Discordance for exposures and disease phenotypes
To what extent can easily-accessible peripheral tissues/cells (e.g. whole blood, saliva, buccal) be used as a proxy for inaccessible tissues/cells?

Biomarkers
Peripheral mechanisms [immune?]
But careful interpretation needed.
Cellular heterogeneity within a tissue

- Infection
- Disease
- Age
- Stress
- Diet
- Exercise
- Circadian rhythm
- Lifestyle exposures
  ...many more...
Profiling Regulatory Variation in the Brain: Methods for Exploring the Neuronal Epigenome

Aaron R. Jeffries and Jonathan Mill

NeuN (neurons)
Sox10, Olig2 (oligodendrocytes)
Pax6, Aldolase C (astrocytes)
Controlling for neural cell differences

Neuronal cell proportions derived from 5mC data

Gene expression data
- ENO2 – neurons
- CD68 – microglia
- OLIG2 – oligodendrocytes
- GFAP – astrocytes
- CD34 – endothelial

Covariate in analysis models
Individual cells can exhibit substantial differences even when derived from an apparently homogenous population.
Applications of epigenetics to personalised medicine???

- Epigenetic biomarkers of disease
- Epigenetic markers of exposure
- Mechanisms of disease
- Refining traditional risk models
- Epigenetic biomarkers of drug response
- Modulation of epigenome via environmental, lifestyle, pharmacological interventions
Targeting the epigenome for drug discovery

Target identification
Connectivity mapping of test compounds
Data resources

Fetal brain DNA modifications (total, 5mC, 5hmC): http://epigenetics.essex.ac.uk/fetalbrain2/

Fetal brain RNA-seq (Nick Bray, Cardiff): http://genex.psycm.cf.ac.uk/FBSeq1/

Fetal brain mQTL: http://epigenetics.essex.ac.uk/mQTL/

GoDMC: http://www.godmc.org.uk (available soon)

Heritability estimates for DNAm sites: www.epigenomicslab.com

mQTL / SMR (for fine-mapping GWAS regions): www.epigenomicslab.com
Schizophrenia-associated hypermethylation at HDAC4

Dempster et al (in prep)
Structural genomic variation
Dup15q copy number variation in autism

**cis-effects:**
Hyper- and hypo-methylation

**trans-effects**
Overlap with idiopathic autism

Wong et al (in review)
Regulatory variation associated with elevated polygenic risk burden

Striatum

Cross-brain region model

Viana et al (2017)
Study designs that have been used in genetic epidemiology not optimal for epigenomics

<table>
<thead>
<tr>
<th>Timing of epigenomic change</th>
<th>Timing of studies of phenotypic consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–1m</td>
<td>Birth</td>
</tr>
<tr>
<td>1m–1y</td>
<td>Infancy</td>
</tr>
<tr>
<td>1–12y</td>
<td>Childhood</td>
</tr>
<tr>
<td>12–18y</td>
<td>Adolescence</td>
</tr>
<tr>
<td>18–40y</td>
<td>Adulthood</td>
</tr>
<tr>
<td>40–90y</td>
<td>Ageing</td>
</tr>
<tr>
<td>90–100y</td>
<td>Extreme longevity</td>
</tr>
</tbody>
</table>

- Cumulative stochastic changes
- Cumulative environmental changes
- Longitudinal cohort studies (including twins)
- Short-term interventions
- Natural experiments
- Birth cohorts (including twins)
- Guthrie cards
- IVF conceptions
- Natural exposures

Mill & Heijmans, Nature Reviews Genetics, 2013
Regulatory genomic signatures of clozapine exposure in schizophrenia

- BUB1
- TMCO6
- S100A4
- DIS3L2
- LCK
- TMEM194A
- TMUB2
- MHC
Methyloomic & transcriptomic analysis of antipsychotic compounds

Add media containing compound (or vehicle) → Harvest Cells → RNA-seq (DNA methylation beadchip)

For each well, cells detached and pelleted for DNA and RNA. Triplicates collected

