Dimorphism Of Cardiomyocyte Size In Adult But Not Fetal Sheep

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Background: The capacity for cardiomyocyte enlargement (necessary for adaptive cardiac growth) influences progression of cardiac disease, a process that differs between men and women.
Objective: Determine how cardiomyocytes differ between the sexes in adulthood, and if those differences are present near birth.

Methods: Adult (male n=4; female n=4) and near-term fetal (138±1 of 145 days of gestation; male n=8; female n=9) sheep hearts were enzymatically dissociated. Cellular dimensions were measured from fixed isolated cardiomyocytes. In cells from fetal hearts, proliferative index (measured by Ki-67 immunoreactivity) and maturational index (measured by binucleation) were quantified.

Results: Adult male sheep were significantly larger than adult females (105±2 vs 69±8 kg) and had larger hearts (444±50 vs 301±40 g). In adult sheep, males had significantly longer (171±9 vs 139±7 μm) and wider (45±3 vs 38±4 μm) left ventricular cardiomyocytes, and longer (176±15 vs 142±20 μm) right ventricular cardiomyocytes. In contrast, neither body weight, heart weight, nor cardiomyocyte dimensions were found to be different between male and female fetuses. Additionally, the proliferative index and cardiomyocyte maturation were similar between male and female fetuses.

Conclusions: Differences in cardiomyocyte dimensions in adult sheep reflect sexual divergence in body (and consequently heart) weights. In contrast, body weight, heart weight, and cardiomyocyte parameters are similar between males and females in late gestation. These results suggest that cardiomyocyte growth in the fetus does not presage adult sexual dimorphisms. Sex differences in cardiomyocyte morphology that are important for adaptive growth in the adult may arise during post-natal maturation.

Compliance with Annual Diabetic Eye Exams Survey (CADEES): Preliminary Results

CR Sheppler, WE Lambert, CL VanAlstine, SL Mansberger

PURPOSE: To evaluate the psychometric properties of the Compliance with Annual Diabetic Eye Exams Survey (CADEES), a new instrument designed to identify the factors related to adherence with yearly eye exams. While diabetic retinopathy is the leading cause of blindness in adults aged 20-74, early diagnosis and treatment can reduce the likelihood of vision loss by 90%. Unfortunately, fewer than 50% of those diagnosed with diabetes receive annual eye exams.

METHODS: The 45-item survey was developed using the framework of the Health Belief Model (HBM). The survey measured health beliefs, demographics, and eye exam history. Participants rated the extent to which they agreed with each health belief statement using a Likert scale.

RESULTS: Participants (n=127) were 47% male and ranged in age from 24 to 83 years (M=56.3, SD=12.1). Reliability analyses on items created to measure specific HBM constructs showed acceptable Cronbach’s alphas (> .70) for two of the six constructs (seriousness and barriers). A principal components analysis supported the presence of four distinct factors, comprising 37% of the variance. Three of the four factors were identifiable within the constructs of the HBM (seriousness, susceptibility, and barriers); however, factor scores did not predict adherence. Based on univariate results comparing those who had an eye exam in the past year with those who had not, 15 health belief items were identified as potential predictors for adherence. A logistic regression model containing these items classified cases with 78% accuracy (Nagelkerke R-Square = .338). The strongest predictors were beliefs about whether diabetic eye disease can be seen with an eye exam (p=.01) and whether insurance covered most of the eye exam cost (p<.01). A logistic regression model containing demographic variables (age, sex, race, insurance status, hemoglobin A1c, and years diagnosed with
diabetes) showed that adherence was associated with a longer duration of diabetes (p=.02) and lower hemoglobin A1c levels (p=.01).

**CONCLUSIONS:** Further research is needed to confirm these preliminary results, and to determine whether the Health Belief Model is the best framework to use when attempting to predict compliance with annual eye exams.

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**Cost-Effectiveness of Telemedicine Screening for Diabetic Retinopathy**

VanAlstine, CL; Kymes, SM; Stwalley, D; Sheppler, CR; Mansberger, SL

**Purpose:** Previous studies have demonstrated that using telemedicine to provide diabetic retinopathy screening exams is cost-saving from the perspective of the provider. We evaluate the cost-benefit of this method from the perspective of the patient, using time as the metric for cost.

**Methods:** We developed a cost-effectiveness survey, which was administered to participants enrolled in a clinical trial. In this trial, screening for diabetic retinopathy was performed using either a nonmydriatic camera placed at the primary care clinic, or by the participant’s eye care provider. Retinal images were taken by a technician and forwarded electronically to an ophthalmologist for evaluation. Imaged participants found to have moderate diabetic retinopathy or worse were referred to an eye care provider for follow up care. We modeled this care process (see figure for model) and compared the time reported by patients using the telemedicine model to the time to obtain screening from their eye care provider. From this we estimated the break-even pretest probability of Diabetic Retinopathy that would make screening by the primary care clinic cost-saving from the patient’s perspective.

**Results:** Participants (n=127) were 47% male and had a mean age of 56 years. In regard to transportation, 61% of participants drove themselves and 28% had someone else drive them to their appointment (the balance walked or used public transportation). The median time to travel to the primary care clinic and obtain a diabetic retinopathy screening image was 40 minutes; the median travel time to and length of an eye care provider visit was 60 minutes. The model showed a cost-savings from the patient perspective, so long as the pretest probability of moderate or worse diabetic retinopathy was less than 33%. Data from the clinical trial shows that 7.7% of participants were diagnosed as having moderate or worse diabetic retinopathy.

**Conclusions:** Our preliminary results demonstrate that in this population, the use of telemedicine to provide diabetic retinopathy screening exams is time saving from a patient perspective. Future work will examine financial impact, long term time savings, the impact of incidental ocular findings, and the importance of patient specific pretest vision status.
CLIMP-63 is a gentamicin-binding protein involved in drug-induced cytotoxicity

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Aminoglycoside-induced nephrotoxicity and ototoxicity is a major clinical problem. To understand how aminoglycosides, including gentamicin, induce cytotoxicity in the kidney proximal tubule and the inner ear, we pulled down gentamicin-binding proteins (GBPs) from mouse kidney cells with gentamicin-agarose conjugates, and identified the GBPs by mass spectrometric analysis. Among several GBPs specific to kidney proximal tubule cells, cytoskeleton-linking membrane protein of 63 kDa (CLIMP-63) was the only protein localized in the endoplasmic reticulum (ER), and was co-localized with gentamicin-Texas Red (GTTR) conjugate after cells were treated with GTTR for 1 hour. In Western blots, kidney proximal tubule cells and cochlear cells, but not kidney distal tubule cells, exhibited a dithiothreitol (DTT)-resistant dimer band of CLIMP-63. Gentamicin treatment increased the presence of DTT-resistant CLIMP-63 dimers in both kidney proximal (KPT11) and distal (KDT3) tubule cells. Transfection of wild-type and mutant CLIMP-63 into 293T cells showed that the gentamicin-dependent dimerization requires CLIMP-63 palmitoylation. Transfection with CLIMP-63 siRNA enhanced cellular resistance to gentamicin-induced toxicity in KPT11 cells. Thus, the dimerization of CLIMP-63 is likely an early step in aminoglycoside-induced cytotoxicity in the kidney and cochlea. Gentamicin also enhanced the binding between CLIMP-63 and 14-3-3 proteins. 14-3-3 siRNA transfection enhanced cellular resistance to gentamicin-induced cytotoxicity, and suppressed caspase-3 activation. Therefore, CLIMP-63 and 14-3-3 proteins are involved in gentamicin-induced cytotoxicity.
Mapping Neural Response to Alcohol Using Optical Imaging Techniques

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The use of functional imaging techniques to study drugs of abuse, especially alcohol, is in its infancy and their use in the mouse brain is severely limited by its small size.

We propose the use of two complementary optical imaging techniques, laser speckle imaging (LSI) and Doppler optical microangiography (DOMAG) to quantitatively and non-invasively map changes in cerebral blood flow and blood volume down to the level of individual vessels (10 micrometers) in the mouse brain. Our preliminary LSI and DOMAG mouse data show that alcohol decreases arterial and venous flow. However, several biophysical and computational issues must be addressed to realize the full potential of these techniques. For example, blood vessel size could differentially impact alcohol induced changes and these changes may be region specific. Furthermore, analysis of the imaging data is computationally intense. Thus only a fraction of the vessels from our dataset have been analyzed.

The purpose of this work is to increase the efficient analysis of optical imaging data in these techniques and to combine whole brain qualitative LSI data with quantitative regional DOMAG data, demonstrating their use in determining the differential response of mice known to have different genetically mediated behavioral responses to alcohol.

Effectiveness of a multi-component, community-based noise-induced hearing loss and tinnitus prevention intervention in an Oregon American Indian community


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The Centers for Disease Control and Prevention reported that American Indian/Alaska Natives (AI/AN) have significantly more moderate to severe hearing loss than other ethnic groups in the U.S. The exact cause is unknown. Survey studies indicate that AI/AN individual’s experience significant noise exposure placing them at risk for noise-induced hearing loss (NIHL) and tinnitus.

Incorporating the established and effective interventions of Oregon’s Dangerous Decibels program, a community-based, multi-component noise-induced hearing loss and tinnitus prevention program called Listen for Life was created and taken to a Native community in the Northwest. This whole-community strategy included a media campaign (incorporating local newspapers and radio for articles, ads, interviews, and public service announcements plus a YouTube video on the tribal website of interviews with several elders and youth in the community), a classroom program (45-minute presentation to fourth and fifth grade students on the reservation), online games (eight activities that are part of the Dangerous Decibels Virtual Exhibit and shown to be effective as a stand-alone intervention), and a community event (an evening event for the community to have dinner, to learn about hearing protection, and to watch
as the elementary students show what they learned in the classroom) with the aim of educating the families as well as the children in schools to protect hearing. Using pre, post, and follow-up questionnaires, elementary students were presented the Dangerous Decibels program in school. Data analysis shows a significant improvement in sustained improvements knowledge, attitudes, and intended behaviors regarding exposure to dangerous sounds. Sustainability was demonstrated by an average increase of 18% across all categories at four months after the interventions and by evidences that the community independently incorporated lessons of hearing health promotion on an ongoing basis during the following year.

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**The effect of moderate and excessive alcohol exposure on male experimental stroke outcomes in mice is androgen-independent**

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**Background and Purpose:** Heavy alcohol intake is a major stroke risk factor while moderate alcohol use may offer neuroprotective benefits. We have previously shown that regardless of sex, moderate ethanol (EtOH) exposure decreases while excessive EtOH exposure increases cortical infarct volume following experimental stroke in mice. Furthermore, the protective effect of acute moderate EtOH exposure in female cortex is lost in ovariectomized mice, indicating female sex steroids (estrogen and progesterone) may be involved in the female response to acute moderate EtOH exposure and experimental stroke. However, little is known about how male sex hormones (androgens) might impact on the effects of varying levels of alcohol exposure on male stroke outcomes. We therefore explored the role of androgens in the male response to EtOH exposure and experimental stroke.

**Methods:** Young adult male mice were castrated (CAST) 7 to 8 days before EtOH exposure and experimental stroke to reduce endogenous androgen levels. Intact and CAST males were given 0.25 g/kg (moderate dose, n=11) or 2.5 g/kg (excessive dose, n=10-11) EtOH i.p. 1 h before 45 min of middle cerebral artery occlusion (MCAO). These EtOH doses mimic moderate (1-2 drinks daily) or excessive (>4 drinks daily) alcohol intake in men based on blood ethanol concentrations. Control male and CAST mice (n=10-11/group) were given vehicle (saline, 0.015 mL/g) i.p. 1 h before MCAO. Brains were collected at 72 h of reperfusion. Infarct volumes (% contralateral cortex or striatum) were determined by digital image analysis of 2 mm thick coronal brain slices stained with 2,3,5-triphenyltetrazolium chloride.

**Results:** In contrast to corresponding saline treated mice (EtOH vs. saline), acute moderate EtOH exposure decreased cortical infarct volumes in intact (41 ± 3% vs. 54 ± 1%) and CAST (23 ± 5% vs. 34 ± 5%) males. Following acute excessive EtOH exposure, cortical infarct volumes were increased in male (62 ± 2% vs. 54 ± 1%) and CAST (47 ± 4% vs. 34 ± 5%) mice compared to corresponding saline treated mice.

**Conclusions:** Clinically, our preliminary data suggest that moderate alcohol consumption may positively, while excessive alcohol consumption may negatively, alter stroke outcomes in men independent of hormonal status. However, future studies will need to address the role of androgens in aging male brain as gradual androgen loss during aging (andropause) may not have a comparable effect on the aged male brain's response to EtOH exposure and experimental stroke as an abrupt loss of androgens from castration did in the young male brain.
Increased striatal iron accumulation in methamphetamine users

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Preliminary evidence from animal and in vitro models suggests that brain iron accumulation plays a role in methamphetamine (MA) toxicity, both as a biomarker of damage and a potential source of oxidative stress. This study utilized magnetic resonance imaging (MRI) to investigate brain iron levels in human subjects with a history of MA dependence. The presence of iron shortens the transverse relaxation time constant (T2) of nearby water protons causing a loss of signal intensity (SI) on T2-weighted images. It was predicted that former MA users would have increased iron concentrations as measured by decreased T2-weighted SI values within basal ganglia structures compared to aged match healthy controls.

**Methods:** T2-weighted images from 37 individuals with a history of MA dependence and 33 healthy control subjects were analyzed. MRI data were acquired on a Siemens 3T TIM Trio and included high-resolution, T2-weighted, whole-brain 3D MPRAGE images and axial 2D double spin echo EPI T2 weighted images. Bilateral, subcortical regions of interest (ROI), including caudate, putamen, and pallidum, were identified on the MPRAGE images using FMRIB's Integrated Registration and Segmentation Tool (FIRST). These identified ROI's were transformed to the individual’s T2-weighted image space and mean signal intensity values within each ROI were obtained. Mixed effects linear models were constructed to assess the effects MA use on T2-weighted signal intensities in each ROI. Iron content estimates were calculated for control subjects using published regression equations from postmortem data based on age and subcortical region. Voxel-wise analysis of the T2-weighted images was carried out using tools from FSL and AFNI as an unbiased confirmation of the ROI approach.

**Results and Discussion:** Individuals with a history of MA dependence had reduced T2 signal intensity values in the caudate (F(1,67)=6.04, p=0.0166) and putamen F(1,67)=8.42, p=0.005), but not in the pallidum (F(1,67)=1.01, p=0.31). These findings were further confirmed with a voxel-wise analysis approach that identified reduced T2-weighted signal intensity measurements in the right striatum of MA users. Iron content estimates obtained by applying regression formulas based on postmortem data of iron content by brain region and age significantly correlated with the measured T2-weighted signal intensity values in control subjects (R^2=0.88, p<0.0001). T2-weighted signal intensity measurements also demonstrated a strong effect of age in the caudate (F(1,67)=22.08, p<0.0001) and putamen (F(1,67)=17.22, p<0.0001), consistent with the established age-related increase in iron seen in these subcortical areas. Taken together, these results provide the first evidence that iron accumulation is increased within the striatum of human MA users.
**Poster Session 3 | 2 - 3 PM Wednesday, May 9**

**Alison Ting** | Optimization of rhesus macaque ovarian tissue vitrification in a closed system

**Allison Lindauer** | Managing Behavior Symptoms of Dementia: What Can We Learn from Black Family Caregivers?

**Andrew Terker** | Adrenergic regulation of the renal sodium chloride cotransporter

**Anke Vermehren-Schmaedick** | Quantum Dots to study live, molecular dynamics of ligand-receptor complexes in neurons

**Anna Wilson** | Adolescents at risk for chronic pain

**Anna Wilson** | Overview of research activities in the Child Development and Rehabilitation Center's Division of Psychology

**Becky Proskocil** | Airway hyperreactivity is potentiated in ovalbumin sensitized guinea pigs treated with chlorpyrifos, but not diazinon or permethrin, and is IL-5 dependent.

**Brett Dufour** | Assessing a global therapeutic strategy for Huntington's Disease: Systemic RNAi therapeutics for central and peripheral symptoms

**David Stein** | Inhibition of dengue virus infections in vitro and in vivo by an siRNA targeting highly conserved sequence.

**Doria Thiele** | Maternal vitamin D transfer to infants via breast milk: An evidence-based literature review.

**Elizabeth Brass** | Maternal Obesity Suppresses Male Dominance of Placental Fatty Acid Uptake

**Eric Earl** | Novel Quantitative Ischemic Lesion Analysis Method in a Non-Human Primate Stroke Model

**Henryk Urbanski** | The Secrets of Healthy Aging: Gaining Insights from Nonhuman Primate Research

**Hongzhe Li** | Gentamicin uptake in acoustically traumatized cochleae

**Jeremy Woods** | Development and application of a polymicrobial, in vitro, biofilm wound model.

**Jesse Lorton** | New eosinophils recruited to the lungs of sensitized guinea pigs after ozone exposure persist in the airway tissues

**Jessica Hebert** | Promoter DNA Hypomethylation in the Fetal Kidneys of Intrauterine Growth Restricted Mice

**Jessica Hebert** | Fetal Programming of White Coat Hypertension in Growth Restricted Male Mice

**Jessica Stanley** | Medical Termination of Pregnancy in Cynomolgus Macaques
Katie Schenning | Ischemic Injury to Glomerular Endothelium: Proposed Mechanism of Hyperglycemic Protection

Kentaro Yomogida | Cell penetrating recombinant Foxp3 protein enhances Treg function, suppresses Th17 cells and ameliorate arthritis

Lance Johnson | Diet-induced Insulin Resistance, ApoE, and Cognitive Function

Laurie King | Comparison of individual, group and home agility boot camp (ABC) exercise program for people with Parkinson's disease?

Maria Manczak | Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage

P. Hemachandra Reddy | Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: implications for selective neuronal damage

P. Hemachandra Reddy | Mitochondria-targeted catalase reduces abnormal APP processing, amyloid beta production, and BACE1 in a mouse model of Alzheimer's disease: Implications for neuroprotection and lifespan extension

P. Hemachandra Reddy | Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease

P. Hemachandra Reddy | Gene Expression Profiles of Mitochondrial Structure/Function and Amyloid Beta Production in Brain Tissues from Alzheimer's Disease Patients: Implications to Neuronal Damage and Cognitive Decline

Rebecca Tempel | Tularemia Vaccine Trials in Nonhuman Primates

Sarah Wicher | Testing whether sustained or bolus delivery of BrdU labels dividing inflammatory cells

Sheetal Bodhankar | Indispensable role of B cells in mediating protective effects of estrogen against EAE

Sudeshna Dutta | The role of Swiss Cheese, the Drosophila homologue of Neuropathy target esterases, in glia.

Tao Wu | Optogenetic control of mouse outer hair cells

Tessa Steel | Environmental Food Balance: The Neighborhood Context of BMI and Diet Among Landless Northwest American Indians

Tetsuhiro Fujiyoshi | Memory dysfunction following cardiac arrest and cardiopulmonary resuscitation in mice is associated with hippocampal inflammation

Tosha Zaback | Summative Evaluation of a Tribal Telemedicine Project

Tracy Zitzelberger | Intelligent Systems for Detection of Aging Changes

Valerie Conrad | TLR4- and TLR9-induced neuroprotection against stroke is mediated by IRF signaling
Optimization of Rhesus Macaque Ovarian Tissue Vitrification in a Closed System

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Objective: Clinical practice for ovarian tissue vitrification uses an open system to allow loading of minimal vitrification solution (VS) for maximum cooling rate, but direct tissue contact with liquid nitrogen (LN2) poses a safety risk of cross contamination. Optimal conditions were determined for vitrification of macaque ovarian tissue in a closed system using sealable high security straws.

Materials and Methods: Macaque (N=4) ovarian cortical pieces were exposed to 1/8, 1/4, 1/2, and 1X VS for 3, 5 or 10 min/step at 37C, room temperature (RT), or 4C, loaded into straws with 1ml VS, heat sealed and cooled in LN2 or LN2 vapor. Various concentrations of permeating (1:1; glycerol, ethylene glycol) and non-permeating (polyvinyl alcohol, polyvinylpyrrolidone, and polyglycerol) cryoprotectants (CPA) were tested as VS. To warm, straws were submerged into a 40C water bath or left at RT then moved into a water bath. CPA was diluted with 1, 0.5, 0.25M sucrose. After histological analysis, tissues vitrified with optimal conditions were cultured with bromodeoxyuridine (BrdU), or used for encapsulated 3-dimensional secondary follicle culture.

Results: Dense stroma and intact preantral follicles were observed using 54% total permeating CPAs with 0.8% polymers, equilibration for 3min at 37C, 5min at RT, or 10min at 4C, cooling in LN2 vapor (0.48±0.02C/sec) and a 2-step warming. Higher cooling and warming rates led to fracturing. BrdU uptake was evident in granulosa, theca and some stromal cells post-thaw. Secondary follicles from vitrified tissues survived (56±8%), grew, and formed an antrum (55±7%) during 5 weeks in vitro.

Conclusions: This is the first demonstration of functional preantral follicles from primate ovarian tissue vitrified in a closed system and offers a safe and efficient method for fertility preservation. Examination of tissue function after heterotopic transplantation is ongoing.

Support: NIH UL1RR024926, R01AHD058293, PL1EB008542, U54 HD018185, P51RR000163

Managing Behavior Symptoms of Dementia: What Can We Learn from Black Family Caregivers?

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Background: The incidence of Alzheimer’s disease in the United States is rapidly growing. In lockstep with this growth is the increased demand on families to provide care to those with dementia. In order to cultivate both personal satisfaction for caregivers and successful management of the aging population in our society, it is essential to identify and use strategies that foster successful adaptation to the caregiving role. Studies on ethnic differences in caregiving suggest that Black caregivers fare better in the caregiving role as indicated by lower depression scores and better life satisfaction scores than White caregivers. What can be learned from Black family caregivers about effective stress and burden management when caring for a person with dementia?

Purpose: The aim of this study is to identify the strategies Black family caregivers used to manage their care-recipients’ behavioral symptoms of dementia. This is a secondary analysis of interviews with Black family caregivers. Earlier research on strategies used by White caregivers identified eleven typical interventions. In this secondary analysis, we expect to find that Black family caregivers employed strategies similar to those used by White family caregivers. Furthermore, we expect to identify new strategies that would help explain how Black family caregivers manage challenging behaviors in their family members with dementia.

Method: 18 Black family caregivers living in Wisconsin were interviewed in the early 1990s. They were asked about how they managed the behavior symptoms of dementia in their care recipients. This current study uses content analysis to identify patterns of intervention types used by these caregivers.

Results: Preliminary findings suggest that these Black family caregivers employed similar management techniques for managing the behavioral symptoms of dementia as White caregivers. Common approaches to managing behaviors included using “convincing” (attempts to change what the care receiver thinks), “going along” (caregiver does not try to change behavior) and “help-seeking” (employing family or formal services for help). Unique approaches used by the family caregivers in this study included “ignoring” and the use of prayer.

Implications: This study adds to the current understanding of how families manage behavioral symptoms of dementia. In order to address the needs of millions of caregivers of persons with dementia, effective interventions for behavioral symptoms are needed. Identifying the strategies Black family caregivers use could inform the development of interventions that foster healthy family caregiver adaptation to the caregiving role.

Adrenergic regulation of the sodium chloride cotransporter

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Essential hypertension affects one billion individuals worldwide, and is believed to be the largest global contributor to premature death; it contributes half the global risk of stroke and ischemic heart disease. The prevalence of essential hypertension is rising, owing, in part, to changes in diet and activity. However, heritability estimates suggest a large genetic component (typically 30-60 percent attributable to genetics). While empiric treatments exist and are widely used clinically, they have substantial side effects and up to 20-30 percent of patients are resistant to these interventions. A major challenge in providing better treatments is our lack of knowledge regarding the molecular mechanisms driving the pathogenesis of essential hypertension.
The kidney’s part in blood pressure homeostasis has been well-studied over the past few decades. The sodium chloride cotransporter (NCC), an electroneutral salt transporter present along the distal convoluted tubule, is of particular importance in this regulation. The key role of NCC in blood pressure regulation is demonstrated clinically by the ability of thiazide diuretics, which inhibit NCC, to lower blood pressure. Additionally, pseudohypoaldosteronism type 2, a Mendelian disease characterized by hypertension and hyperkalemia, results from overactivity of NCC\(^3\). Despite these observations and intense investigation of the molecular regulation of NCC over the past 10 years, details remain unclear.

Using both in vivo and in vitro model systems, we have shown for the first time that NCC in regulated acutely by adrenergic stimulation. Specifically, kidneys from wild-type mice treated with norepinephrine experience acute (30 minutes post-treatment) upregulation of NCC activity as demonstrated through increased phosphorylation of the cotransporter. The timing of this effect precludes involvement of aldosterone, a known hormonal regulator of NCC that is also downstream of adrenergic stimulation. This effect can be reproduced with isoproterenol treatment (\(\beta_1\)- and \(\beta_2\)-adrenergic receptor selective agonist), but not with phenylephrine (\(\alpha_1\)-adrenergic receptor selective agonist). This suggests that \(\beta\)-adrenergic signaling regulates NCC activity along the distal nephron. Consistent with this, HEK293 cells transiently transfected with NCC also demonstrate increased phospho-NCC after stimulation of \(\beta_2\)-adrenergic receptors for 30 minutes, but not if \(\beta_1\)-adrenergic receptors are stimulated simultaneously or in isolation. Additionally, phenylephrine stimulation of these cells does not reproduce the effect. These data indicate that this novel regulatory mechanism of NCC proceeds through a \(\beta_2\)-mediated pathway.

