Pennsylvania State University

Annual Progress Report: 2006 Nonformula Grant

Reporting Period

June 1, 2010 – May 31, 2011

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.

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 18-county high risk area of central/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)
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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

The most important milestone that was attained over the last year was to surpass our expectations in terms of both case and control recruitment for this study. As shown in *Table 1*, we recruited a total of 1,195 incident colorectal cases (705 males, 590 females) and 1,205 controls (616 cases, 589 females) into this study, which surpasses the proposed and expected recruitment of 1,000 cases and 1,000 controls. All cases were recruited within 24 months of their diagnosis for colorectal cancer. Controls were randomly selected using random-digit dialing and were well-matched in a ~1:1 ratio to cases by gender and age (within 5 years for both genders). For the first 22 months of the study, recruitment was performed solely in a 9-county region of northeastern PA whose cancer case registry was served primarily by the Northeast Regional Cancer Institute (NRCI). As case recruitment was significantly slower than expected, we modified our recruitment strategy in the spring of 2009 to utilize the PA State Cancer Registry and to recruit over a wider region, encompassing an 18-county region in central and northeastern PA. To accomplish this, a second recruitment site in addition to the one at NRCI was established at the Penn State College of Medicine in Hershey, PA, enabling interviewers at both sites to recruit subjects living as far away as a 100 mile diameter of each site and allowing coverage of the entire region.

In addition to completing out a demographics/lifestyle questionnaire in an interviewer-guided interview, all subjects completed a detailed central/northeastern PA-specific dietary questionnaire which took into account potential dietary habits native to this region. Extensive personal, lifestyle, demographic, and dietary information was therefore collected on all subjects recruited into these studies.

We also obtained a series of tissue specimens from recruited subjects:

1. Oral buccal cell swabs – This was obtained from all subjects recruited into the study to allow for genomic DNA collection and genetic studies. For many of the subjects (n=374; 202 cases, 172 controls), buccal cells were collected using the Oragene DNA Collection kit as a second potential source of genomic DNA.

2. Urine – A one-time urine was collected from 870subjects (147cases, 723controls) at the time of interview. The purpose was to potentially assess dietary or tobacco metabolites in cases vs controls as biomarkers of exposure and correlative analysis with pertinent genotypes.

3. Blood – A 10 cc draw of blood was taken from all consenting subjects (n=106; 58 cases, 48 controls) recruited at the Hershey recruitment site (NRCI was not equipped for blood draws and blood fractionation). Blood was fractionated within 1 h of collection, separated into plasma (for future metabolite and biomarker measurements for phenotyping assays), buffy coat (ie, lymphocytes; for cell culture work, a source of RNA for expression analysis, or another source of genomic DNA) and red blood cells (for potential hemoglobin adduct measurements). All fractions were aliquoted and stored at -80C (plasma and RBCs) or liquid nitrogen (lymphocytes).

4. Saliva – Saliva was collected separate from oral buccal cell swabs from 852 subjects (508 cases, 344 controls) for the purposes of metabolite assays (including nicotine metabolites) for phenotyping experiments.

Therefore, we have established a rich bank of data and specimens for this large case-control study on colorectal cancer targeting the population of central and northeastern PA.

In the past year, we have performed and are currently working on several studies relevant to Aims 1-5 of the proposal.

<u>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.</u> A 96-SNP chip was designed and purchased from Illumina to perform genetic association studies of the UGT family of carcinogen metabolizing genes. Genotyping was performed for a total of genomic DNA specimens from ~1800 subjects recruited into this study. Statistical analysis examining the relationship between UGT SNPs and colorectal cancer risk is currently being performed in collaboration with Drs Liao and Berg of the Penn State Cancer Institute Bioinformatics Core.

In addition to this SNP Chip, a 2nd 96-SNP Chip has been designed and ordered from Illumina to analyze haplotypes and missense SNPs in genes related to folate and vitamin D biosynthesis, metabolism and mechanism of action. Given our exciting new data regarding the UGT2A1 gene and its potential importance in metabolizing PAHs, we also added SNPs present in the UGT2A1 gene in this chip. The designing of this chip was in response to data described below demonstrating significant associations between increased consumption of vitamin D and folate with decreased risk for colorectal cancer. The actual genotyping of this chip and the analysis of potential associations with colorectal cancer will take place over the course of the next few months (after the DoH grant has ended) and will serve as preliminary results for a NIH R01 application in February 2012.

