24 and 72 hours post treatment. We looked for genetic loci significantly linked to inter-strain cellular responses to treatment and found a 1.2 Mb locus on Chr X that was significantly linked to variable cytotoxic responses to a known mitochondrial toxicant, rotenone (-logP >4.0). Within this putative locus is cytochrome b-245, beta polypeptide (Cybb) gene, which encodes for a voltage-gated H(+) channel that mediates pH in the mitochondria. We conducted a series of experiments to examine the role of Cybb in mediating toxic responses to rotenone in vivo, given that mitochondrial dysfunction has been shown to underlie idiosyncratic adverse drug reactions. We found that strains belonging to different Cybb haplotypes scored differently in the treadmill exercise stress test for aerobic endurance after chronic treatment with rotenone. Our study demonstrates that cell-based genetic assays using MEFs are an effective tool for identifying genes underlying drug and chemical toxicity. Importantly, mouse strains that exhibit differential in vitro sensitivity, from a cell-based screen, also display a differential in vivo phenotype. This in vitro-to-in vivo validation is difficult, but a fundamental step toward recognition of cell-based toxicity screens.

PS 187 P-Glycoprotein Transport in the Disposition of Neurotoxicants.

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Background: P-glycoprotein (P-gp), encoded by the *ABCB1* (or *MDR1*) gene, is an efflux xenobiotic transporter expressed in many tissues important in xenobiotic disposition. P-gp is highly expressed at the blood-brain-barrier and protects the brain from substances circulating in the blood. Although the importance of P-gp in drug disposition is clear, its role in disposition of environmental neurotoxicants is not well understood. Our goal is to investigate the role of P-glycoprotein in neurotoxicant accumulation in the brain, particularly pesticides that have been associated with Parkinson's disease such as rotenone, maneb, paraquat, and MPP+. Methods: We used polarized kidney epithelial control cells, LLC-PK1, and *ABCB1*-transfected cells, LLC-*MDR1*, to characterize pesticides as substrates or inhibitors of P-gp using flow cytometry, cytotoxicity, and transepithelial permeability assays. P-gp-stimulated ATPase activity was also measured to evaluate compounds as P-gp substrates in a membrane-based system. Results: We observed weak inhibition of rhodamine-123 (R123) efflux in *ABCB1*-expressing cells by flow cytometry in the presence of 100 μM rotenone or maneb, 16.2 ± 1.53 and 11.6 ± 4.53%, respectively. Paraquat and MPP+ showed no R123 inhibition. ATPase assays showed that rotenone is a P-gp substrate with a Km = 26.7 ± 12.9 μM and Vmax = 35.8 ± 6.5 nmol Pi/mg protein/min. This compares to the known P-gp substrate verapamil with kinetic constants of Km = 8.22 ± 3.79 μM and Vmax = 42.9 ± 5.5 nmol Pi/mg protein/min. Paraquat, maneb, and MPP+ showed no ATPase stimulation. Conclusions: In combination these data suggest that rotenone acts both as a substrate and a weak inhibitor of P-gp, whereas maneb acts only as a weak inhibitor. MPP+ and paraquat are neither substrates nor inhibitors of P-gp. We will further confirm these results using cytotoxicity and transepithelial permeability studies. Our studies will provide data to show the role of P-gp in the disposition of pesticides associated with Parkinson's disease.

PS 188 Polymorphic Enzymes, Urinary Bladder Cancer Risk, and Structural Change in the Local Industry.

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In the 1990s, the highest percentage of glutathione S-transferase M1 (GSTM1) negative urinary bladder cancer cases (70%) ever reported was observed in the greater Dortmund area. The question arose whether this uncommonly high percentage of GSTM1 negative urinary bladder cancer cases was due to environmental and/or occupational exposure decades ago. Thus, 15 years later, another study on urinary bladder cancer was performed in the same area after the coal, iron and steel industries had finally closed in the 1990s. In total 196 bladder cancer patients from a local department of urology and 235 controls with benign urological diseases were investigated by a questionnaire and genotyped for GSTM1, GSTT1 and the N-acetyltransferase 2 (NAT2) tag SNP rs1495741. The frequency of the GSTM1 negative genotype was 52% in bladder cancer cases and in the controls as well and thus much lower, compared to a previous study performed from 1992-95 in the same area (70%). NAT2 genotypes were distributed equally among cases and controls (63% slow acetylators). Less GSTT1 negative genotypes were present in cases (17%) than in controls (20%). The normal frequency of the GSTM1 negative genotype in bladder cancer cases in the present study supports the assumption that the highly increased percentage of GSTM1 negative bladder cancer patients observed in the preceding study may be related to past occupational and environmental exposures decades ago.