References:

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**Quantum Dots to study live, molecular dynamics of ligand-receptor complexes in neurons**

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Growth factor ligands, such as brain-derived neurotrophic factor (BDNF), are highly expressed in the brain where they play key roles activating downstream signaling cascades that result in brain development, learning and memory. However, in spite of their clinical importance, the molecular mechanisms underlying their signaling and trafficking remain largely unknown since current technologies are population based, and do not offer visualization of individual receptor complexes to study their movements in time and space within cells. In order to better understand the spatiotemporal dynamics of ligand-receptor signaling, we use quantum dot technology to track individual ligand-receptor pairs inside live cells. Quantum dots (QDs) are bright, resistant to photobleaching nanocrystals that have been used as fluorescent probes for biomedical applications, making them ideal candidates for live animal targeting and imaging. We have recently developed and characterized QD-BDNF probes (biotinylated BDNF protein conjugated to streptavidin-QD655) that will help us elucidate the complex spatiotemporal organization of cells during...
signaling. In the present study, we have validated the QD-BDNF probe’s use in live cells by testing the specificity of its binding to their BDNF receptor TrkB, and QD-BDNF bioactivity, using in plate assays and cultured neurons. In addition, we have demonstrated that, compared to Alexa Fluor-labeled BDNF which shows faint fluorescence at concentrations in the nM range, our QD-BDNF probes allow us to quantify individual ligand/receptor complexes at concentrations in the pM range. Some of the functional applications of our QD-BDNF probes so far include localizing the ligand/receptor complex within the cell at different time points, and tracking their translocations within the live neuron with high spatial and temporal resolutions.

Adolescents at risk for chronic pain

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Background & Aims. Family history of chronic pain increases the risk for the development of chronic pain in children and adolescents. This ongoing study will identify risk and protective factors associated with pain reactivity and somatic symptoms in children who are living with a biological parent with chronic pain. The long-term goal is to prevent the development of adult chronic pain and related disability. The first aim is to examine individual and parental correlates of pain and somatic symptoms, physical functioning, and psychological functioning within two groups: 1) children of adult caregivers with chronic pain, and 2) children of healthy parents without chronic pain. The second aim is to follow this cohort of children over a one-year period in adolescence to identify risk and protective factors that predict pain and somatic symptoms longitudinally and examine the role of child sex. Parental, psychological, and health-related risk factors will be examined. The third aim is to compare parental responses during laboratory pain tasks in the two groups to better understand the influence of parent behaviors on children’s pain experiences. This innovative study utilizes clinical and laboratory pain assessment techniques to provide new information about an at risk population, and will inform the development of preventive interventions.

Preliminary Results. As of February 2012, 59 parent-child dyads (118 participants total; n=28 parents with chronic pain; n=31 healthy parents) have been enrolled and have completed baseline data collection. Adolescent participants are 11-15 years of age (M age=13.76 years), 60% female, and child age and sex is evenly distributed across the two study groups. Preliminary analyses conducted with available data indicate that the frequency of pain experienced by adolescents who have a parent with chronic pain is significantly higher than the frequency of pain experienced by adolescents who have a healthy parent, t(56) = -2.78, p = .008, with 37% of adolescents who have a parent with chronic pain reporting pain occurring once per week or more frequently, compared to just 16% of adolescents who have healthy parents. Additional preliminary results on baseline pain prevalence, psychological functioning, and laboratory pain responses in the two groups will be presented at Research Week.

Funded by: NIH/NICHD, K23HD064705 (PI: Wilson)

Overview of Research Activities in the Child Development and Rehabilitation Center’s Division of Psychology

Anna C. Wilson PhD*, Kurt A. Freeman PhD, Michael A. Harris PhD, Danny C. Duke PhD, Sage Saxton PsyD, Kimberly Guion PhD
Faculty members in the Division of Psychology in the CDRC are involved in a range of clinical research activities. These studies largely focus on the health and psychological well-being of children and adolescents with chronic health conditions and developmental disabilities. This poster will highlight ongoing individual and collaborative research activities in the Division, and outline areas of faculty research expertise. Providing information to the larger OHSU research community about the role that pediatric psychologists play in collaborative research may stimulate future collaborations across the university. Projects that will be highlighted include:

**National Spina Bifida Registry Project** (PI: Freeman). The goal of this project is to contribute to a national database on outcomes for individuals with spina bifida through systematic and longitudinal data collection. Data are already being used to investigate variability in outcomes across clinical programs and identify variables that may account for variability.

**Improving Health Through Skype™: Family-Based Intervention for Teens with Poorly Controlled Diabetes** (Co-PI’s: Harris & Freeman, Co-I: Duke). This is an American Diabetes Association funded study examining the use of internet-based video-conferencing (Skype™) to deliver an empirically supported family-based intervention to improve adherence in youth (ages 12-19) with type 1 diabetes.

**Type 1 Diabetes Grows Up.** (PI: Jennifer Raymond, MD, Pediatrics; Co-I’s: Duke & Harris) The focus of this ADA-funded investigation is to improve the transition of youth with type 1 diabetes from pediatric to adult care through a provider-based learning protocol. Targeted is optimizing the transition process and clarifying responsibility for delivering information that is important during the developmental period corresponding with transition to adult care.

**Research on Smith Lemli-Optiz Syndrome** (PI: Robert Steiner, MD, Pediatrics; Co-I: Freeman). Dr. Freeman collaborates on two NIH-funded projects focused on behavioral phenotyping of individuals with SLOS, investigation of natural history of aberrant behavior in this population, and investigation of response of aberrant behavior to dietary and medical intervention.

**Adolescents at Risk for Chronic Pain** (PI: Wilson). This NICHD-funded study is part of Dr. Wilson’s K23 career development award. The goal is to characterize pain responses, psychological functioning, and clinical outcomes over time in two groups of 11-15 year old adolescents: youth who have a parent with chronic pain and youth with healthy parent(s). Dr. Wilson also has an MRF grant examining neurobiological characteristics of these youth (Co-I: Bonnie Nagel, PhD, Psychiatry)

**Improving Executive Function in Young Children Born Prematurely** (PI: Patricia Blasco, PhD, CDRC; Co-I: Saxton). This project aims to better identify executive functioning deficits in young children who were born prematurely, and identify methods to improve these executive functioning deficits in the preschool years.

**Parenting stress among parents of children with a cochlear implant** (PI: Saxton).

**Program evaluation of pediatric psychology inpatient consultation services** (PI: Guion).

**Pediatric pain clinic study** (PI: Wilson, Co-I: Jeffrey Koh, MD, Anesthesiology). 

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Airway hyperreactivity is potentiated in ovalbumin sensitized guinea pigs treated with chlorpyrifos, but not diazinon or permethrin, and is IL-5 dependent.

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Both organophosphorus (OP) pesticides and sensitization to allergen contribute to human asthma. We have previously shown that doses of the OP parathion that do not inhibit acetylcholinesterase (AChE) cause airway hyperreactivity. Guinea pigs sensitized to ovalbumin (but not challenged) are more sensitive to low doses of parathion and have increased airway hyperreactivity compared to non-sensitized animals. Treatment with the antibody to interleukin-5 (IL-5) that inhibits eosinophils prevents airway hyperreactivity in sensitized but not non-sensitized animals. Here we tested whether other OP pesticides (diazinon and chlorpyrifos) and a non-OP pesticide (permethrin) similarly enhance airway hyperreactivity in sensitized guinea pigs. Guinea pigs were sensitized with ovalbumin (20 mg/kg, i.p.) over 3 alternating days. Three weeks later, sensitized and non-sensitized guinea pigs were injected s.c. with 0.3 ml pesticide containing 0.75-75 mg/kg diazinon or 15-150 mg/kg permethrin resuspended in peanut oil or 0.7-70 mg/kg chlorpyrifos resuspended in peanut oil/ethanol. Control animals received equal volumes of either peanut oil or peanut oil/ethanol, respectively. Both non-AChE inhibiting and AChE inhibiting doses of OP pesticides were tested. 24hr after pesticide administration, guinea pigs were anesthetized, paralyzed, and ventilated, and bronchoconstriction was measured in response to electrical stimulation of the vagus nerves and to i.v. acetylcholine. Permethrin did not cause airway hyperreactivity in either non-sensitized or sensitized guinea pigs. Diazinon (75 mg/kg), significantly inhibited lung AChE activity and significantly increased airway hyperreactivity equally in sensitized and non-sensitized guinea pigs (probably mediated by AChE inhibition). Chlorpyrifos (0.7-7 mg/kg) caused airway hyperreactivity in both non-sensitized and sensitized animals that was not different with sensitization. However, a higher dose of chlorpyrifos (70 mg/kg) potentiated airway hyperreactivity significantly more in sensitized guinea pigs. Furthermore, antibody to IL-5 prevented chlorpyrifos-induced airway hyperreactivity in sensitized but not in non-sensitized guinea pigs suggesting, like parathion, eosinophils have a role in chlorpyrifos-induced airway hyperreactivity in sensitized guinea pigs. These data show that sensitization changes the mechanism of hyperreactivity to some but not all OP pesticides. Funding: NIH ES014601 and ES017592

Assessing a global therapeutic strategy for Huntington's Disease: Systemic RNAi therapeutics for central and peripheral symptoms

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Huntington’s disease (HD) is a rare, fatal, autosomal dominant neurological disorder affecting approximately 1 in 10,000 individuals worldwide. HD is caused by a trinucleotide (CAG) expansion in the gene HTT, which encodes the protein huntingtin. The mutation renders huntingtin with an abnormally long glutamine stretch at its N-terminus, which causes protein misfolding and inappropriate cellular processing. Mutant huntingtin leads to dramatic inclusion formation, cell...
loss and gliosis in numerous regions throughout the brain including the frontal and motor cortex, striatum, globus pallidus, substantia nigra, thalamus, hypothalamus and hippocampus. In addition, several peripheral tissues including the liver, skeletal muscle, pancreas and gonads show mutant-huntingtin inclusion formation and altered production of tissue specific hormones and enzymes. The resulting phenotype includes severe motor, cognitive, psychiatric, hormonal and metabolic disturbances. Symptom onset is typically in the fourth decade of life and invariably progresses to death within 10-15 years. Gene therapy for HD is currently undergoing pre-clinical trials, which utilizes RNA-interference (RNAi) as a method for suppressing the expression of the mutant huntingtin gene (mHTT). RNAi therapeutics involve transducing cells/tissues with a short-interfering RNA (siRNA) that are designed to silence expression of a specific endogenous gene. To confer long-term expression of these silencing constructs, they are expressed from safe, non-replicating viral vectors that are injected directly into tissue or brain regions of interest. Studies investigating gene therapy in HD have successfully utilized direct injections into the striatum, a brain region affected by HD that is involved in initiating and refining motor movements, to prevent neurodegeneration and associated symptoms. However, since mHTT is expressed ubiquitously and affects a variety of brain a peripheral tissues, RNAi therapy should target numerous affected regions to elicit the highest clinical benefit. In the current set of studies, we assessed the efficacy of a global therapeutic strategy for HD and hypothesized that systemically administered RNAi would more effectively treat HD symptoms by blocking the degenerative sequelae of mutant huntingtin expression throughout the CNS and peripheral tissues. We first assessed the biodistribution of an adeno-associated viral vector encoding green fluorescent protein (AAV9-GFP) delivered systemically (via the jugular vein) to transduce affected cells in the CNS and in peripheral organs in a mouse model of HD. We found that AAV9 vector transduces CNS regions associated with HD neurodegeneration including the cortex, striatum, globus pallidus, hippocampus, and hypothalamus. Peripheral tissues affected in HD were also robustly transduced, including the liver, testes, pancreas, and skeletal muscle. In a follow-up study, we tested the hypothesis that pre-treatment with 25% mannitol, which temporarily disrupts the blood brain barrier, will increase transduction of cells in the brain by AAV9-GFP. Mannitol pre-treatment increased transduction in regions associated with HD, including the striatum, globus pallidus and hypothalamus. In a third study, we tested the hypothesis that systemic delivery of AAV9 expressing RNAi constructs would reduce behavioral, hormonal and metabolic symptoms in our mouse model. Again, we observed robust AAV9-RNAi transduction in the liver, pancreas, skeletal muscle, and other tissues. AAV9-RNAi treatment successfully prevented alterations in several associated blood-borne metabolites including reduced urea nitrogen and ALT, and elevated triglycerides. Ongoing analyses are assessing the level of huntingtin suppression in tissues throughout the brain and periphery via qPCR. Despite observing a moderate level of transduced neurons and glia in the aforementioned brain regions, transduction levels were not sufficient in preventing the formation of neither motor nor weight disturbances inherent to the HD mouse model. A future study is planned to repeat the systemic administration of AAV9-RNAi using a higher viral titer, with the hopes that increasing the dose will transduce enough cells throughout the brain to prevention the formation of a motor phenotype. Together, these data support the feasibility of RNAi as a potential therapy for HD and are a first step towards achieving a global delivery strategy for this devastating neurological disorder.

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**Inhibition of dengue virus infections in vitro and in vivo by an siRNA targeting highly conserved sequence**

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The dengue viruses (DENV) consist of multiple genotypes representing 4 antigenically distinct serotypes. Various strains of DENV from each serotype can cause severe disease and dengue disease threatens public health in subtropical and tropical regions worldwide. No licensed antiviral to treat DENV infections is currently available and there is a pressing need for the development of novel therapeutics. We designed siRNA against DENV genomic RNA using sequence alignments to select target sites showing high conservation across all four serotypes. One siRNA (DC-3) produced >100-fold reduction in the titer of a representative strain from each DENV serotype in Huh7 cell culture evaluations. To determine the feasibility of targeting the same region in an in vivo setting, the DC-3 siRNA sequence was re-synthesized as “In Vivo Ready” siRNA (Ambion Inc.) and administered to AG129 mice at 1 day before and 1 and 3 days after infection with DENV-2 strain S221 in the presence of DENV-specific antibody (antibody-dependent enhancement model). Infected control groups included mock-treated and negative control siRNA treated mice. All infected control mice succumbed to DENV infection around day 5, whereas DC-3-treated mice lost little weight before day 12 and survived for over 15 days. These results suggest that DC-3 siRNA may represent a useful reagent for research against genetically diverse strains of DENV and a novel approach to the development of therapeutics against DENV infections.

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**Maternal vitamin D transfer to infants via breast milk: An evidence-based literature review**

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**Problem:** Maternal vitamin D insufficiency during lactation contributes to levels of vitamin D in breast milk that are inadequate to meet nutritional needs in infancy. Current evidence suggests that mothers who are vitamin D sufficient can pass optimal amounts of vitamin D to their infants, though recommendations for maternal vitamin D intake to achieve breast milk transfer necessary to meet infant needs are not clearly defined.

**Purpose:** The objective of this inquiry is to review the literature regarding evidence for best practices in achieving maternal vitamin D status that promotes vitamin D transfer from mother to infant exclusively from breast milk.

**Search Strategy:** PubMed and CINAHL databases were searched using the terms “Lactation” or “Breastfeeding” or “Milk, Human” and “Vitamin D”. The resulting papers were further limited to: published within the last 10 years, clinical trial, randomized controlled trial, humans, and English.

**Results:** The search yielded 13 studies, 4 of which provide level 1 evidence for maternal intake of vitamin D and the correlation with exclusively breastfed infants’ serum vitamin D level.

**Synthesis of Evidence:** There was a strong positive correlation between maternal vitamin D intake during exclusive breastfeeding and adequate infant serum vitamin D levels when maternal vitamin D intake reached 4000-6400 IU daily. There was strong evidence that the currently recommended dosage of 600 IU vitamin D intake daily was insufficient to achieve optimal levels of vitamin D for both mother and infant. No participants or infants in the studies had any markers of vitamin D toxicity (hypercalcemia, hypercalcuria). A gap in the literature exists regarding vitamin D intake and status of mothers and infants when vitamin D supplementation is initiated prenatally, as current evidence is limited by studies initiating supplementation during the postpartum period.
Implications for Practice: There is support to conclude that when maternal vitamin D intake is sufficient to achieve blood levels of > 45 ng/ml, vitamin D transfer via breast milk is adequate for infant needs. Recommendations for maternal vitamin D supplementation should be individualized, targeting the achievement of optimal maternal serum vitamin D and breast milk transfer. Further research is needed to refine the dose and gestational timing of maternal vitamin D supplementation to optimize breast milk transfer to infants.

Maternal Obesity Suppresses Male Dominance of Placental Fatty Acid Uptake

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OBJECTIVE: The fetus is dependent upon the placenta for its supply of long chain polyunsaturated fatty acids (LCPUFA), which are essential in fetal growth and development. Previous work suggests that males have greater fatty acid requirements and placental uptake than females during development. We hypothesized that male placental fatty acid uptake would be more sensitive to maternal body mass index (BMI) compared to females.

METHODS: Women were recruited upon admission to Labor & Delivery for cesarean section (n=25). At delivery, placental samples were collected for fatty acid uptake studies using ¹⁴C-labelled oleic acid (OA), arachidonic acid, (AA) and docosahexanoic acid (DHA) in placental explants. Uptake was calculated as nmol fatty acid/mg protein at 15 minutes. Results were stratified by fetal sex and maternal first trimester BMI (normal BMI<25 or obese BMI>26). Women with significant co-morbidities were excluded. Dichotomous outcomes were analyzed using 1 way ANOVA followed by Tukey post-hoc testing; p<0.05 was used to indicate statistical significance.

RESULTS: Placental fatty acid uptake of OA and AA in males of obese women was decreased 62% and 60% respectively compared to normal BMI women (p<0.001). In females, placental fatty acid uptake was not suppressed in the setting of obesity (Figure). Placental fatty acid uptake of OA and AA in females of normal BMI women was reduced 49% and 48% respectively compared to its male cohort (p<0.01). There was no difference in DHA uptake between sex or BMI groups.

CONCLUSION: Male placentas of normal weight women took up LCPUFAs at a higher rate than female placentas per gram of tissue. As predicted, placentas from males with obese mothers had suppressed uptake of two LCPUFAs, while uptake in females was unaffected by maternal BMI. This data suggest that males born to high BMI mothers may have inadequate LCPUFA acquisition. Males may pay a higher developmental price in the setting of maternal obesity.
Magnetic resonance imaging is commonly used to view cerebral stroke damage and to quantify the extent of infarcted tissue. Measuring cerebral stroke damage can be complicated and time-consuming, as past methodologies have involved selecting individual regions of interest, which can be subjective. Our newly developed semi-automated system for infarct analysis provides an efficient and robust alternative that can be applied to large data sets for more precise and less subjective delineation of water-containing lesions.

The Secrets of Healthy Aging: Gaining Insights from Nonhuman Primate Research

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In their natural habitat rhesus macaques (*Macaca mulatta*) rarely survive into old age, but with the care and attention that they receive at the ONPRC they can live well into their third decade of life. Consequently, macaque monkeys represent an outstanding animal model for research into the causes of normal and pathological human aging. Moreover, non-invasive technologies, such as bone densitometry, magnetic resonance imaging, and remote activity measurements enable ONPRC scientists to closely monitor age-related changes in cognition, sleep, behavior and other physiological functions.
An important objective of the ONPRC Biology of Aging Working Group is to establish biomarkers of normal and pathological aging that have clinical potential. Ongoing studies are laying the foundation for more effective and safer alternatives to hormone replacement therapy for postmenopausal women. Other studies are helping to determine if diet modification can retard age-related decline in immune function, learning and memory. Such multi-disciplinary approaches make effective use of a valuable animal resource and have the potential to disclose important inter-related mechanisms that underlie human aging.

One important finding from these nonhuman primate studies is that many hormones, such as melatonin, dehydroepiandrosterone (DHEA), leptin, and testosterone, have a circadian pattern of release which changes significantly during aging. Furthermore, gene profiling studies have shown the existence of circadian clock mechanisms in several peripheral organs, including the adrenal gland, liver, kidneys and spleen. Taken together, the results suggest that age-related changes in circadian physiology may severely undermine the body’s ability to function normally in a changing environment. Consequently, a deeper understanding of the underlying mechanisms should provide insights into lifestyle changes that promote healthy aging. They should also help with the development of novel therapies for age-related disorders, such as sleep perturbation and cognitive decline.

Gentamicin uptake in acoustically traumatized cochleae

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Exposure to intense sound or high doses of aminoglycoside antibiotics can increase hearing thresholds, induce cochlear dysfunction, disrupt hair cell morphology and promote hair cell death, leading to permanent hearing loss. When the two insults are combined, synergistic ototoxicity is evident both physiologically and morphologically. The underlying mechanism of this synergism remains unknown. One hypothesis is that sound exposure enhances hair cell uptake of cationic aminoglycosides, such as gentamicin. We found that prolonged sound exposure increased gentamicin uptake by murine hair cells. To preclude pathological changes induced by acoustic trauma, we examined whether acute concurrent narrow-band sound exposure increased gentamicin uptake over short periods. We observed negligible changes in hair cell uptake of gentamicin, implying that concurrent moderate-to-intense sound exposure does not directly increase gentamicin uptake. Additional experiments with prolonged sound exposure revealed increased gentamicin permeation across the strial blood-labyrinth barrier, suggesting that changes in strial physiology and/or integrity can lead to increased hair cell uptake of gentamicin, and thus may represent one mechanism of synergistic ototoxicity due to acoustic trauma followed by drug exposure. Therefore, the site of ototoxic synergy could reside in the stria vascularis, in the sensory epithelium, or in both locations.

Development and application of a polymicrobial, in vitro, biofilm wound model.

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Aims: The goal of this investigation was to develop an in vitro, polymicrobial, wound biofilm capable of supporting the growth of bacteria with variable oxygen requirements.

Methods and Results: The strict anaerobe Clostridium perfringens was isolated by cultivating wound homogenates using the drip-flow reactor (DFR), and a three-species biofilm model was established using methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas aeruginosa and Cl. perfringens in the colony-drip-flow reactor model. Plate counts revealed that MRSA, Ps. Aeruginosa and Cl. perfringens grew to 7.39 ± 0.45, 10.22 ± 0.22 and 7.13 ± 0.77 log CFU per membrane, respectively. The three-species model was employed to evaluate the efficacy of two antimicrobial dressings, Curity™, AMD and Acticoat™, compared to sterile gauze controls. Microbial growth on Curity™ AMD and gauze was not significantly different, for any species, whereas Acticoat™ was found to significantly reduce growth for all three species.

Conclusions: Using the colony-DFR, a three-species biofilm was successfully grown, and the biofilms displayed a unique structure consisting of distinct layers that appeared to be inhabited exclusively or predominantly by a single species.

Significance and Impact of the Study: The primary accomplishment of this study was the isolation and growth of an obligate anaerobe in an in vitro model without establishing an artificially anaerobic environment.

New eosinophils recruited to the lungs of sensitized guinea pigs after ozone exposure persist in the airway tissues

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Rationale: Eosinophils are clustered around parasympathetic nerves in patients with asthma and in animal models of airway hyperreactivity. Sensitized and non-sensitized animals exposed to ozone are hyperreactive three days later, but the mechanism differs between them. Previously, we have shown that depleting eosinophils makes hyperreactivity worse three days post-ozone exposure in non-sensitized animals, where 83% of BAL eosinophils are new (BrdU+). However depleting eosinophils prevents hyperreactivity in sensitized animals, where only 5% of BAL eosinophils are new. Thus, the role of eosinophils in ozone-induced hyperreactivity at three days changes from beneficial in naïve animals to destructive in sensitized animals.

Methods: Guinea pigs were exposed to either air or 2ppm ozone for 4 hours. Some guinea pigs were sensitized to ovalbumin 3 weeks before ozone or air exposure. Migration of recently divided eosinophils after a single exposure to ozone was tracked using 5-bromo-2′-deoxyuridine (BrdU, 100mg/kg, ip) to label dividing cells. Bronchoalveolar lavage (BAL) and lungs were collected three days later and analyzed by immunocytochemistry.

Results: In non-sensitized guinea pigs, ozone did not increase total BrdU+ or nerve-associated BrdU+ eosinophils around the airways, although BrdU+ eosinophils in the BAL were significantly increased. In contrast, in sensitized guinea pigs where BrdU+ eosinophils do not appear in the BAL, ozone significantly increased them in the airways. In sensitized animals, total BrdU+ eosinophils increased from 14.4 eosinophils/mm² (3%) in air-exposed animals to 72.4 eosinophils/mm² (16%) in ozone-exposed animals. Nerve-associated BrdU+ eosinophils increased from 7.2 eosinophils/mm² (3%) in air-exposed animals to 35.0 eosinophils/mm² (19%) in ozone exposed animals.

Conclusions: In non-sensitized animals, ozone exposure recruits BrdU+ eosinophils to the lungs but they don’t appear to linger in the airway tissues and, instead, pass through to the BAL. In sensitized animals, ozone recruits BrdU+ eosinophils to the lungs where they are found in the airway tissues and around parasympathetic nerves.

This project was funded by NIH grants ES014601, ES017592, HL061013, AI092210
Promoter DNA Hypomethylation in the Fetal Kidneys of Intrauterine Growth Restricted Mice

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Background: We have recently shown significant differences in fetal kidney renin-angiotensin system (RAS) expression in our mouse model of intrauterine growth restriction (IUGR). Similar to rat dams fed low protein diets whose progeny down-regulate renal renin expression, male progeny in our IUGR model also showed decreased renin and angiotensinogen (AGT) expression. Conversely, female IUGR progeny showed significantly increased AGT transcription. This may be significant because IUGR males have fewer nephrons and develop adult onset hypertension, while females do not. We hypothesize differences in renal RAS expression may be related to promoter methylation. Our objective was to characterize DNA methylation in fetal kidneys from our IUGR mice and controls.

Methods: Transgenic B6.129P2-Tg (Agtdup) mice (Jackson labs) were backcrossed for at least five generations into a wild-type C57BL/6 background, yielding female mice with 3-copies of the murine AGT gene. We harvested pregnant females near term (day 17) and collected fetal kidneys from five litters of each maternal genotype [3-copy; WT ]. Fetal sex and genotype were determined by PCR. Only fetuses with 2-copies of the AGT gene were included for analysis. Global promoter methylation status was determined using methylated DNA immunoprecipitation coupled with microarray analysis (MeDIP-chip, Nimblegen). According to published criteria, promoters with DNA methylation levels lower than 1.3-fold of the genome-wide median methylation level were considered hypomethylated. Control (IgF2 DMR1) and RAS candidate genes were further analyzed by bisulfite pyrosequencing.

