Pennsylvania State University

Annual Progress Report: 2006 Nonformula Grant

Reporting Period

July 1, 2009 – June 30, 2010

Nonformula Grant Overview

The Pennsylvania State University received $4,206,097 formula funds for the grant award period June 1, 2007 through May 31, 2011. Accomplishments for the reporting period are described below.

Research Project: Project Title and Purpose

Gene-Environment Interactions for Colorectal Cancer in Northeast PA - Colorectal cancer (CRC) is the fourth most commonly diagnosed cancer in the U.S., and is particularly high in the northeastern section of PA. The goal of the present study is to examine how genetics and the environment combine in northeastern PA to increase risk for CRC. In particular, we are examining modifiable risk factors, that is, lifestyle factors such as cigarette smoking and specific patterns of diet that could be changed to lower the risk of disease. The specific genes and their variants that will be examined are those involved in either the activation or elimination of many of the harmful substances in smoking or diet that can cause CRC. The findings will result in a significant advance in our understanding of CRC and help to identify high-risk individuals for the development of CRC screening and prevention strategies in PA and nationwide.

Anticipated Duration of Project

6/1/2007 - 5/31/2011

Project Overview

The overall goal of the present study is to determine the relationship between haplotypes of relevant Phase I and Phase II metabolizing enzymes that are responsible for the activation or detoxification of polycyclic aromatic hydrocarbons (PAHs), heterocyclic amines (Has) and nitrosamines and the risk of CRC. In particular, we will examine whether there is an interactive effect between exposure to specific environmental factors and haplotype tagging SNPs (htSNPs) in linkage disequilibrium with metabolizing gene variants. This approach will enable us to determine the risks associated with known SNPs, and also will allow us to identify new potentially functional SNPs that are in linkage disequilibrium with htSNPs that capture the full haplotype information. Another important aspect of our approach is that we are conducting a population-based study in a contiguous 9-county high risk area of northeastern PA. Thus, our results will be directly generalizeable to the residents of this area, but the scientific findings should be pertinent to all PA and U.S. residents. We will enroll newly diagnosed incident cases

identified directly from the participating tumor registries that serve in this region of PA within ~6-9 months since cancer diagnosis. Controls will be frequency-matched to cases by sex, age (within 5 years) and race and will be identified by random-digit dialing (RDD). Our pilot studies have demonstrated the feasibility of this approach, and interestingly have shown increased risks associated with meat consumption and cigarette smoking. The specific aims of this study are:
(1) Determine the association between haplotypes of genes that are involved in the metabolism of PAH, HA and nitrosamine, and colorectal cancer risk in northeastern PA.
(2) Determine the association between red meat consumption, meat cooking methods, dietary carcinogens, and cigarette smoking with the risk for colorectal cancer in northeastern PA.
(3) Measure the interaction between these haplotypes and diet and smoking on the risk of colorectal cancer.
(4) Sequence those haplotypes associated with colorectal cancer risk from aim 3, in order to identify novel functional SNPs.
(5) Confirm the functional capacity of newly identified high-risk genotypes identified in aim 4.

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Gregory R. Harper, MD, PhD - employed by Lehigh Valley Hospital (LVH)
Anna K. Hull, PhD, Delroy Louden, PhD - employed by Lincoln University

Expected Research Outcomes and Benefits

It has been recognized that dietary factors are important in the cause of CRC. However, typical diets that promote good health, such as a high consumption of fruits and vegetables, do not appear to be important for colorectal cancer. Instead, there is growing evidence that highly cooked meats and certain carcinogens that are formed during the cooking/browning process are important causes of colorectal cancer. We are exploring this issue using state of the art databases that provide us with estimates of the actual level of these meat-based carcinogens. Further, the effects of meat cooking likely vary by an individual's metabolic capacity to metabolize meat carcinogens. If specific meat cooking practices increase CRC risk, it will be prudent to launch new public health campaigns emphasizing the role of cooked meats in causing cancer. Thus it will be of significant public health value to inform the public that the ways in which they cook meat increases their risk of CRC. Similarly, it seems surprising that the role of cigarette smoking in causing colorectal cancer has really only been recognized in the last few years although the

evidence is not yet definitive. This study will add to our knowledge of the smoking risks and if confirmed, public knowledge that smoking causes CRC will add further impetus to smoking cessation programs, smoking bans and decisions to take up or quit smoking. By identifying individuals with high risk genetic variants, these programs and knowledge can be targeted to those who can especially benefit from quitting. These are important goals in theory, but in reality require the identification of all important genetic variants in smoking and meat carcinogen metabolism. By using the new human genome project HapMap and associated software, combined with new high throughput gene sequencing ability we will be able to accomplish these tasks. Finally, an understanding of the effects of genetic variability is needed to fully comprehend the proposed research. The identification of a mutation will be carried out by carefully controlled laboratory studies to examine the actual extent to which that mutation affects metabolism.

