

mild—peak AST < 100 IU/L; moderate—peak AST 100–1000 IU/L; severe—peak AST > 1000 IU/L). For subjects with more than one sample available for analysis, the peak APAP-CYS value was used for comparison to other parameters.

**RESULTS:** 71 patients had acute single ingestions of APAP (median reported dose 272 mg/kg), while 4 had chronic ingestions. One subject required a liver transplant, while all others had spontaneous recovery. All but one subject received treatment with N-acetylcysteine. 40 subjects had no to mild; 16 had moderate and 19 had severe toxicity. APAP-CYS was detected in all 207 samples. Peak APAP-CYS (median [range] nmol APAP-CYS/mL serum) was higher in subjects with severe toxicity (2.53 [0.44–23.91]) compared to those with moderate toxicity (0.35 [0.09–2.14];  $p = 0.0001$ ) and peak APAP-CYS was higher in those with moderate toxicity compared to those with no to mild toxicity (0.24 [0.04–1.22];  $p = 0.016$ ). For subjects in whom the time of the overdose was known ( $n = 52$ ), APAP-CYS was higher in those at risk for toxicity (Rumack nomogram;  $n = 44$ ), compared to those at no risk ( $n = 8$ ;  $p = 0.008$ ). Peak APAP-CYS did not correlate with prolongations of prothrombin time. Peak APAP-CYS was also compared to peak values for interleukin (IL)6, IL8, IL10 and monocyte chemoattractant protein 1 (MCP-1). Peak APAP-CYS was higher in patients with an elevated MCP-1 level ( $p = 0.008$ ).

**CONCLUSION:** APAP-CYS levels in serum correlate with the severity of liver injury in children and adolescents with APAP overdose.

### OI-C-IV

A DRUG BURDEN INDEX TO DEFINE THE FUNCTIONAL BURDEN OF MEDICATIONS IN OLDER PEOPLE. S. N. Hilmer, MD, PhD, D. E. Mager, PharmD, PhD, E. M. Simonsick, PhD, Y. Cao, MB, S. M. Ling, MD, B. G. Windham, MD, T. B. Harris, MD, MS, J. T. Hanlon, PharmD, MS, S. M. Rubin, MPH, R. I. Shorr, MD, MS, D. C. Bauer, MD, MPH, D. R. Abernethy, MD, PhD, University of Sydney, University at Buffalo, SUNY, National Institute on Aging, Johns Hopkins Medicine, University of Pittsburgh, University of California, San Francisco, University of Tennessee, Memphis, Sydney, Australia.

**BACKGROUND/AIMS:** Older people carry a high burden of illness for which medications are indicated along with increased risk of adverse drug reactions. We developed an index to determine drug burden based on pharmacological principles of anticholinergic and sedative drugs and evaluated the relationship of this index to physical and cognitive performance apart from disease indication and relative to simple counts of specific and total drugs concurrently taken.

**METHODS:** Data from the Health, Aging and Body Composition (Health ABC) study on 3075 well functioning community dwelling persons aged 70–79 were used to assess the cross-sectional association of drug burden index score with a validated composite continuous measure of physical function with scores ranging for 0 to 4, and cognitive performance with the Digit-Symbol Substitution Test (DSST). For both measures, higher values indicate better function. Associations were evaluated using multiple linear regression.

**RESULTS:** Use of anticholinergic and sedative medications was associated with poorer physical performance score: (anticholinergic exposure [2.08 vs 2.21,  $p < 0.0001$ ]; sedative exposure [2.09 vs 2.19,  $p < 0.0001$ ]) and cognitive performance shown by DSST score (anticholinergic exposure [34.5 vs 35.5,  $p < 0.05$ ]; sedative exposure [34.0 vs 35.5,  $p < 0.05$ ]). Associations were strengthened when pharmacological principles of dose exposure and response were used to calculate exposure with an increase of one unit in the drug burden index associated a deficit of 0.15 points ( $p < 0.0001$ ) on the physical function scale and 1.5 points ( $p < 0.01$ ) on the DSST. These values were three to four times of that associated with a single comorbid illness.