Results: Global promoter methylation was significantly less in IUGR female progeny compared to their IUGR male siblings and controls (p<0.0001). As expected, renal IgF2, H19, and XIST were hypermethylated in all fetal kidneys. There were no promoter methylation differences in renin, ACE, or the angiotensin II type-1 receptor between groups. However, the type 2 receptor (AT2) and ACE2 promoters were hypomethylated in IUGR progeny compared with controls. The AGT promoter was hypomethylated only in IUGR females.

Conclusions: Similar to recently published placenta data from human and mouse studies, we observed significantly less promoter methylation in the kidneys of our IUGR mouse model. There were also significant gender effects. IUGR female progeny tended to have less promoter methylation than their siblings and controls.

Fetal Programming of White Coat Hypertension in Growth Restricted Male Mice

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Background: Accumulating evidence suggests uteroplacental insufficiency and fetal growth restriction (IUGR) may cause reduced nephrogenesis and an increased risk of adult onset hypertension. This effect appears to be more pronounced in males than females. We study a transgenic mouse model designed to simulate a common human angiotensinogen (AGT) promoter variant associated with uteroplacental insufficiency and IUGR. Our objective was to test whether the wild-type progeny from transgenic dams have gender-based differences in nephrogenesis and develop adult onset hypertension.
Methods: Transgenic B6.129P2-Tg (Agtdup) mice (Jackson labs) were backcrossed for five generations into a wild-type C57BL/6 background, yielding female mice with 3-copies of the murine AGT gene. The progeny from 3-copy dams with wild-type (WT) AGT genotype (n=10 males and 12 females from five litters) were compared to transgenic 3-copy male positive controls (n=7) and gender-matched progeny from wild-type negative controls (n=22). Blood pressure was measured by radio telemetry in adult progeny (12 weeks old) at rest (2 hour mean arterial pressure [MAP]) and under mild stress for 30 minutes (loud hard-rock music, so-called music torture). Kidneys were harvested, weighed, and the number of glomeruli per kidney were determined by stereometry.

Results: Newborn pups from transgenic mothers were smaller (p<0.05), but caught up to controls by six weeks of age. Telemetry showed that IUGR males, but not IUGR females, had significantly elevated MAP when stressed, but not at rest compared with negative controls (WTm and WTI) (Figure). 3-copy male positive controls showed a similar increase in MAP, especially when stressed. Preliminary stereometric analysis suggested mean glomeruli/kidney counts may be significantly less in growth restricted males (p<0.05).

Conclusions: We observed adult onset increases in MAP in the IUGR male progeny from our transgenic mouse model, but only when these animals were stressed. The etiology may be related to differences in nephrogenesis, but we hypothesize there may also be differences in systemic sympathetic tone.

Medical Termination of Pregnancy in Cynomolgus Macaques

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Although pregnancy is expected during studies of novel contraceptives in nonhuman primates, gestation, delivery and lactation remove females from groups for prolonged intervals. Since the macaque cervix does not facilitate transcervical surgical termination of pregnancy, we sought to establish a safe and effective medical termination protocol. Evaluation was based on a case study of on-going procedures done within the Nonhuman Primate Contraceptive Core at the Oregon National Primate Research Center. Treatment regimens were based on the current clinical practices used for medical termination of pregnancy in women. Efficacy of treatment and the time to uterine resolution (no retained products of conception), which are needed for future conception viability, were evaluated using ultrasound. Outcomes
were determined according to gestational age, medication, dose, and route of administration. A total of 36 cynomolgus macaques enrolled in contraceptive trials, as controls or those receiving test contraceptive treatments, underwent 66 medical terminations. Oral and intramuscular mifepristone with misoprostol (orally, buccally, or vaginally) were evaluated. Pregnancies in over 95% of animals were terminated at ≤35 days of gestation following the most effective treatment of mifepristone (20 mg intramuscularly, followed by misoprostol 200 mcg buccally). Pregnancy termination was less effective when mifepristone was initially administered at gestational ages >35 days. With intramuscular mifepristone, uterine resolution occurred within 21 days when no retained products of conception remained following treatment. The time to uterine resolution increased to 41 days if products of conception were still observed one week following mifepristone and misoprostol treatment. A safe and effective salvage protocol, following unsuccessful treatment with mifepristone, was also evaluated. An intrafetal injection of methotrexate (50 mg/m²) under ultrasound guidance administered one week after unsuccessful treatment with mifepristone was found to be the most successful method. Thus, medical termination of pregnancy for macaques is safe, effective and does not impair subsequent establishment of pregnancy. We recommend a protocol with mifepristone 20 mg IM and misoprostol 200 mcg buccally as initial treatment.

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Ischemic Injury to Glomerular Endothelium: Proposed Mechanism of Hyperglycemic Protection

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Introduction: Acute kidney injury (AKI) is a common perioperative complication that leads to high mortality. Unfortunately, there are no effective therapies. The most common cause of perioperative AKI is ischemia-reperfusion injury (IRI). Glomerular endothelium is a critical part of the glomerular filtration barrier, and ischemic injury to this barrier leads to increased microvascular permeability. Interestingly, animal investigations have shown that acutely, hyperglycemia (HG) protects against IRI in cardiomyocytes and retinal cells.(1,2) We hypothesize that HG at the time of ischemia protects the function of glomerular endothelial cells (gENC) via activation of a protective cellular signaling pathway, the sphingosine kinase-1/sphingosine-1-phosphate (SK1/S1P) pathway.

Methods: gENC were cultured on transwells in osmotically controlled, normoglycemic (5.5 mM glucose+20 mM mannitol) or HG conditions (25.5 mM glucose). After 7 days, gENC were subjected to a model of IRI: 8h of oxygen-glucose deprivation (OGD) and 12h of reoxygenation/glucose repletion(RGR). Transendothelial electrical resistance (TEER) and permeability to macromolecules (Fluorescein isothiocyanate conjugated to 70 kD Ficoll) were used as measures of gENC monolayer integrity, and were assessed prior to OGD (baseline value) and after 12h of RGR. Following OGD/RGR, total RNA was extracted from both control and HG gENC and real-time, quantitative PCR was performed to quantify sphingosine kinase-1 mRNA levels. Levels were normalized to RPL32, a reference gene. Statistical analysis was performed using 2-tailed Student’s t-test.

Results: At baseline (prior to OGD), normoglycemic gENC had higher TEER (decreased permeability) compared to HG gENC (24±1Ω; p<0.05, n=6). Following OGD/RGR, HG gENC were more resistant to OGD than control gENC as evidenced by higher TEER (Fig. 5, 85±4% of baseline in HG gENC; 70±2% baseline in control gENC; p<0.05, n=3) and decreased Ficoll flux (p=0.16). Using real-time quantitative PCR we found that sphingosine kinase-1 was increased relative to RPL32 following OGD/RGR in HG gENC when compared to control gENC.
Conclusions: These data suggest while chronic HG is detrimental to the integrity of gENC monolayers at baseline, acute HG protects against OGD-induced injury. Our data suggest that HG upregulates sphingosine kinase-1 in ischemic gENCs. Elucidating the mechanism of HG-induced protection could provide a focus for the development of protective therapies for AKI that do not expose patients to the potential adverse effects of HG.

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Cell penetrating recombinant Foxp3 protein enhances Treg function, suppresses Th17 cells and ameliorate arthritis

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Background: Foxp3 is the key transcription factor for T regulatory (Treg) cell differentiation and function. Deficiency in Foxp3 results in severe autoimmune inflammation. Foxp3 induces itself expression in Treg cells and negatively regulates Th17 cell differentiation by repressing RORγ. SKG mice have impaired Treg function and develop chronic arthritis closely resembling human rheumatoid arthritis. Arthritis in SKG mice is dependent on Th17 cells. This study aimed to test the therapeutic potential of cell penetrating recombinant Foxp3 protein in treating arthritis.

Methods: Recombinant Foxp3 protein was fused to a cell penetrating polyarginine (11R) tag to facilitate intracellular transduction. Arthritis was induced in SKG mice by intraperitoneal (i.p.) injection of 2 mg zymosan. Foxp3-11R was injected i.p. daily one week after arthritis induction and continued for seven days. Severity of arthritis was assessed by using a score system and followed up for 8 weeks. Treg function was assessed by inhibition of CD4+ T effector cell proliferation using CFSE dilution assay. Th1 and Th17 cells in lymph nodes and spleens from mice treated with Foxp3-11R were detected by intracellular cytokine staining.

Results: In vitro Foxp3-11R treated CD4+ cells showed a 50% increase in suppressive function compared with control protein treated cells. Severity of arthritis in Foxp3-11R treated SKG mice was reduced compared with those treated with a control protein (see Table 1). CD4+ T cells of lymph nodes and spleen from Foxp3-11R treated mice showed increased levels of Foxp3 expression compared with those of control protein treated with mean fluorescence intensity 38 ±3 vs 30 ±4 in draining lymph nodes and 40 ±2 vs 30 ±5 in the spleen (p<0.05). The number of IL-17 producing CD4+ T cells in the spleen was decreased in Foxp3-11R treated mice with 1.3% vs 2.5% in control protein treated. However, the number of IFN-γ producing cells was similar between Foxp3-11R and control protein treated groups.

Conclusion: These results demonstrate that Foxp3-11R can enhance T cell suppressive function, suppress Th17 cells and ameliorate experimental arthritis. The data suggest that cell penetrating recombinant Foxp3 is a potentially useful agent in therapy of arthritis.

Table 1. Arthritis score in SKG mice treated with Foxo3-11R or a control protein.

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<th>Week after</th>
<th>4</th>
<th>6</th>
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Diet-induced Insulin Resistance, ApoE, and Cognitive Function

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An emerging body of work suggests that the pathologies of diabetes and dementia are strongly linked. The APOE gene encodes for three isoforms in the human population (E2, E3, and E4). Compared to the E3 isoform, the E4 isoform is a major genetic risk factor for Alzheimer’s Disease (AD). Type 2 diabetes, commonly a result of diet-induced obesity, is also a risk factor for AD and other dementias. Interestingly, epidemiological studies have suggested a synergistic effect between E4 and diabetes in risk to develop AD. However, the shared cellular and molecular mechanisms by which APOE genotype might contribute to the pathophysiological effects of a high fat diet on cognitive dysfunction remain to be determined. Therefore, we propose to induce diabetes using a high fat diet in a mouse model of human apoE isoforms in order to examine the joint effect of impaired insulin signaling and human apoE isoforms on brain function. In mice with endogenous mouse apoE, a high fat diet has been shown to decrease cognitive performance in transgenic amyloid precursor protein mouse models of Alzheimer’s disease. In addition, our preliminary data show that compared to mice with human E3, mice with human E4 display glucose intolerance when challenged with a high fat diet. We hypothesize that diet-induced diabetes will impair cognitive performance and affect neurodegenerative processes and that these effects will be more pronounced in the presence of E4 than E3. To determine the role of apoE isoform in effects of diet-induced diabetes on brain function, E2, E3 and E4 mice will be fed a high fat diet (60% kcal from fat) similar to that consumed by many Western cultures. The degree of obesity, glucose tolerance and insulin resistance will be determined following four months on the diet. Cognitive performance and behavior will then be analyzed using a battery of tests. Following behavioral testing, the brains of these mice will be analyzed for potential alterations in cholesterol and insulin metabolic pathways and measures of synaptic pathology.

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Comparison of individual, group and home agility boot camp (ABC) exercise program for people with Parkinson’s disease?

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Objectives: To investigate the most successful manner of delivery of physical rehabilitation (individual, group session or home exercise program) for people with Parkinson’s disease (PD).
**Design:** A randomized intervention study.

**Setting:** The study was conducted at a university outpatient balance disorders laboratory. Participants: Twenty-eight people (mean age 64± 8 years; UPDRS motor 34± 11) with PD were included in this pilot study.

**Interventions:** People were randomized into one of three modes of delivery of the same exercise program. The ABC program is based on a previously published protocol for improving mobility in people with PD. Intervention was 1 hour long, 3 times/week for 4 weeks. Pre-and-post exercise testing was administered by a blinded examiner.

**Main Outcome Measure(s):** the primary outcome was balance performance in the mini-BESTest. Secondary measures include Activities of Balance Confidence (ABC), Self-Efficacy for Exercise (SEE) and Geriatric Depression Scale (GDS). Results: We found significant improvement in the mini-BESTest for the individual instruction only, (t=4.1; p=0.003) with no significant improvement in the home or group class. However, we found significant improvements in perceived balance confidence (ABC: t = 3.1; p=0.007), self-efficacy for exercise (SEE: t=3.1; p =0.02;) and depression (GDS: t=2.3; p=0.05) in the group exercise class only.

**Conclusions:** The data from this pilot study suggests that individual exercise instruction may be more beneficial than the current standard of care (home exercise program) or a group class for improving balance but a group class may be better for patient reported improvements.


*Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage*

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The purpose of our study was to better understand the relationship between mitochondrial structural proteins, particularly dynamin-related protein 1 (Drp1) and amyloid beta (Aβ) in the progression of Alzheimer's disease (AD). Using qRT-PCR and immunoblotting analyses, we measured mRNA and protein levels of mitochondrial structural genes in the frontal cortex of patients with early, definite and severe AD and in control subjects. We also characterized monomeric and oligomeric forms of Aβ in these patients. Using immunoprecipitation/immunoblotting analysis, we investigated the interaction between Aβ and Drp1. Using immunofluorescence analysis, we determined the localization of Drp1 and intraneuronal and oligomeric Aβ in the AD brains and primary hippocampal neurons from Aβ precursor protein (AβPP) transgenic mice. We found increased expression of the mitochondrial fission genes Drp1 and Fis1 (fission 1) and decreased expression of the mitochondrial fusion genes Mfn1 (mitofusin 1), Mfn2 (mitofusin 2), Opa1 (optic atrophy 1) and Tomm40. The matrix gene CypD was up-regulated in AD patients. Results from our qRT-PCR and immunoblotting analyses suggest that abnormal mitochondrial dynamics increase as AD progresses.
Immunofluorescence analysis of the Drp1 antibody and the Aβ antibodies 6E10 and A11 revealed the colocalization of Drp1 and Aβ. Drp1 immunoprecipitation/immunoblotting analysis of Aβ antibodies 6E10 and A11 revealed that Drp1 interacts with Aβ monomers and oligomers in AD patients, and these abnormal interactions are increased with disease progression. Primary neurons that were found with accumulated oligomeric Aβ had lost branches and were degenerated, indicating that oligomeric Aβ may cause neuronal degeneration. These findings suggest that in patients with AD, increased production of Aβ and the interaction of Aβ with Drp1 are crucial factors in mitochondrial fragmentation, abnormal mitochondrial dynamics and synaptic damage. Inhibiting, these abnormal interactions may be a therapeutic strategy to reduce mitochondrial fragmentation, neuronal and synaptic damage and cognitive decline in patients with AD.

Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: implications for selective neuronal damage

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The purpose of our study was to determine the relationship between mutant huntingtin (Htt) and mitochondrial dynamics in the progression of Huntington’s disease (HD). We measured the mRNA levels of electron transport chain genes, and mitochondrial structural genes, Drp1 (dynamin-related protein 1), Fis1 (fission 1), Mfn1 (mitofusin 1), Mfn2 (mitofusin 2), Opa1 (optic atrophy 1), Tomm40 (translocase of outer membrane 40) and CypD (cyclophilin D) in grade III and grade IV HD patients and controls. The mutant Htt oligomers and the mitochondrial structural proteins were quantified in the striatum and frontal cortex of HD patients. Changes in expressions of the electron transport chain genes were found in HD patients and may represent a compensatory response to mitochondrial damage caused by mutant Htt. Increased expression of Drp1 and Fis1 and decreased expression of Mfn1, Mfn2, Opa1 and Tomm40 were found in HD patients relative to the controls. CypD was upregulated in HD patients, and this upregulation increased as HD progressed. Significantly increased immunoreactivity of 8-hydroxy-guanosine was found in the cortical specimens from stage III and IV HD patients relative to controls, suggesting increased oxidative DNA damage in HD patients. In contrast, significantly decreased immunoreactivities of cytochrome oxidase 1 and cytochrome b were found in HD patients relative to controls, indicating a loss of mitochondrial function in HD patients. Immunoblotting analysis revealed 15, 25 and 50 kDa mutant Htt oligomers in the brain specimens of HD patients. All oligomeric forms of mutant Htt were significantly increased in the cortical tissues of HD patients, and mutant Htt oligomers were found in the nucleus and in mitochondria. The increase in Drp1, Fis1 and CypD and the decrease in Mfn1 and Mfn2 may be responsible for abnormal mitochondrial dynamics that we found in the cortex of HD patients, and may contribute to neuronal damage in HD patients. The presence of mutant Htt oligomers in the nucleus of HD neurons and in mitochondria may disrupt neuronal functions. Based on these findings, we propose that mutant Htt in association with mitochondria imbalance and mitochondrial dynamics impairs axonal transport of mitochondria, decreases mitochondrial function and damages neurons in affected brain regions of HD patients.
Mitochondria-targeted catalase reduces abnormal APP processing, amyloid beta production, and BACE1 in a mouse model of Alzheimer’s disease: Implications for neuroprotection and lifespan extension

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The purpose of this study was to investigate the protective effects of the mitochondria-targeted antioxidant catalase (MCAT) and lifespan extension in mice that express Aβ. Using immunoblotting and immunostaining analyses, we measured the production of full-length APP, soluble APPα, C-terminal fragments CTF99 and CTF83, monomeric and oligomeric Aβ, Aβ deposits, BACE1, in different stages of disease progression in MCAT/AβPP and AβPP mice. Using qRT-PCR, and immunostaining analyses, we studied the expression of catalase, BACE1, and the AD markers, synaptophysin, APP, neprilysin, insulin degrading enzyme, transthyretin in MCAT, AβPP, MCAT/AβPP, and wild-type mice. Using HPLC analysis of 8-hydroxy-deoxyguanosine, we measured oxidative DNA damage in the cerebral cortical tissues from MCAT, AβPP, MCAT/AβPP, and wild-type mice. We found that the AβPP transgenic mice that carried the human MCAT gene lived 3.5 months longer than did the AβPP mice. We also found that the overexpression of MCAT in brain sections from the MCAT/AβPP transgenic mice significantly correlated with a reduction in the levels of full-length APP, CTF99, BACE1, Aβ levels (40 & 42), Aβ deposits, and oxidative DNA damage in brain sections from the AβPP mice. Interestingly, we found significantly increased levels of soluble APPα and CTF83 in the MCAT/AβPP, mice relative to the AβPP mouse. These data provide direct evidence that oxidative stress plays a primary role in AD etiopathology, and that in MCAT mice that express Aβ, MCAT prevents abnormal APP processing, reduces Aβ levels, and enhances Aβ-degrading enzymes in mice at different ages, corresponding to different stages of disease progression. These findings indicate that mitochondria-targeted molecules may be an effective therapeutic approach to treat patients with AD.

Impaired mitochondrial biogenesis, defective axonal transport of mitochondria, abnormal mitochondrial dynamics and synaptic degeneration in a mouse model of Alzheimer's disease

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Increasing evidence suggests that the accumulation of amyloid beta (Aβ) in synapses and synaptic mitochondria causes synaptic mitochondrial failure and synaptic degeneration in Alzheimer’s disease (AD). The purpose of this study was to better understand the effects of Aβ in mitochondrial activity and synaptic alterations in neurons from a mouse model of AD. Using primary neurons from a well-characterized Aβ precursor protein transgenic (AβPP) mouse model (Tg2576 mouse line), for the first time, we studied mitochondrial activity, including axonal transport of mitochondria,
mitochondrial dynamics, morphology and function. Further, we also studied the nature of Aβ-induced synaptic alterations, and cell death in primary neurons from Tg2576 mice, and we sought to determine whether the mitochondria-targeted antioxidant SS31 could mitigate the effects of oligomeric Aβ. We found significantly decreased anterograde mitochondrial movement, increased mitochondrial fission and decreased fusion, abnormal mitochondrial and synaptic proteins and defective mitochondrial function in primary neurons from AβPP mice compared with wild-type (WT) neurons. Transmission electron microscopy revealed a large number of small mitochondria and structurally damaged mitochondria, with broken cristae in AβPP primary neurons. We also found an increased accumulation of oligomeric Aβ and increased apoptotic neuronal death in the primary neurons from the AβPP mice relative to the WT neurons. Our results revealed an accumulation of intraneuronal oligomeric Aβ, leading to mitochondrial and synaptic deficiencies, and ultimately causing neurodegeneration in AβPP cultures. However, we found that the mitochondria-targeted antioxidant SS31 restored mitochondrial transport and synaptic viability, and decreased the percentage of defective mitochondria, indicating that SS31 protects mitochondria and synapses from Aβ toxicity.

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Gene Expression Profiles of Mitochondrial Structure/Function and Amyloid Beta Production in Brain Tissues from Alzheimer’s Disease Patients: Implications to Neuronal Damage and Cognitive Decline

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Alzheimer’s disease (AD) is a late-onset neurodegenerative disorder that is characterized by the progressive decline of memory and cognitive functions. Recent mitochondrial and amyloid beta studies of AD revealed that amyloid beta is localized to mitochondrial membranes and causes abnormal mitochondrial dynamics. The purpose of our study was to measure mRNA expression levels in genes related to mitochondrial structure/function and genes related to amyloid beta production and clearance in different stages of AD progression. Using quantitative real-time RT-PCR, we measured mRNA abundance and protein levels of mitochondrial structural genes; the fission genes Drp1 and Fis1, and the fusion genes Mfn1, Mfn2, Opal, and Tomm40. We also measured the mitochondrial matrix proteins HSP60 and CypD in the frontal cortex of a large number of Braak stage I/II (early AD) and Braak stage IV-VI (definite AD) patients, and in control subjects. To determine the genes responsible for amyloid beta production/clearance in AD progression in relation to mitochondrial structure, we measured mRNA and protein levels of BACE1, BACE2, Neprilysin, insulin degrading enzyme, and transthyretin in patients with early AD and definite AD, and control subjects. Using immunohistochemistry, we examined the localization of proteins related to mitochondrial structure and amyloid beta in different brain regions of patients with early and definite AD. In brain specimens from patients with early AD and definite AD compared to control subjects, we found increased expression levels of the mitochondrial fission genes Drp1, and Fis1; and decreased expression levels of the mitochondrial fusion genes Mfn1, Mfn2, Opal, and Tomm40. We found the matrix genes CypD, Bcl2, and HSP60 were upregulated in early and definite AD patients. Our immunoblotting analysis of fission, fusion, and matrix proteins concurred with our real-time RT-PCR findings, indicating that abnormal mitochondrial dynamics is present in patients with AD. We also found increased mRNA expression of BACE1 and BACE2, and decreased expression of neprilysin, an insulin-degrading enzyme, in both early and definite AD patients. Our immunohistochemistry analysis revealed that abnormal expression of proteins related to mitochondrial structure and amyloid beta production/clearance are confined to brain regions affected with AD, suggesting that the increased accumulation of amyloid beta may cause abnormal mitochondrial dynamics in AD. Increased production and decreased clearance of
amylod beta levels may contribute to abnormal mitochondrial dynamics, and synaptic damage in neurons from AD patients.

Tularemia Vaccine Trials in Nonhuman Primates

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Francisella tularensis is the Gram-negative bacterial pathogen that causes tularemia, a debilitating and potentially fatal disease. Inhalation of as few as ten bacteria can produce a severe pneumonic illness in humans. Despite its high infectivity, classification as a category A select agent, and use as a biological weapon, there is currently no FDA-approved vaccine against tularemia.

Our previous studies identified several Francisella genes that, when mutated, give rise to attenuated strains in cell culture and mouse models of infection. Deletion derivatives of two of these strains, ΔdmbB and ΔdpdB, in Francisella Schu S4 provided protection against wild-type challenge in mice, indicating the potential of these genes to be included in a live attenuated vaccine.

Here, we describe testing these strains in the nonhuman primate (NHP) rhesus macaque. Groups of three NHPs were inoculated subcutaneously with ΔdmbB (10⁴ CFU) or ΔdpdB (10⁴ CFU). Four weeks after the primary inoculation, the NHPs were boosted with 10¹⁰ CFU of the respective attenuated strain. Thirty-three days post-boost, 10⁴ CFU of wild-type Schu S4 were inoculated into the lung. Two vehicle-only control NHPs were concurrently challenged with 10⁴ CFU of wild-type Schu S4. The challenge dose was chosen based on our results that intrabronchial inoculation of 10⁴ CFU resulted in tularemia-related death nine and 18 days after infection. In contrast, naive NHPs infected with 100 CFU survived until the end of the study (day 23).

Upon necropsy, organ, blood, lymph node, bone marrow, and bronchoalveolar lavage samples were obtained to determine the bacterial load. O₂ saturation, CBC panels, weight, and temperature were monitored to assess the health of the NHPs. While there have been several studies describing protective strains of Francisella in mice, these are the first such studies in a nonhuman primate model of infection.

Testing whether sustained or bolus delivery of BrdU labels dividing inflammatory cells

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Patients with asthma have both airway hyperreactivity (increased contraction of the airways) and airway inflammation characterized by eosinophils. In an animal model of acute asthma exacerbation, preliminary data suggest that
eosinophils mediate hyperreactivity at an early time point, but play a protective role three days later. To test whether different populations of eosinophils are involved, we plan to measure whether eosinophils have recently undergone cell division by treating guinea pigs with 5-bromo-2'-deoxyuridine (BrdU), which is intercalated into the DNA of dividing cells, and can be subsequently identified histologically. However, BrdU is degraded rapidly following injection with a half-life of 2 hours. Here we tested whether continuous BrdU administration by osmotic minipump results in greater labeling of cells versus bolus dosing by ip administration. To implant minipumps, guinea pigs were anesthetized with 5% isoflurane followed by maintenance with 3% isoflurane. Minipumps were implanted subcutaneously on the back, the incision sealed with skin glue, and guinea pigs were treated with 0.05 mg/kg buprenorphine for analgesia. Alternatively, 75 mg/kg BrdU was administered by ip injection twice daily, starting one hour before ozone exposure. Three days later animals were killed and bone marrow, peripheral blood, and bronchoalveolar lavage were collected and analyzed by immunohistochemistry. There were no differences in percent of BrdU positive eosinophil populations in bone marrow or blood among any group regardless of whether BrdU was administered by perfusion or bolus injection. However, in bronchoalveolar lavage there were significantly more total inflammatory cells, including eosinophils, in minipump versus ip treated animals. Additionally, very few inflammatory cells were BrdU positive in bronchoalveolar lavage from animals with the osmotic minipump. These data demonstrate that animals with minipumps have increased airway inflammation and decreased newly divided cells in the lungs, suggesting that either inhalational anesthesia, subsequent analgesic, or minipump implantation altered the inflammatory response in lungs. Thus, bolus dosing of BrdU ip, is sufficient to label dividing inflammatory cells in the bone marrow and bronchoalveolar lavage without altering the base line inflammatory response.