Summary of Research Completed

We have performed several studies relevant to Aims 1-5 of the proposal.

Aim 1. Determine the association between haplotypes of genes that are involved in the metabolism of PAH, HA and nitrosamine, and colorectal cancer risk in northeastern PA. A large SNP panel comprising ~1300 SNPs is currently being designed to perform genetic association studies of carcinogen metabolizing genes as described in the original proposal using the same approach described in last year's report for the UGT SNP panel.

Aim 2. Determine the association between red meat consumption, meat cooking methods, dietary carcinogens, and cigarette smoking with the risk for CRC in northeastern PA. We are currently in the process of examining dietary and lifestyle factors and risk for colorectal cancer. Two additional studies were undertaken within the past year:

1. Comparison of our modified DHQ with the Block Dietary questionnaire. Food-frequency questionnaires (FFQs) are commonly used in large epidemiological studies of diet and health to estimate long-term usual intake of total energy, nutrients, and other dietary components. The 1998 version of the Block FFQ (Block) and the NCI Diet History Questionnaire (DHQ) are two widely used self-administered FFQs. We used the Block questionnaire to measure usual dietary intake in a previous sample of adult residents of Northeast Pennsylvania (NE PA) participating in a colorectal cancer case-control study, and we are currently using a modified DHQ in a sample recruited from the same NE PA population in an ongoing population-based colorectal cancer case-control study. The DHQ used in this study was modified to capture the meat eating patterns of NE PA using previously collected 24-hour dietary recall data from a separate sample. Examples of meat items identified from the 24-hour recall data that were added to the DHQ include chipped and dried beef as well as corned beef. We conducted a study to compare dietary intake data collected by the Block questionnaire and the modified DHQ. We recruited 50 healthy adult residents of both genders aged 40 to 80 years to complete the two FFQs in random order on separate occasions 4 weeks apart. The Block questionnaire was provided along with several additional questions concerning preferred meat-cooking practices, whereas the DHQ incorporated a comprehensive meat-cooking practices module with an extensive list of questions designed to capture detailed meat intake information (Meats Module Questionnaire developed by

NCI Investigators). The FFQs were self-administered and reviewed by trained interviewers during in-person interviews. Our preliminary findings suggest that the DHQ provides more detailed information on total meat and relevant meat subtype intake (e.g., processed meat), as well as usual meat-cooking practices. This more detailed information will provide better estimates of meat-related carcinogens of interest. This study was presented at the International Conference on Diet and Activity Methods in Washington D.C. in 2009 (Miller et al).

2. Analysis of different methods to assess dietary factors in colorectal cancer risk. Previous studies have derived patterns by measuring compliance with pre-established dietary guidance or empirical methods such as principal components analysis (PCA). Our objective was to examine colorectal cancer risk associated with patterns identified by both methods. Our study included 431 incident colorectal cancer cases (225 men, 206 women) and 726 healthy controls (330 men, 396 women) from our population-based case-control study. PCA identified sex-specific dietary patterns and the Healthy Eating Index-2005 (HEI-05) assessed adherence to the 2005 Dietary Guidelines for Americans. Three dietary patterns among men (fruits and vegetables, meat and potatoes, alcohol and sweetened beverages) and two among women (fruits and vegetables, meat and potatoes) were identified. After adjusting for potential confounders, the fruits and vegetables pattern was inversely associated with colorectal cancer among both men (OR = 0.38, 95% CI = 0.21-0.69 for the highest compared to the lowest quartile; *P* for trend = 0.006; Table 1) and women (OR = 0.35, 95% CI = 0.19-0.65; *P* for trend = 0.031; Table 2). The meat and potatoes pattern was positively associated with colorectal cancer in women (OR = 2.20, 95% CI = 1.08-4.50; *P* for trend = 0.070). Men and women with greater HEI-05 scores had a significantly reduced risk of colorectal cancer (OR = 0.56, 95% CI = 0.31-0.99; *P* for trend = 0.004; OR = 0.44, 95% CI = 0.24-0.77; *P* for trend <0.001, respectively). Following the Dietary Guidelines or a dietary pattern lower in meat and potatoes and higher in fruits and vegetables may reduce colorectal cancer risk. These studies are in press in the Journal of Nutrition (Miller et al).