**CONCLUSION:** Use of the drug burden index indicates that anticholinergic and sedative drug exposure is associated with poorer

functional status in community-dwelling older people. This pharmacologically based approach provides a useful evidence-based tool for assessing the functional impact of exposure to medications in this population.

### OI-D-I

A NOVEL GENOME-WIDE APPROACH TO IDENTIFY GENETIC VARIANT THAT CONTRIBUTE TO CHEMOTHERAPY-INDUCED CYTOTOXICITY. R. S. Huang, PhD, S. Duan, PhD, W. K. Bleibel, E. O. Kistner, PhD, T. A. Clark, T. X. Chen, A. C. Schweitzer, J. E. Blume, M. E. Dolan, PhD, University of Chicago, Affymetrix Inc., Chicago, IL.

**BACKGROUND/AIMS:** The polygenic nature of the sensitivity to drugs has limited the success of candidate gene approaches. We present a novel genome-wide model utilizing human lymphoblastoid cell lines from the International HapMap consortium, of which extensive genotype information is available, to identify genetic variants that contribute to chemotherapeutic agents-induced toxicity.

**METHODS:** Our model was based on molecular biology, integrated genotype, gene expression and phenotype as measured by sensitivity of HapMap cell lines to chemotherapeutic drug. Cell lines derived from 30 trios of European descent (CEU) and 30 trios of African descent (YRI) were utilized. Cell growth inhibition with increasing concentrations of etoposide for 72 h was determined using alamarBlue<sup>®</sup> assay. Gene expression in these 180 cell lines was determined using the Affymetrix GeneChip<sup>®</sup> Human exon 1.0 ST Array. SNP genotypes and etoposide IC<sub>50</sub> were linked through whole genome association in cell lines from combined and independent CEU and YRI populations. A second association test was performed between SNP genotype and gene expression and linear regression was utilized to evaluate the correlation between gene expression and etoposide IC<sub>50</sub>.

**RESULTS:** Among the 387,417 SNPs tested, 49, 122 and 51 SNPs were significantly associated with etoposide IC<sub>50</sub> in combined, CEU and YRI population, respectively ( $p < 0.0001$ ). Among them, 7, 56 and 6 SNPs were also significantly associated with gene expression of 6, 21 and 40 genes in combined, CEU and YRI population (Bonferroni corrected  $p < 0.05$ ). The expression of 3, 18 and 24 genes whose expression were associated with SNP genotypes also significantly correlated with etoposide IC<sub>50</sub> in the three tested populations ( $p < 0.05$ ). These include genes that may play a role in cancer (AGPAT2, IL1B and WNT5B) and genes not yet known to be associated with sensitivity to etoposide.

**CONCLUSIONS:** Using our genome-wide approach, we identified previously unknown genetic variants that contribute to cell sensitivity to etoposide-induced cytotoxicity. These genetic variants contribute to drug-induced toxicity through their effect on gene expression. This novel, unbiased method can be used to elucidate genetic variants contributing to a wide range of cellular phenotypes induced by chemotherapeutic agents.

### OI-D-II

GENOME-WIDE ASSOCIATION STUDY TO IDENTIFY RISK FACTORS FOR DEVELOPING THERAPY-ASSOCIATED (SECONDARY)LEUKEMIA. A. A. Shinde, MD, PhD, G. Hayes, PhD, R. A. Larson, MD, R. A. Larson, MD, N. J. Cox, PhD, K. Onel, MD, PhD, University of Chicago, Chicago, IL.

**BACKGROUND/AIMS:** Therapy-related acute myelogenous leukemia (t-AML) is the most serious long-term complication of cancer chemotherapy. Only a subset of patients treated for cancer develop t-AML suggesting a genetic predisposition. We conducted a genome-wide association (GWA) study to investigate genetic factors associated with t-AML risk.