**Indispensable role of B cells in mediating protective effects of estrogen against EAE**

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Increased remissions in multiple sclerosis (MS) during pregnancy suggest that elevated levels of sex steroids exert immunoregulatory activity. Estrogen (E2=17-estradiol) protects against experimental autoimmune encephalomyelitis (EAE), but the cellular basis for E2-induced protection remains unclear. Studies demonstrate that depletion of B cells prior to induction of EAE exacerbates disease severity, implicating regulatory B cells. We thus evaluated pathogenic and E2-induced protective mechanisms in B cell deficient (MT−/) mice. EAE-protective effects of E2 were abrogated in MT−/− mice, with no reduction in disease severity, cellular infiltration or pro-inflammatory factors in the CNS compared to untreated controls, indicating a crucial role of B cells in E2-mediated protection. E2-treated WT mice demonstrated a selective increase in the percentage of IL-10-producing CD1dhighCD5+ regulatory B cells and an up-regulated expression of PD-L1 on B cells. Upregulation of PD-L1 was critical for E2-mediated protection since E2 did not inhibit EAE in PD-L1−/− mice. B cells directly treated with E2 significantly reduced proliferation of MOG35-55-specific T cells and this inhibition required the presence of ERα on B cells. Adoptive transfer of total B cells from MOG-immunized WT mice to E2-treated 

MT−/− mice was sufficient to restore E2-mediated protection and protection was attributed to the presence of ERα on the donor B cells, since the transfer of B cells from ERα−/− mice failed to protect the recipient MT−/− mice. These results demonstrate for the first time a requirement for B cells in E2-mediated protection against EAE involving direct E2 effects on regulatory B cells mediated through ERα and the PD-1/PD-L1 negative co-stimulatory pathway. E2-primed B cells may
The role of Swiss cheese, the *Drosophila* homologue of Neuropathy target esterase, in glia.
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Neuropathy target esterase (NTE), a molecular target of organophosphates (OP) found in pesticides and nerve gases induce delayed neuropathy (OPIDN) in humans. OPIDN is characterized by axonal degeneration mainly of motoneurons. Similarly, loss of the *Drosophila* homologue of NTE, Swiss Cheese (SWS) causes progressive neurodegeneration in flies but also glial degeneration. Previously we have shown a cell autonomous requirement of SWS in both neuronal and glial cell types in the adult brain of *Drosophila*. Using cell type specific down regulation of SWS, we can now specifically address its requirement in glia. Our recent findings in mouse show presence of SWS/NTE in astrocytes in the sciatic nerve, suggesting a conserved role of SWS in glia in higher vertebrates. These studies, using both *Drosophila* and mouse model systems, will help us to understand the importance of the SWS protein in glia, its role in axonal-glial interaction and its pathogenic function in inherited spastic paraplegia and OPIDN in humans.

Optogenetic control of mouse outer hair cells

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Normal hearing in mammals depends on both sound detection by inner hair cells (IHCs) and sound amplification by outer hair cells (OHCs). The voltage-dependent somatic motility of OHCs is a major component of the cochlear amplification process and is driven by membrane potential. Since the first historical discovery in 1986, OHC motility has been the focus of hearing research for over two decades due to its critically important role in increasing sensitivity and frequency selectivity in normal mammal hearing and human hearing diseases. So far, there are still numerous undiscovered mysteries in this field such as the origin of traveling wave and acoustic emission, etc. In addition, a number of hearing diseases, such as sensory hearing loss caused by age, noise and ototoxin, involve OHC function impairment and consequently have reduced cochlear amplification and frequency tuning. For these patients, only amplifying sound is not enough. It is a dream idea to control OHC function in a none-invasive, cell specific and precisely localized and timed manner for both laboratory study and the clinical application in the future. So far there are varieties of methods that have been applied to study OHC motility. However, all these techniques have considerable limitations, such as being invasive, lack of cellular precision and temporospatial resolution, which limited their applications. To overcome these limitations, we used an optogenetic approach based on channelrhodopsin 2 (ChR2), a direct light-activated non-selective cation channel (NSCC) originally from Chlamydomonas Reinhardtii. In the current study, we specifically transferred ChR2 gene to the mouse cochlea OHCs through in uterus injection of adenovirus vector with ChR2 in fusion with fluorescent marker tdTomato. We also transected ChR2(H134R), a point mutant of ChR2, with plasmid to an auditory cell line (HEI-OC1). With whole cell recording, we found that blue light (470 nm) elicited the typical NSCC
current of ChR2 with reversal potential around zero in both mouse OHCs and HEI-OC1 cells and generated significant depolarization in both cell types. In addition, pulsed light stimulation (10 Hz) elicited a 1:1 repetitive depolarization and ChR2 Currents in mouse OHCs and HEI-OC1 cells respectively. The time constant of depolarization in OHCs is 1.45 ms, 10 times faster than HEI-OC1 cells, which allows faster light stimulation to be applied to mouse OHCs. This is the first demonstration that ChR2 was successfully expressed in mouse OHCs and HEI-OC1 cells and presented a typical light-sensitive current and depolarization.

Supported by NIDCD DC 00105 and 00141

Environmental Food Balance: The Neighborhood context of BMI and Diet among Landless Northwest American Indians

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Introduction: American Indians and Alaska Natives (AI/AN) endure many of the worst health outcomes in the nation, with notably high rates of obesity, diabetes, and heart disease. Simultaneously, research geared to the health of AI/AN groups is limited and often inaccurate, particularly with regard to the 60% of AI/AN people who do not live on reservations. Using the Retail Food Environment Index (RFEI) system, this study evaluates the influence of neighborhood food environment on body mass index (BMI) and diet among members of Tribe A, a non-reservation-based AI community.

Methods: Working collaboratively with Tribe A, we conducted a Behavioral Risk Factor Surveillance System (BRFSS) telephone survey of 392 tribal members living in Oregon and Washington states. Participants were asked to report their height, weight, dietary habits, and the US Postal Service ZIP code of their home address. We used US Census ZIP Code Business Patterns data from 2009 to calculate a RFEI score for each participant by dividing the total number of limited service restaurants and convenience stores by the total number of grocery stores and produce vendors in each participant’s ZIP code “neighborhood.”

Results: BRFSS telephone questionnaires were completed during the summer of 2009 and 2010. We attempted to contact all tribal members at least 18 years of age living in Washington and Oregon; ultimately, 215 women and 176 men agreed to participate. The average age of tribal participants was 55 years. The average BMI score was 30.6. RFEI data collection and score calculations were completed during the winter of 2012. The mean RFEI score was 5.1, signifying that on average, tribal members reside in ZIP code neighborhoods with approximately 5.1 times as many limited service restaurants and convenience stores as grocery stores and produce vendors.

Conclusion: We expect the balance of unhealthy versus healthy food options facing tribal members has significant health implications, particularly with regard to maintaining healthy diets and healthy BMIs. We will test this relationship using multiple linear regression and logistic regression models. Through this work, our study aims to clarify the complex interplay between food environment and obesity, while highlighting the unique circumstances of a group of people who are critically affected by obesity and its sequelae conditions, yet chronically under-recognized.
Memory dysfunction following cardiac arrest and cardiopulmonary resuscitation in mice is associated with hippocampal inflammation

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Introduction: Many survivors of cardiac arrest (CA) and cardiopulmonary resuscitation (CPR) are disabled by severe loss of memory and executive cognitive function. Much of this memory dysfunction is thought to be caused by ischemia/reperfusion injury to the hippocampus and the subsequent loss of hippocampal neurons. The brain's inflammatory response to ischemia can exacerbate injury and provides a potential treatment target that remains understudied. While activated microglia, the brain's resident immune cells, are thought to contribute to injury after ischemic stroke, little is known about their role after global ischemia during CA/CPR. We hypothesized that microglia are activated by CA/CPR and contribute to neuronal loss and functional deficit.

Method: Adult male C57BL6 mice (20 – 25 gram body weight) were anesthetized with isoflurane and orally intubated. An internal jugular catheter was placed and cardiac arrest was induced by injection of potassium chloride. After 8 minutes of CA, CPR was performed by injection of epinephrine and chest compression. Return of spontaneous circulation (ROSC) was achieved within 2.5 minutes of CPR. Sham operated mice underwent anesthesia, intubation, and catheter placement, but not CA. Mice were sacrificed 1, 3, or 10 days after CA/CPR. Behavior was assessed by neuroscore on day 3. Microglial activation in the hippocampus was assessed by immunostaining for the activation marker Mac-2 at 1, 3, and 10 days after CA/CPR. Expression of inflammatory cytokines in hippocampal tissue was measured by quantitative RT-PCR. Dying CA1 hippocampal neurons were counted at 3 or 10 days. Delay fear conditioning was used to test memory 10 days after CA/CPR.

Result: Delayed death of ischemia-sensitive hippocampal CA1 neurons was evident as early as 3 days after CA/CPR (52% dead and dying neurons) and continued to day 10 (12% dead and dying neurons). Activated (Mac-2 positive) microglia appeared in the hippocampus as early as 1 day after CA/CPR, before significant neuronal death was present, and persisted at 10 days. Concurrently, expression of pro-inflammatory tumor necrosis factor (TNF)-α and interleukin (IL)-1β increased. All animals after CA/CPR exhibited abnormalities of gait, strength and coordination (wire grasping), and executive function (nest building), on day 3 (neuroscore 5 after CA/CPR vs 0 after sham surgery). Context, but not cued, freezing after delay fear conditioning was severely compromised 10 days after CA/CPR (42±8% after CA/CPR vs 72±5% in sham-operated animals), indicating loss of hippocampus-dependent memory acquisition.

Discussion and Conclusion: We present a mouse model of hippocampal memory dysfunction after CA/CPR. Inflammation and microglial activation in the hippocampus were pronounced after CA/CPR and preceded neuronal death. This model allows us to further investigate the differential contribution of inflammation to neuronal death and memory dysfunction after CA/CPR. Future studies will test whether delayed intervention that targets microglial activation and inflammation can improve memory function and reduce disability in survivors of CA/CPR.

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Summative Evaluation of a Tribal Telemedicine Project

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Background: The Tribal Visual Impairment Prevention Program was designed to provide preliminary information regarding prevalence of eye diseases in NW American Indian communities, to measure the quality of life benefits of providing eyeglasses, and to measure the impact of non-mydriatic cameras and telemedicine on preventing blindness.
from diabetic retinopathy. The evaluation was designed to inform future research projects in our community-university partnership.

**Methods:** We conducted a summative evaluation of the project using qualitative methods to assess community-based research experiences, effectiveness of the research protocol, adoptability and diffusion of the project. We conducted document review, evaluated open-ended questionnaires from university researchers and one-on-one interviews with community members, study participants, community health representatives, researchers, partner organizations, and community research assistants.

**Results:** Overall, the project demonstrated a successful collaborative approach with the intervention communities. Both the university and community researchers anticipated the relationship would persevere through subsequent studies. The university had been approached by several rural communities to replicate the work and plans for future research into the cost-effectiveness of telemedicine in eye care were underway.

Recruitment, staffing, and technical issues slowed down the project initially, and could be minimized in future projects by enlisting the support of the tribes to hire staff and develop position descriptions and procedures. We also learned that prior to project implementation data tracking systems should be developed by university staff and then adapted by each site; technical issues should be planned for in the early phases. Importantly, future projects should address sustainability in the initial proposal phase.

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**Intelligent systems for detection of aging changes**


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Our rapidly aging population will result in an increasing number of people at risk for loss of independence through dementia, frailty and other syndromes of aging. Evolving sensor and other technologies now provide a means of early detection and intervention minimizing morbidity and cost. We hypothesize that integrated, continuous and unobtrusive home monitoring of activity (motor and cognitive) can detect transitional or early signal events important for maintaining cognitive and physical health. To test our hypothesis and to further develop the resulting new clinical paradigm, lead institution Oregon Health & Science University (OHSU) established a novel Bioengineering Research Partnership (BRP) including OHSU's Layton Aging & Alzheimer's Disease Center, Biomedical Engineering Department, and Oregon Roybal Center for Aging & Technology, and industry partners Spry Learning and Intel. This BRP is dedicated to developing and testing in real world environments unobtrusive intelligent systems for integrating activities and clinical status and ultimately providing the key feedback necessary for instituting appropriate health maintenance, and illness prevention or intervention strategies. Thus our specific aims are to: 1) Determine if continuous, unobtrusive monitoring of motor and cognitive activities detects incident cognitive decline in seniors living in typical community settings; 2) Develop novel algorithms and assessment techniques for detecting motor and cognitive change in these community settings and in the context of the ongoing BRP, to test evolving sensor technology; and 3) Identify the monitoring needs of, and optimal communication channels, for lay individuals and health care professionals. As a result of this research this BRP will: establish a community living laboratory of homes outfitted with integrated sensing systems to determine early cognitive decline and identify the earliest points of cognitive change using this methodology; identify the optimal predictors and data fusion that will result in early detection; establish a fast track system for unobtrusively field-testing new sensor systems while an ongoing longitudinal study is conducted; and create a shared resource of data, expertise
and community attitudes about the conduct and application of these continuous assessment techniques for future proactive application in health care.

**TLR4- and TLR9-induced neuroprotection against stroke is mediated by IRF signaling**

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Stroke is a leading cause of mortality and morbidity in the United States, yet there is a dearth of treatments for this disease. Preconditioning with either the TLR4 ligand lipopolysaccharide (LPS) or the TLR9 ligand unmethylated CpG oligodeoxynucleotides elicits a neuroprotective response to subsequent brain ischemia; however, the mechanism underlying this phenomenon is unclear. Our goal was to elucidate the neuroprotective response to stroke in the context of TLR preconditioning. To examine this response, mice were preconditioned with LPS or CpG 72 hours prior to ischemia induced via a 45-minute transient middle cerebral artery occlusion (MCAO). RNA was isolated from brains harvested 24 hours after MCAO and analyzed using microarrays or quantitative PCR. The brain transcriptomes of mice preconditioned with LPS or CpG were compared to those of control animals. Computational analysis revealed that animals pretreated with LPS or CpG had a distinct transcriptional profile following stroke; this converged on the upregulation of 13 genes that were not regulated in untreated animals. PAINT analysis of the promoter regions of these 13 genes revealed that binding sites for interferon regulatory factors (IRFs) were over-represented. This suggests a dominant role for IRF-mediated signaling in the neuroprotective response to stroke. The roles of IRF3 and IRF7, the major IRFs downstream of TLR signaling, were assessed by examining the effect of LPS and CpG preconditioning on the response to MCAO in mice deficient in either IRF3 or IRF7. Neither IRF3- nor IRF7-deficient mice were protected by either preconditioning agent, demonstrating that both IRF3 and IRF7 are required for neuroprotection. Quantitative PCR analysis revealed that many of the 13 interferon (IFN)-associated genes seen in wild-type mice were still upregulated in mice deficient in IRF3 or IRF7 at 3 hours following preconditioning; however, these genes were not upregulated following MCAO. Thus, the interplay of IRF3 and IRF7 may be required following stroke to produce this potentially neuroprotective IFN-associated gene expression pattern. This research strongly implicates IRF signaling as a major effector of TLR-preconditioning-induced neuroprotection and suggests that IFN-associated genes may participate in ameliorating the damage caused by cerebral ischemia.

**Intrastriatal B cell administration limits infarct size after stroke in B cell deficient mice**

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**Background and Purpose:** Recent evidence emphasizes B cells as a major regulatory cell type that can play an important role in the pathogenesis of ischemic stroke. We have previously shown that intraperitoneal administration of IL-10-
secreting B cells reduces infarct volume following experimental stroke in mice. The aim of the current study was to determine the effects of CD19+ B cell restoration and treatment on infarct size in B cell deficient µMT−/− mice by stereotaxic cell delivery.

**Methods:** A novel method of stereotactic cell delivery was utilized to deliver CD19+ B cells into the striatum 24 hours before middle cerebral artery occlusion (MCAO) so as to bypass the blood brain barrier. Cortical and striatal infarct volumes in brain were compared in untreated B-cell deficient µMT−/−, B-cell-transferred µMT−/− and medium (vehicle)-transferred µMT−/− male mice 48 hours following 60 min of MCAO.

**Results:** No differences in cortical and striatal infarct volume were observed between untreated and medium (vehicle)-transferred µMT−/− male mice 48 hours after 60 min of MCAO. However, cortical, but not striatal, infarct volumes were significantly decreased in B-cell-transferred µMT−/− compared to untreated µMT−/− mice and medium-transferred µMT−/− mice.

**Conclusions:** These findings suggest that B-cells may be a novel therapeutic target in acute stroke for modulating the immune response in central nervous system inflammation.

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**Intravital microscopy imaging of cochlear lateral wall in live mice through a thinned otic capsule**

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Measuring cochlear blood flow in response to various physiological stresses has remained challenging due to the lack of a sensitive detection methods and difficulty accessing the organ. Here we report a new approach using a “thinned otic capsule window” method in combination with intravital microscopy for studying cochlear blood flow and pathophysiology of various lateral wall cell types in murine models. Although surgery on the mouse cochlea is difficult due to its small size, the benefit of this newly established method is high-resolution imaging of fluorescently labeled vessels and cells. It provides a way to investigate the cellular biology of lateral wall cell populations in-vivo using murine models. Most significantly, imaging through a thinned capsule provides an intact lateral wall minimizing the disruption of the delicate homeostatic balance. Perforation of the cochlear otic capsule causes changes in cochlear pressure due to the loss of perilymphatic fluid. This new method is a minimally invasive approach for studying structural and functional changes in cochlear blood flow and lateral wall biology under physiological and pathological conditions.
Aaron Grossberg | Inflammation-induced lethargy is mediated by suppression of orexin signaling.

Abby Floeter | Adherence to guidelines for heart failure with preserved ejection fraction (HFPEF) at the Portland Veterans Affairs Medical Center (PVAMC)

Aimee Mooney | A Brain Computer Interface using the RSVP keyboard for users who are locked-in

Amy Trevor | Children with Severe Early Childhood Caries: Identification of Cariogenic Mutans Streptococci Genetic Strains Harbored Within Carious and White Spot Lesions

Anders Goranson | Evaluation of a Home Based Telemental Health (HBTMH)

Andy Barnett | Curative versus palliative therapy for patients with colorectal cancer presenting to the emergency department

Anita Cservenka | Gender differences in the neural substrates of emotional conflict during adolescence.

Beth Darnall | Medication taking attitude/behavior is influenced by relationships: Results from the CARE Scale online survey

Carmem Pfeifer | Conversion-dependent shrinkage in (meth)acrylates as a function of irradiance

Carolina Glogowski | Physiology of unipolar brush cells in the dorsal cochlear nucleus

Carrie Farrar | CTRC Study Coordinator Unit

Cheng Fang | Inhibition of axonal transport is an early event following exposure to reactive oxygen species

Clive Woffendin | OCTRI Core Laboratory

Corinne Stevens | Functional brain connectivity in infants with prenatal risk for ADHD

Daniel Kriz | Navigating the Unstandardized Approaches to Cortical Mapping

David Grayson | Altered Structural Connectivity in Youth with ADHD

David Grayson | Resting-state Functional Connectivity MRI Reveals Large-Scale Differences Between Monkeys Raised on High vs Low Omega-3 Fatty Acid Diets

Diana Parrish | Proneurotrophins cause peri-infarct sympathetic denervation

Eli Schwarz | Dental Care Needs Among Underserved Oregonians - the Oregon Mission of Mercy
Ian Tagge | Population-Generalized vs. Individual-Specific AIF in Human Prostate DCE-MRI Pharmacokinetic Analysis

Izabela Chamot | Variability in the diagnostic criteria to evaluate heart failure with preserved ejection fraction (HFPEF) in a veteran affairs (VA) patient population

Jacob Pearson | The Effect of Maternal Low Protein Diet on Indoxyl Sulfate and Oat1/3 Expression

Joannah Vaughan | How Effective is the PediaVision SO9 in Detecting Amblyopic Risk Factors in Children, Ages 3-5 Years?

John Mitchell | Flexural strength and modulus of bioactive glass-containing, anti-microbial dental composites

John Muschler | Loss of cell-surface laminin anchoring promotes tumor growth and is associated with poor clinical outcomes.

Joseph Haynes | Dental Implants Placed from 2002-2009 in an Advanced Specialty Education Program in Periodontics: A Radiographic and Clinical Review

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Junan Zhang | A method of improving the effective spatial resolution for small field IMRT QA with 2D-array device

Kristen Mackiewicz Seghete | White matter microstructure and cognitive control in a developmental sample

Lani Doser | Perinatal Mortality of Planned Out of Hospital Births Transferred to an Oregon Hospital, 2004-2008

Lillian Nail | Describing the Experience of Hair Loss with Cancer Chemotherapy

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Matthew Brush | The Reagent Ontology: an Integrated Resource for Curation of Biomedical Research Reagents

Megan O'Brien | Development of a Non-Human Primate Special Care Nursery

Monica Rincon | Ovarian torsion in pregnancy: ultrasound characteristics and histopathology

Nicole Vasilevsky | The eagle-i research resource discovery system

Pamela Levine | Validation of electronic medical record data for the retrospective identification of skin and soft tissue infections in primary care settings
Inflammation-induced lethargy is mediated by suppression of orexin signaling

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Background: Lateral hypothalamus orexin neurons regulate physiologic arousal in response to environmental cues. Normal circadian increases in wakefulness and locomotor activity (LMA) are absent following inflammatory challenge. We hypothesize that inflammation induces lethargy by suppressing central orexin signaling.

Methods: Male Sprague-Dawley (LPS) and F344 (tumor) rats were used in all experiments. Subcutaneous Minimitter telemetry was used to monitor LMA. Ip LPS (250 μg/kg) 90 min before lights off and syngeneic tumor implantation were used as inflammatory insults. Gene expression and location was measured using in situ hybridization. Neuron activity was assessed by presence of cFos immunoreactivity (IR) 90 min after lights off. Lateral ventricle cannulation was used for central replacement of Orexin-A (OxA) following LPS. CSF [OxA] was measured by radioimmunoassay. Rats were food restricted (FR) to activate the orexin system independent of circadian influence and LMA and cFos IR were quantified during peak of food-anticipatory activity (FAA).

Results: Dark phase LPS-treated and tumor-bearing rats show reduced dark phase LMA associated with decreased orexin mRNA in the medial LH (tumor) and decreased CSF [OxA] (LPS). Dark-phase LPS treatment reduced cFos IR in orexin neurons, particularly in the perifornical region. Acute bolus OxA replacement both prevented onset of lethargy and reversed existing lethargy. Subchronic OxA replacement increased LMA throughout dark phase in LPS-treated rats with no effect on food intake. LPS suppressed FAA in FR rats and suppressed the increase in cFos IR observed in vehicle treated FR rats.
Conclusion: Inflammation suppresses circadian- and FR-induced orexin neuron activation, leading to diminished arousal. This system is regulated independently of feeding, suggesting that parallel CNS inflammatory pathways are responsible for these elements of sickness behavior. That OxA replacement restores total and crepuscular peaks in LMA and FR animals exhibit a similar response indicates that circadian clock neurons are not responsible for diminished orexin neuron activity. This work also defines an anatomically distinct subpopulation of inflammation-sensitive orexin neurons, which mediate physiologic arousal.

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Adherence to guidelines for Heart failure with preserved ejection fraction (HFPEF) at the Portland Veterans Affairs Medical Center (PVAMC)

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Introduction: No pharmacologic therapy has been shown to reduce mortality in patients with HFPEF. According to the American College of Cardiology and American Heart Association (ACC/AHA) guidelines, management of HFPEF focuses on controlling blood pressure, heart rate, myocardial ischemia and fluid management with diuretics. However, the presence of non-cardiac comorbidities can complicate care of these patients. While many studies to date have evaluated the frequency of such comorbidities, none have either assessed adherence to guidelines or comorbidity management.

Objective: To evaluate the adherence to guideline-based recommendations for HFPEF and treatment of competing comorbidities in patients with HFPEF at the PVAMC.

Methods: A list of patients with documented ICD-9 codes for heart failure (HF) was generated from the Veterans Affairs data warehouse. Electronic medical records of these patients were screened for an Ejection Fraction (EF) > 50%. Patients were excluded if they had a reduced EF (<50%), were under hospice care, or no longer seen at the PVAMC. A chart audit form was developed to extract data on patient demographics, non-cardiac comorbidities affecting HF management (i.e., diabetes, chronic obstructive pulmonary disease (COPD), and sleep apnea), and components of care related to adherence to the four key ACC/AHA guidelines. Data were analyzed using descriptive statistics. Frequencies and percentages were calculated for demographics and the incidence of co-morbidities.

