Molecular Basis of Inter-Individual Variability in CYP2D6-Mediated Drug Metabolism

M. Ning¹, H. Jeong²
¹University of Illinois -Chicago, ²University of Illinois

Purpose
Cytochrome P450 (CYP) 2D6 is a major hepatic drug-metabolizing enzyme, responsible for eliminating ~25% of marketed drugs, including many drugs with narrow therapeutic windows. Of note, CYP2D6 exhibits very large inter-individual variability, which can lead to unintentional drug over- or under-dosing. Previous studies have found genetic polymorphisms of CYP2D6 as a potential contributor to this variability. For example, subjects carrying CYP2D6 polymorphisms linked with nonfunctional CYP2D6 enzyme exhibit poor metabolizer (PM) phenotype. Notably, PM constitutes only a small fraction (~5-10%) of population, and predicting CYP2D6 activity levels in an individual for the rest of population still remains a challenging task. Recently, our laboratory identified small heterodimer partner (SHP) as a novel repressor of CYP2D6 expression. SHP represses CYP2D6 expression by inhibiting hepatocyte nuclear factor (HNF) 4α transactivation of CYP2D6 promoter. Here, we hypothesize that differential transcription of CYP2D6 regulated by SHP contributes to inter-individual variability in CYP2D6 activity. The purpose of this study is first to examine the correlation between CYP2D6 transcription and its protein and activity level in human liver tissues; secondly to examine the role of SHP expression/activity in CYP2D6 expression in different individuals.

Methods
Snap frozen adult human liver tissues without gross pathology were obtained from Liver tissue and cell distribution system (n=65) and Corning Inc. (n=50). Total RNA was extracted from the livers using Trizol reagent. Relative mRNA levels of CYP2D6, HNF4α, SHP, and CYP8B1 (a gene whose expression is regulated via SHP-HNF4α pathway) are determined by quantitative real-time PCR (qRT-PCR) using HPRT-1 as reference gene. CYP2D6 copy number variation (CNV) was determined using Taqman assays and genomic DNA prepared from the livers. CYP2D6 protein levels in liver S9 fraction were determined using semi-quantitative western blotting. CYP2D6 activity in liver S9 fraction was determined using dextromethorphan as a substrate.

Results
In our cohort of human liver tissues (n=115), the number of subjects carrying 0, 1, 2 and more than 2 copies of CYP2D6 were 1, 9, 94 and 9 respectively. CYP2D6 copy numbers were positively correlated to CYP2D6 mRNA expression, as expected, with mean relative expression of 0, 0.37, 0.84 and 1.80 for CNV of 0, 1, 2 and over 2, respectively (p<0.0001). Notably, CYP2D6 activity highly correlates with its protein level (r = 0.85) in liver S9 fraction regardless of CYP2D6 copy numbers. The correlation between CYP2D6 mRNA and S9 protein levels was also significant (r = 0.56). Since CYP2D6 transcription is regulated by SHP-HNF4α pathway, we examined whether CYP2D6 mRNA level correlate to those of SHP, HNF4α and CYP8B1. In liver samples with two copies of CYP2D6 (n = 94), qRT-PCR results showed that CYP2D6 mRNA expression strongly correlated to that of CYP8B1 (r = 0.64), suggesting co-regulation of expression for these two genes. Both CYP8B1 and CYP2D6 (CNV=2) mRNA levels are also positively correlated to mRNA expression of HNF4α (r= 0.63 and 0.58, respectively). Interestingly, SHP mRNA expression also showed significant positive correlation (r=0.46) with that of CYP2D6 in livers of CNV =2, likely due to the fact that SHP is also a target gene of HNF4α.

Conclusion
This study showed that CYP2D6 activity highly correlates with its protein and mRNA levels in healthy human liver tissues, suggesting that CYP2D6 activity is governed at the level of mRNA expression. Positive correlation between the expression levels of CYP2D6 and HNF4α, as well as CYP2D6 and CYP8B1 expression suggest that the SHP-HNF4α regulatory pathway may determine CYP2D6 expression and activity in human livers. Further analysis to examine relative contribution of this regulatory pathway to CYP2D6 variability as compared to genetic polymorphisms of CYP2D6 is currently ongoing.