<u>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.</u> A preliminary study examining the relationship between red meat consumption, meat cooking methods, dietary carcinogens, and cigarette smoking and risk for colorectal cancer was undertaken for 431 incident colorectal cancer cases (225 men, 206 women) and 726 healthy controls (330 men, 396

women) from our population-based case-control study in October 2009 and were published in *J Nutr (Miller et al, 2010; 140:1267-73)* and in a thesis by Paige Miller, PhD (working at Penn State University with Dr. Terry Hartman). Positive associations for increases in colorectal cancer risk were observed in both males and females for red meat intake, exposure to polycyclic aromatic hydrocarbons (PAHs), and intake of nitrosamines from processed meats. In contrast, the consumption of fruits and vegetables was inversely associated with colorectal cancer in both sexes. In addition, both men and women with greater Healthy Eating Index-2005 (HEI-05) scores (which assesses adherence to the 2005 Dietary Guidelines for Americans) had a significantly reduced risk of colorectal cancer.

In a full assessment of environmental and demographic risk factors for the entire population of 2400 recruited subjects (~1200 cases and ~1200 controls), age, BMI, physical activity, education (probably linked with BMI &/or physical activity), and family history of colorectal cancer were found to be risk factors for colorectal cancer (see *Table 2*). Similar to that observed in previous studies, NSAID use was protective against colorectal cancer in this population.

In terms of diet, the following were found to be preventive for colorectal cancer (*Table 3*):

1. Decreased alcohol consumption
2. Low-fat dairy product consumption
3. High fruits and vegetables consumption
4. High fiber
5. High vitamin D intake
6. High calcium consumption
7. Decreased consumption of BaP

In terms of meat intake, the following associations were observed:

1. Nitrate & nitrate consumption: They were signficantly correlated with risk for both colon and rectal cancers, but this appears to be driven by the effect seen with consumption of nitrites. This association may be confounded by an interaction with fruit/vegetable consumption, BMI and/or NSAID use. For nitrites, no overall effect was observed for colorectal cancer risk, but a possible effect was observed for early stage colon cancer.

2. MeIQx and DiMeIQx consumption are risk factors for early-stage colon and rectal (strongest) cancer, but were not significant for late-stage colon or rectal cancers.

3. PhIP consumption appears to be a risk factor for rectal but not colon cancer.

4. While no significant effect was observed for BaP consumption on colorectal cancer risk by odds ratio analysis, this is not consistent with the overall significant increase risk associated with BaP intake in cases vs controls observed in *Table 3*. Further analysis is being conducted to better evaluate the potential role of BaP in risk for colorectal cancer.

5. Mutagenic activity is not a good biomarker for colorectal cancer risk.

6. Current smoking was not a risk factor for colorectal cancer. More detailed analysis of

the interaction between pack-years of smoking and risk for colorectal cancer are currently on-going.

Aim 5. Confirm the functional capacity of potentially important high-risk genotypes. An important goal has been to focus primarily on the UGT family of enzymes which have been extensively studied by Dr. Lazarus and which are thought to play a major role in PAH and HA detoxification. We will determine the effect of high-risk UGT genotypes on, (i) UGT enzyme activity in UGT-over-expressing cell lines, (ii) UGT promoter activity, (iii) the UGT activities in a panel of colorectal or liver microsomal specimens, (iv) altered enzyme RNA levels in the same liver or colorectal tissues, and (v) correlate the urinary ratios of HA metabolites/HA and PAH metabolites/PAH with UGT and other potentially other genotypes.