**METHODS:** A GWA scan using Affymetrix Mapping 10K arrays was performed on germline genomic DNA from 81 Caucasian individuals with t-AML and compared to previously genotyped 150 healthy Caucasian controls. Cases were adults with clinically diagnosed and cytogenetically characterized t-AML followed at the

University of Chicago. Controls were 150 previously genotyped unrelated healthy Caucasian individuals. After applying quality control and checking for departure from Hardy-Weinberg equilibrium (HWE), 6600 SNP markers were available for analysis. Association p values were computed using Fisher's exact test and the false discovery rate (FDR) was estimated by permutations. The extent of LD and differences in LD between cases and controls were computed using the CCOLDD (case control LD difference). The CCOLDD method is a novel computational method developed by our group, which contrasts differences in the extent of LD between cases and controls.

#### RESULTS:

SNP ID	Chromosome	p Value*	Gene
rs1394384	17q12	2.55×10 <sup>-5</sup>	ACCN1 (intronic)
rs719293	2p16.3	3.40×10 <sup>-05</sup>	NRXN1 (intronic)
rs1335546	10p12.1	1.94×10 <sup>-4</sup>	GAD2
rs2133508	4p15.2	1.98×10 <sup>-4</sup>	SLA/LP
rs1394605	5q33.3	2.27×10 <sup>-4</sup>	SGCD (intronic)
rs1374284	2q13	2.81×10 <sup>-4</sup>	IL-1 gene family
rs1199098	10q21.1	6.27×10 <sup>-4</sup>	IPMK
rs2255408	15q24.2	6.39×10 <sup>-4</sup>	ETFA
rs1351865	3p26.3	8.28×10 <sup>-4</sup>	CHL1

**Table 1.** Markers associated with t-AML susceptibility. P value = association p value by Fisher's exact test

Of the candidate genes identified by this analysis, none have previously been studied in t-AML, but several participate in cellular functions that have been directly implicated in leukemogenesis. The CCOLDD method detected differences in the extent of LD in the region in several gene loci important in carcinogenesis. Notable, these were—Fragile histidine triad gene (FHIT) that has been implicated as a tumor suppressor gene, Estrogen receptor 1 ESR1, CYP39A1 and JMJD2C.

**CONCLUSION:** The prior identification of individuals with a genetic susceptibility for developing t-AML would be invaluable in patient selection for chemotherapy and variants identified in this study may prove to be translationally useful biomarkers in subsequent prospective clinical studies.

### OI-D-III

GENE RE-SEQUENCING AND FUNCTIONAL GENOMICS OF FOLYLPOLYGLUTAMATE SYNTHASE (FPGS). T. A. Leil, PhD, A. A. Adjei, PhD, C. Endo, MD, O. E. Salavaggione, MD, G. K. Dy, MD, J. M. Reid, PhD, M. M. Ames, PhD, A. A. Adjei, MD, PhD, Mayo Clinic, Rochester, MN.

**BACKGROUND:** FPGS is a key enzyme in folate and anti-folate metabolism. It is present in both cytosolic and mitochondrial forms, both of which catalyze the polyglutamation of pteroyl-glutamate. This allows folate and anti-folate compounds to be retained within the cell and increases their affinity for target enzymes in the folate pathway. Genetic variation in the human FPGS gene has potential to impact anti-folate therapeutic efficacy in patients and folate utilization in the general population. We have re-sequenced the FPGS gene from 240 individual DNA samples and characterized the functional genomics of three non-synonymous coding single nucleotide polymorphisms (cSNP's).

**METHODS:** Re-sequencing of the FPGS gene was performed on Coriell DNA samples from 240 individuals of four different ethnic populations. Three non-synonymous cSNP's of FPGS were expressed in the cytosolic form of the protein in AuxB1 cells and numerous functional parameters were measured.

