## PII-25

CHARACTERIZATION OF DUPLICATED CYP2D6 ALLELES: CORRELATION OF ALLELIC FREQUENCIES WITH ETHNICITY. K. G. Butz, MS, R. S. Muir, B. D. Read, L. S. Clark, PhD, M. P. Murphy, MS, P. J. Nakhle, PhD, Gentris Corporation, Morrisville, NC.

**BACKGROUND/AIMS:** Cytochrome P450 enzymes play an important role in the therapeutic effect of currently prescribed drugs including antiarrhythmics, antidepressants, and morphine derivatives. The highly polymorphic *CYP2D6* (*2D6*) gene locus manifests variations in enzymatic activity that affect phenotype. Ultrarapid metabolizers are a target of study as they often exhibit therapeutic failure or inefficacy. The purpose of this study was to characterize the allelic variants found within *2D6* gene duplications and to derive allelic frequency data across different ethnic groups.

**METHODS:** 227 subjects were screened for the presence of a *2D6* gene duplication using long-range PCR. DNA from subjects who were *2D6* duplication-positive was subjected to a second long-range PCR specific for the duplicated region, which was then used as a template for PCR and/or sequencing for allelic identification of the *2D6* duplication.

**RESULTS:** We have identified several different *2D6* allelic duplications. Further analysis has revealed subjects that have duplicated non-functional alleles including **4xN* and **6xN*. Continued testing is currently underway to establish frequency information for each type of duplication and to correlate frequencies among different ethnic groups.

**CONCLUSIONS:** In order to fully understand the effect that *2D6* gene duplications have on phenotype, it is important to know which allelic variant is being duplicated. For the subjects examined, we have identified which alleles are duplicated.

## PII-26

LACK OF ASSOCIATION OF THE ANGIOTENSIN II TYPE I RECEPTOR (AGTR1) 1166A>C POLYMORPHISM WITH CARDIOVASCULAR AND CEREBROVASCULAR OUTCOMES IN A SUBGROUP OF PATIENTS OF THE INTERNATIONAL VERAPAMIL SR/TRANDOLAPRIL STUDY (INVEST). J. A. Johnson, PharmD, J. H. Karnes, M. Brunner, MD, Y. Gong, PhD, T. Y. Langaee, PhD, R. M. Cooper-DeHoff, PharmD, C. J. Pepine, MD, University of Florida, College of Pharmacy, Center for Pharmacogenomics, University of Florida, College of Medicine, Gainesville, FL.

**BACKGROUND:** Genetic association between adverse cardiovascular (CV) outcomes and AGTR1 1166A>C as been studied by several groups, with conflicting results. We tested association of this SNP and a composite of adverse CV outcomes (death, nonfatal MI, nonfatal stroke) in the genetic substudy of INVEST, a trial of hypertensive coronary artery disease (CAD) patients randomly assigned to a calcium antagonist or β-blocker strategy, to either of which the ACE inhibitor (ACEI) trandolapril and/or the diuretic hydrochlorothiazde could be added for blood pressure (BP) control. Previous studies did not include stroke as an outcome.

**METHODS:** 270 patients with adverse CV outcomes during the study and 777 age-, sex- and race-matched controls were genotyped by pyrosequencing. Genotype, age, gender, body mass index, percentage of visits with BP under control, race, previous MI or stroke, history of heart failure or diabetes, smoking, study drug use and an interaction term between ACEI use and genotype were included in a logistic regression model to differentiate cases and controls.

**RESULTS:** Allele frequencies were 0.77 and 0.23 for the A and C allele, respectively. Neither genotype nor the interaction term of genotype and ACEI use were significantly associated with adverse CV outcome. Odds ratio of adverse CV outcome for A/A vs. C carriers was 1.09 (95% CI 0.80-1.48).

**CONCLUSIONS:** These data suggest that the AGTR1 1166A>C genotype is not associated with adverse CV and cerebrovascular outcomes in hypertensive CAD patients.

## PII-27

GENETIC POLYMORPHISM OF HNF-4 ALPHA IN A KOREAN POPULATION: IDENTIFICATION OF A NOVEL FUNCTIONAL HNF-4 ALPHA VARIANT WITH ALTERED TRANSACTIVATION ACTIVITY TOWARD CYP2D6. J. Shin, MD, PhD, S. Lee, PhD, E. Cha, MS, K. Liu, PhD, J. Shon, MD, Department of Pharmacology and PharmacoGenomics Research Center, Busan, Republic of Korea.

It has been well known that hepatic CYP expression widely varies in general population and large part of this variability may be attributed to genetic factors. Although the presence of genetic variations in CYP genes has been extensively explored, it is well agreed that the individual variation in CYP expression and activity cannot be expected solely from CYP genetic variations. Recent studies have revealed that hepatocyte nuclear factor-4 alpha (HNF-4A) is an essential transcription regulator of many CYPs such as CYP2D6, CYP3A4, etc. We hypothesized that the genetic variation of HNF-4A may contribute to the individual variability of CYPs. In the present study, the genetic polymorphism of HNF-4A was investigated in 50 Korean subjects through direct sequencing of all exons, exon-intron boundaries, and promoter region of HNF-4A gene. Twenty SNP, including two novel nonsynonymous SNPs in exon 2, were identified. One novel HNF-4A variant was tested for its functional alteration by its trans-activation activity of CYP2D6 promoter since this promoter contains functional DNA element for HNF-4A binding. The variant showed the total loss of function to regulate CYP2D6 promoter activity. We also confirmed that the mutant HNF-4A protein could not bind to CYP2D6 promoter region through EMSA experiment. The allelic frequency of this mutant HNF-4A in Koreans was about 2%. Our results suggest that natural HNF-4A variant may play a role in the interindividual variability of CYP2D6 expression.

## PII-28

THE NANOCHIP MOLECULAR BIOLOGY WORKSTATION ELECTRONIC MICROARRAY FOR CYP2D6 GENOTYPING. L. D. Lewis, MB BCh, MD, H. K. Lee, PhD, B. C. Schurr, MT, P. J. Jannetto, PhD, S. H. Wong, PhD, G. J. Tsongalis, PhD, K. J. Yeo, PhD, Dartmouth Medical School, Medical College of Wisconsin, Hanover, NH.

**BACKGROUND:** The Phase I drug metabolizing enzyme, CYP2D6, is highly polymorphic and metabolizes approximately 20% of therapeutic drugs. The objective of this study was to validate a CYP2D6 genotyping assay using the Nanochip® Molecular Biology Workstation (Nanogen Inc., San Diego, CA).

**MATERIALS AND METHODS:** Following IRB approval, eighty DNA samples from anonymous patients were obtained. PCR primers, ratio-references and reporters for SNP detection were provided by Nanogen Inc. For the Nanochip® microarray multipad addressing and duplicate runs were performed to test the intra- and inter-pad, as well as within and between run precision and accuracy for the defined genotypes.

**RESULTS:** All 80 DNA samples were genotyped for CYP2D6 *3, *4, *5, *6, *7 and *8 using both Pyrosequencing (Biotage Inc., Uppsala, Sweden) and the Nanochip® microarray. There was 99.4% concordance in the genotype results for these SNPs between both methods. We found one discordant call each for the *5 , *6 and *7 SNPs. DNA sequencing for the discordant *6 SNP confirmed the Nanochip® genotype and is pending for the discordant *5 and *7 SNPs. The intra- and inter-pad studies and within run studies were 100% concordant for the Nanochip® microarray.

**CONCLUSIONS:** This validation study of the Nanochip® CYP2D6 microarray genotyping system suggested it was accurate and reproducible.