1. *Studies characterizing UGT1A variants in human tissue*. The UGT1A gene cluster encodes nine UGT1A family members via splicing of individual first exons to common exons 2 through 5. Each of these 9 UGT1As can also undergo alternative splicing at their 3' ends using an alternate exon 5, resulting in 27 different UGT1A mRNA species with each UGT1A gene encoding three different combinations of 5A and 5B UGT1A exons. To examine the importance of UGT1A exon 5 splice variants on overall UGT1A activity, a nested, quantitative PCR assay was developed to accurately assess the combined expression of exon 5 splice variants (termed 'v2/v3') versus the expression of wild-type (termed 'v1') for each specific UGT1A. v1 mRNA expression was 16-, 17-, 57- and 29-fold higher than observed for the combined levels of v2/v3 mRNA for the hepatic UGTs 1A1, 1A4, 1A6, and 1A9, respectively, in normal human liver specimens. In a series of 58 normal human liver specimens, the expression of both UGT1A1 v1 and v2/v3 mRNAs were significantly correlated with bilirubin glucuronidation activity in corresponding microsomes prepared from the same specimens ($p<0.0001$, $r^2=0.4571$; $p=0.0031$, $r^2=0.1455$, respectively), with their expression found to be significantly lower with increasing copies of the UGT1A1*28 allele ($p<0.001$). UGT1A1 variants from livers from subjects homozygous for the UGT1A1*28 allele were significantly lower (42% for v1; 53% for v2/v3) than livers from subjects homozygous for the wild-type UGT1A1*1 allele. The low relative abundance of UGT1A v2/v3 mRNA and its positive correlation with hepatic glucuronidation activity suggests that proteins encoded by these mRNAs are not exerting a negative regulatory role on UGT1A activity in human liver. As multiple UGT1A enzymes are well-expressed in colon, these studies are directly relevant to this proposal. Further studies examining UGT1A splice variant expression in colon are currently underway. The above studies have been submitted for publication.

2. *Characterization of other UGT2A enzymes and their potential importance in colorectal cancer risk.* In addition to UGT2A1, UGT2A2 and 2A3 comprise the UGT2A sub-family of enzymes. Like UGT2A1, few studies had examined their expression patterns in human tissues, examined their activity against carcinogens, or assessed the effect of potentially functional SNPs on enzyme activity or cancer risk. We have performed a series of preliminary experiments to examine UGT 2A2 and 2A3 expression and activity. As shown in *Figure 1*, both enzymes are expressed in multiple tissues including UGT2A3 in colon. Similar to that observed for UGT2A1, there appears to be a splice variant within UGT2A2 resulting in a second PCR-amplified band, a pattern consistent with the fact that UGTs 2A2

and 2A1 are located within the same gene loci and share exon 1 sequences. This is currently being examined in greater detail. In activity studies of UGT2A2, glucuronidation activity was observed against PAHs like naphthol and 1-hydroxy-pyrene, and like UGT2A1, UGT2A2 exhibited high levels of glucuronidation activity against multiple PAHs (*Table 4*). We are in the process of examining UGT2A3 activities against multiple carcinogens. Together, these data suggest that several members of the UGT2A family of enzymes could potentially play a role in colorectal cancer susceptibility.

3. *Studies examining the glucuronidation pathway and the metabolism of the highly carcinogenic PAH, (DB[a,l]P).* DB[*a,l*]P has been identified in tobacco smoke and is found in our environment due to incomplete combustion of organic matter. Its metabolites are known to form stable DNA adducts in bacteria and mammalian cells, and can lead to tumors in animal models. Glucuronidation of major metabolites of DB[*a,l*]P by the uridine-5'-diphosphate glucuronosyltransferase (UGT) family of enzymes is an important route of detoxification of this pro-carcinogen. We examined the glucuronidation of the pro-carcinogenic enantiomers DB[*a,l*]P-(+)-*trans*-11S,12S-diol and DB[*a,l*]P-(-)-*trans*-11R,12R-diol. Glucuronidation assays with HEK293 cell lines over-expressing individual human UGT enzymes demonstrated that UGTs 1A1, 1A4, 1A7, 1A8, 1A9, 1A10, and 2B7 exhibited glucuronidation activity against both DB[*a,l*]P-diol enantiomers. Three glucuronide conjugates were observed in activity assays with UGTs 1A1 and 1A10, while two glucuronides were formed with UGTs 1A7, 1A8, 1A9, and 2B7, and one glucuronide was made by UGT1A4. Enzyme kinetic analysis indicated that UGT1A9 exhibited the highest k_{cat}/K_m for both the (+)-DB[*a,l*]P-11-Gluc and (-)-DB[*a,l*]P-11-Gluc while UGTs 1A1 and 1A10 exhibited the highest k_{cat}/K_m values for the (+)-DB[*a,l*]P-12-Gluc (*Table 5*). Incubations with human liver microsomes showed formation of three diastereomeric glucuronide products: (+)-DB[*a,l*]P-11-Gluc, (+)-DB[*a,l*]P-12-Gluc, and (-)-DB[*a,l*]P-11-Gluc (Figure 1). The (-)-DB[*a,l*]P-11-Gluc product was formed in largest quantity, which supports the evidence that its precursor DB[*a,l*]P-(-)-*trans*-11R,12R-diol is formed in excess in human tissues. Human bronchus and trachea tissue homogenates demonstrated glucuronidation activity against both DB[*a,l*]P-*trans*-11,12-diol enantiomers, with both tissues producing the (+)-DB[*a,l*]P-11-Gluc and (+)-DB[*a,l*]P-12-Gluc while only bronchus made the (-)-DB[*a,l*]P-11-Gluc. Overall activity in these tissues was lower than HLM. These results indicate that multiple UGTs are involved in the stereospecific glucuronidation of DB[*a,l*]P-*trans*-11,12-diol in a pattern consistent with their expression in respiratory tract tissues, and that glucuronidation may be an important first-line detoxification mechanism of DB[*a,l*]P. These studies are highly relevant for colorectal cancer since DB[*a,l*]P is among the potent PAH carcinogens, and PAHs are a major source of carcinogen exposure in grilled foods and other dietary components. These studies are in press in *Chem Res Toxicol (Olson et al, in press).*