**RESULTS:** Of the 34 SNP's identified by gene re-sequencing, five were non-synonymous cSNP's that resulted in alteration of the FPGS protein sequence: F13<sup>Mit</sup>L, V22<sup>Mit</sup>I, R466<sup>Mit</sup>/424<sup>Cyt</sup>C, A489<sup>Mit</sup>/447<sup>Cyt</sup>V, S499<sup>Mit</sup>/457<sup>Cyt</sup>F. When expressed in AuxB1 cells,

the A447V variant was similar to WT FPGS in nearly all functional parameters, while the R424C and S457F variants were reduced approximately 2-fold in protein expression. The in vitro catalytic efficiency of these two FPGS allozymes was also reduced: by 4.7 fold (R424C) and 2.8 fold (S457F) with glutamic acid as a substrate; and by 2.2 (R424C) fold and 2.3 fold (S457F) with methotrexate (MTX) as a substrate. Additionally, the in vitro enzyme velocity at saturating pemetrexed (PMX) concentrations was reduced by 1.6 fold for the R424C variant, and 2.6 fold for the S457F variant. AuxB1 cells harboring the cytosolic forms these two FPGS isoforms displayed a 4.3 fold increase in the EC50 for folic acid.

**CONCLUSIONS:** Here we describe the first comprehensive re-sequencing and functional genomic study conducted on the FPGS gene. We discovered five cSNP's, two of which alter the in vitro kinetics of the FPGS enzyme and affect folic acid utilization of cells expressing the allozymes in culture. Individuals carrying these polymorphisms may be at higher risk for folic acid deficiency and for toxicity during anti-folate therapy.

### OI-D-IV

AROMATASE GENOTYPE PREDICTS SURVIVAL AFTER ACUTE CORONARY SYNDROME. C. X. Ma, MD, S. Cresci, MD, J. Wu, MS, M. Minton, BS, P. G. Jones, MS, M. A. Province, PhD, H. L. McLeod, PharmD, J. A. Spertus, MD, MPH, A. L. Beitelshoes, PharmD, MPH, Washington University, Mid America Heart Institute, St. Luke's Hospital, Mid America Heart Institute, St. Luke's Hospital, St. Louis, MO.

**BACKGROUND/AIMS:** Aromatase catalyzes the conversion of androgens to estrogens and is encoded by the *CYP19A1* gene. Given the postulated link between sex hormones and cardiovascular disease, we evaluated 4 SNPs in *CYP19A1* for their association with outcomes after acute coronary syndrome (ACS).

**METHODS:** INFORM is a prospective cohort study of ACS patients with 3-year follow-up. A total of 714 DNA samples were available and genotyped for *CYP19A1*-81371 C>T, M201T, and R264C, and 32266 G>T, by pyrosequencing. Cox proportional hazards models containing age, race, ACS type (unstable angina, non-ST elevation myocardial infarction [MI], and ST-elevation MI) and treatment, diabetes, heart failure, ejection fraction, and genotype (coded as -1, 0, or 1) were conducted separately by sex to evaluate survival. Sexes were pooled if positive genotype findings trended in the same direction for men and women. In addition, analyses were subsequently conducted separately by race to minimize the effects of population stratification.

**RESULTS:** The average age of the cohort was 61 ± 12 years and was comprised of 35% female subjects. Minor allele frequencies were 0.22, 0.04, and 0.03, and 0.30 for -81371 C>T, M201T, and R264C, and 32266 G>T, respectively. -81371 C>T was associated with mortality in men, HR 1.85 (95% CI 1.16–2.97) for increasing copies of the T allele. M201T was associated with mortality in men, HR 5.66 95% CI 2.08–15.38 for increasing copies of the T allele, with a trend in women, HR 3.35 95% CI 0.9–12.5. In the overall population the HR for increasing copies of 201T alleles was 4.12 (95% CI 1.89–9.00), which remained significant in the Caucasian and African American subgroups (Table).