<u>Aim 5. Confirm the functional capacity of potentially important high-risk genotypes.</u>

1. Characterization of UGT1A9 polymorphisms. In studies reported in last year's Annual Report, we identified novel polymorphisms in UGT1A9 and characterized their effect on glucuronidation activity. These studies were published in Drug Metabolism and Disposition (Olson et al, 2009).

2. Characterization of the UGT2A1 family of enzymes and their potential importance in colorectal cancer risk. As described above, UDP-glucuronosyltransferases (UGTs) play an important role in the metabolism and excretion of various endogenous and xenobiotic compounds, including carcinogens and chemotherapeutic agents. While various studies have examined the UGT1A and 2B enzyme families in carcinogenesis, virtually no studies have been performed on the UGT2A family of enzymes. The goal of the present study was to examine the expression of UGT2A1 in human tissues, assess its glucuronidation activity against various carcinogens, and assess the potential functional role of UGT2A1 missense SNPS on UGT2A1 enzyme activity [a manuscript describing this study (Bushey et al) has been submitted to Carcinogenesis]. As determined by reverse-transcription polymerase chain reaction, UGT2A1 was expressed in a variety of aerodigestive tract tissues including trachea, larynx and tonsil, and was also expressed in lung & colon; no expression was observed in breast, whole brain, pancreas, prostate, liver or esophagus. Real-time PCR suggested that UGT2A1 exhibited highest expression in all of these tissues in the following order: lung > trachea > tonsil > larynx > colon > olfactory tissue (Figure 1). Cell homo-

genates prepared from wild-type UGT2A1^{75Lys308Gly}-over-expressing HEK293 cells showed significant glucuronidation activity, as observed by reverse-phase UPLC, against a variety of polycyclic aromatic hydrocarbons (PAHs) including, 1-hydroxy-benzo(a)pyrene, benzo(a)pyrene-7,8-diol, and 5-methylchrysene-1,2-diol (Figure 2). No activity was observed in UGT2A1-over-expressing cell homogenate against substrates that form *N*-glucuronides, such as NNAL, nicotine, or *N*-OH-PhIP. A significant ($p<0.05$) ~25% decrease in glucuronidation activity (V_{max}/K_M) was observed against all PAH substrates for the UGT2A1^{75Arg308Gly} variant as compared to homogenates from wild-type UGT2A1^{75Lys308Gly} (Table 3); no activity was observed for cell homogenates over-expressing the UGT2A1^{75Lys308Arg} variant for all substrates tested. These data suggest that UGT2A1 is an important detoxification enzyme in the metabolism of PAHs within target tissues for PAHs, a family of carcinogens strongly implicated in colorectal cancer risk and functional polymorphisms in UGT2A1 may play a role in colorectal cancer risk. This will be determined in our subsequent genotyping analysis of our colorectal cancer case-control study.

3. Studies of UGT genotypes and nicotine metabolism phenotypes. Smoking is a risk factor for colorectal cancer. Glucuronidation is an important pathway in the metabolism of nicotine, with previous studies suggesting that ~22% of urinary nicotine metabolites are in the form of glucuronidated compounds. Recent *in vitro* studies have suggested that the UGTs 2B10 and 2B17 play major roles in nicotine glucuronidation with polymorphisms in both enzymes shown to significantly alter the levels of nicotine-, cotinine-, and *trans*-3'-hydroxy-cotinine (3HC)-glucuronides in human liver microsomes *in vitro*. In the present study, the relationship between the levels of urinary nicotine metabolites and functional polymorphisms in UGTs 2B10 and 2B17 were analyzed in urine specimens from 104 Caucasian smokers. Based on their percentage of total urinary nicotine metabolites, the levels of nicotine-glucuronide and cotinine-glucuronide were 42% ($p<0.0005$) and 48% ($p<0.0001$), respectively, lower in the urine from smokers exhibiting the UGT2B10 (*1/*2) genotype and 95% ($p<0.05$) and 98% ($p<0.05$), respectively, lower in the urine from smokers with the UGT2B10 (*2/*2) genotype as compared to the urinary levels in smokers having the wild-type UGT2B10 (*1/*1) genotype (Figure 3). The levels of 3HC-glucuronide was 42% ($p<0.001$) lower in the urine from smokers exhibiting the homozygous UGT2B17 (*2/*2) deletion genotype as compared to the levels in urine from wild-type UGT2B17 subjects. These data are consistent with previous *in vitro* studies and demonstrate that UGTs 2B10 and 2B17 play important roles in the glucuronidation of nicotine, cotinine and 3HC and suggest that the UGT2B10 codon 67 SNP and the UGT2B17 deletion significantly reduce overall glucuronidation rates of nicotine and its major metabolites in smokers. These studies are in press in Cancer Research (Chen et al).

