Deriving novel gene discovery from families with multiple members affected by Connecting clinical findings to pathophysiology in neurodegeneration with brain iron accumulation (NBIA)

PI: Kruer, Michael, MD

Abstract

The aim of the current project is to fill this crucial knowledge gap. Using an autozygosity mapping/candidate gene sequencing approach, we predict that by identifying novel genes that lead to NBIA, we will successfully implicate novel signaling pathways in the pathogenesis of the disorder and thereby identify new therapeutic targets. Our preliminary studies have already led to new insight regarding the role of a novel NBIA gene in the genesis of the disorder (see preliminary data), and available evidence indicates that several additional genes await identification. It is our overriding goal to use the insight gained from the current effort to inform future studies and contribute to rational therapy development in NBIA.

Specific Aim 1: Using highly informative multiplex consanguineous families, we will use autozygosity mapping to identify critical genomic regions that contain NBIA candidate genes. We will genotype multiple affected and unaffected members of six multiplex, consanguineous families with NBIA, negative for mutations in *PANK2*, *PLA2G6*, or *MIN,* using the Illumina platform. The Illumina Genome Viewer software suite will then be used to identify blocks of homozygosity conserved among affected individuals, but absent in family members unaffected by NBIA, localizing candidate genes to small haplotype blocks. Copy number analysis will also be performed to probe for any deleterious copy number variants that might lead to the observed phenotype.

Specific Aim 2: Using a candidate gene approach, DNA sequencing will be performed in affected and unaffected family members in order to identify causative mutations that segregate with disease.

Candidate genes will be ranked and sequenced in order to identify novel pathogenic mutations. DNA sequencing will be performed using a capillary DNA Sequencer and compared to reference sequence to identify potentially pathogenic sequence alterations.

Specific Aim 3: We will establish the pathogenicity of putative mutations by sequencing appropriate controls and performing appropriate *in vitro* studies.

Ethnically-matched controls will be identified and the relevant gene sequenced to demonstrate that the putative pathogenic sequence variant is not found in unaffected individuals. Subsequently, we will use quantitative PCR, Western blotting, and/or immunohistochemistry to determine the effect(s) of the mutation identified on transcript abundance, protein expression, and cellular localization. Effects on signaling function or enzymatic activity will be determined using an appropriate reporter assay.