Results: A total of 475 potential heart failure patients were identified from the data warehouse. To date, the electronic medical records for 199 patients were screened and, of those, 70 (35%) met the inclusion criteria and were included in the analysis. The majority of patients were male (99%), white (63%) and obese (BMI > 30, 56%) and were seen in the past six months by their primary care provider (PCP) (93%). Of the 70 patients assessed, 99% had hypertension, 47% had atrial fibrillation, 79% had hyperlipidemia, 63% had diabetes, 43% had COPD or asthma, 47% has obstructive sleep apnea, 30% had chronic kidney disease, and 37% had anemia. The 69 patients who had hypertension had a median of 5 (interquartile range (IQR), 3-6) blood pressure readings in the past 6 months. Only 50% of these patients were at the target systolic blood pressure of <130 mmHg. Of the 33 patients with diagnosed atrial fibrillation, all had controlled heart rates (<110 bpm). Most of the patients were assessed for coronary artery disease (71%) and 44% of patients had coronary revascularization procedures. Of the 53 patients prescribed diuretics, 42 (79%) had been evaluated for volume
overload in the last 6 months. Non-cardiac comorbidities were appropriately evaluated: diabetes (98%), COPD (70%), sleep apnea (91%).

**Conclusion:** To our knowledge, our analysis is the first to evaluate the management of HFPEF in the veterans population. The guidelines recommend that management of HF must include a strong focus on diagnosis and treatment of comorbidities. Our data suggests that the majority of our patients were evaluated for comorbidities and had follow up appointments within the last six months. Our results also show that the majority of our patients were screened and treated for class I recommendations according to the practice guidelines. We believe that HFPEF patients at the PVAMC have received appropriate evaluation in controlling this challenging clinical syndrome.

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**Initial design of an augmentative communication device with non-invasive brain-computer interface, adaptive language modeling and Rapid Serial Visual Presentation (RSVP)**

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We are designing a portable communication device that relies on a non-invasive brain-computer interface (BCI) with optimized language modeling for literate individuals who are functionally locked-in. We use single trial P3 detection for binary selection of single characters in a rapid serial visual presentation (RSVP). The innovative BCI has three essential, unique features: (1) linguistic components ranging from letters to words to phrases that are presented on a computer screen one at a time in rapid succession; (2) a detection mechanism that employs multichannel electroencephalography (EEG) and/or other suitable response mechanisms that can reliably indicate the *binary intent* of the user and adapt based on individualized neurophysiologic data of the user; and (3) an open-vocabulary natural language model with a capability for accurate predictions of upcoming text. The collaborative nature of the proposed translational research is expected to yield new knowledge for both BCI development and clinical augmentative communication use.

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**Children with Severe Early Childhood Caries: Identification of Cariogenic Mutans Streptococci Genetic Strains Harbored Within Carious and White Spot Lesions**

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**Objectives:** Dental caries is induced by cariogenic microorganisms in the oral cavity and represents one of the major health concerns in children. Our laboratory proposes to understand the factors affecting the genetic diversity and potential selection of mutans streptococci (MS) strains during the development of dental caries. The specific objectives
of this study, using a cohort of 20 children, are to identify distinct MS genetic strains that are harbored within carious and white spot lesions (WSLs) vs. non-carious enamel surfaces.

**Published Findings:** Genetic fingerprints of oral MS isolates (n=828) were identified within 7 children exhibiting severe-early childhood caries (S-ECC), before and after comprehensive dental treatment. Children underwent full-mouth dental rehabilitation using general anesthesia and were subjected to restorative procedures, use of antimicrobial rinse and application of fluoride varnish. MS isolates were also characterized for cariogenicity markers, including acid generation, acid tolerance and xylitol resistance. We identified 39 genetic strains of MS, and found that caries restorative therapy in some patients resulted in population shifts to highly acidogenic or acid-tolerant MS strains, with single dominant MS strains appearing at 1-year post-therapy.

**Methods:** Dental plaque from children (n=20; ages 3-5) diagnosed with early childhood caries was collected from tooth enamel surfaces, cavitated carious lesions and incipient non-cavitated white spot lesions (WSLs). MS isolates (n=10) from each collection site were selected on mitis salivarius agar containing bacitracin and characterized by polymerase chain reaction (PCR) for verification as MS and arbitrarily primed-PCR for assignment within genotypic strains.

**Results:** Prior to comprehensive dental treatment, children contained 1-7 MS strains. Nine out of 20 patients contained dominant MS strains in carious lesions or WSLs that were distinct from strains isolated from sound enamel surfaces.

**Conclusions:** Diverse MS strains found within carious lesions and WSLs may retain potential differences in cariogenicity and treatment response. Well-accepted practices for caries prevention and treatment should be more closely examined for efficacy.

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**Evaluation of a Home Based Telemental Health (HBTMH) Pilot Program**

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Since February 2010, the VISN 20 Home Based Telemental Health Pilot Program (HBTMH) was implemented with the intention of eliminating barriers to care by meeting Veterans where they're at. This patient centric / provider empowered program is aimed at serving the mental health needs of rural Oregon Veterans whose access to care is restricted by geography, limited resources or who are home-bound due to psychiatric and/or medical conditions. During a period of 18 months, providers delivered various mental health services utilizing a web-cam, secure and encrypted software (MOVI) on the Veteran's side. The poster will be presenting clinical findings, patient safety measures, clinician perspectives, and areas for improvement.

(6 Providers treating 40 patients dispersed throughout Oregon. There are currently 9 Veterans who continue to receive services).

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**Curative versus palliative therapy for patients with colorectal cancer presenting to the emergency department.**

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**Background:** Colorectal cancer (CRC) is the second leading cause of cancer death in the United States for men and women combined, and can present emergently with symptoms such as abdominal pain, bleeding, and obstruction. Emergency presentation as the first indication of colorectal cancer is generally thought to be associated with advanced disease and poor outcome. The specific aim of this analysis was to describe characteristics of patients (pts) presenting to the Emergency Department (ED) at their index diagnosis, and to determine whether emergency presentation precludes treatment with curative intent.

**Methods:** We performed an IRB-approved retrospective cohort analysis. We queried the prospectively maintained institutional tumor registry to identify pts diagnosed with CRC from 2008-2010. EMRs were reviewed to identify which pts presented to the ED with acute symptoms of CRC as the initial sign of their illness. The primary outcome variable was treatment plan (curative vs. palliative). Secondary outcome variables included demographics, tumor type and location. Descriptive statistics were conducted for major variables. $\chi^2$ and Fisher’s exact tests were used to detect the association between categorical variables. Two-sample t-test was used to identify the association between continuous and categorical variables.

**Results:** Between Jan 1 2008 and Dec 31 2010, 376 pts were identified with CRC. 214(57%) were male and 162(43%) were female, with mean age 60.6; SD: 13.3. 33 (8.8%) pts initially presented to the ED, of which 5 (15.5%) received palliation. Of 339 pts who initially presented elsewhere, 69 (20.5%) received palliation. Acute ED presentation with CRC symptoms did not preclude treatment with curative intent ($p = 0.47$). Pts who presented emergently were more likely to be female (64% vs male 41%; $p=0.01$), and older (65 vs. 60; $p=0.02$). There was no statistically significant relationship between age, gender, tumor location or type and treatment approach.

**Conclusions:** Pts with CRC may present to the ED with acute symptoms, which ultimately leads to the diagnosis. Emergent presentation of CRC does not preclude patients from receiving therapy with curative intent.

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**Gender differences in the neural substrates of emotional conflict during adolescence**

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Adolescence is a developmental period characterized by heightened emotional reactivity and immature top-down executive control, which may underlie vulnerabilities associated with the emergence of psychiatric disorders during this time. In particular, gender differences in brain functioning may contribute to gender-specific vulnerabilities to psychopathology seen during adolescence. Thus, the goal of the current study was to investigate adolescent gender differences in brain functioning during functional magnetic resonance imaging of an emotional processing task requiring inhibitory control (Etkin at al., 2006). During the task, participants were required to indicate the emotion of facial expressions while disregarding emotion-congruent or incongruent words printed across the faces. In order to examine gender differences in emotional inhibition, 28 age-matched participants were divided into adolescent girls (n=16) and boys (n=12) (mean age=13.37±.31). No gender differences in task performance were present. Using an analysis of covariance, covarying for pubertal status, we found that girls had greater brain activity in bilateral precuneus/cuneus and left insula/superior temporal gyrus (STG) during correct congruent vs. incongruent trials compared with boys ($p<0.05$, voxel/clusterwise corrected). In the face of distraction by emotion-incongruent stimuli, girls showed significantly weaker brain activity compared with emotion-congruent face processing, a distinction not present in boys.
Overall, girls also showed much greater brain response in these regions on congruent trials compared with boys. The precuneus/cuneus and insula/STG are brain areas implicated in visual attention and emotional processing, respectively. The results of this study suggest that girls allocate greater attentional resources and display increased affective brain response when processing emotional faces in the presence of emotion-congruent words compared with boys. These findings highlight differences in attention and affect-related brain activity to emotional faces between adolescent girls and boys, which could confer vulnerability for the emergence of internalizing symptoms and psychiatric disorders, such as anxiety and depression, in a gender-specific fashion.

This research was supported by the Oregon Health & Science University Graduate Research Scholarship (Cservenka) and the Dana Foundation (Nagel).

**Medication Taking Attitude/Behavior is Influenced by Relationships: Results from the CARE Scale Online Survey**

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**Introduction**: The impact of close relationships on pain management is understudied in chronic pain populations. However, daily activities and self care are integral to effective pain management and may predict pain medication usage. We examined whether relationships, self-care and limit setting capacity were related to endorsement of pain medication use; sex differences were also tested.

**Materials and Methods**: A survey was posted online and advertised via chronic pain websites. Those with pain for ≥3 months were invited to take the anonymous survey comprised of the 22-item CARE Scale (comprised of 4 subscales: Locus of Care orientation (Internal and External), Limit Setting Capacity, and Relationship Guilt/Fear related to pain) and items for sex, marital status, dependents, and primary caretaker status. Using multiple rank regression, response to the item: “If I push myself too hard, I can always take medication for the pain” was regressed on the components of each subscale group. The study was IRB approved.

**Results**: The chronic pain sample was 495 adults (mean age =48.3, SD = 12.8, 86.5% female), predominantly married (56.5%), with dependents (54.6%), and primary caretaker status (70.9%). Pain medication taking behavior related to poor pacing was significantly predicted by each subscale: Internal Locus of Care (R²= 0.05), External Locus of Care (R²=0.07), Limit Setting Capacity (R²=0.18) and Relationship Guilt/Fear (R²=0.09); all p-values <0.0001. Significant sex differences were found for each subscale.

**Conclusions**: Relationship factors significantly impact pacing and medication taking attitude/behavior, particularly for women. Future research will validate these findings in a pain clinic sample.

**Conversion-dependent shrinkage in (meth)acrylates as a function of irradiance**

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**Objectives:** The objective of this study was to evaluate, at different irradiances, the conversion-dependent shrinkage behavior of (meth)acrylates as a function of monomer reactivity, resulting network stiffness and hydrogen bonding potential.

**Methods:** Triethylene glycol dimethacrylate (TEGDMA), polyethylene glycol dimethacrylate (and acrylate; PEGDMA/PEGDA, respectively), urethane dimethacrylate (UDMA), glycerol dimethacrylate (GlyDMA) and 1,14-tetradecanediol dimethacrylate (C14DMA) were mixed with 0.1 wt% 2,2-dimethoxy-2-phenylacetophenone (λ<sub>max</sub>=365 nm). Shrinkage (linometer) and conversion (near-IR) were followed simultaneously during photopolymerization (3 or 30 mW/cm²). Conversion (DC) and shrinkage (VS) at the point of maximum rate of shrinkage development (R<sub>vmax</sub>) were analyzed with 2-way ANOVA/Tukey’s test (alpha=5%).

**Results:** When comparing glassy x rubbery methacrylates of similar structure (TEGDMA x PEGDMA), R<sub>vmax</sub> is similar at both irradiances, but TEGDMA presents much greater DC/VS@R<sub>vmax</sub> as irradiance decreases than does the rubbery polymer, pointing to the much greater free volume entrapment at higher irradiances for glassy networks. For rubbery networks, free volume is independent of reaction rate, as is evidenced by the greatest increase in VS@R<sub>vmax</sub> with the decrease in irradiance presented by PEGDA in comparison to PEGDMA. For glassy methacrylates, as the hydrogen bonding potential increases (C14DMA<TEGDMA<UDMA<GlyDMA), the increase in DC/VS@R<sub>vmax</sub> for higher irradiances is greater, due to additional mobility restrictions imposed by stronger secondary intermolecular interactions.

<table>
<thead>
<tr>
<th>Monomer</th>
<th>R&lt;sub&gt;vmax&lt;/sub&gt; (%)</th>
<th>DC@R&lt;sub&gt;vmax&lt;/sub&gt; (%)</th>
<th>VS@R&lt;sub&gt;vmax&lt;/sub&gt; (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>High I&lt;sub&gt;0&lt;/sub&gt;</td>
<td>Low I&lt;sub&gt;0&lt;/sub&gt;</td>
<td>High I&lt;sub&gt;0&lt;/sub&gt;</td>
</tr>
<tr>
<td>TEGDMA</td>
<td>0.5±0.0</td>
<td>0.6±0.1</td>
<td>13±2</td>
</tr>
<tr>
<td>PEGDMA</td>
<td>0.3±0.0</td>
<td>0.3±0.0</td>
<td>22±3</td>
</tr>
<tr>
<td>PEGDA</td>
<td>2.1±0.1</td>
<td>1.1±0.2</td>
<td>80±5</td>
</tr>
<tr>
<td>GlyDMA</td>
<td>1.6±0.1</td>
<td>0.4±0.1</td>
<td>32±2</td>
</tr>
<tr>
<td>UDMA</td>
<td>2.7±0.1</td>
<td>0.7±0.2</td>
<td>13±1</td>
</tr>
<tr>
<td>C14DMA</td>
<td>0.1±0.0</td>
<td>0.2±0.0</td>
<td>14±1</td>
</tr>
</tbody>
</table>

**Conclusions:** The delay in shrinkage in relation to conversion has been demonstrated to be dependent on irradiance, monomer reactivity, secondary intermolecular interactions and stiffness of the network. NIH/NIDCR R01DE014227

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**Physiology of Unipolar Brush Cells in the Dorsal Cochlear Nucleus**

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The dorsal cochlear nucleus (DCN) integrates auditory input with multisensory signals from various brain regions. Multisensory signals are conveyed by mossy fibers, which terminate in the deep layer of the DCN and whose main targets are granule cells. However, a large subset of granule cells also receives multisensory input through a feedforward excitatory pathway mediated by an excitatory interneuron called the unipolar brush cell (UBC). UBCs have a peculiar morphology, with a single short dendrite terminating in a brush-like structure that interdigitates with a single pre-
synaptic mossy terminal. UBCs are also prominent in the vestibular cerebellum. One cerebellar model suggests that the large irregular synapse onto UBCs entraps the transmitter glutamate and slows diffusion. Thus, the prolonged glutamate transient leads to a characteristic slow-decaying post-synaptic current (EPSC), which mediates repetitive firing in UBCs. DCN UBCs share homology with their cerebellar counterparts. Although they are one of the primary targets of mossy fibers carrying multisensory signals, their impact on granule cell activity in either DCN or cerebellum has never been directly investigated. We have made recordings from UBCs in slices from 3-week old mouse DCN, characterizing the basic intrinsic and synaptic properties of these neurons. We find that DCN UBCs exhibit bimodal firing (tonic vs burst mode) dependent on the resting membrane potential. Evoked EPSCs have a striking biphasic decay, with a slow component lasting hundreds of milliseconds. We also observed that EPSC amplitudes show strong synaptic depression and have very low trial- to-trial variability, suggestive of a high probability of transmitter release. These features are consistent with a model in which the large UBC synapse may function to amplify signals from mossy fibers, thereby increasing the salience of a subset of non-auditory signals.

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**CTRC Study Coordinator Unit: Program and Services**

CTRC Study Coordinator Unit: Carrie Farrar, MPH, CCRP; Troy Lubianski, RN, CCRP; Lissy Powell, BS; Whitney Drew, BS; Matt French, BS; Andrea Kuchler, BS

The Study Coordinator unit at the *Oregon Clinical and Translational Research Institute* (OCTRI) offers support to adult and pediatric studies, investigator initiated or industry sponsored, in any indication across the OHSU and Portland Veteran’s Administration Medical Centers. We also have experience working with Kaiser Permanente and Community-based studies. Our team is made up of highly motivated, experienced, professional individuals who receive extensive training in regulatory compliance and study coordination. Study Coordinators work collaboratively with investigators and study staff, and clinical personnel to ensure the integrity and success of the research study. In 2011, we coordinated clinical research studies in ADHD, bleeding disorders, cardiology, digestive health, mental health, neurology, otolaryngology, pediatric urology, pharmacology, rare diseases, surgery, and VA studies.

**Oregon Clinical and Translational Research Institute**

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**Inhibition of axonal transport is an early event following exposure to reactive oxygen species**

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Axonal transport defects have been suggested to contribute to the axonal degeneration observed in several neural diseases. We are exploring the possibility that elevated levels of reactive oxygen species produced during inflammation inhibit axonal transport and that this contributes to axonal degeneration seen in MS.

Mitochondria and Golgi-derived vesicles are the two principal organelle populations that undergo fast anterograde axonal transport. We used live cell imaging to assess the transport of these organelles in the axons of cultured hippocampal neurons before and at varying times after adding hydrogen peroxide, a common reactive oxygen species. Addition of 100µM hydrogen peroxide significantly reduced the transport of both Golgi-derived vesicles and mitochondria within 30-60 minutes. Mitochondrial transport was inhibited more rapidly than that of Golgi-derived
vesicles, suggesting that it is particularly sensitive to reactive oxygen damage. Transport was inhibited before any changes in mitochondrial shape and long before the earliest morphological signs of axonal damage. It is known that mitochondrial function is compromised in MS, which can lead to reduced ATP production. As axonal transport is ATP-dependent, we examined whether the effects of hydrogen peroxide could be mimicked by sodium azide, an ATP synthesis blocker. Both reagents inhibited axonal transport similarly, but the effects of blocking ATP synthesis were fully reversible. In contrast, mitochondrial transport remained depressed following hydrogen peroxide treatment and axons eventually degenerated. These results suggest that in addition to inhibiting ATP synthesis, hydrogen peroxide may activate signaling pathways that selectively target some components of the axonal transport machinery. These results also show that reactive oxygen species directly damage axons, independent of the damage to myelin.

OCTRI Core Laboratory

Clive Woffendin PhD*

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Overview: The OCTRI Core Laboratory is specifically designed and equipped to perform both basic and specialized research assays and sample processing in support of translational research studies and trials. Laboratory staff have extensive experience in the performance of a wide variety of analytical and genetic based assays and procedures.

Laboratory Services: Some of the most essential components of successful clinical research projects are sample integrity, timely sample processing, and effective sample storage all of which facilitate correct interpretation of subsequent assay data. A major role of the OCTRI Core Laboratory is to perform clinical specimen processing, storage and shipping.

The Laboratory can perform over 60 different laboratory assays and analyses, including hormones, inflammatory markers, obesity factors, cardiac biomarkers and bone markers. Additionally, an extensive menu of lipid analyses can be performed by the OHSU Lipid lab.

The Laboratory also performs genetic analyses and procedures, including DNA & RNA extraction and purification, DNA sequencing and genotyping methodologies.

Laboratory staff is also available for consultations on assay selection, assay development and experimental design & consultation.

The OCTRI Core Laboratory is specifically designed and equipped to perform both basic and specialized research assays and sample processing in support of translational research studies and trials.

Functional brain connectivity in infants with prenatal risk for ADHD


Oregon Health & Science University, Portland OR
**Background:** MR imaging indicates that the neonatal brain is only about half the volume of the adult brain, and grows to about 90% adult brain volume by the end of the second year of life (Shi 2011). As such, this time period lends heightened vulnerability to environmental insults (Gilmore 2008). Attention deficit hyperactivity disorder (ADHD) is a heterogeneous disorder with known heritability that manifests as an interaction between genes and environment. We hope to learn how environmental toxicants, metal contaminants (lead, cadmium, manganese), key nutrients (essential fatty acids, iron), stress, genetics, and other prenatal health measurements relate to functional connectivity in neonates and infants with and without a first degree family history of ADHD.

**Method:** This is a longitudinal study evaluating the relationship between genetics, nutrition, stress, toxins and ADHD development. The subjects consist of a cohort with either a first degree relative (biological mother, father, or sibling) with ADHD and a second control cohort without a family history of ADHD. To date 12 offspring (14 days to 18 months) have completed MR brain imaging while asleep without sedation. Here we present results from our 1st 5 neonatal scans (14days - 30days). For our initial pilot analysis resting state functional connectivity MR (rs-fcMRI) measurements of the default network were related to dietary intake of the mothers during the 3rd trimester of pregnancy.

**Results:** We see decreased functional connectivity of the posterior cingulate cortex to the Nucleus Accumbens and the ventral medial prefrontal cortex/rostral cingulate as a function of increased saturated fat intake. Among several functions, these links are thought to be involved in impulse control, which is a key feature of the ADHD phenotype.

**Discussion/Conclusion:** In this study we report preliminary results on (a) correlations between dietary intake and functional brain connectivity in offspring at risk for ADHD, and (b) feasibility of collecting fMRI data on infants without sedation. We have shown that rs-fcMRI analyses in infants are feasible. Results suggest that environmental factors, such as nutrition during pregnancy, have an effect on early development of functional brain connectivity and may be a precursor to the ADHD phenotype.

**Funding:** Research was supported by the Oregon Health & Science University Department of Behavioral Neuroscience, Department of Psychiatry, OHSU Advance Imaging Research Center, R00 MH091238 (Fair), R01 MH59105 (Nigg), OHSU Neuropsychiatric Institute (Nigg).

**References:**

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**Navigating the Unstandardized Approaches to Cortical Mapping**

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*Presenting author

Electrocorticography and cortical stimulation are generally considered to be the gold standard in functional cortical mapping for the surgical treatment of epilepsy. These procedures require meticulous examination and delineation of cortical functions and utilize a broad range of tasks to determine these functions. Unfortunately, despite widespread
employment of these strategies, there currently exists no standard battery of language and motor tasks for implementation in the use of cortical stimulation. Previous investigations into the utilization of test batteries indicate that due to the complexity of cortical organization, especially with regard to language organization, the application of multitask batteries during electrical stimulation is essential for the preservation of language and motor functioning. Although most research in this field has focused on adult populations, some studies have identified differences in cortical organization amongst pediatric, adolescent, and adult populations, thus suggesting an enhanced need for thorough delineation of cortical functioning when utilizing electrical stimulation procedures in the treatment of pediatric epilepsy. The goal of the current study is to provide an overview of current task batteries utilized for cortical mapping and provide suggestions for the creation of a comprehensive, efficient, and standardized battery for use with pediatric epilepsy patients.

Altered Structural Connectivity in Youth with ADHD

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Introduction: In recent years, an increasing number of neuroimaging studies have aimed to delineate the functional and structural alterations that may be linked with attention-deficit hyperactivity disorder (ADHD). Previous studies have demonstrated microstructural alterations in the white matter, as determined by voxel-wise analyses of fractional anisotropy and mean diffusivity. However, thorough analyses of the structural connectivity between regions of the cortex via whole-brain tractography have been lacking. This study uses deterministic fiber-tracking along with graph theoretical analysis to characterize the structural organization of the cortex in the typical developing population and in children with ADHD.

Methods: Using diffusion tensor imaging, deterministic fiber-tracking was performed throughout all voxels of white-matter. A high-resolution parcellation of cortical gray-matter was then used to generate a whole-brain structural connectivity matrix for each subject. Several graph-theoretical measures were applied on an average matrix and on single subject matrices. Comparisons between 27 children with ADHD and 33 healthy children (age 7-11 years) were conducted.

Results: Our results indicate altered patterns of connectivity at several key regions along the midline of the brain, including the anterior cingulate cortex (ACC) and the cuneus cortex (CC). We observe alterations in the overall strength of connections to these regions across multiple measures, including fractional anisotropy, inverse apparent diffusivity, and number of streamlines.

Discussion: Consistent with previous work regarding functional connectivity in ADHD, these results suggest that childhood ADHD may be linked with alterations in the pattern and strength of structural connectivity of key midline regions with the rest of the brain.

Resting-state Functional Connectivity MRI Reveals Large-Scale Differences Between Monkeys Raised on High vs Low Omega-3 Fatty Acid Diets
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**Introduction:** Resting-state functional connectivity MRI (rs-fcMRI) is an emerging neuroimaging method used to examine the functional organization of the human brain. Several distinct functional systems have been already been identified using this tool, including the default-mode network, the cingulo-opercular control network, and the frontoparietal task network. To further our understanding and increase the translational potential of this method, extending this tool towards the study of animal brain function will be critical, and promises to shed new light on the way brain organization differs across species. In this study, we use rs-fcMRI to assess differences in brain organization in the adult rhesus macaque monkey as a result of differences in omega-3 fatty acid intake over the lifetime. Omega-3 fatty acids are essential for normal retinal development and have been implicated in a variety of neurodevelopmental disorders. Docosahexanoic acid (DHA) is a long-chain omega-3 fatty acid that comprises a large portion of the phospholipid bilayer in the membranes of the retina and brain. Specifically, we aim to compare rs-fcMRI results in monkeys raised on a diet high in DHA with those raised on a diet deficient in omega-3 fatty acids. While previous work has primarily supported a role for DHA in the healthy functioning of the visual system, we hypothesize that omega-3 deficiency will lead to large scale changes in functional brain organization as well.

**Methods:** Experiments were first done to show that a low dosage of isoflurane is an optimal sedation parameter for acquiring the rs-fcMRI signal. rs-fcMRI scans then were acquired under 1% isoflurane anesthesia on 10 rhesus monkeys with lifelong high and low omega-3 fatty acid status. Functional connectivity analyses were performed using the ‘Lewis and Van Essen 2000’ functionally defined brain areas. We assessed the modular organization of the whole brain as well as the functional connectivity maps for individual seed regions. Comparisons were then drawn between six monkeys (aged 17-19) raised on a diet deficient in omega-3 fatty acids which was previously shown to result in substantially reduced brain DHA levels, and four monkeys (aged 17-19) raised on a diet high in DHA.