<u>Publications (2010-2011) citing this grant</u>

1. Miller PE, Lesko SM, Muscat JE, <u>Lazarus P</u>, Hartman TJ. Dietary Patterns and Colorectal Adenoma and Cancer Risk: A Review of the Epidemiological Evidence. Nutr Cancer 2010; 62:413-24.

2. Chen G, Giambroni N, Dluzen D, Muscat JE, Berg A, Gallagher C, <u>Lazarus P</u>.

Glucuronidation genotypes and nicotine metabolic phenotypes: importance of functional UGT2B10 and UGT2B17 polymorphisms. Cancer Res. 2010; 70:7543-52.

3. Gallagher CJ, Balliet RM, Sun D, Chen G, Lazarus P. Sex differences in UDP-glucuronosyltransferase 2B17 expression and activity. Drug Metab Dispos. 2010; 38:2204-9.

4. Bushey RT, Chen G, Blevins-Primeau AS, Krzeminski J, Amin S, Lazarus P. Characterization of UDP-glucuronosyltransferase 2A1 (UGT2A1) variants and their potential role in tobacco carcinogenesis. Pharmacogen Genomics 2011; 21:55-65.

5. Olson KC, Sun D, Chen G, Sharma AK, Amin S, Ropson IJ, Spratt TE, Lazarus P. Characterization of dibenzo[*a,l*]pyrene-*trans*-11,12-diol glucuronidation by UDP-glucuronosyltransferases. Chem Res Toxicol (*in press*).

Table 1. Demographic data of cases versus controls in the Pennsylvania Colorectal Cancer Gene-Environment Interaction Study.

	Cases	Controls	Totals
Males			
White	578	601	1179
White Hispanic	11	4	15
Black or African American	15	10	25
Black Hispanic	0	0	0
Asian	1	1	2
American Indian	1	0	1
Native Hawaiian	0	0	0
2 or More Races	0	1	1
Other	1	0	1
Total	**607**	**617**	**1224**
Females			
White	564	560	1124
White Hispanic	6	13	19
Black or African American	14	8	22
Black Hispanic	1	1	2
Asian	1	1	2
American Indian	0	1	1
Native Hawaiian	1	0	1
2 or More Races	1	0	1
Other	2	1	3
Total	**590**	**585**	**1175**
Both Males and Females			
White	1142	1161	2303
White Hispanic	17	17	34
Black or African American	29	18	47
Black Hispanic	1	1	2
Asian	2	2	4
American Indian	1	1	2
Native Hawaiian	1	0	1
2 or More Races	1	1	2
Other	3	1	4
Total	**1197**	**1202**	**2399**

Table 2. Descriptive characteristics of cases and controls (n = 2,022)