<u>Publications citing this grant</u>

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susceptibility loci at chromosomes 15q25, 5p15, and 6p21: a pooled analysis from the International Lung Cancer Consortium. J Natl Cancer Inst. 2010, 102:959-971.

Chirumbole M, Gusani N, Howard A, Leonard T, Lewis P, Muscat J. A comparison of stage of presentation for pancreatic and colorectal cancer in Pennsylvania 2000-2005. Anticancer Res. 2009, 29:3427-3431.

Balliet RM, Chen G**,** Dellinger RW, Lazarus P. UGT1A10: Activity against the tobacco-specific nitrosamine, NNAL, and a potential role for a novel UGT1A10 promoter deletion polymorphism in cancer susceptibility. Drug Metab Dispos 38:484-90, 2010.

Miller PE, Lazarus P, Lesko SM, Muscat JE, Harper G, Cross AJ, Sinha R, Ryczak K, Escobar G, Hartman T. Diet Index-Based and Empirically-Derived Dietary Patterns are Associated with Colorectal Cancer Risk. J Nutrition *In press*.

Chen G, Giambroni N, Dluzen D, Gallagher C, Lazarus P. Role of UGT2B10 and UGT2B17 knock-out polymorphisms in urinary excretion of nicotine metabolites Cancer Res *In press*.

Directly Pertinent Meeting Presentations

Bushey R, Chen G, Blevins-Primeau AS, and Lazarus P. Characterization of the activity and expression of UDP-Glucuronosyltransferase 2A1 variants and potential role in tobacco carcinogenesis. Proc Amer Assoc Cancer Res, 2010 (Washington DC).

Miller PE, Lazarus P, Lesko SM, Muscat JE, Harper G, Cross AJ, Sinha R, Ryczak K, Escobar G, Hartman TJ. Examination of two approaches to assess dietary patterns in relation to colorectal cancer risk [abstract]. Proc Amer Assoc Cancer Res, 2010 (Washington DC).

Table 1. Odds ratios (95% CI) of colorectal cancer risk according to quartiles of dietary pattern and diet index scores in men.

Dietary pattern scores	Cases/controls	Age-adjusted OR (95% CI)	Multivariate-adjusted[1] OR (95% CI)
Fruits and vegetables pattern			
Quartile 1	65/82	1.00 (ref)	1.00 (ref)
Quartile 2	70/83	0.96 (0.60-1.54)	0.89 (0.53-1.49)
Quartile 3	50/82	0.76 (0.46-1.24)	0.64 (0.37-1.10)
Quartile 4	40/83	0.54 (0.32-0.90)	0.38 (0.21-0.69)
P for trend		0.046	0.006
Meat and potatoes pattern			
Quartile 1	48/83	1.00 (ref)	1.00 (ref)
Quartile 2	64/82	1.37 (0.83-2.25)	1.39 (0.83-2.32)
Quartile 3	47/82	1.08 (0.65-1.83)	1.03 (0.58-1.81)
Quartile 4	66/83	1.92 (1.15-3.20)	1.56 (0.84-2.90)
P for trend		0.005	0.070
Alcohol and sweetened beverages pattern			
Quartile 1	51/83	1.00 (ref)	1.00 (ref)
Quartile 2	53/82	1.07 (0.65-1.77)	1.13 (0.67-1.91)
Quartile 3	69/83	1.39 (0.86-2.52)	1.37 (0.83-2.27)
Quartile 4	52/82	1.14 (0.69-1.89)	1.02 (0.58-1.80)
P for trend		0.161	0.441
HEI-05			
Quartile 1	60/83	1.00 (ref)	1.00 (ref)
Quartile 2	80/82	1.23 (0.77-1.97)	1.24 (0.76-2.03)
Quartile 3	49/82	0.72 (0.44-1.19)	0.74 (0.43-1.26)
Quartile 4	36/83	0.52 (0.31-0.88)	0.56 (0.31-0.99)
P for trend		0.002	0.004

[1] Adjusted for age, BMI, education, energy intake, family history of colorectal cancer, NSAID use, physical activity, and smoking.

Table 2. Odds ratios (95% CI) of colorectal cancer risk according to quartiles of dietary pattern and diet index scores in women.

