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Characterization of rare genetic variation in CYP2D6 using human liver microsomes
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Patient-specific genetic information is increasingly important in clinical settings. An individual's specific genetic makeup influences if particular drugs will be effective or if they will have potentially life threatening side effects, and helps physicians select a proper dose. A major source of variability in patients' responses to medications is due to variable activity in drug-metabolizing enzymes called cytochrome P450s (CYPs). Genetic variation in the genes that encode CYP enzymes is a major focus of current research. We are specifically interested in one CYP enzyme, CYP2D6, that metabolizes 25% of commonly used drugs and has widely variable activity among individuals. Specific single nucleotide polymorphisms (SNPs), either alone or in combination, alter the ability of CYP2D6 to metabolize drugs. These changes in metabolic activity, defined as the phenotype, are often predictable based on the genotype of the individual. At one extreme, a poor metabolizer phenotype may have buildup of excess drug within their body while on the other extreme, an ultrarapid metabolizer phenotype may eliminate the drug quickly before it can have an effect. *CYP2D6* is a complicated gene however, and the genotype/phenotype relationship is not as straightforward. Within the same genotype class, there are often individuals who are phenotypic outliers. *CYP2D6* is a highly polymorphic gene and novel, rare SNPs are regularly discovered, although their functional effect is not always known. Our hypothesis is there may be rare, uncharacterized genetic variation that is responsible for this variability in the genotype/phenotype relationship.

To test the hypothesis that rare, uncharacterized SNPs are responsible for phenotypic outliers, we will investigate the effect of rare variants on *CYP2D6* interindividual variability using human liver microsomes (HLMs) from the University of Washington Liver Bank. The livers (n=370) will be sequenced on the PGRNSeq platform to assign the common *CYP2D6* genotype and characterize any novel coding variants. PGRNSeq was developed by the Pharmacogenomics Research Network (PGRN) to perform high-throughput sequencing on 84 "VIP" genes related to drug response and toxicity and includes the protein coding region of each gene as well as the regions 2 kb upstream and 1 kb downstream of the genes. CYP2D6 activity will be evaluated by performing HLM incubations with four different CYP2D6 probe drugs at low concentrations within the linear range (less than K_m). HLMs are endoplasmic reticulum fractions that contain active drug-metabolizing enzymes, including CYP2D6. CYP2D6 activity can be evaluated in HLMs by incubating them with probe drugs, which are drugs metabolized by one specific enzyme of interest to produce a specific metabolite. We can calculate an intrinsic clearance (CL_{int}) of parent probe drug conversion to the metabolite at a single point from an incubation in the linear range of the enzymatic reaction, expressed as rate of elimination over concentration of parent probe drug. We are currently performing optimization experiments using bufuralol, dextromethorphan, metoprolol, and codeine as probe drugs. Bufuralol, dextromethorphan, and metoprolol are well established in their use as CYP2D6 probe drugs while codeine is not commonly used but has clinical significance because the efficacy of codeine is dependent upon CYP2D6 activation to morphine. We have selected probes that include both classic and clinically relevant drugs that are involved in different pharmacokinetic pathways. We will quantitate substrate depletion and metabolite formation by liquid chromatography-mass spectrometry (LC-MS), and the CL_{int} of parent to

metabolite will be obtained for each HLM sample. Genotype-phenotype associations will be made by categorizing samples by the common genotype classification and identifying rare variants that could explain outlying samples. We expect that these rare variants may contribute significantly to the observed phenotype of one of more probe drugs.

This work is important because once rare SNPs are discovered and characterized, it will be possible to refine the criteria for what SNPs contribute to a particular genotype and improve phenotype predictions. This will allow physicians to make better decisions about the proper drug and dosage for a patient, bringing us closer to an age of personalized medicine.