Characteristic	Cases[a] (*n* = 989)		Controls[a] (*n* = 1,033)		*P*-Value[b]
	No.	%	No.	%	
Age, years					
<50	93	9.4	172	16.7	<0.001
50-59	183	18.5	245	23.7	
60-69	292	29.5	367	35.5	
≥70	421	42.6	249	24.1	
Sex					
Men	493	49.9	516	50.0	0.963
Women	496	50.2	517	50.1	
Race					
White	956	96.7	1010	97.8	0.128
Non-white	33	3.3	23	2.2	
Education					
< High school	110	11.1	47	4.6	<0.001
High school/some college	644	65.3	633	61.3	
College graduate/advanced degree	233	23.6	352	34.1	
Body mass index[c]					
<25	91	21.9	213	29.3	<0.001
25-29.9	139	33.5	264	36.4	
≥30	185	44.6	249	34.3	
Smoking status					
Never	459	46.4	484	46.9	0.030
Former	426	43.1	404	39.1	
Current	104	10.5	145	14.0	
Past regular NSAID use[d]					
Yes	466	47.1	571	55.3	<0.001
No	523	52.9	462	44.7	
Vigorous physical activity					
<1 hour/week	725	73.3	641	62.1	<0.001
≥1 hour/week	264	26.7	392	38.0	
Family history of colorectal cancer					
Yes	173	17.5	125	12.1	<0.001
No	816	82.5	908	87.9	
Stage					
Early stage	400	40.4	--	--	--
Late stage	481	48.6	--	--	
Unknown	108	10.9	--	--	
Multivitamin use					
Yes	409	41.4	445	43.1	0.433
No	580	58.7	588	56.9	
Supplemental calcium use					
Yes	204	20.6	246	23.8	0.085
No	785	79.4	787	76.2	
Supplemental vitamin D use					
Yes	99	10.0	108	10.5	0.742
No	890	90.0	925	89.6	

Abbreviations: NSAID, nonsteroidal anti-inflammatory drug

[a] Numbers were smaller for some risk factors due to missing data.

[b] *P*-values for differences in means were calculated with *t* tests and differences in proportions were calculated with χ^2 tests.
[c] Weight (kg)/height (m)^2.
[d] NSAID use is defined as ever having been a regular user ($\geq$ 3 time/week for at least 1 year prior to the interview for controls and diagnosis for cases).

Table 3. Dietary intakes of cases and controls.

Dietary variable	Cases (*n* = 989) Mean (SD)	Controls (*n* = 1,033) Mean (SD)	*P*-Value
Total energy intake, kcal/day	1,870 (871)	1,781 (762)	0.116
Alcohol, g/day	9.3 (30.5)	9.7 (25.2)	<0.001
Red meat, g/1,000 kcal	36.5 (24.2)	35.8 (25.0)	0.089
Processed meat, g/1,000 kcal	15.4 (14.3)	14.7 (14.6)	0.104
Fruit and vegetables, servings/1,000 kcal	3.1 (1.8)	3.4 (1.9)	<0.001
Low-fat dairy products, servings/1,000 kcal	0.28 (0.41)	0.33 (0.43)	0.001
Whole grains, servings/1,000 kcal	0.62 (0.58)	0.67 (0.63)	0.086
Saturated fat, g/1,000 kcal	12.8 (3.4)	12.7 (3.5)	0.241
Fiber, g/1,000 kcal	9.2 (3.5)	9.9 (3.5)	<0.001
Total folate, DFE[a]	808 (377)	832 (381)	0.109
Total vitamin D[a]	10.5 (7.4)	11.4 (8.3)	0.023
Total calcium, mg[a]	934 (430)	1012 (489)	<0.001
Meat-derived nitrites, µg/1,000 kcal	123 (117)	118 (126)	0.095
Meat-derived nitrates, µg/1,000 kcal	228 (220)	209 (187)	0.028
Meat-derived nitrites and nitrates, µg/1,000 kcal	351 (307)	327 (289)	0.021
DiMeIQx, ng/1,000 kcal	1.6 (1.9)	1.4 (1.8)	0.043
MeIQx, ng/1,000 kcal	18.3 (20.8)	16.5 (19.3)	0.089
PhIP, ng/1,000 kcal	44.3 (67.2)	45.9 (64.5)	0.094
Benzo[a]pyrene, ng/1,000 kcal	9.7 (17.1)	11.2 (17.5)	0.001
Total mutagenic activity, revertant colonies/1,000 kcal	2,713 (3,887)	2,688 (3,291)	0.912

Abbreviations: DFE, dietary folate equivalents; DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; SD, standard deviation.
[a] Dietary intakes were energy-adjusted by the residual method and then combined with supplemental intake

Table 4. Enzyme kinetics summary of UGT2A2 activity against PAH substrates.