Dietary pattern scores	Cases/controls	Age-adjusted OR (95% CI)	Multivariate-adjusted[1] OR (95% CI)
Fruits and vegetables pattern			
Quartile 1	76/99	1.00 (ref)	1.00 (ref)
Quartile 2	45/99	0.52 (0.31-0.85)	0.46 (0.27-0.78)
Quartile 3	47/99	0.53 (0.33-0.87)	0.45 (0.26-0.76)
Quartile 4	38/99	0.47 (0.28-0.79)	0.35 (0.19-0.65)
P for trend		0.108	0.031
Meat and potatoes pattern			
Quartile 1	30/99	1.00 (ref)	1.00 (ref)
Quartile 2	64/99	2.51 (1.45-4.33)	2.34 (1.32-4.17)
Quartile 3	51/99	2.34 (1.33-4.14)	2.02 (1.09-3.75)
Quartile 4	61/99	2.85 (1.63-4.97)	2.20 (1.08-4.50)
P for trend		0.007	0.170
HEI-05			
Quartile 1	69/99	1.00 (ref)	1.00 (ref)
Quartile 2	55/99	0.78 (0.48-1.26)	0.80 (0.48-1.33)
Quartile 3	50/99	0.61 (0.37-0.99)	0.59 (0.35-0.99)
Quartile 4	32/99	0.37 (0.21-0.62)	0.44 (0.24-0.77)
P for trend		<0.001	<0.001

[1] Adjusted for age, BMI, education, energy intake, family history of colorectal cancer, postmenopausal hormone use, NSAID use, physical activity, and smoking.

Table 3. Enzyme kinetics summary of UGT2A1 activity against PAH substrates.

	UGT2A1^{75Lys308Gly}		
	K_m		K_m
Substrate	(μM)	Substrate	(μM)
1-hydroxypyrene	90.8 ± 7.2	300 ± 17	3.3 ± 0.2
1-naphthol	29.9 ± 3.6	182 ± 11	6.1 ± 0.4
5-methylchrysene-1,2-diol	269.2 ± 23.9	100 ± 7.2	0.37 ± 0.04
dibenzo(a,l)pyrene-11,12-diol	283.7 ± 26.6	26 ± 1.0	0.09 ± 0.004
benzo(a)pyrene-7,8-diol	397.2 ± 32.7	109 ± 14	0.28 ± 0.04
1-OH benzo(a)pyrene	246.9 ± 11.7	70 ± 4.4	0.28 ± 0.01
3-OH benzo(a)pyrene	271.5 ± 14.9	84 ± 3.6	0.30 ± 0.008
7-OH benzo(a)pyrene	260.9 ± 35.0	58 ± 4.6	0.22 ± 0.01
8-OH benzo(a)pyrene	308.0 ± 20.4	76 ± 6.6	0.24 ± 0.008

	UGT2A1^{75Arg308Gly}		
	K_m	V_{max}	V_{max}/K_m
Substrate	(μM)	(pmol/min/mg)[a]	(μL/min/mg)[a]
1-hydroxypyrene	111 ± 19	269 ± 18	2.5 ± 0.2[b]
1-naphthol	44 ± 11[b]	177 ± 20	4.1 ± 0.7[b]
5-methylchrysene-1,2-diol	381 ± 37[b]	87 ± 7.4	0.23 ± 0.02[b]
dibenzo(a,l)pyrene-11,12-diol	307 ± 8.9	22 ± 0.4[b]	0.07 ± 0.001[b]
benzo(a)pyrene-7,8-diol	426 ± 53	86 ± 8.1[b]	0.20 ± 0.009[b]
1-OH benzo(a)pyrene	263 ± 29	61 ± 1.4[b]	0.23 ± 0.02[b]
3-OH benzo(a)pyrene	278 ± 21	74 ± 1.6[b]	0.27 ± 0.014[b]
7-OH benzo(a)pyrene	334 ± 20	49 ± 2.4[b]	0.15 ± 0.004[b]
8-OH benzo(a)pyrene	368 ± 51	60 ± 5.1[b]	0.16 ± 0.02[b]

[a] Data expressed as mg of total protein homogenate, corrected for relative UGT2A1 protein. K_m, V_{max}, and V_{max}/K_m represent the mean of three independent experiments.
[b] Denotes $p<0.05$ versus corresponding value for UGT2A1^{75Lys308Gly}.