PS 189 CTNNA3 (α-Catenin) Gene Variants Are Associated with Diisocyanate Asthma in Occupationally-Exposed Workers.

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A genome-wide association study conducted recently in Korean subjects identified three CTNNA3 (alpha-T catenin) single nucleotide polymorphisms (SNPs) (rs10762058, rs7088181, and rs4378283) associated with diisocyanate induced occupational asthma (DA). We conducted a candidate gene association study to replicate these findings in Caucasian workers. Genotyping was performed on genomic DNA, using a 5' nuclease PCR assay. Genotyping of these SNPs was performed in 410 diisocyanate-exposed and predominantly Canadian workers including: 132 workers with DA confirmed by a specific inhalation challenge (DA+); 131 symptomatic workers in whom DA was excluded by a negative challenge (DA-); and 147 HDI-exposed asymptomatic workers (AWs). CTNNA3 rs7088181 and rs10762058 SNPs were significantly associated with DA+ when compared to AWs ($p \leq 0.05$) but not in comparison to DA- workers. After adjusting for potentially confounding variables of age, smoking status and duration of exposure, minor allele homozygotes of rs7088181 and rs10762058 SNPs were at increased risk for DA compared with AWs [OR= 9.05 (95% CI:1.69, 48.54) and OR = 6.82 (95% CI:1.65, 28.24), respectively]. In conclusion, we replicated association between two closely linked CTNNA3 gene SNPs and DA in Caucasian workers. These findings suggest that genetically altered expression of CTNNA3 might influence cellular adherence and epithelial barrier function in the airways and play a role in the pathogenesis of DA.
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PS 190 Prevalence and Functional Characterization of the NADH Cytochrome b_5 Reductase I1M+6C>T Intronic Variant.

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Women exposed to cigarette smoke may have an increased risk of breast cancer, although this is controversial. In addition, African American, but not Caucasian, women showed an association with smoking and breast cancer in the Carolina Breast Cancer Study (CBCS) population. We hypothesized that this could be due to race-associated genetic variability in the pathways that detoxify tobacco carcinogens such as 4-aminobiphenyl (4-ABP) and 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP). Both are mammary procarcinogens that are bioactivated to hydroxylamine metabolites, which form DNA adducts and are thought to initiate cancer. Cytochrome b_5 (*CYB5A*) and NADH cytochrome b_5 reductase (*CYB5R3*) comprise a detoxification pathway that reduces 4-ABP and PhIP hydroxylamine metabolites back to their parent compounds. The purpose of this study was to determine whether *CYB5A* and *CYB5R3* polymorphisms were over-represented in African American women in the CBCS, and to evaluate their role in the association with smoking and breast cancer risk. Several *CYB5A* and *CYB5R3* single nucleotide polymorphisms (SNPs) were more prevalent in African Americans than in non-African Americans in the CBCS population. One intronic SNP in *CYB5R3*, I1M+6C>T, previously found in tissue samples with low b5 reductase protein expression, was found with a minor allele frequency that was 100-fold higher in African American subjects (MAF = 0.0428, P < 0.0001). Among smokers, this variant was significantly over-represented in African American women with breast cancer compared to same-race controls (OR 2.10, 95% CI, 1.08-4.06). The I1M+6C>T *CYB5R3* variant is being functionally characterized for promoter and repressor function using a dual-luciferase reporter assay. These studies suggest that the I1M+6C>T intronic variant in *CYB5R3* may increase the risk of breast cancer among African American women that smoke.

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