SNP	Hazard Ratios and 95% Confidence Intervals					
	Females	Males	Combined	Sex* genotype interaction p	Caucasian	African American
-81371 C>T	0.63 (0.24–1.7)	1.85 (1.2–3.0)	–	0.05	–	–
M201T	3.3 (0.9–12.5)	5.7 (2.1–15.4)	2.5 (1.21–5.05)	0.59	2.7 (1.1–7.0)	3.4 (1.1–10.9)
R264C	1.09 (0.2–5.2)	0.37 (0.05–2.7)	–	0.31	–	–
32266 G>T	1.1 (0.6–2.3)	0.78 (0.49–1.2)	–	0.58	–	–

**CONCLUSIONS:** Our results suggest that *CYP19A1* genotypes are associated with mortality, in a sex-specific manner after ACS. If confirmed, these findings would represent the first report of such an association and could not only contribute to our understanding of sex-based differences in cardiovascular disease but may also provide novel targets for treatment of ACS.

### PI-1

DEVELOPMENT OF AN ASSAY FOR THE QUANTIFICATION OF LEUKOTRIENE E<sub>4</sub> (LTE<sub>4</sub>) IN HUMAN URINE USING LIQUID CHROMATOGRAPHY-ELECTROSPRAY IONIZATION MASS SPECTROMETRY. G. L. Milne, PhD, H. Yin, PhD, J. D. Morrow, MD, Vanderbilt University, Nashville, TN.

**BACKGROUND:** Leukotrienes (LTs) are important inflammatory mediators generated from the enzymatic oxidation of arachidonic acid by 5-lipoxygenase. LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub> are cysteinyl conjugates. These molecules are potent pro-inflammatory molecules that have been implicated in the pathophysiology of many diseases including allergic asthma, adult respiratory distress syndrome, inflammatory bowel disease, and, more recently, cardiovascular disease. Thus, establishment of a biomarker assay to measure *in vivo* LT production is of important clinical interest. Urinary LTE<sub>4</sub> is an index systemic of cysteinyl LT production as LTC<sub>4</sub> and LTD<sub>4</sub> are metabolized *in vivo* to LTE<sub>4</sub> which is then excreted into the urine. Previous methods reported to quantify this analyte suffer from being complex and are not robust. Herein, we report an improved, highly precise, and accurate assay to quantify LTE<sub>4</sub> in human urine utilizing liquid chromatography-electrospray ionization tandem mass spectrometry (LC-MS/MS).

**METHODS:** Briefly, after addition of the d<sub>3</sub>-LTE<sub>4</sub> internal standard, urine (5 mL) is acidified and then purified using a C-18 solid phase extraction column. The elutant is then subjected to LC-MS/MS analysis during which the instrument is operated in the negative ion mode monitoring the transition of the native LTE<sub>4</sub> precursor ion *m/z* 438 to product ion *m/z* 333 and the equivalent transition for d<sub>3</sub>-LTE<sub>4</sub>.

**RESULTS:** We found this method to be highly robust and accurate measure of LTE<sub>4</sub> excretion in urine. The calibration curve is linear over more than a 100-fold concentration range and the assay is highly precise. The lower limit of detection is approximately 9 pg. Normal concentrations of urinary LTE<sub>4</sub> in healthy humans range from 17–52 pg/mg creatinine. The average concentration in women is 29 pg/mg creatinine (n = 5) and in men is 31 pg/mg creatinine (n = 6). Further, we have been able to show that administration of certain pharmacological agents markedly increases production of urinary LTE<sub>4</sub>.

**CONCLUSIONS:** In conclusion, this assay provides a sensitive, robust, and accurate method to assess endogenous LT formation that can be used to further explore the role of these inflammatory mediators in human disease.

### PI-2

PHARMACOKINETIC AND PHARMACODYNAMIC EFFECTS OF THE INTRAVENOUSLY ADMINISTERED CB1 RECEPTOR AGONIST ORG 28611 IN HEALTHY MALE VOLUNTEERS. L. Zuurman, MD, MSc, P. C. Passier, PhD, M. de Kam, MSc, H. J. Kleijn, MSc, A. F. Cohen, Professor, MD, J. M. van Gerven, Professor, MD, CHDR, NV Organon, Leiden, The Netherlands.