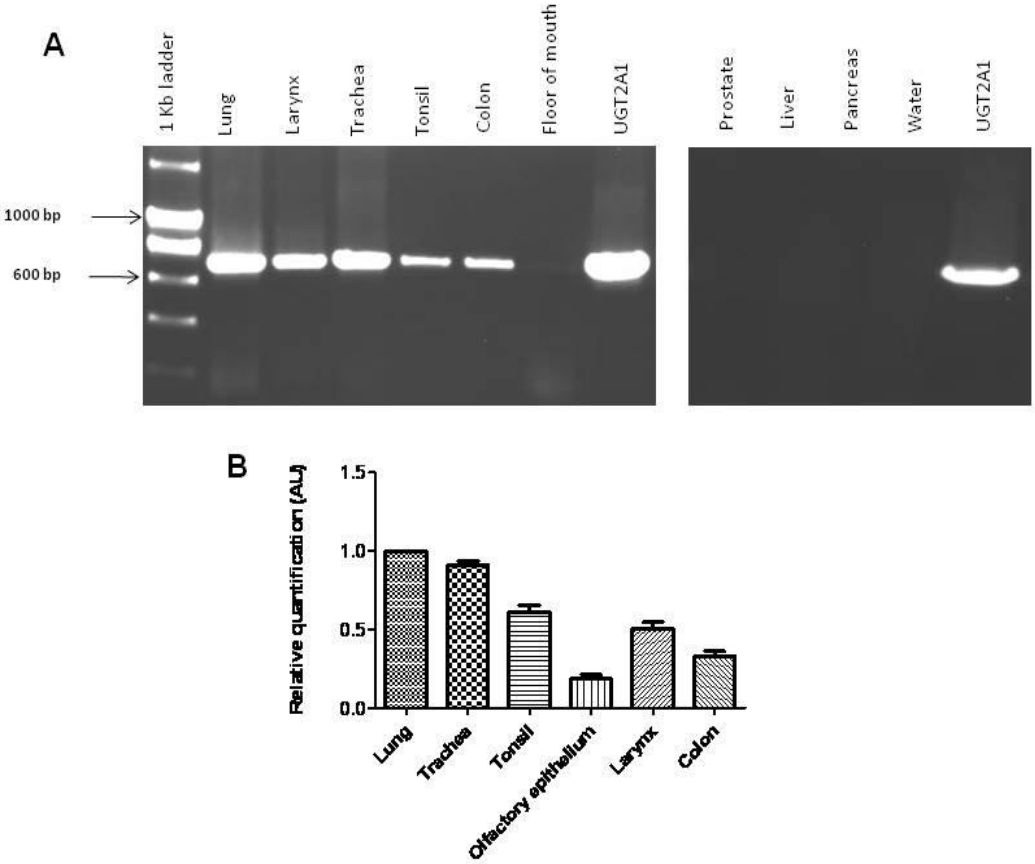

Fig. 1. Characterization of UGT2A1 tissue expression. **(A)** Initial characterization of UGT2A1 expression in multiple human tissues using RT-PCR. RNA pooled from at least three individuals was used to assess overall UGT2A1 expression. RNA from HEK293 cell lines over-expressing wild-type UGT2A1 was used as a positive control; water in place of cDNA was used as a negative control. **(B)** Quantitative analysis of UGT2A1 expression in human tissues. RNA from tissues exhibiting UGT2A1 expression in Fig. 1A was used in conjunction with a UGT2A1-specific real-time PCR assay (ABI) to quantitatively assess UGT2A1 expression. Relative UGT2A1 mRNA tissue expression was determined by comparing mRNA levels in each tissue to that observed in the tissue with the highest UGT2A1 expression (i.e., lung). Results, expressed as the mean ± SD of triplicates, were normalized to RPLPO mRNA expression in each tissue.