Substrate	UGT2A2 K_m (μM)	V_{max} (pmol/min/mg)[a]	V_{max}/K_m (μL/min/mg)[a]
1-hydroxypyrene	100 ± 15	38 ± 2.1	0.38 ± 0.07
1-naphthol	106 ± 15	26 ± 1.5	0.25 ± 0.04
1-OH benzo(a)pyrene	125 ± 24	3.3 ± 0.2	0.03 ± 0.006
3-OH benzo(a)pyrene	332 ± 54	7.4 ± 0.5	0.02 ± 0.008

[a] Data expressed as mg of total protein homogenate. K_m, V_{max}, and V_{max}/K_m represent the mean of three independent experiments.

Table 5. Kinetic analysis of glucuronide formation against DB[a,l]P by individual UGTs.

	k_{cat}/K_m ($mM^{-1} \cdot min^{-1}$)[a]		
	DB[a,l]P-(+)-trans-11-Gluc[b]	DB[a,l]P-(+)-trans-12-Gluc[b]	DB[a,l]P-(-)-trans-11-Gluc[c]
UGT1A1	6.1 ± 1.5	3.1 ± 0.6	0.72 ± 0.07
UGT1A7	0.23 ± 0.11	<0.001	0.18 ± 0.03
UGT1A8	0.80 ± 0.12	<0.001	0.46 ± 0.03
UGT1A9	2980 ± 146	<0.001	3290 ± 59
UGT1A10	9.5 ± 2.0	2.6 ± 0.5	13 ± 1.3
UGT2B7	0.38 ± 0.01	<0.001	<0.001

[b] Glucuronides produced from DB[*a*,*l*]P-(+)-trans-11,12-diol enantiomer incubated with individual UGTs.
[c] Glucuronide produced from DB[*a*,*l*]P-(-)-trans-11,12-diol enantiomer incubated with individual UGTs.
[a] k_{cat} was adjusted for UGT expression according to Western Blot analysis.

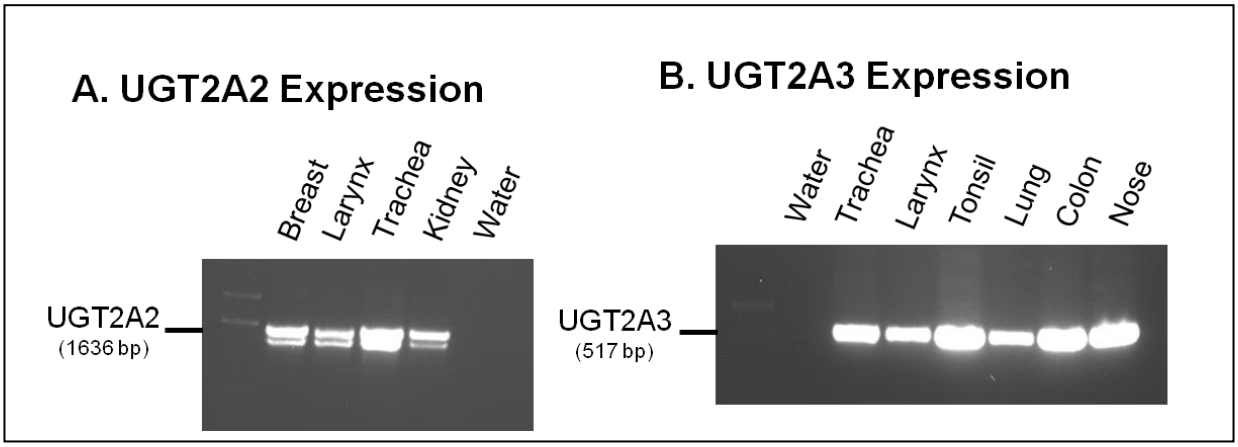

Figure 1. Characterization of UGT2A2 and UGT2A3 expression. **(A)** Initial characterization of UGT2A1 expression in multiple human tissues using RT-PCR. RNA pooled from at least three individuals was used to assess overall UGT2A1 expression.