**BACKGROUND/AIMS:** Like THC (active ingredient of cannabis), newly developed CB1 agonists are expected to be analgesic, sedative, amnesic, and anti-emetic, but will probably not adversely affect respiratory function. Org 28611, a CB1 agonist, will be developed as an analgesic with or without sedative properties for surgeries in outpatient or day-care cases. Here we describe the safety, tolerability and pharmacodynamic effects following intravenous administration of this compound in the first human exposure study.

**METHODS:** During a double-blinded five-way, placebo controlled, cross-over study, ascending doses of Org 28611 were for the first time intravenously administered to 20 healthy volunteers. Midazolam was used as a positive control. Volunteers were admitted for one week in a specialized phase I unit. Subjects with a life-time cannabis use of more than five times were excluded. In addition, it was not allowed to use cannabis for at least one month before screening. Subjects with a personal or family history of psychosis were excluded from the study.

**RESULTS:** Up to a dose of 1 µg/kg Org 28611 no effects were observed. Higher doses demonstrated dose related effects. The observed sedation after administration of 3-10 µg/kg Org 28611 (indicated by observers rating scale) differed from the sedation observed after administration of midazolam. Although subjects reported a reduction on the visual analogue scale of alertness, in contrast to midazolam subjects were awake and reacted quickly to stimuli. In addition, unlike midazolam (-20: 95% CI-27,-12) a dose of 3 µg/kg Org 28611 (-14: 95% CI-21, -7) did not affect saccadic eye movements (an indication for sedation). Midazolam impaired both imprinting and active and passive delayed recall of learned words. In contrast, Org 28611 primarily reduced active recall, but (passive) recognition was unimpaired. Doses higher than the maximum tolerated dose of 3 µg/kg (either administered as a 25-minute infusion or a bolus) caused untoward psychiatric (anxiety, hallucinations) and psychotropic ('high') effects.

**CONCLUSION:** Org 28611 does not have the pharmacologic profile to be developed as a sedative analgesic. However, doses up to 3 µg/kg Org 28611 are well tolerated and may be useful for reduction of pain or nausea.

### PI-3

EVIDENCE BASED REGULATORY GUIDANCE, TO FLARE OR NOT TO FLARE IN THE OSTEOARTHRITIS PAIN MODEL. M. Averbuch, MD, M. Katzper, PhD, Tel Aviv University Medical Center, Tel Aviv, Israel, CDER, FDA, Silver Spring, MD.

**BACKGROUND:** There is reason to question the approach used for studying new medications for the treatment of osteoarthritis pain. In the standard approach the on-going treatment is withdrawn before the new medication is given. Usually a pain flare up occurs. The test medication is administered to see how well it reduces the flared pain. Should we judge medication relative to a flared baseline? Is flaring predictive of response?

**METHODS:** Data for a 12-week randomized double-blind placebo-controlled trial were analyzed. Using the hip osteoarthritis (OA) flare-up pain model three doses of a test medication, naproxen sodium (500 mg BID) and placebo were administered. Pain was measured on both visual analog and categorical scales Pain scores were compared with pre-flare scores.

**RESULTS:** Analysis shows that there is not a relationship between flare intensity and efficacy of the drug when measuring reduction of pain versus flare. Looking at pain scores versus the pre-flared pain score indicates that for all the tested doses a large fraction of the subjects does not return to the lower pre-flared pain state.

**CONCLUSIONS:** We suggest that there is place for reassessment of the osteoarthritis flare-up pain model. These results indicate that flaring may be an ethically questionable procedure.

### PI-4

IDENTIFICATION OF A NOVEL CLASS OF DIOXOLANE-ISOPROSTANES FROM EICOSAPENTAENOIC ACID *IN VIVO*. J. D. Brooks, H. Yin, PhD, J. D. Morrow, MD, Vanderbilt University Medical Center, Nashville, TN.

**BACKGROUND/AIMS:** Free radical-catalyzed peroxidation of polyunsaturated fatty acids (PUFAs) occurs in intact phospholipids *in vivo* and the accumulation of these oxidized species has been implicated in a number of diseases. Previously, we reported the formation of a novel class of isoprostanes containing an additional ring, termed the dioxolane-isoprostanes, generated from the oxidation of