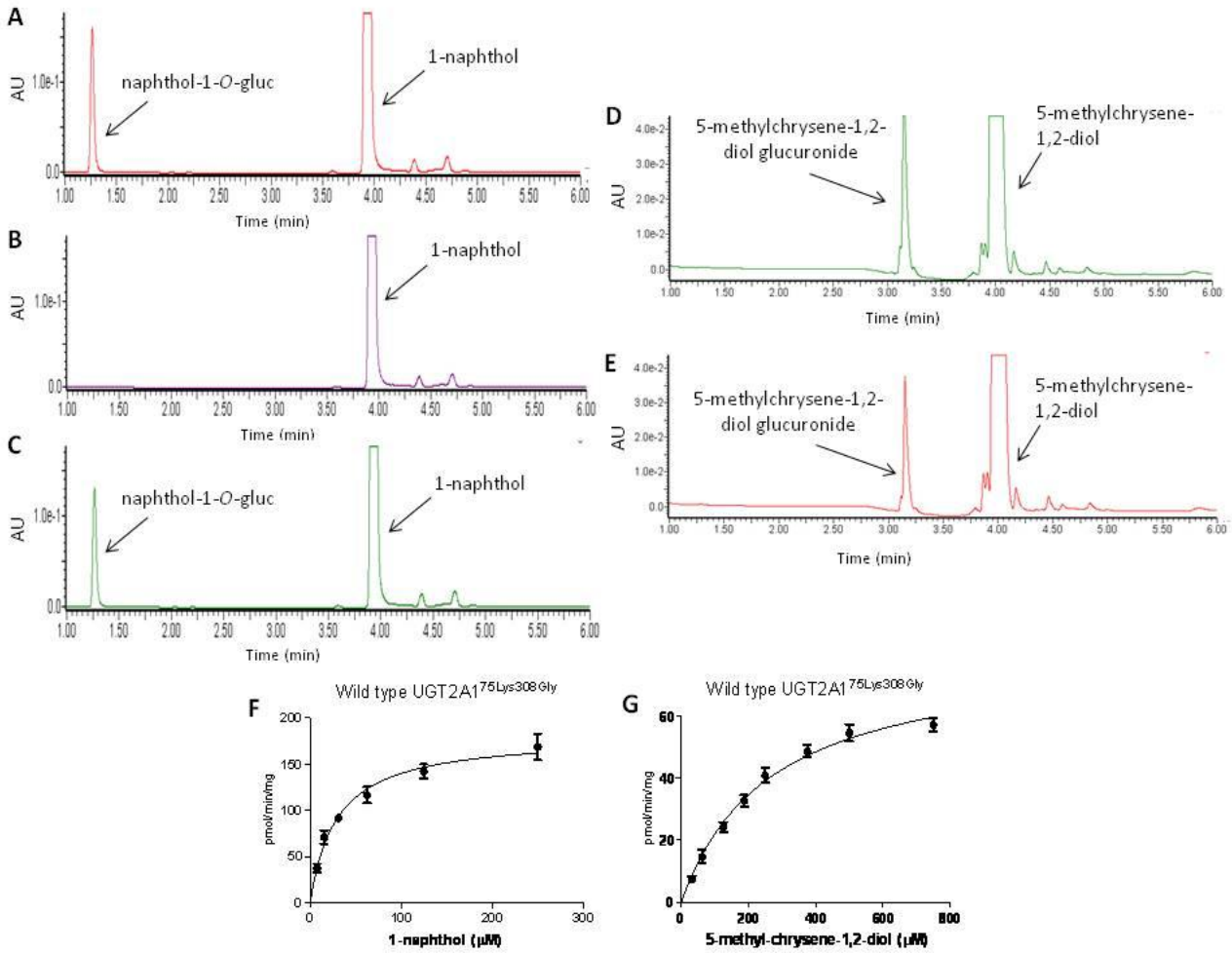

Fig. 2. UPLC analyses of UGT2A1 over-expressing cell line glucuronidation activities. **(A)** UPLC trace of 1-naphthol-*0*-glucuronide formation following incubation with wild-type UGT2A1 cell homogenate. **(B)** UPLC trace of reaction from top panel following treatment with 1000 units of *E. Coli* β-glucuronidase. **(C)** UPLC trace of 1-naphthol-*O*-glucuronide formation using homogenate from cell line over-expressing the UGT2A1^{75Arg308Gly} variant. **(D)** UPLC trace of 5-methylchrysene-1,2-diol glucuronide formation following incubation with wild-type UGT2A1 cell homogenate. **(E)** UPLC trace of 5-methylchrysene-1,2-diol glucuronide formation using homogenate from cell line over-expressing UGT2A1^{75Arg308Gly}. **(F)** Michaelis-Menten kinetic curve for 1-napthol glucuronidation by wild-type UGT2A1. **(G)** Michaelis-Menten kinetic curve for 5-methyl-chrysene-1,2-diol glucuronidation by wild-type UGT2A1.