Connectivity mapping → Gene regulation networks

Reference compounds

<table>
<thead>
<tr>
<th></th>
<th>Description</th>
<th>Protein targets</th>
<th>Conc low</th>
<th>Conc high</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clozapine</td>
<td>Atypical Antipsychotic</td>
<td>D2/multiple GPCRs</td>
<td>10nM</td>
<td>100nM</td>
</tr>
<tr>
<td>CRF</td>
<td>Corticotrophin releasing factor</td>
<td>CRHR1</td>
<td>100nM</td>
<td>500nM</td>
</tr>
<tr>
<td>Haloperidol</td>
<td>Antipsychotic</td>
<td>D2/multiple GPCRs</td>
<td>10nM</td>
<td>100nM</td>
</tr>
<tr>
<td>LY341495</td>
<td>Agonist for group II metabotropic glutamate receptors</td>
<td>mGlu2</td>
<td>100nM</td>
<td>1uM</td>
</tr>
<tr>
<td>LY379268</td>
<td>Antagonist for group II metabotropic glutamate receptors</td>
<td>mGlu2/3</td>
<td>100nM</td>
<td>500nM</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Calcium antagonist (used for hypertension)</td>
<td>Cav1.2</td>
<td>100nM</td>
<td>1uM</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>Calcium antagonist (used for hypertension)</td>
<td>Cav1.2/Cav3</td>
<td>100nM</td>
<td>1uM</td>
</tr>
<tr>
<td>Olanzapine</td>
<td>Atypical Antipsychotic</td>
<td>D2/multiple GPCRs</td>
<td>10nM</td>
<td>100nM</td>
</tr>
<tr>
<td>Sauvagine</td>
<td>Neuropeptide similar to CRF</td>
<td>CRHR1/CRHR2</td>
<td>100nM</td>
<td>500nM</td>
</tr>
<tr>
<td>Sodium valproate</td>
<td>Anticonvulsant and mood stabilizer</td>
<td>HDACs</td>
<td>600nM</td>
<td>1uM</td>
</tr>
<tr>
<td>STO-609</td>
<td>Selective inhibitor of calmodulin-dependent protein kinase kinase</td>
<td>CAMKK/CAMK1D</td>
<td>500nM</td>
<td>5uM</td>
</tr>
<tr>
<td>Vorinostat</td>
<td>Potent HDAC inhibitor primarily used in T-cell lymphoma treatment</td>
<td>HDACs</td>
<td>500nM</td>
<td>1uM</td>
</tr>
</tbody>
</table>
Clozapine-induced transcriptional networks in the zebrafish brain – module 1 (455 genes)
Clozapine-induced transcriptional networks in the brain – module 1 (455 genes)

394 human gene homologues
Critical windows of environmental sensitivity in schizophrenia across the life-course

Embryo/fetus

- Paternal age
- Maternal infection
- Perinatal events
  - Nutrition
  - Hypoxia

Childhood trauma

- Maternal stress
- Urban environment
  - Rearing environment
  - Migration

Subclinical experiences

- Cannabis

Major psychotic disorder
**Gene regulation: relevance to disease**

**Scenario ‘A’**

The DNA sequence determines what specific mRNA molecules are synthesized.

Epigenetic regulation determines how much of the mRNA is made, and where and when it is synthesized.

Even genes without any disease-causing mutation/polymorphism may be pathogenic...

**Scenario ‘B’**

No protein
Parallel trajectories of 5mC and 5hmC

Interaction between trajectories of 5mC and 5hmC

Variation in 5-hydroxymethylcytosine across human cortex and cerebellum

Lunnon et al.
Not differentiating between different DNA modifications in the brain can majorly confound associations with disease.

Standard sodium bisulfite approaches cannot distinguish 5mC from 5hmC – may be critical in analysis of CNS phenotypes.

5hmC: corr = 0.45
DNAmod: corr = 0.03
5mC: corr = -0.47

Smith et al (in review)
Study of Holocaust survivors finds trauma passed on to children's genes

New finding is clear example in humans of the theory of epigenetic inheritance: the idea that environmental factors can affect the genes of your children

had either been interned in a Nazi concentration camp, witnessed or experienced torture or who had had to hide during the second world war.