**Results:** The modular organization of the brain differed substantially between the two groups of monkeys. Interestingly, the high-DHA monkeys showed many similarities with the normal resting-state human brain, such as high correlations between regions of the default-mode network, including the ventromedial prefrontal cortex, medial parietal cortex, and inferior temporal cortex. By contrast, the omega-3 deficient monkeys showed an unusual pattern of organization, including markedly less coherence within the default-mode regions, and unexpectedly high integration of V1 with the ventral visual pathway. Large differences were also observed in the connectivity maps of specific seed regions within the default-mode network.

**Discussion:** These results indicate that omega-3 fatty acid intake substantially affects the pattern of functional brain organization in the rhesus macaque. Furthermore, the findings presented here suggest that DHA consumption influences large-scale functional brain organization. While the work here is still preliminary, the effort highlights the potential for resting-state functional connectivity to serve as a surrogate brain marker that can be measured in both humans and non-human primates.

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**Proneurotrophins cause peri-infarct sympathetic denervation**

Diana C. Parrish¹, Christina U. Lorentz¹, Barbara L. Hempstead², Anders Nykaer³, and Beth A. Habecker¹
Altered sympathetic innervation of the heart is a major contributor to arrhythmias following myocardial infarction (MI). Changes to the innervation include denervation of the proximal peri-infarct myocardium. Proneurotrophin signaling through a heterodimer of the p75 neurotrophin receptor and the sortilin receptor leads to axon degeneration, and we asked if the denervation of peri-infarct myocardium was caused by proneurotrophins. Sympathetic innervation density was quantified by tyrosine hydroxylase immunohistochemistry in 12-18 week old p75⁻/⁻, sortilin⁻/⁻, and wild-type control mice (C57Bl6/J) that were subjected to sham or ischemia-reperfusion surgery. 24 hours post-MI, the peri-infarct zone was largely denervated in wild-type mice. In contrast, peri-infarct denervation was absent in both sortilin⁻/⁻ and p75⁻/⁻ mice. Additionally, both sortilin⁻/⁻ and p75⁻/⁻ mice exhibited hyperinnervation in more distal peri-infarct areas. Pro- and mature nerve growth factor (NGF) were detected in the infarcted left ventricle by immunohistochemistry, with proNGF being the more abundant species. Pro- and mature brain derived neurotrophic factor (BDNF) were quantified in the left ventricle of wild-type mice 24 hours following MI by western blotting. ProBDNF was elevated in the infarcted left ventricle compared to sham-operated controls, while mature BDNF was not detected. Application of proBDNF to sympathetic ganglia explants reduced axon length in wild type ganglia but not in sortilin⁻/⁻ ganglia. These data suggest a role for proneurotrophins in acute peri-infarct sympathetic denervation of the left ventricle.

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Dental care needs among underserved Oregonians – the Oregon Mission of Mercy

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Background. The United States is in the midst of a major dental crisis. There are 130 million Americans who have no dental insurance. One-fourth of adults aged 65 or older have lost all their teeth. Only 45% of Americans age 2 and older had a dental visit in the last 12 months, and more than 16 million low-income children go each year without seeing a dentist. Lack of access is a national problem, but those most affected are people who are low-income, racial or ethnic minorities, pregnant women, older adults, those with special needs, and those who live in rural communities. Simply put, the groups that need care the most are the least likely to receive it (Sanders 2012). Across the country dental groups have organized large scale, but short term, charitable dental care events under the name Mission of Mercy to alleviate some of the problems.

Objectives. (1) Describe the oral health problems and demographic characteristics of a population rarely presenting in the organized dental care system; (2) describe the dental care provided to them; (3) analyze selected barriers for their dental care access; and (4) assess whether demographic and behavioral factors were associated with the type of care received.

Methods. The Mission of Mercy is a project organized by the Oregon Dental Association. Free dental care was provided during a two-day period in November 2011. Hundreds of dental care providers supported by around 1,000 students and community volunteers offered dental care in portable dental stations in a large public arena, the Portland Convention Center. People in need presenting for treatment completed a health information form with a unique identifier and were
triaged to various dental service stations according to the character and acuity of their dental problem. The form served as a dental record which was completed as the patient went through various treatment sessions. The information was scanned into a computerized data base for analysis. The session included oral hygiene education and a computer-based exit interview. We examined the relationship between three dental procedures, namely tooth extractions, tooth restorations (fillings), and preventive services and selected demographic and behavioral variables.

**Results.** 2,023 individuals were seen; after data cleaning 1,840 individual records were available for analysis due to incomplete information (e.g. gender or age). Almost 10,000 dental services were provided, predominantly x-rays (30%), extractions (20%), and restorations (16%). Interviews revealed that 80% came from within 20 miles; only 26% had seen a dentist within the last year due to lack of insurance (75%) and affordability (70%). 53% had been in pain prior to the event, half for 6 months, half for a year or more. 78% of respondents had nowhere to continue dental care. Dental fillings and preventive services were more likely to be provided to women than to men; to Hispanics more than to Whites and Blacks; and to non-smokers more than to current smokers. On the other hand, men, Whites, Blacks, and smokers had more tooth extractions performed than women, Hispanics, and non-smokers respectively.

**Conclusions.** This charitable project revealed immense oral care needs among mostly uninsured adults. Although the population is self selected it represents a cross section of the most vulnerable and underserved individuals in the society. The distribution of restorative, preventive and surgical services in this underserved population implies that men, Whites, Blacks, and smokers had more urgent needs for dental care. To approach a solution will require innovative long term strategies engaging both public and private resources.

IRB approval: IRB00007916

**SOURCE OF FUNDING:** Kaiser Family Foundation Community Benefit

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**Population-Generalized vs. Individual-Specific AIF in Human Prostate DCE-MRI Pharmacokinetic Analysis**

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**Introduction.** Dynamic-contrast-enhanced magnetic resonance imaging (DCE-MRI) has shown promise in diagnostic medicine, particularly as applied to breast cancer screening [1]. Pharmacokinetic parameter determination relies on arterial input function (AIF) validity. However, reliable AIFs are not easily obtained and often cannot be; this is particularly true when the image field-of-view does not contain large arteries. Thus, it is often necessary to rely on an averaged, population AIF. The latter is also desired for data post-processing simplification. Here, the standard model (SM) [2] and first generation “shutter-speed” model (SSM) [3] are used to assess the impact of a generic AIF on the pharmacokinetic parameter $k^{\text{trans}}$ (volume contrast reagent (CR) transfer constant) estimation in human prostate studies.
**Methods.** DCE-MRI data sets were obtained for each of 9 adult male subjects (some of which with biopsy-proven cancer), each comprising 100 time points with 6.3 s intersampling intervals. RF transmitting was through the whole body coil and RF receiving was with a combination of Spin Matrix and flexible Body Matrix RF coils. The DCE-MRI sequence employed a 3D TurboFLASH sequence with a 256*144*16 matrix size and a 360*203 mm2 field of view, resulting in an in-plane resolution of 1.4 * 1.4 mm2. Other parameters are: slice thickness: 3 or 3.2 mm; TR/TE/FA: 5.0 ms/1.57ms/15o. Femoral arterial time-course data were obtained from all subjects, a subset of which (subjects 1-6) were temporally aligned and averaged to generate the generic AIF. Regions-of-interest (ROIs) were selected to include the entire prostate. Pharmacokinetic modeling (SM and SSM) was performed for all nine subjects on a pixel-by-pixel basis in each ROI using the subject-specific AIF. The analyses were repeated for all subjects using the generic AIF. In each case, the respective AIF was amplitude adjusted using obturator muscle as reference tissue [4].

**Results.** When the generic AIF was used, both SM and SSM experienced parameter overestimation as compared with use of the individual AIF for most cases. Most of the time, use of the individual AIF also yields higher precision and is reflected by smaller SD.

**Discussion.** While the generic AIF is amplitude adjusted such that the total area under the curve (AUC) is approximately equal to that of the individual AIF, the peak of the former is generally lower than that of the latter. The washout rate is often seen to be shallower than that of the individual AIF. Overall, the generic AIF takes on a somewhat different shape from the individual AIF and thus results in the elevated $K_{\text{trans}}$ values in general. However, these differences may appear visually small. Since AIFs from subjects 1-6 were averaged to generate the generic AIF, it is expected that at least some of these data sets would display decreased $K_{\text{trans}}$ values from use of the generic AIF (subjects 1, 3) and some would increase (subjects 2, 4 - 6). Even so, the trend of elevated $K_{\text{trans}}$ values holds for the remaining population (subjects 7-9) whose individual AIFs did not contribute to the generic AIF. As seen in Figure 3, both approaches find hot spots in the same prostate areas, which correlates with that of a biopsy proven lesion. The hot spots seem better defined when the individual AIF is used, and are hotter when the SSM is used. This suggests that an individual AIF is preferable whenever one is possible, especially for large $K_{\text{trans}}$ applications as in the prostate.

Grant Support: NIH: RO1-NS40801, RO1-EB00422, and RO1-CA120861, Medical Research Foundation of Oregon.


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**Variability in the diagnostic criteria to evaluate heart failure with preserved ejection fraction (HFPEF) in a veteran affairs (VA) patient population**

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**Purpose:** The prevalence of heart failure with preserved ejection fraction (HFPEF) continues to increase. Despite these epidemiological observations the diagnosis and treatment of HFPEF is frequently challenging. To date, four sets of guidelines have been published to diagnose HFPEF. The standardized diagnostic criteria for HFPEF requires simultaneous presence of signs and/or symptoms of heart failure (HF), ejection fraction (EF) >50%, accompanied by objective evidence
of resting structural or functional heart abnormalities. The purpose of this study is to evaluate the variability of the diagnostic criteria used to identify patients with HFPEF at the Portland Veteran Affairs Medical Center (PVAMC).

**Methods:** This study received approval as a quality assurance/quality improvement project from the PVAMC institutional review board. A modified diagnostic criteria checklist was developed based on the current guidelines. The checklist consisted of signs and symptoms based on the Framingham study, left ventricular ejection fraction, and surrogate markers of diastolic left ventricle dysfunction. Electronic medical records for 116 patients followed by primary care providers (PCP) from the PVAMC Heart Failure Clinic were screened for HF ICD-9 codes. Patients with an EF >40% were included in the study and charts were reviewed for data including active comorbidities, current medications, and HFPEF diagnostic criteria. The use of different diagnostic criteria for HFPEF was summarized using frequencies and percentages.

**Results:** Overall, 41 patients for three PCPs met the inclusion/exclusion criteria. Within the sample population, 38 (92.7%) of the patients had an EF > 40% documented within the proximity of diagnosis and of those, 34 (82.9%) had an EF >50%. Only 25 (60.9%) of these patients met the diagnostic criteria for symptoms of CHF. Also, only 25 (60.9%) patients had at least one surrogate marker of diastolic left ventricle dysfunction. These surrogate markers included: left ventricular hypertrophy documented in 16 (39%) patients, left atrial enlargement observed in 16 (39%) patient, atrial fibrillation in 15 (36.5%) patients, and elevated NT-proBNP in 18 (43.9%) patients. Cardinal symptoms of HF including dyspnea and peripheral edema were observed in 29 (70.7%) patients. Other symptoms of HF such as jugular vein distension, paroxysmal nocturnal dyspnea, and/or 53 gallop was observed in 20 (48.7%) patients. In addition, only 16 (39%) patients received intravenous furosemide to control symptoms of HF.

**Conclusion:** These results demonstrate a lack of unified diagnostic criteria for HFPEF, which leads to variable population characteristics. Therefore, this heterogeneity within a study population must be considered, as quality and standards of care continue to evolve in HFPEF treatment.

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**The Effect of Maternal Low Protein Diet on Indoxyl Sulfate and Oat1/3 Expression**

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**Purpose:** To examine the effect of maternal low protein diet (LPD) during gestation and lactation on Oat mediated indoxyl sulfate elimination.

**Methods:** Pregnant and lactating rats were fed either a purified control diet (19% casein) or a low protein diet (8% casein) throughout pregnancy and lactation. Offspring were weaned onto lab chow on postnatal day 28. On postnatal day 120, a 24 hour urine collection and single blood draw was performed on one male and one female offspring from each litter. On day 150 post-birth one male and one female offspring from each litter were sacrificed, and kidneys and brains were collected and snap frozen. Total RNA from kidney samples was isolated using the TRIZOL method, cDNA was synthesized using iScript CNDA synthesis kit from Bio-Rad, and RT-PCR was carried out using Bio-Rad iQ SYBR Green supermix. Western Blotting was conducted using polyclonal Oat1 and Oat3 antibodies on crude plasma membrane fractions. High Performance Liquid Chromatography was utilized to measure indoxyl sulfate concentrations in tissue, serum, and urine.
Results: A significant increase in the serum concentration of indoxyl sulfate was found between LPD and control females. No differences were observed in the renal excretion of indoxyl sulfate or the expression of renal transporters Oat1 and 3. Renal tissue concentration of indoxyl sulfate also remained unaltered indicating that indoxyl sulfate was not accumulating in the kidney, presenting the possibility of accumulation in another organ. Due to the encephalopathy associated with increased serum indoxyl sulfate, we measured concentrations in brain tissue. Contrary to our expectations, a significant decrease in brain indoxyl sulfate was observed in females. In addition to this, a significant increase in brain OAT3 was observed, indicating the difference in brain tissue concentration may be due to increased efflux of indoxyl sulfate across the blood brain barrier.

Conclusion: Maternal LPD increases serum indoxyl sulfate concentration in female offspring without altering the urinary excretion of indoxyl sulfate, expression of renal transporters, or renal tissue concentration of indoxyl sulfate. Decreased brain tissue concentration, coupled with an increase in brain transporter expression, indicates organ specific changes in LPD offspring.

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How Effective is the PediaVision SO9 in Detecting Ambylopic Risk Factors in Children, Ages 3-5 Years?

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Purpose: PediaVision SO9 (manufactured by PlusOptix, GmbH) is a hand held auto refracting vision screener. This study aims to determine the referral accuracy of the PediaVision SO9 in detecting the amblyopic risk factor of significant refractive error in children ages 3 to 5 using the factory default settings.

Methods: Five hundred children age 36-60 months were screened using the PediaVision SO9 screening device. Comprehensive cycloplegic examinations were performed on 192 children. PediaVision SO9 results (113 passes, 53 referrals) were compared with cycloplegic refraction for the 166 children who completed the protocol.

Results: PediaVision SO9 correctly identified 31 of 45 children with significant refractive error using the factory default settings (68.9% sensitivity, 81.8% specificity), and 21 of 26 if settings were adjusted to AAPOS screening guidelines (80.8% sensitivity, 79.3% specificity). Of the 14 children diagnosed by the clinician as an amblyopia suspect during the masked, complete cycloplegic examination, 13 were correctly failed by factory default settings (92.9% sensitivity, 73.7% specificity), 11 by AAPOS referral criteria (78.6% sensitivity, 74.3% specificity).

Discussion: Significant refractive error by examination was defined by the referral criteria to which it was being compared. Diagnosis of amblyopia suspect instead of true amblyopia was made as best corrected acuity measure was not possible in this mobile setting. Sensitivity and specificity for each refractive condition will be reported.

Conclusion: For the preschool population, PediaVision SO9 has a high level of accuracy detecting the amblyopia risk factor of significant refractive error using factory default settings.
Flexural Strength and Modulus of Bioactive Glass-containing, Anti-microbial Dental Composites

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The principal reason for replacement of dental composites is recurrent decay of the tooth due to bacterial colonization at the interface or below the restoration.

Objectives: In this project, we prepared novel dental polymer composite restorative materials containing sol-gel bioactive glasses (BAGs) as fillers. These BAGs may render the restoration more resistant to bacterial colonization via the demonstrated anti-bacterial effect of the BAG and the release of fluoride.

Methods: Two types of BAG were synthesized in our lab: 65 wt% silica - 31 wt% calcia - 4 wt% phosphate; and 61 wt% silica - 31 wt% calcia - 4 wt% phosphate - 4 wt% flouride. These were incorporated in three proportions (5, 10 and 15 wt%) along with silane-treated strontium glass into a 50:50 bisGMA/TEGDMA visible light-cured resin. Test bars for flexure testing (strength and E) in 3-point bending (25mm x 2mm x 2mm) were prepared. Composite containing the glass only (no BAG) served as a control. Test bars were aged for 24 hours in water, and for two months in a bacterial culture system of brain-heart infusion (BHI) media containing Streptococcus mutans (strain 25175), a known acid-producing microbe. Bars were also immersed in BHI without bacteria as a control. Samples were incubated at 37ºC, 5%CO2 and agitated daily; media was changed every other day. Bacterial growth and colonization of the bars was confirmed throughout the test period.

Results: Samples with BAG had comparable strength and flexural modulus to the control samples - no significant differences were seen after any of the treatments (ANOVA/Tukey’s; α=0.05).

![Graph showing Flexural Strength and Modulus](image_url)
Conclusion: The inclusion of anti-bacterial BAG as a filler component did not diminish the initial strength or the stability over 2 months of these new composite materials, which may ultimately increase longevity and service life of dental composite restorations.

Loss of cell-surface laminin anchoring promotes tumor growth and is associated with poor clinical outcomes

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Extracellular matrix (ECM) molecules are assembled into higher-order structures, and perturbation of these assemblies contributes to progression of numerous diseases. The anchoring of laminins at the cell surface enables assembly and signaling of many ECMs, but its roles in cancer progression are largely unexplored. We investigated the prominence and origins of defective laminin anchoring in cancer cells, and its association with cancer subtypes and clinical outcomes. Loss of laminin anchoring is widespread in cancer cells, originating from several distinct defects all of which lead to dysfunctional glycosylation of the laminin receptor dystroglycan. In aggressive breast cancer and glioblastoma cells, defective laminin anchoring is often due to suppressed expression of like-acetylglcosaminyltransferase (LARGE). Reduced expression of LARGE is characteristic of a broad array of human tumors and is associated with aggressive cancer subtypes and poor clinical outcomes. Notably, this defect is a robust predictor of poor survival in patients with brain cancers. Restoring LARGE expression repaired anchoring of exogenous and endogenous laminin and modulated cell proliferation and tumor growth, suggesting that restoration of laminin anchoring can be therapeutic. Thus, defects of laminin anchoring are prominent in cancer cells, are characteristic of aggressive cancer subtypes, and are potentially important drivers of disease progression.

Dental Implants Placed from 2002-2009 in an Advanced Specialty Education Program in Periodontics: A Radiographic and Clinical Review

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Background: The adoption into mainstream dental therapy of endosseous dental implants manufactured from medical grade titanium has revolutionized the treatment of edentulous dental sites while demonstrating excellent success and survival percentages. Few studies have evaluated dental implant survival or success within a university residency program. There are even fewer studies that have analyzed implant outcomes by radiographic survey. The primary objective of this study were to evaluate survival and success of dental implants placed by periodontal residents at Oregon Health and Science University (OHSU) between the years 2002 and 2009 and to assess residual bone height as
assessed radiographically, around these implants. A novel radiographic parameter described as dental implant radiographic residual bone height (DIRRBH) ; a measurement of dental implant success. We also measured dental implant radiographic crestal bone loss (DIRCBL). Secondary aims of this study include determining if various demographic and patient health parameters or if the residency year of the surgeon placing the implant fixture(s) may affect DIRRBH or dental implant survival.

Methods: A retrospective clinical chart review was performed of patients at OHSU who had one or more implants placed by periodontology residents in the years 2002-2009. Subjects were invited to participate in a recall examination, at which time a limited clinical assessment was performed on the restored dental implant and a single digital periapical radiograph was taken of each dental implant. Patient demographic, health, and dental implant data were collected from the chart review and verified at the clinical appointment. An anonymous survey was collected of all participants in order to evaluate patient satisfaction with the dental implant experience and final result. Radiographs were then analyzed by two calibrated and independent evaluators. Statistical data analysis was performed using the cox proportional hazard regression model and Kruskal Wallis test. Results were considered significant where the p-value was less than 0.05.

Results: The case series included 79 patients with 167 dental implants that had been placed. The mean follow-up period was 5.11 years from implant placement with a range of two to nine years. The study population consisted of 56% female and 44% male subjects. Subject age ranged from 17 years to 85 years, with a mean of 60.3 years. Implant lengths ranged from 6 to 16 millimeters, with a median length of 11.5 millimeters. Dental implant radiographic residual bone height (DIRRBH) as dental implant fixture length was 90.7%. Females retained a mean of 90% and males 91%; healthy patients retained 91% while diabetics retained 88% of the DIRRBH per dental implant fixture length. DIRRBH per dental implant fixture length was 91% for never smokers however current and historical smokers retained 89.4 and 89.6%, respectively. Cumulative average radiographic crestal bone loss on the dental implant fixture is 1.03 millimeters (s = 0.882; range 0 – 5.5). Cumulative survival rate over the nine year period was 96.8%.

Conclusion: This study reports that endosseous dental implants placed from 2002-2009 achieve survival rates that achieve accepted published standards for dental implant survival. Age, gender, diabetes, smoking, osteoporosis, osteopenia do not significantly affect the combined DIRRBH or overall dental implant survival. There was slightly less non-significant DIRRBH for dental implants placed in diabetics and smokers. Finally, the level of experience of the periodontology resident was not shown to have an impact on dental implant survival.

Bionutrition Resources for Clinical Research
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The Bionutrition Unit, part of the Oregon Clinical and Translational Research Institute (OCTRI), has been assisting OHSU investigators with clinical research for over 30 years. Our Bionutritionists are Registered Dietitians experienced in nutrition research techniques, including the design and implementation of research diets, education and counseling of study participants on dietary modifications or interventions, and the estimation of usual nutrient intake using a variety of methodologies. We specialize in controlled outpatient feeding studies, using food prepared in our state-of-the-art research kitchen, lasting days, weeks, or even months. Our Body Energy and Composition Core (BECC) Unit offers body composition measurements using the BOD POD, Dual Energy X-Ray Absorptiometry (DEXA), Bioelectrical Impedance Analysis (BIA), and anthropometrics. We also utilize indirect calorimetry, treadmill testing, and activity monitoring, to assess energy expenditure at rest, after food consumption, and during activity.
A method of improving the effective spatial resolution for small field IMRT QA with 2D-array device

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Purpose: To improve the effective spatial resolution of small field IMRT QA with 2D-array device and therefore the accuracy of 3D dose estimation.

Methods and Materials: When small field IMRT QA is measured with 2D-array device, the spatial resolution (5-10mm) may be considered too sparse to accurately verify dose over high gradient area. Reducing the grazing angle (i.e. the angle between beam and detector plane) can improve spatial resolution along the transverse direction of the beam eye view (BEV). However, angular dependency and electron scattering may potentially degrade measurement accuracy. In this study, a Scandidos Delta4 device was used for IMRT QA measurements. It has two orthogonal dose planes with 5mm spatial resolution. We measured IMRT plans with two settings: one with 40 and 50 grazing angles and the other with 15 and 75 grazing angles. The measured 2D dose planes were compared with the ones calculated by TPS. 3D doses were estimated by interpolating two measurement points along beamlets based on the PDD curve and compared with the one from TPS.

Results: Calculation showed that for single detector plane, the spatial resolution along the transverse direction of the BEV is improved from 3.21 mm (for the 40 degree) to 1.29 mm (for the 15 degree). When both orthogonal planes were considered, the spatial resolution was improve from 1.75 mm to 1.02 mm. The QA measurements showed that the average passing rate with 2% dose deviation criterion was 74% for 15-degree grazing angle, only slightly lower than the passing rate of 80% for the 40-degree grazing angle. On the other side, the 15/75 setting reconstructed 3D dose more accurately than the 40/50 setting due to higher spatial resolution.

Conclusion: A moderately small grazing angle can improve effective spatial resolution without sacrificing accuracy.

White matter microstructure and cognitive control in a developmental sample

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Studies have established networks of brain regions recruited for cognitive control during development, but less is known about how white matter microstructure in children and adolescents relates to cognitive control. This study explored the relationship between white matter microstructure and inhibition in a sample of 88 children and adolescents (age 10-16, mean = 13.10; 41 female). Fractional anisotropy (FA) values, indexing water diffusivity in white matter, were derived from diffusion tensor imaging using Tract-Based Spatial Statistics. Youth completed the Color-Word Interference task from the Delis-Kaplan Executive Function System, a modification of the Stroop paradigm. A composite measure of cognitive control was computed using normed reaction time on the Inhibition and Color Naming subtests and errors on the Inhibition subtest, with higher scores representing relatively worse cognitive control. Composite scores were regressed on the FA white matter skeleton, controlling for age and IQ. Results demonstrated greater FA in the right splenium of the corpus callosum and right corona radiata were associated with lower composite scores (p<.01, whole-
These results suggest greater structural integrity of fibers tracts connecting anterior cortical regions with other cortical and subcortical regions, as well as fiber tracts interconnecting parietal and cortical regions, are involved in directing attention towards task-relevant processes/representations and inhibiting task-irrelevant processes/representations in children and adolescents. Furthermore, these results are also consistent with hierarchical models of cognitive control and inhibition suggesting the importance of cortical-cortical communication and the importance of directing communication within parietal and occipital regions towards task-relevant processes and representations.

Perinatal Mortality of Planned Out of Hospital Births Transferred to an Oregon Hospital, 2004-2008

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Introduction: In Oregon planned out of hospital birth outcomes are not consistently documented through vital statistics and either licensed or unlicensed providers may attend these births.

Methods: A five-year (1/1/2004 to 12/31/2008) retrospective study was conducted to examine the outcomes of maternal and neonatal cases transferred to an Oregon tertiary care referral center during a planned home or birth center birth.

Results: 229 cases were identified. Of the 223 cases with documented neonatal outcomes, eight deaths were found. One infant died at greater than seven days of life; thus, eight of the 223 neonates died between 28 weeks gestation and seven days of life. Findings suggest a perinatal mortality rate for planned out of hospital births among cases transferred to the study hospital of 3.59 (CI 95%, 1.56 to 6.95) percent or approximately 36 [(15.61 to 69.46) CI 95%] per 1,000. Of the eight deaths, one infant had congenital anomalies that were not compatible with life. The following higher risk conditions were associated with the seven other deaths: breech presentation (three cases), pregnancy-induced hypertension (PIH) or preeclampsia (four cases), and postdates gestation (two cases). Seven of the eight deaths had licensed direct entry midwives and an unlicensed midwife cared for the one case with anomalies.