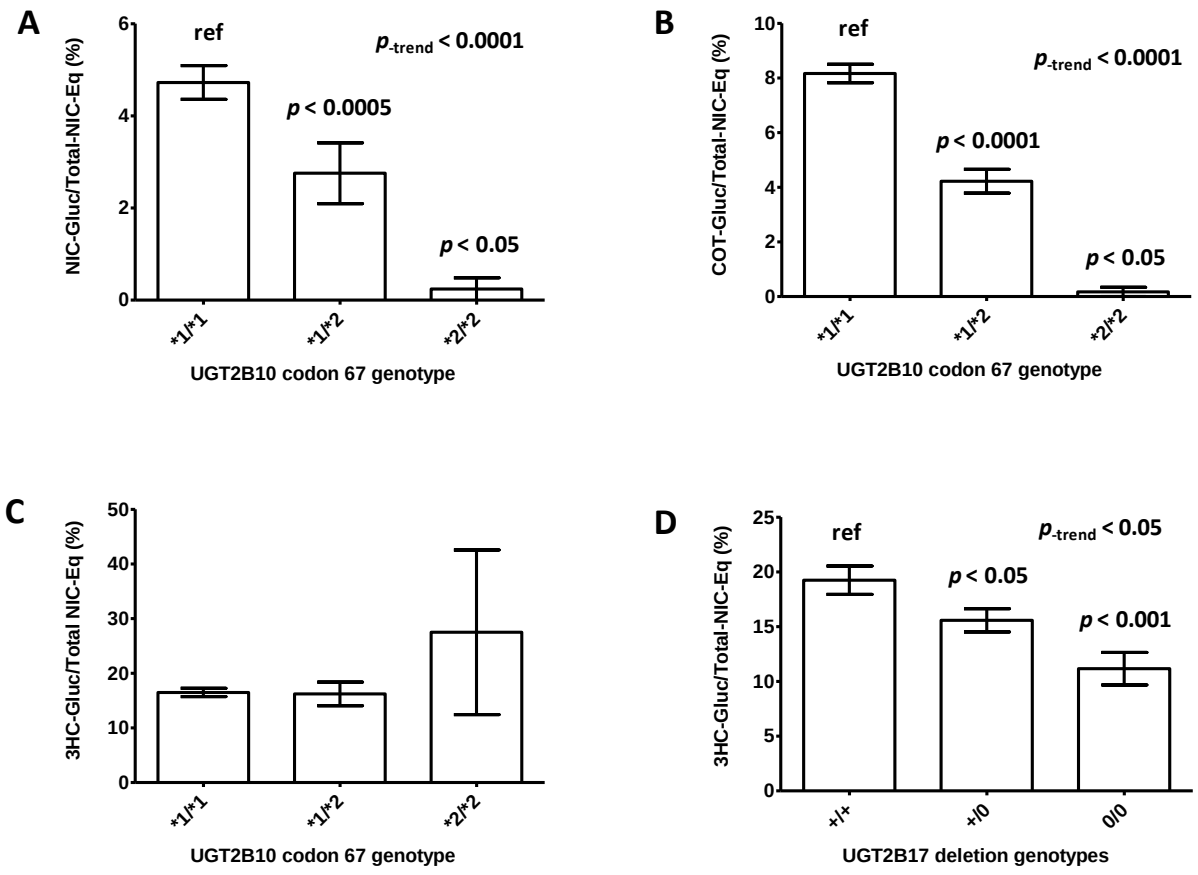

Figure 3. Effect of UGT2B10 and UGT2B17 polymorphisms on nicotine-Gluc, cotinine-Gluc, and 3HC-Gluc levels in urine samples from smokers. Shown are the percentage of Total-NIC-Eq for urinary nicotine and nicotine metabolite glucuronides for subjects stratified by UGT2B10 codon 67 or UGT2B17 deletion genotypes. Panel A, UGT2B10 genotype vs. urinary nicotine-Gluc; panel B, UGT2B10 genotype vs. urinary cotinine-Gluc; panel C, UGT2B10 genotype vs. urinary 3HC-Gluc; panel D, UGT2B17 genotype versus urinary 3HC-Gluc. The Mann-Whitney *U* test was used to compare urine from subjects with the UGT2B10 (*2/*2) or (*1/*2) genotypes to specimens from subjects with the wild-type UGT2B10 (*1/*1) genotype, and the Jonckheere-Terpstra trend test was used to examine the overall effect of UGT genotypes on nicotine-, cotinine-, and 3HC-glucuronide levels determined as a percentage of Total-NIC-Eq. The Students t-test was used to compare urine from subjects with the UGT2B17 (0/0) or (0/+) genotypes to specimens from subjects with the wild-type UGT2B17 (+/+) genotype, and the trend test was performed to examine the overall effect of UGT genotypes on nicotine-, cotinine-, and 3HC-glucuronide levels determined as a percentage of Total-NIC-Eq. Data are shown as the mean ± standard error.