They also analysed the genes of their children, who are known to have increased likelihood of stress disorders, and compared the results with Jewish families who were living outside of Europe during the war. “The gene changes in the children could only be attributed to Holocaust exposure in the parents,” said Yehuda.

His team’s work is the clearest example in humans of the transmission of trauma to a child via what is called “epigenetic inheritance”—the idea that environmental
LAMARCK’S GIRAFFE

Original short-necked ancestor

Keeps stretching neck to reach leaves higher up on tree

and stretching and stretching until neck becomes progressively longer

Driven by inner “need”
Fetal mQTLs in schizophrenia-associated regions have larger effects on DNA methylation during neurodevelopment than the in adult brain

CER $P = 0.00998$
Clozapine-induced behavioural changes

Clozapine exposure had a striking dose-dependent effect on spatial position in the tank.

**General activity**
- No increase in stress
- No hypoxia

**Feeding**
- Social hierarchy maintained
- No overt physiological effects

**Spawning**
Widespread gene expression changes associated with clozapine exposure

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Gene symbol</th>
<th>log2 fold change (low)</th>
<th>log2 fold change (high)</th>
<th>P-value</th>
<th>Human orthologue gene symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>ENSDARG00000035458</td>
<td>atp2a1l</td>
<td>3.13</td>
<td>0.61</td>
<td>1.59E-06</td>
<td>-</td>
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<tr>
<td>ENSDARG00000007377</td>
<td>odc1</td>
<td>-0.37</td>
<td>-0.43</td>
<td>3.43E-06</td>
<td>ODC1</td>
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<tr>
<td>ENSDARG00000036028</td>
<td>arrdc3b</td>
<td>-0.60</td>
<td>-0.83</td>
<td>1.26E-05</td>
<td>-</td>
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<tr>
<td>ENSDARG00000035859</td>
<td>angptl4</td>
<td>-0.39</td>
<td>-0.83</td>
<td>1.70E-05</td>
<td>ANGPTL4</td>
</tr>
<tr>
<td>ENSDARG00000104687</td>
<td>slc16a9b</td>
<td>-0.39</td>
<td>-0.58</td>
<td>2.36E-05</td>
<td>SLC16A9 / MCT9</td>
</tr>
</tbody>
</table>
Ornithine decarboxylase is an enzyme which catalyses ornithine to putrescine. Higher blood concentration of ornithine has been reported in the plasma of schizophrenia patients (He et al., 2012). Increased expression of ornithine decarboxylase in the hippocampus of a rat model of schizophrenia (Bernstein et al., 1999).
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GoDMC: [http://www.godmc.org.uk](http://www.godmc.org.uk)

Other databases: [www.epigenomicslab.com](http://www.epigenomicslab.com)
Analysis of DNA Methylation in Young People: Limited Evidence for an Association Between Victimization Stress and Epigenetic Variation in Blood

Sarah J. Marzi, Ph.D., Karen Sugden, Ph.D., Louise Arseneault, Ph.D., Daniel W. Belsky, Ph.D., Joe Burrrage, Ph.D., David L. Corcoran, Ph.D., Andrea Danese, M.D., Ph.D., Helen L. Fisher, Ph.D., Eilis Hannon, Ph.D., Terrie E. Moffitt, Ph.D., Candice L. Odgers, Ph.D., Carmine Pariante, M.D., Ph.D., Richie Poulton, Ph.D., Benjamin S. Williams, B.Sc., Chloe C.Y. Wong, Ph.D., Jonathan Mill, Ph.D., Avshalom Caspi, Ph.D.

FIGURE 1. Association Between Adolescent Polyvictimization and DNA Methylation

A
DNA methylation age of human tissues and cell types
Horvath

This insurance company wants to analyze your saliva to predict when you’ll die

A life insurance company is turning to the hot, but still unproven, field of epigenetics to try to bet on how long you’re likely to live. Now, one company is turning to the hot, but still unproven, field of epigenetics to try to make that bet more scientific.

GWG Life, which buys life insurance policies from people who don’t want or can’t afford them anymore, last month started requiring those people to turn over a saliva sample. Its
...but evidence for an accelerated epigenetic clock in neurodegeneration.