Conclusion: This is the first published data in Oregon examining planned out of hospital births transferred to a tertiary care facility. Our findings suggest that more research is needed to assess the maternal/fetal risk factors or provider-related factors that may have contributed to the higher incidence of perinatal mortality.

Describing the Experience of Hair Loss with Cancer Chemotherapy

Lillian M. Nail, PhD, RN, FAAN, Frances Lee-Lin, MN, RN, OCN, CNS, Jennifer Scherer, MPH Presenting

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Background: Cancer chemotherapy-induced hair loss (CIHL) is estimated to affect 250,000 adults in the U.S. each year. CIHL is distressing to patients and to their friends and families. Some patients report that the prospect of HL made them consider refusing treatment. In addition to disrupting self-image, CIHL is a symbol of cancer that discloses the diagnosis to others and also serves as a personal reminder of the disease. Despite the large number of people who experience CIHL loss and the distress generated by it, there is little research on the experience of CIHL. Most published studies report tests of interventions to prevent or reduce scalp CIHL, none of which were successful in preserving appearance. None of the earlier studies report on the extent to which strategies suggested by cancer care experts, cancer advocacy and information organizations, and hair stylists for dealing with HL are adopted, describe how HL influences daily life, or provide information about perceptions of hair regrowth.

Purpose: To describe selected aspects of the experience and responses to scalp CIHL in adult cancer survivors who have completed chemotherapy.

Framework: Cognitive appraisal theory was used to guide selection of the CIHL experience variables addressed in this study including appraisal, use of CIHL-specific coping strategies, emotional and social responses, and the match between expectations and actual experience.

Methods: Retrospective descriptive study using a 50 item investigator-developed self-administered survey instrument. Volunteer subjects were recruited through advertisements posted in clinical settings, announcements at support groups, and displays at cancer survivorship events.

Subjects: The sample (N=211) was mostly middle aged (median age 53), female (95%), Caucasians (85%), who received chemotherapy for breast cancer (70%).

Results: 86% reported that they either all of their scalp hair fell out or that they clipped their hair off when it began to fall out. Most (73%) had at least one wig, and over half wore the wig all or most of the time they were out of their home. Wigs were described as hot (71%), itchy (47%), unnatural in appearance (45%), scratchy (36%), too tight (33%), and hard to secure (27%). Other head coverings such as scarves (31%) or hats (38%) were used with 55% reporting that they never left their house without wearing a wig or other head covering. 31% wore a head covering all or most of the time while sleeping. Psychosocial issues included expense of wigs and other head covers, dealing with upsetting questions from others, changes to self-image, and experiencing greater than expected distress due to HL (27%). There was wide variation in the time it took hair to regrow and on the match between the color, thickness, texture, and grayness of the regrown hair and prechemotherapy hair. Among women, age was significantly negatively related to amount of money spent on head coverings, frequency of strangers noticing CIHL, questions about CIHL, and level of confidence that hair would regrow.

Conclusions: The results of this study demonstrate wide variation in the experience of CIHL and responses to CIHL. The results provide information that will be useful in preparing others for the experience of CIHL, including addressing issues and problems reported by subjects which are not included in current standard of care information for people likely to experience CIHL. Additional research is needed to explore potential explanations of the variation in scalp hair regrowth including partial or patchy regrowth following CIHL and to develop coping skills training to minimize distress due to unexpected CIHL-related comments and questions.

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Age is an important part of the disease context for lung cancer—the majority of patients are over 65 and the median age at diagnosis is 70. Older adults with lung cancer have a poorer prognosis due to co-morbidities and age-related factors, which both complicate and hastens the end of life process for people who already struggle with an aggressive disease. The patient’s ability to determine their desired plan of care depends upon strong social support systems, effective decision-making, and minimized family conflict. The aim of this investigation is to describe the role of age in aspects of the coping process, including social support, decision-making, and family conflict, among lung cancer patients. Data obtained from 115 lung cancer patients ranging in age from 31 to 95, and their family caregivers, showed that adults with lung cancer over the age of 75 have significantly less social support than their counterparts aged 75 and under. Across all ages, having a spouse as the primary caregiver was significantly associated with less family conflict, overall greater social support, and an easier decision-making process (e.g. more listening and cooperating, and less tension and guilt). Cognitive impairment was not significantly correlated with decision-making, family conflict or social support. Findings will be discussed in regard to identifying and supporting the families most at risk for discordance during the end of life process.

Decreased Cellular Energetics in Multiple Sclerosis Gray Matter: a 7T Phosphorus Spectroscopy Study

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²Department of Neurology, Oregon Health & Science University, Portland, OR
³Siemens Healthcare
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Objective: The focus of this study was to investigate phosphorous metabolite differences between healthy control and MS subjects while accounting for contributions from gray matter (GM), white matter (WM), lesions (LE) and skeletal muscle (SM).

Background: The association between mitochondrial dysfunction and neurodegeneration in multiple sclerosis (MS) is unclear. Neurodegeneration as evidenced by GM loss in an early feature of MS. ³¹P magnetic resonance spectroscopy and imaging (MRSI) provides a non-invasive measure of high energy phosphates and may serve as a surrogate marker for altered cerebral metabolism.

Methods: Eleven healthy controls (HC) [6 women and 5 men, 48±10 years] and nine MS subjects [7 women and 2 men, 47±10 years, RRMS, EDSS scores: 2-6] were studied. High resolution anatomic, quantitative T₁ maps and 3D ³¹P MRSI data were acquired on a 7T Siemens Magnetom system. Quantitative ¹H₀ T₁ maps were used to segment brain and surrounding tissue into GM, WM, CSF, LE and SM. Spectral fittings of ³¹P data from a volume of interest (~160 mL) in
parietal supra-ventricular region were analyzed using a linear mixed-effects regression model for tissue type dependence.

**Results:** There was a significant reduction in average phosphate metabolite levels in MS (21%) compared to HC. Tissue dependence analysis found significant \( p=0.01 \) decrease in several phosphorus metabolites including phosphocreatine (17%), adenosine triphosphate (43%), inorganic phosphate (Pi) (27%) and phosphorylcholine (22%) in GM in MS whereas WM showed no significant changes. There are no significant differences in quantitative \(^1\text{H}_2\text{O} \ T_1\) values between the groups.

**Conclusions:** These significant changes in energy metabolites in GM, but not WM, may identify tissue at risk for, or in the process of, degeneration as evidenced by the reduced cortical thickness or GM volume in larger MS population in literature. It may act as a surrogate marker of early neurodegeneration.

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**The Clinical and Translational Research Center**

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The Clinical and Translational Research Center (CTRC), part of the Oregon Clinical and Translational Research Institute (OCTRI), has been supporting OHSU investigators with patient-oriented research for over 45 years. The CTRC includes four major units: The nursing unit, which includes inpatient and outpatient space and registered nurses and research assistants; the study coordinator unit, with mobile staff who can travel throughout the campus and community to conduct research procedures; the bionutrition unit, which includes research bionutritionists, a metabolic kitchen, and a core for measuring body composition and energy expenditure; and a core laboratory with facilities and staff for the processing, storage and assay of numerous analytes and genetic tests. The CTRC supports over 100 protocols in diverse areas of clinical research, with particular expertise in neuroscience, metabolism, genetic disorders, and early phase clinical trials. In addition to supporting established investigators, the CTRC has an emphasis on assisting career development awardees with their clinical research projects.

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**The Reagent Ontology: an Integrated Resource for Curation of Biomedical Research Reagents**

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The volume of information available to biomedical researchers has far surpassed levels amenable to human access and comprehension. Accordingly, we are becoming increasingly reliant on informatics tools to discover and exploit such information. These tools must bridge diverse research domains that rely on specialized vocabularies and data models. In recent years, ontologies have emerged as a valuable approach for dealing with such semantic and syntactic data
heterogeneity, with community-consortiums such as the Open Biomedical Ontologies (OBO) Foundry leading the way. Here, we describe development of the Reagent Ontology (ReO), and its potential to support linking and inferencing across data sources that describe the people, resources, and publications comprising the research network.

ReO models the domain of biomedical research reagents, considered broadly to include materials applied 'chemically' in scientific techniques to facilitate generation of data (examples include antibodies, constructs, cell lines, biochemicals). ReO was developed to be deeply integrated into the biomedical landscape, importing classes from a diverse set of ontologies and encoding their relation to reagents through rich semantic axioms. By providing a shared, computationally-tractable resource for modeling reagents, ReO can support applications aimed at research resource discovery, knowledge extraction from experimental data, and evaluation of scientific expertise and productivity.

Development of a Non-Human Primate Special Care Nursery

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In the United States, 1 in 8 babies (12.8% of live births) are born too early (1). The most prevalent underlying cause of preterm labor is intra-amniotic infection caused by *Ureaplasma* species (2). Previous studies at the Oregon National Primate Research Center have focused on early detection and interventional therapy for the prevention of fetal organ injury and preterm labor (3). In order to develop our studies into a neonatal model it has been necessary to expand the scope of newborn infant care at the primate center by establishing a Special Care Nursery which is analogous to a human neonatal intensive care unit (4). Our team of investigators includes neonatologists, perinatologists, neonate registered nurses, clinical/surgical veterinarians and primate neurobehavioral specialists. We have successfully survived the first ever prematurely born non-human primate neonates exposed to *Ureaplasma* species and antenatal antibiotic therapy. Careful consideration was taken to integrate both human neonatal and veterinary based equipment and care guidelines to ensure low morbidity and mortality (5). This unique special care nursery can now accommodate prematurely born infants that require 24hr intensive care, respiratory support (i.e., oxygen supplementation, mechanical ventilation and/or continuous positive airway pressure), continuous intravenous fluids, and drug administration specific to the needs of the neonate.

Ovarian torsion in pregnancy: ultrasound characteristics and histopathology

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²Maternal and Fetal Medicine, University of Hawaii, Honolulu, HI, USA

Objectives: Adnexal masses during pregnancy carry risk for ovarian torsion (OT). Our objective was to identify ultrasound (US) and pathology findings associated with OT.

Methods: A cohort of cases with adnexal mass > 5 cm was studied from University of Hawaii and Oregon Health & Science University (1/01-1/09). 4 reviewers used a standardized data collection form. Cases were divided into 2 groups,
OT and no ovarian torsion (NOT). Variables studied are listed in table. Data analysis performed with t test, Wilcoxon test and Fischer exact.

**Results:** 181 cases with adnexal masses > 5 cm were identified, 60 with ovarian pathology (table).

**Conclusions:** 18.3% masses > 5 cm with pathology diagnosis had OT. Teratoma is the most frequent diagnosis for both groups. No significant association between pathology diagnosis and OT was noted, however, masses complicated by OT were larger. The OT group contained two normal enlarged ovaries. The most common US characteristic for OT was complex cyst. OT group women were younger and at higher risk for delivery < 35 wks.

**Results:** 181 cases with adnexal masses >5 cm were identified, 60 cases with ovarian histopathology.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ovarian Torsion</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (N=60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean Age 29.3 ± 65.7 (mean ± Std) (Range: 13-43 yo)</td>
<td>49 (81.7%)</td>
<td></td>
</tr>
<tr>
<td>Nulliparous (29, 49.2%) GA at 1st US (6 - 37.8 wks)</td>
<td>29.3 ± 5.7</td>
<td>24.4 ± 6.5</td>
</tr>
<tr>
<td>GA at 1st US (6 - 37.8 wks) Median (ITQ)</td>
<td>22 (45.8 %)</td>
<td>7 (63.6%)</td>
</tr>
<tr>
<td>GA at delivery Median (ITQ)</td>
<td>17 wks (12.0, 25.0)</td>
<td>13.4 wks (6.0, 29.0)</td>
</tr>
<tr>
<td>Median (ITQ)</td>
<td>38 (37, 39)</td>
<td>37 (34, 38)</td>
</tr>
<tr>
<td>Less than 35 wks</td>
<td>5 (10.4%)</td>
<td>4 (36.4%)</td>
</tr>
<tr>
<td>Ultrasound Findings</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adnexal mass size: Median (ITQ)</td>
<td>6.6 cm (5.4, 7.7)</td>
<td>8.0 cm (6.5, 11.9)</td>
</tr>
<tr>
<td>Mass characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilocular</td>
<td>17 (34.7 %)</td>
<td>2 (18.2%)</td>
</tr>
<tr>
<td>Complex</td>
<td>13 (26.5%)</td>
<td>7 (63.6%)</td>
</tr>
<tr>
<td>Septations</td>
<td>9 (18.4%)</td>
<td>5 (45.5%)</td>
</tr>
<tr>
<td>Nodularity</td>
<td>7 (14.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Solid only</td>
<td>6 (12.2%)</td>
<td>2 (18.2%)</td>
</tr>
<tr>
<td>Mixed cystic and solid</td>
<td>13 (26.5%)</td>
<td>2 (18.2%)</td>
</tr>
<tr>
<td>Pathological diagnosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teratoma</td>
<td>20 (40.8%)</td>
<td>3 (27.3%)</td>
</tr>
<tr>
<td>Serous cystadenoma</td>
<td>7 (14.3%)</td>
<td>1 (9.1%)</td>
</tr>
<tr>
<td>Mucinous cystadenoma</td>
<td>7 (14.3%)</td>
<td>2 (18.2%)</td>
</tr>
<tr>
<td>Borderline or malignant tumors</td>
<td>3 (6.1%)</td>
<td>0%</td>
</tr>
<tr>
<td>Other*</td>
<td>12 (24.5%)</td>
<td>5 (45.5%)</td>
</tr>
</tbody>
</table>

Mean (std) or median (ITQ, inter-quartile range) used for continuous variables and frequency (%) used for categorical variables.

As the volume of biological information has grown, so has a corresponding need to strengthen the practice of data collection, sharing, reuse, and preservation. While the need for improved data and resource management is apparent, the available tools and adoption from the researcher community is lacking. eagle-i aims to help solve this problem by making scientific research resources more visible and uniquely identifiable via a national federated network of institutional repositories. The pilot project began with 9 universities and has recently expanded to 14 additional institutions. The Network has developed a discovery platform using an ontology-driven approach for identifying biomedical resources. At OHSU, information about over 1,400 biomedical resources has been collected, including services offered at core laboratories, instrumentation at research labs, model organisms, reagents, software, biospecimens and protocols. Information about over 45,000 research resources in the central eagle-i repository is searchable via www.eagle-i.net. eagle-i provides a much-needed mechanism to help researchers obtain and share biomedical research resources that are rarely published or shared.

Validation of electronic medical record data for the retrospective identification of skin and soft tissue infections in primary care settings

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Purpose: Epidemiologic studies often identify SSTIs retrospectively using electronic medical record (EMR) data, such as International Classification of Disease, Ninth Revision (ICD-9) codes, but the validity of this method has not been assessed. We evaluated the positive predictive value (PPV) associated with the use of EMR data to retrospectively identify SSTIs.

Methods: A retrospective cross-sectional study was conducted among primary care (family medicine, internal medicine, and pediatrics) outpatients at Oregon Health & Science University. Encounters in January, April, July, and October 2010 (sampled to account for seasonal variation) were included if they met any of the following criteria: SSTI ICD-9 code [035, 680-684, 686,704.8], incision and drainage (IND) current procedural terminology (CPT) code [10060/1, 10080/1, 10120/1, 10140, 10160, 10180], positive microbiology wound culture. Chart review was performed to establish gold standard diagnosis of SSTIs based on standardized definitions. PPVs and 95% confidence intervals (CI) were calculated for all SSTIs and for cellulitis/abscess cases [ICD-9 codes 681-2] among all encounters, as well as among initial encounters only. Descriptive statistics were calculated to describe SSTI type, treatment, and causative pathogens.

Results: Of the 697 encounters included, 486 (69.7%) were initial encounters; 390 (56.0%) were cellulitis/abscess cases. When the presence of an ICD-9 code, CPT code, or positive culture was used to identify an SSTI, 584 encounters were considered true positives, yielding a PPV of 83.8% [95% CI: 81.1–86.5%]. The PPV for use of ICD-9 codes alone to identify SSTIs was 90.4% [95% CI: 88.1-92.7%]. Limiting analysis to initial encounters, the PPV was 88.3% [9 % CI: 85.2-91.3%]. For encounters with a cellulitis/abscess code, the PPV was 97.9% [95% CI: 96.5-99. %]. Antibiotics were prescribed in 62.3% of encounters. In 33.7% of encounters, no IND was performed nor antibiotic prescribed. Of the 67 positive wound
cultures, 11 (16.4%) were positive for *Streptococcus* species and 43 (64.2%) for *Staphylococcus aureus*, with 46.5% of those being methicillin-resistant *S. aureus*.

**Conclusion:** Use of ICD-9 codes may be used to retrospectively identify SSTIs with a high PPV; additional use of microbiology data and CPT codes for SSTI identification only attenuates the PPV. PPVs may vary by type of SSTI, and future applications of this identification method should take this into consideration.

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**Dynamic establishment of vesicular neurotransmitter content at a mixed Glycine/GABA synapse**

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The concentration of neurotransmitter inside pre-synaptic vesicles reflects the availability of cytosolic transmitter and the properties of vesicular transporters. However, little is known regarding the endogenous concentrations of inhibitory neurotransmitters in nerve terminals or the dynamics of vesicle refilling following exocytosis. We addressed these issues by recording from synaptically coupled pairs of glycine/GABA co-releasing interneurons in acute brain slices. We found that cytosolic glycine concentrations required constitutive activity of the plasma membrane glycine transporter GlyT2, whereas GABA was almost entirely supplied by glutamic acid decarboxylase. Surprisingly, vesicular glycine/GABA content was remarkably labile. Vesicles equilibrated within a few minutes upon acute increases or decreases in cytosolic neurotransmitter, and changes in vesicle filling were independent of previous exocytosis. Furthermore, by experimentally manipulated pre-synaptic glycine concentrations and assessing its effects on postsynaptic responses we found that the endogenous glycine concentration in nerve terminals is \( \sim 5 \text{ mM} \). Thus, nerve terminal transmitter availability dynamically regulates the strength of inhibitory synapses in an activity-independent manner. We suggest that cytosolic transmitter concentration may represent a key site of regulation for the short- and long-term control of inhibitory synaptic transmission.

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**Understanding the Functional Connectivity of Brain’s Bladder Control Network**


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**Objective:** To characterize the brain’s functional architecture involved in bladder control using resting state functional connectivity MRI (rs-fcMRI).

**Background:** Understanding the functional integration of brain regions involved in normal bladder control is a necessary first step in recognizing pathologic states. Prior functional MRI studies have identified brain activity in several discrete areas of the brain in response to repeated bladder filling and emptying. These regions are presumed to be involved in bladder control; however, how they interact with other control systems in the brain is poorly defined. In this study, we use rs-fcMRI to further inform normal bladder functioning by examining the regional brain interactions while subjects are at rest with empty and full bladder.
**Methods:** We performed functional MRI and connectivity sequences for 8 subjects with normal bladder control (ages 52-64) before and after bladder filling. High-resolution T1-weighted structural images and BOLD-weighted functional data were acquired. Analysis of rs-fcMRI were accomplished by generating resting BOLD timeseries of 5 a-priori defined brain regions of interest (see figure 1a) and correlating each timeseries with all other voxels in the brain. Voxels with high correlation are considered “functionally connected” to that region. Comparisons between the FULL and EMPTY conditions were conducted for each region. To identify common functional circuitry involved in bladder control related to the a-priori regions of interest (ROIs), fixed effects analysis on the comparison maps (FULL vs EMPTY) was used.

**Result:** Fixed effect images of the comparison maps across the five ROIs shows significantly increased functional connectivity with the medial prefrontal cortex, temporal cortex and the cerebellum (p<0.05) with full bladder compared to empty bladder. Bladder filling was also associated with significantly reduced functional connectivity with Pons and Intraparietal sulcus (part of the dorsal frontoparietal network).

**Conclusion:** The medial prefrontal cortex, which is thought to be involved in decision making for voiding becomes more engaged, or connected, with the bladder control circuit when the bladder is full, and less so when the bladder is empty. Interestingly, the pons, which contains the pontine micturition center is less functionally connected to our regions of interest with bladder filling perhaps due to the ability of normal subjects to disengage micturition when in a socially inappropriate setting. This is also true for intraparietal sulcus (IPS) postulated to be involved in attention and initiation of stimulus response. It is possible that these normal functional brain responses are atypical in patients with poor bladder control function.

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**OCTRI Community & Practice Research Program: Partners and Research Methods**

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The Community & Practice Research (CPR) program at the Oregon Clinical and Translational Research Institute (OCTRI) catalyzes the translation of biomedical science to improve community health. The goal of the Community & Practice Research (CPR) program is to address translation challenges, which include transferring research findings to routine community practice; engaging communities in defining their own health and research needs; including diverse populations in clinical research; and reducing well-recognized health disparities that occur along racial, educational, income, and urban/rural fault lines. The CPR program facilitates the implementation and dissemination of research, and helps to build the community partnerships needed to conduct meaningful health research. Community-based participatory research (CBPR) and practice-based research networks (PBRNs) are synergistic research approaches that enhance the efforts of the CPR program to define priorities, interpret findings, and disseminate results.

Among the CPR program’s primary partners are the Oregon Rural Practice-based Research Network (ORPRN), an active clinical research network spanning rural communities throughout the state of Oregon; OCHIN, a non-profit organization that provides a unified electronic medical record to safety net clinics up and down the west coast and in six other states; Let’s Get Healthy!, an interactive education and research exhibit that allows community members to learn important information about their bodies while contributing to science; and a Community Partnership Board made up of local and state community leaders and community researchers. The Community & Practice Research staff is available for
consulting on community and practice research issues and projects. They also actively support relationship building between investigators and local communities and research networks.

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**CSPG-dependent Inhibition of sympathetic axon outgrowth by infarcted myocardium.**

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Sympathetic axons fail to regenerate into infarcted myocardium after cardiac ischemia-reperfusion injury. This phenomenon is similar to what is seen in injury of the CNS, where chondroitin sulfate proteoglycans (CSPG) and other molecules of the extracellular matrix are expressed in the scar tissue and inhibit axon growth. We investigated whether CSPGs are present in the infarct after ischemia-reperfusion, and if CSPGs play a role in inhibition of sympathetic axon outgrowth. Immunohistochemistry and western blot analysis indicate that CSPGs are present in the cardiac scar but not outside of the infarct. To date, inhibition of axon outgrowth by CSPGs has only been shown in neurons of the CNS and sensory neurons. To determine if CSPGs inhibit sympathetic axon outgrowth, dissociated mouse neonatal sympathetic neurons were treated with increasing concentrations of CSPGs. CSPGs caused dose-dependent inhibition of sympathetic axon outgrowth. When sympathetic ganglia were co-cultured with either healthy or infarct cardiac tissue, we found that growth of axons toward infarcted tissue was significantly attenuated compared to growth toward healthy tissue. Treatment of co-cultures with Chondroitinase ABC—a bacterial enzyme that degrades CSPG—fully rescues axon growth to the level seen in co-cultures with healthy myocardium. Recently, protein tyrosine phosphatase sigma (PTPRS) was identified as a receptor for CSPG. When ptprs −/− ganglia are co-cultured with infarcted myocardium, infarct-dependent inhibition of axon growth is abolished. Collectively our data indicate that, similar to CNS injury, CSPGs are upregulated in infarcted myocardium and may prevent sympathetic axon regeneration into the infarct after cardiac ischemia-reperfusion.

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**Incidence and Duration of Breast Feeding Infants with Phenylketonuria in the United States and Canada**

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**Introduction:** Breast milk is the healthiest milk for infants. Some clinics encourage mothers of infants with PKU to continue breast feeding whereas others discourage breast feeding after diagnosis. Although breast feeding and PKU have been discussed in the literature, there is a lack of research describing patterns of breast feeding infants with PKU.

**Purpose:** Describe incidence and duration of breast feeding among mothers who have infants with PKU in the US and Canada.

**Sample:** The participants were mothers meeting the criteria: 1) at least twenty-one years of age, 2) able to read and write in English, 3) had a child with PKU, and 4) lived in the US or Canada.

**Method:** Descriptive statistics were used to describe data from the internet survey.
Results: 89 mothers (86%) started breast feeding and 14 mothers (14%) began bottle feeding immediately after delivery. Of 89 mothers who breast fed after delivery, 75 (84%) were from the US and 14 (16%) were from Canada. After diagnosis, 60 mothers (80%) from the US continued to breast feed while 15 mothers (20%) switched to bottle feeding. In contrast, only two Canadian mothers switched to bottle feeding after the diagnosis of PKU. McNemar’s test was performed to assess the difference between the proportion of mothers’ breast feeding immediately after delivery to mothers who continued breast feeding after PKU diagnosis. Significantly fewer mothers breast fed after diagnosis (McNemar’s $\chi^2 = 30.33, p < .000; n = 89$ versus $n = 72$). This significant reduction in breast feeding mothers was a function of women from the US (McNemar’s $\chi^2 = 27.48$ $p < .000; n = 60$ versus $n = 75$), and not from Canada.

Duration of breast feeding among mothers ($n = 89$) in the survey ranged from less than one month ($n = 18, 18\%$) to nineteen to twenty-four months ($n = 5, 5\%$). The mean duration of breastfeeding was from seven to nine months ($n = 15, 15\%$) with the mode from ten to twelve months ($n = 19, 18\%$).

Several variables were assessed in relation to duration of breast feeding using chi square analysis. Only one variable, when standard commercial infant formula was added to the diet replacing breast feeding or pumped expressed mothers’ milk, was significantly associated with duration of breastfeeding infants with PKU, $\chi^2 (42, n = 73) = 88.13, p < .000$. Of those identifying when standard commercial formula was added to the infant’s diet, 25% ($n = 21$) had added by 6 months. Thirty-three percent of mothers ($n = 34$) never introduced standard commercial infant formula to their infant’s diet, rather transitioned from breast feeding to the introduction of solid foods with phenylalanine free formula. The following variables were not associated with duration of breast feeding: maternal age, marital status, education level of mother, maternal ethnicity, hours employed per week, total number of children in the family, country of residence, mode of delivery, type of insurance, annual household income, infant’s initial phenylalanine level, and initiation of dietary therapy.

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Endoscopic Treatment for Vesicoureteral Reflux: How Important is Technique?

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Purpose: The endoscopic injection of dextranomer/hyaluronic acid (Dx/HA) by subureteric transurethral injection (STING) or hydrodistention implantation technique (HIT) for the treatment of vesicoureteral reflux (VUR) has variable results with the HIT technique reporting better outcomes. We determined our outcomes with each technique comparing reflux resolution rates and evaluating predictors of treatment failure and success.

Methods. Univariate and multivariate logistic regression analysis was used to compare results in 163 patients (246 ureters) who underwent a single endoscopic Dx/HA injection from December 2001 to April 2010. 50 patients underwent Dx/HA injection by the STING method and 113 by the HIT technique. The mean patient age was 6.6 years (range 10 months to 23 years). Data on the presence or absence of voiding dysfunction, reflux grade, injection volume, endoscopic appearance, and calculated post-operative Dx/HA volume were collected prospectively in an IRB approved
Results. Ureter resolution rates were 79.75% and 80.84% for STING and HIT, respectively (p=0.86). Patient resolution rates were 70.0% and 74.3% for STING and HIT, respectively (p=0.57). Multivariate logistic regression analysis based on ureteral resolution for the entire cohort revealed pre-op grade (I & II vs. IV) (p=0.004) and injected Dx/HA volume (0.80-1.00cc) (p=0.039) as positive predictors of resolution. Negative predictors of resolution were calculated ellipsoid volume (CEV) <0.20cc (p=0.002) and CEV/actual-injected-volume <25% (p=0.006). Multivariate logistic regression analysis based on patient resolution revealed age at surgery <6yrs (p=0.03) as a significant negative predictor for the entire cohort. Mound morphology for STING (p=0.004) and pre-op grade (I & II vs. IV) for HIT (p=0.015) were positive predictors of procedure success. HIT negative predictors included CEV <0.20cc (p=0.012) and age at surgery <6 (p=0.037).

Conclusions. We found no differences in ureter or patient resolution between endoscopic Dx/HA injection techniques STING or HIT. However, injected Dx/HA volume (0.8-1.0cc) and CEV >25% of original Dx/HA injected volume were predictors of success regardless of technique.

The placebo response in neuropathic pain trials: it’s not what you think

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Background: The placebo response varies across neuropathic pain trials making it challenging to demonstrate a treatment effect and difficult to compare trials of different medications.

Objective: The primary objective was to determine the study-level characteristics which predict the placebo response in trials of painful diabetic neuropathy. A second objective was to identify characteristics that are associated with changes in relative effect, independent of the placebo response.

Methods: A systematic review of the English-language literature was performed and fair-good quality, randomized, placebo-controlled trials measuring ≥50% pain relief from baseline as an outcome, were identified. Medications included the tricyclic antidepressants, the serotonin-norepinephrine reuptake inhibitors, gabapentin, pregabalin, and other anticonvulsants. Meta-regression techniques were employed to identify predictors of placebo response and predictors of relative effect while controlling for the placebo response. Covariates included: drug studied, publication year, trial duration, size and design, gender distribution, age, duration of diabetes and neuropathy, number of treatment groups, presence of a washout period, use of additional pain medications, and rate of patient recruitment.

Results: Twenty-five trials of diabetic neuropathy were included. The best predictor of placebo rate was whether the study was conducted in the United States or Europe versus another country or region (p=0.053). After adjusting for placebo response, predictors of relative risk were year of publication and length of trial. For example, assuming a 20% placebo rate, a 6-week trial published in 1997 is 1.7 times more likely to demonstrate pain relief versus placebo than a 12-week trial published in 2005. In contrast to previous research, neither baseline pain levels nor recruitment rate were found to predict placebo response.
Conclusion: Although the placebo response in neuropathic pain trials is an important consideration for systematic reviewers, trial duration and publication year may be equally important.

Comparative Study of Oral Health of Rural Children in the Khumbu Valley, Nepal

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Background. Dental caries (tooth cavities) is recognized as one of the most prevalent childhood diseases. Considerable concern has been expressed as to whether low-income countries will experience the level of oral disease as that experienced by high-income countries.

Objectives. To investigate the prevalence of dental caries in children in two rural tourist towns along the route to Mount Everest in Nepal and in two non-tourist rural towns off the tourist track; to analyze the caries severity in relation to selected variables; and to compare the oral health situation with data from a study conducted in the same locations 25 years earlier.

Methods. This study surveyed 136 students in the Khumbu valley in four different towns. Two of the towns chosen were along the Mt. Everest trekking route, and two of the towns chosen were not along the route. The study participants were divided into two groups: Five and six year-olds and eleven and twelve year-olds. Each child was surveyed using the World Health Organization’s DMFT criteria, the Visible Plaque Index (VPI), and a short questionnaire about their dental hygiene and eating habits.

Results. There was a significant difference between the four towns with the rate of decay being higher in the towns close to the tourist routes. Compared with earlier data dental caries had increased in the five-six year-old group but decreased in the 11-12-year-old group. A correlation was found between those children with a high plaque index and the rate of decay. Most of the decay was untreated.

Conclusions. The findings of the study confirmed the negative side effects of exposing an unprepared rural population to Western style snacks and identified a need for more access to dental care in this region of Nepal. Public health measures such as fluoridating the water would also help prevent the severity of the decay.

IRB approval: IRB000007597

Acknowledgments. We gratefully acknowledge OHSU Global Health Center, OSHU School of Dentistry, and World Medical Foundation for their generous travel grants to make this study possible. Colgate-Palmolive is gratefully acknowledged for their material support of toothpaste and oral health materials for the village children. The extensive local community support by the dental therapist in Namche Bazaar, Ms. Nawang Dkoha, without whose assistance we could not have conducted our study, is greatly appreciated.
Pain, Fatigue, Sleep, Depression, and Quality of Life in Children (age 7 to 18 years) during the first 18 months of Chemotherapy for Acute Lymphoblastic Leukemia (ALL)

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Education Learning Objective: Description of pain, sleep, fatigue, depression, and quality of life in children with acute lymphoblastic leukemia (ALL) after outpatient chemotherapy.

Significance: Little is known about the pattern of symptoms, depression, and quality of life (QoL) in children receiving chemotherapy for ALL. This information will serve as the basis for future intervention studies.

Problem and Purpose: To describe the pattern of selected symptoms and child outcomes over the first 18 months of treatment.

Theoretical / Scientific Framework: UCSF Model of Symptom Management

Methods and Analysis: This prospective, longitudinal study included six time points (T1-T6) (Induction, Consolidation, Interim Maintenance, Delayed Intensification, twice in Maintenance). Subjects completed ratings of intensity of sleep, fatigue each evening and morning for three days beginning the evening of treatment. Total sleep time (TST) and wake time after sleep onset (WASO) were computed from 3 day Actiwatch® monitoring; data on depression (CDI-S) and quality-of-life (PedQL-core) were obtained for the week prior to chemotherapy; and clinical data were extracted from medical records. On average, subjects (N=45) were school-age (mean=11.6±3.8), treated as high-risk (57.7%), white (76.6%), and male (69.4%). Within-subjects ANOVAs were based on subjects without missing data. Results of analyses of the full sample had similar findings. Symptom 3-day means were used; grand means (GM) and standard deviations computed across T1-T6 were reported on non-significant time effect findings.

Findings and Implications: Evening (p=.003; T1 MaximumM=1.09±78; T5 MinimumM=36±.57)) and morning pain (p=.02; T1 MaximumM=.60±.72; T5 MinimumM=.15±.42, decreased over time (N=27). WASO decreased (N=21; F(5,100)=2.48, p=.04 (T3 MaximumM=79.80±25.78, T6 MinimumM=56.06±23.58). PedQL-core improved over time (N=23; T1 MinimumM=51.94±12.97; T6 MaximumM=79.95±14.88). There were no changes over time in evening fatigue (N=21; GM=2.56±.88), morning fatigue (N=27; GM=2.09±.75), TST (N=21; GM=483.03±70.29), and CDI-S scores (N=19; GM=45.40±5.88). Similar to the prior work, pain and fatigue were evident during chemotherapy but the overall level of pain varies among studies. Fragmented sleep emerged as an important variable that has received little attention in this population. Subjects averaged 8 hours of sleep, but their average wake time after falling asleep was four to five times the 15 minutes reported for healthy children with normal sleep (Sadeh, 2000). These findings suggest that research on strategies for improving sleep in children receiving chemotherapy for ALL is needed.

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Hitting the Streets: Bike Commuting Injuries in Portland, Oregon


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The City of Portland, Oregon has made considerable investments into bicycle infrastructure, and the number of cyclists commuting to work in the metro area continues to increase. While the number of bike crashes appear to decrease annually, limited data exist on the types and incidence of injuries sustained during bike commuting. We conducted prospective surveillance of self-reported injuries in a cohort of 962 adults for a one-year period. A baseline questionnaire and monthly surveys were completed online. Riders commuted by bike an average of 10 days per month, with an average roundtrip distance of 11 miles. A total of 164 riders (18%) reported 192 injury events and 49(5%) reported 50 events in which a physical injury was severe enough to require medical attention (“serious injury”). The most common sites of injury were the legs and arms, and the skin and soft tissue. The annual incidence rate for all injury events was 15.0 per 100,000 miles travelled (95% CI, 13.2-17.5), and for serious injury events was 3.9 per 100,000 miles travelled (95% CI, 2.9-5.1). We failed to demonstrate differences in injury risk by age, gender, safety practices, and commuting experience. However, use of helmets at the time of an injury event was associated with a lower risk of serious injury (unadjusted OR = 0.32, 95% CI 0.12-0.89). Poor roadway conditions and motor vehicles were reported in 20% and 48% of serious injury events, respectively, suggesting that environmental factors could be important contributors to serious injury.
Who is Attending? End-of-Life Decision Making in the ICU


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Purpose: Traditional expectations of the single attending physician who manages a patient’s care do not apply in today’s ICUs. Although many physicians and other professionals have adapted to the complexity of multiple attendings, ICU patients and families often expect the traditional, single physician model, particularly at the time of end-of-life decision making (EOLDM). Our purpose was to examine the role of ICU attending physicians in different types of ICUs and the consequences of that role for clinicians, patients, and families in the context of EOLDM.

Methods: Prospective ethnographic study in a university hospital, tertiary care center. We conducted 7 months of observations including 157 interviews in each of four adult critical care units.

Results: The term “attending physician” was understood by most patients and families to signify an individual accountable person. In practice, “the attending physician” was an ICU role, filled by multiple physicians on a rotating basis or by multiple physicians simultaneously. Clinicians noted that management of EOLDM varied in relation to these multiple and shifting attending responsibilities. The attending physician role in this practice context and in the EOLDM process created confusion for families and for some clinicians about who was making patient care decisions and with whom they should confer.

Conclusions: Any intervention to improve the process of EOLDM in ICUs needs to reflect system changes that address clinician and patient/family confusion about EOLDM roles of the various attending physicians encountered in the ICU.
Hypertonic reconstituted lyophilized plasma is an effective low volume hemostatic resuscitation fluid for trauma

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Introduction: Hemostatic resuscitation is superior to crystalloid resuscitation in restoring blood volume, correcting coagulopathy, minimizing dysfunctional inflammation, and acidosis. Resuscitation with ‘full volume’ lyophilized plasma (LP) reduces blood loss, corrects coagulopathy, and decreases inflammation in a swine polytrauma and hemorrhagic shock model. This study compared the efficacy of ‘full volume’ LP to ‘low volume’ hypertonic LP.

Methods: Plasma was lyophilized following whole blood collection from anesthetized swine. Plasma was analyzed for electrolyte content, osmolarity, and coagulation factor activity. Twenty swine were anesthetized and subjected to a validated model of polytrauma and hemorrhagic shock (including a Grade V liver injury), then randomized to resuscitation with LP reconstituted to either 100% (100%LP) or half (50%LP) the original plasma volume. Physiologic data were monitored, and blood loss and hematocrit were measured. Coagulation was evaluated using thrombelastography (TEG).

Results: There were 10 swine in each group. Hypertonic LP had higher electrolyte concentrations, osmolarity, and increased coagulation factor activity levels by volume compared to 100%LP (p < 0.05). Blood loss, hematocrit, mean arterial pressure, and heart rate did not differ between groups at any time point (all p > 0.05). TEG parameters were not different between groups (R time, K, α – angle, or MA, p > 0.17).

Conclusion: Hypertonic LP resuscitation was well tolerated and equally effective to 100%LP with respect to physiologic and hemostatic properties. The smaller volume of fluid necessary to reconstitute hypertonic LP makes it logistically superior to 100%LP for first responders and may reduce adverse effects of large volume resuscitation.

Cryopreserved Deglycerolized Blood is Safe and Achieves Superior Tissue Oxygenation Compared to Standard Liquid Red Blood Cells

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Introduction: During preservation, donated liquid red blood cells (RBC’s) experience multiple functional and structural changes known as the storage lesion. Increased RBC age is associated with increased infection rates, organ failure and mortality. Cryopreserved RBC’s have been demonstrated to retain quality and function independent of storage duration for up to 10 years.

Methods: This prospective, randomized, double-blinded study enrolled stable trauma patients who required RBC
transfusion. Patients were randomly assigned to receive standard or cryopreserved RBC’s. Patient characteristics were recorded. Continuous tissue oxygenation (StO₂) monitoring was performed during the peri-transfusion period. Hematocrit and thromboelastography (TEG) pre and post transfusion were evaluated. Patients were monitored for transfusion reactions and clinical outcomes (length of stay, respiratory failure, multiple organ failure, transfusion reactions, deep venous thrombosis, and mortality). Blood smears were assessed after transfusions.

**Results:** Fifty seven patients were randomized and groups were well-matched for demographics and injury severity scores. No statistically significant differences were noted in hematocrit change, TEG parameters, blood smear findings, transfusion reactions, or clinical outcomes. StO₂ was found to be greater in the cryopreserved group during transfusion and up to 3 hours after transfusion (p=0.03).

**Conclusions:** No adverse complications were associated with the transfusion of cryopreserved RBC’s. Transfusion of cryopreserved RBC’s resulted in superior tissue oxygenation compared to standard RBC’s. Cryopreservation of RBC’s extends the lifespan of RBCs to 10 years allowing preservation of a precious resource while preventing the storage lesion. These findings have the potential to drive a paradigm shift in transfusion practices.

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**Timing of Cranioplasty after Decompressive Craniectomy for Trauma**

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**Background:** The optimal timing of cranioplasty after decompressive craniectomy for trauma is not known.

**Methods:** A retrospective cohort study was undertaken comparing complication rates in early (within 12 weeks of craniectomy) and late (≥ 12 weeks since craniectomy) cranioplasty. Logistic regression analysis was used to determine characteristics that would predict complications.

**Results:** Baseline characteristics were similar between the early and late cohorts. The complication rate in both early and late cohorts was 35%. The operative time for cranioplasty in the early cohort was shorter than the late cohort (102 minutes early, 125 minutes late; p=0.048) and was statistically significant. In the early cohort infection rates were lower (7.7% early, 14% late) as was bone graft resorption (15% early, 19% late). In the late cohort hydrocephalus rates (7.7% early, 1.3% late) and post-operative hematoma incidence (3.9% early, 1.3% late) were lower. None of these differences were statistically significant. In logistic regression analysis, the only predictor of any complication was age. Patients that were < 18 years of age were at higher risk of bone graft resorption than adults ≥ 18 years of age (OR 3.32, 95% CI 1.25 – 8.81; p=0.0162).

**Conclusion:** Cranioplasty after craniectomy for trauma performed early (within 12 weeks of craniectomy) had similar rates of infection, hydrocephalus, epidural hematoma, or bone resorption than cranioplasty performed later. Delaying cranioplasty results in longer operative times and may increase cost. Children are at higher risk for bone resorption after cranioplasty than adults.

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**Treatment of Severe Extremity Injury and Compartment Syndrome Using Autologous Bone Marrow Mononuclear Cells in a Large Animal Model**
Abstract withheld at authors request.

Re-Injury among Veterans with Traumatic Brain Injury Discharged from VA Polytrauma Rehabilitation Centers

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Objective: We examined prevalence of, and potential risk factors for, nonfatal injuries among Veterans with traumatic brain injury (TBI) post-discharge from Veterans Affairs (VA) inpatient polytrauma rehabilitation programs.

Methods: We surveyed caregivers of patients who had military service anytime from 2001-2009, sustained polytrauma including TBI, received VA inpatient care from 2001-2009, were discharged at least three months before the study, and were alive when the study was fielded about caregiver and patient health, including patients’ medically treated “accidents/new injuries” since discharge. We examined prevalence and source(s) of subsequent injuries and estimated patients’ injury risk in reference to hypothesized risk factors. Odds ratios and 95% confidence intervals were calculated using multivariate logistic regression.

Results: Caregivers reported that nearly one-third (32%) of patients incurred medically treated injuries after discharge; most were associated with falls (49%) and motor vehicles (37%). Odds of subsequent injury were associated with select demographics, initial injury characteristics, and post-discharge health and functioning. Characteristics of caregivers, including physical and mental health, were also associated with patients’ odds of subsequent injury.

Conclusions: A significant number of caregivers reported subsequent nonfatal injuries among patients treated for TBI/polytrauma in inpatient rehabilitation settings. Enhanced injury prevention efforts may be needed for this population.
Evidence-based decision aids in women’s health improve the decision making process

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Background and Objective: To address information needs of patient-centered care, we have developed three evidence-based decision aids to help women make informed healthcare decisions. One decision aid is designed to help women with a prior cesarean make a decision about a future childbirth delivery; another helps average risk women ages 38 – 48 decide about whether to have a screening mammogram; and the final decision aid helps abused women plan for safety.

Methods: All three computerized decision aids were developed based on medical evidence. The decision aids included educational components and activities to help the women set priorities for the respective healthcare decision. Eligible women (women with a prior cesarean; average risk women for breast cancer or abused women) were recruited to use the appropriate decision aid and participate in an evaluation study. No women participated in more than one evaluation study. In all three studies, the women reported their levels of decisional conflict (a measure of the decision making process) around the respective decision before and after using the decision aid.

Results: Participants in all three studies reported significant reduction in decisional conflict after using a decision aid (p<0.05). The women felt more informed, had more clear priorities around the health decision, felt more supported and/or reported less overall conflict around the decision.

Conclusions: The results of these three studies suggest that evidence-based computerized decision aids improve the participant’s decision making process, as demonstrated by reduced decisional conflict after only one session with a decision aid.

Factors Associated with PAP Testing Practices Among Vietnamese American Immigrant Women: Community Based Participatory Research

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Objective: To identify factors influencing Pap test receipt (having received at least once within a lifetime) and adherence to national screening guidelines (within the past 3 yrs) for Vietnamese American immigrant women (VIW, foreign-born).

Significance and Background: Vietnamese American women (foreign-born and United States-born [U.S.]) were diagnosed with cervical cancer and died at twice the rate of White non-Hispanic women and at higher rates than four other larger subgroups of Asian women (Chinese, Filipino, Japanese, Korean). Vietnamese American women were also more often diagnosed with late-stage cervical cancer compared to White non-Hispanic, Korean, and Japanese Asian women subgroups. Across studies, only 62–82% of Vietnamese American women reported Pap histories that indicated adherence to cervical cancer screening guidelines. Cervical cancer screening rates are low compared to the Healthy People 2010 and 2020 Objectives. Few data exist that might explain differences among VIW in terms of factors influencing Pap testing. What little is known includes perceiving cancer as a fatal diagnosis, preferring not to know about something that could not be changed, and not wanting to look for problems unless there was a strong reason.

Theoretical Framework: Health educators at the Immigrant & Refugee Community Organization identified cervical cancer as a priority problem for the Vietnamese community in the Portland, Oregon metropolitan area. The Ecological Model guided our understanding of influencing factors in obtaining a cervical Pap test.

Purpose: The purpose of this descriptive, community based participatory research was to explore factors potentially influencing Pap test receipt and adherence to national screening guidelines and to explore which of these factors are predictors among VIW. The following were examined as potential influencing factors: demographics, awareness, having requested a doctor/nurse practitioner for a Pap test, knowledge, Pap testing health beliefs, cultural barriers, confidentiality issues, and external factors. We also described VIW’s awareness of local community cervical cancer screening resources.

Methods & Analysis: Based on a power analysis, a sample size of 211 VIW, at least 21 years or older, was needed and recruited. Data were collected using a self-administered questionnaire. Items included demographics, Revised Susceptibility, Benefits, and Barriers Scale, Cultural Barriers to Screening Inventory, Confidentiality Issues Scale, Quality of Care from the Health Care System Scale, and other influencing factors. The questionnaire was translated using a team approach and pretested. Purposive sampling recruited VIW from 12 Asian community organizations. Descriptive statistics and logistic regression analyses (P < 0.10) were used.

Results: Sample characteristics were a mean age of 50 years (SD = 14), lived in the U.S. a mean of 15 years (SD = 9.15 yrs), 44% reported English speaking ability as average, 67% were married or living with a partner, 40% < high school education, and 33% < $15,000 annual household income. VIW were a mean age of 35 years when immigrated to the U.S. (SD = 15). Approximately 74% of VIW participants who completed the survey reported having received at least one Pap test, and 69% reported Pap testing history that adhered to national screening guidelines. In the final multivariate model for predicting Pap test receipt, the factor most strongly associated was suggestion from a friend (OR = 3.42, 90% CI [1.41-8.27]), followed by longer residency in the U.S. (OR = 1.09, 90% CI [1.05-1.14]), lower perceived common barriers (OR = 0.91, CI [0.85-0.98]), and lower perceived cultural barriers such as lack of family support (OR = 0.88, 90% CI [0.80-0.98]) and use of Eastern/Asian medicine (OR = 0.78, 90% CI [0.66-0.92]). In the final multivariate model for predicting guideline adherence, the factor most strongly associated was having health insurance (OR = 4.97, 90% [1.05-23.41].
followed by a doctor’s or nurse practitioner’s recommendation (OR = 4.92, 90% CI [1.20-20.18]). About 30.3% of VIW participants knew of cervical cancer screening programs in the community. Only 11.4% knew where to go to get a free or low-cost Pap test. Only 10.4% had ever attended a forum on cervical cancer or Pap testing.

Implications/Conclusion: These results indicated that there are multiple influencing factors. Health care providers can influence Pap testing rates among VIW by providing health education through outreach programs targeted at lay health workers and their social networks; identifying at-risk patients such as recently immigrated women; reducing perceived common and cultural barriers to Pap testing; and helping women seek alternative payment options if they lack health insurance. The low Pap testing adherence rate among VIW is a major health concern. As it is well supported that regular Pap testing increases the likelihood of early detection of pre-cancerous and cancerous growths, it stands to reason that routine cervical cancer screening could prevent the deaths of many VIW. Primary health care providers should be reminded of their crucial role in increasing adherence to cervical cancer screening guidelines.

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The Fertility Preservation Decision-Making Process of Adolescent and Young Adult Women with Cancer

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Purpose: The purpose of this study was to characterize and describe the decision-making process related to fertility preservation of adolescent and young adult (AYA) women with cancer.

Methods: This is a qualitative research study based in grounded theory methodology. A purposive sample of AYA women aged 15-39, who had made or were making a fertility preservation decision within the past 2 years was recruited from the cancer center at an academic medical institution in the United States. Participants were interviewed in person for one hour using a semi-structured interview schedule. Results were analyzed using the NVivo software package.

Results: Twenty-six AYA women participated, diverse in diagnosis (34.6% Hodgkin’s lymphoma, 23.1% Leukemia, 15.4% Breast and 7.7% sarcoma and colorectal, 3.8% adrenal, carcinoma and multiple myeloma), fertility preservation decisions (42.3% pursued fertility preservation), time since diagnosis at interview (mean 15.4 months) and family composition at the time of decision-making (19% had children and 42% were married). A theoretical model of decision-making emerged as a result of the analysis. The model addresses important developmental benchmarks (identity development, independence, focus on peers), types and sources of support, factors for consideration (moral issues, religion, money, the desires of others) and external forces (the information provided to them, age and developmental maturity, diagnosis and prognosis) that influence decisions. The model is iterative and illustrates how each decision-making experience affects identity and therefore shapes the next experience.
**Conclusions**: There is a process of decision-making common to AYA women facing fertility preservation decisions and informative to the development of supportive interventions. Healthcare professionals can use these results to better inform and support AYA women with cancer who are making decisions about fertility preservation through awareness of the process AYA women use to make decisions and knowledge of the specific factors patients consider that were identified in the model.

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**Polidocanol Foam for Female Permanent Contraception**

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**Objective**: To demonstrate the feasibility of a nonsurgical method of female permanent contraception using a macaque model

**Setting**: Primate Research Center

**Methods**: Reproductive age female rhesus macaques underwent laparoscopy under general anesthesia. The cervix was visualized, and small dilators were used to guide transcervical placement of a small catheter. Following confirmation of tubal patency by chromopertubation with Ingigo Carmen dye, Polidocanol foam (using a 5% solution and the technique of Tsafari) was administered directly into the uterine cavity until it was observed to spill freely into the peritoneal cavity. Animals were re-examined after 30 days; the reproductive tract was removed for histologic analysis if tubal occlusion was noted. If one or both tubes was patent, the procedure was repeated.

**Results**: A total of 9 animals were examined. Of these, 4 were unsuitable for the protocol due to cervical stenosis (3) or baseline tubal occlusion (1). Five females underwent successful transcervical treatment. Of these, 2 were noted to have bilateral tubal occlusion following two treatments, and 3 had bilateral occlusion after three treatments. Detailed histologic analysis localized the area of tubal damage to the intramural segment. There were no adverse non-target effects noted.

**Conclusions**: Polidocanol foam is a promising candidate agent for nonsurgical female permanent contraception. Further refinements are needed to develop an effective single treatment approach suitable for Phase I studies in women.