$r = 0.89, P < 1.0 \times 10^{-16}$

$N = 1,366$

$P = 0.00343$

$P = 0.0004$
Genomic profiling of autism brain (BA9, BA41/42, cerebellum) – consistent DNA methylation differences across cases

Differentially regulated autism genes strongly enriched for neurodevelopmental pathways.
Methylomic variation associated with dup15q autism (BA9, BA41/42, cerebellum)
Despite striking dup15q cis-effects, highly-correlated DNA methylation alterations in iASD and dup15q patients.
SMR results across a GWAS region on chromosome 12 where differential DNA methylation (DNAm) is associated with schizophrenia.

Hannon et al., in prep
Overlap of mQTL in GWAS regions

Not sufficient to show causal or even pleiotropic effects
Tests for association between DNAm and trait

mQTL datasets – blood, human fetal brain, adult brain regions

In development: matched eQTL and H3K27ac/etc QTL datasets (brain)
A suite of epigenetic modifications act to fine-tune genomic function

Zhou et al (2011)
Stage 1

Trait

DNA methylation

SNP

\( b_{GWAS} \)

(Estimated in GWAS)

\( b_{mQTL} \)

(Estimated in mQTL study)

EWAS

\( b_{EWAS} = \frac{b_{GWAS}}{b_{mQTL}} \)

Stage 2

Pleiotropy – no heterogeneity

Causal SNP

\( b_{GWAS} \)

\( b_{mQTL} \)

\( \alpha \)

\( \alpha b_{GWAS} \)

\( \alpha b_{mQTL} \)

tag SNP

DNA methylation

\( b_{EWAS} = \frac{\alpha b_{GWAS}}{\alpha b_{mQTL}} \)

Causal SNP

\( \beta \)

\( \beta b_{mQTL} \)

DNA methylation

\( b_{GWAS} \)

\( b_{mQTL} \)

\( b_{EWAS} = \frac{\alpha b_{GWAS} \beta b_{mQTL}}{\alpha b_{mQTL}} \)
Bayesian Co-localisation

Do the same genetic variants influence risk of schizophrenia and DNA methylation? i.e. do the GWAS signals overlap?

H₀: there exist no causal variants for either trait;
H₁: there exists a causal variant for one trait only, schizophrenia;
H₂: there exists a causal variant for one trait only, DNA methylation;
H₃: there exist two distinct causal variants, one for each trait;
H₄: there exists a single causal variant common to both traits.
Differential aging effects across different regions of the human brain

Hannon et al (in preparation)
Highly convergent H3K27ac profiles in Alzheimer’s Disease cortex

- 4,162 of 182,065 peaks with FDR < 0.05
- 2,687 AD-hypoacteylated peaks [1.5% of total]
- 1,475 AD-hyperacetylated peaks [0.8% of total]

Hyperphosphorylation of the tau protein precipitates the neurofibrillary tangles associated with the pathogenesis of AD.
Stochastic autosomal monoallelic expression is common in neural cell lineages

- 3.4% of all expressed autosomal genes showed random monoallelic expression

  - Location:
    - Extracellular region
    - Plasma membrane
  - Functions:
    - Neurodevelopmental genes
    - Axon Guidance
    - Cell adhesion

Candidate gene lists from evidence based weighted matrices

\[ p \text{-values (Fisher’s Exact test)} = 0.004 \text{ and } 0.025 \text{ respectively} \]
Erasure and re-establishment of stochastic MA

NSC = Neural Stem Cell
iPSC = Induced Pluripotent Stem Cell
Stochastic monoallelically-expressed loci are associated with promoter hypermethylation.
The brain is a mosaic of clonal cell-lineages characterized by stochastic patterns of monoallelic gene expression.

- A necessity for correct development?
- Factor in phenotypic discordance between monozygotic twins?
Novel isoform / splicing diversity in the developing human brain

Evidence of alternative splicing at 95% of human genes
Particularly prevalent in the CNS
Developmentally dynamic
Functional diversity, potentially antagonistic functional effects