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The influence of genetic polymorphisms in *Ahr*, *CYP1A1, CYP1A2, CYP1B1, GST M1, GST T1* and *UGT1A1* on urine 1-hydroxypyrene glucuronide concentrations in healthy subjects from Rio Grande do Sul, Brazil

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Polymorphisms in genes encoding polycyclic aromatic hydrocarbon (PAH) metabolizing enzymes may alter metabolism of these carcinogens and contribute to inter-individual difference in urine concentrations. We investigated the influence of genetic polymorphism on PAH metabolism in urine from 199 healthy subjects from Southern Brazil. We measured urine 1-hydroxypyrene glucuronide (1-OHPG) concentrations using immuno-affinity chromatography and synchronous fluorescence spectroscopy and genotyped subjects using standard methods. Genetic variants in CYP1B1 (rs1056827, rs1800440, rs10012) were strongly associated with urine 1-OHPG with *P*-values < 0.010. Variants in aryl hydrocarbon receptor (Ahr) (rs4986826), CYP1A1 (rs1799814) and CYP1A2 (rs2069514) were also, although less strongly, associated with changes in urine 1-OHPG concentrations. These variants had *P*-values of 0.074, 0.040 and 0.025, respectively. The median urine 1-OHPG concentrations (pmol/ml) in the homozygous wild-type and homozygous variants for CYP1B1 (rs10012) and the Ahr, CYP1A1 and CYP1A2 variants listed above were 2.16 and 0.10, 2.16 and 0.41, 2.03 and 0.46, 2.19 and 2.79, respectively. We found no effect of deletions in GST M1 or GST T1, or different alleles of UGT1A1*28. Adjusting for age, sex, place of residence, tobacco smoke exposure, maté drinking, cachaça and barbeque preparation had only a minor impact on the associations. A model containing just exposure variables had an r^2 of 0.21; a model with single genotypes for Ahr, CYP1A1, CYP1A2 and CYP1B1 had an r^2 of 0.10; and a model combining both exposure and genotype information had a total r^2 of 0.33. Our results suggest that CYP1B1 genotypes are strongly associated with urine 1-OHPG concentrations in this population.

Abbreviations: Ahr, aryl hydrocarbon receptor; B[*a*]P, benzo[*a*]pyrene; 1-OHPG, 1-hydroxypyrene glucuronide; IQR, interquartile range; PAH, polycyclic aromatic hydrocarbon; SNPs, single nucleotide polymorphisms.

Introduction

Exposure to polycyclic aromatic hydrocarbons (PAHs) may adversely affect human health because this class of chemicals includes toxicants and carcinogens. Human exposure routes include inhalation of smoke (tobacco, wood, coal, auto exhaust, etc.) (1), eating foods cooked at high temperature (2) or contaminated by smoke (3) and through the therapeutic use of coal-tar. Many studies use benzo[*a*]pyrene (B[*a*]P) as a prototypical PAH because it is a major component of many PAH mixtures and because it can be metabolized to a mutagenic intermediate. The urine metabolite of B[*a*]P, 1-hydroxypyrene glucuronide (1-OHPG), may reflect recent exposure to PAHs (4).

Metabolism of PAHs to more hydrophilic compounds facilitates excretion (5). A major PAH biotransformation pathway follows two steps. First, functionalization by Phase I metabolizing enzymes such as cytochromes P450 followed by conjugation by Phase II metabolizing proteins to glucuronide, glutathione or other moieties, to increase water solubility. Phase I and II metabolizing enzymes exhibit distinct but overlapping substrate specificities. In humans, B[*a*]P is thought to be metabolized primarily by P450 1A1, 1A2 and 1B1 in Phase 1 and by glutathione-*S*-transferase and UDP-glucuronosyltransferase in Phase II. PAHs may also stimulate their own metabolism through feedback mechanisms. B[*a*]P and other similar compounds can bind and activate the aryl hydrocarbon receptor (Ahr), which subsequently upregulates transcription of some of these genes. Human genetic variation in each of these genes may play a role in inter-individual differences in the metabolism of PAHs.

The *in vivo* effect of human genetic variation in genes encoding PAH-metabolizing enzymes may alter the adverse health consequences of PAH exposure. Important considerations for these studies include the route of exposure, the mix of PAHs and the genetic background of the exposed individuals. To aid our understanding of the impact of genetic polymorphisms on PAH metabolism we examined the association between selected polymorphisms in a number of genes that encode PAH-metabolizing enzymes and urine 1-OHPG concentrations in a group of healthy Brazilians with elevated PAH exposures from some population-specific sources such as maté (6).

Materials and methods

Subjects

Participants were volunteers from Santa Maria, a city in the central region of Rio Grande do Sul Province in Southern Brazil, an area with esophageal squamous cell carcinoma rates of ~20/100 000/year (7). Healthy subjects from the outpatient unit at the University Hospital were invited to take part; >90% of the invited individuals chose to participate. The study included 200 healthy adult subjects, half males and half females, half current smokers and half non-smokers. Informed consent was obtained from each participant.

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Subjects completed a brief questionnaire to elicit information about demographic variables, habits and potential exposures to PAH and also provided a buccal cell sample for DNA using the ISOCODE system (Schleicher & Schuell, Keene NH). The study was approved by the Ethical Committee on Research of the Health Sciences Center of the University of Santa Maria, RS, Brazil. The analysis of extant anonymized data and samples was exempted from review by the Office of Human Subjects Review of the National Institutes of Health, Bethesda, MD.

Urine 1-OHPG measurements

Each participant was recruited in the morning and asked to provide a 10 ml urine sample. The urine samples were collected in a sterile container, frozen at −80°C and shipped on dry ice to the National Cancer Institute. Urine samples were assayed in the laboratory of Dr Strickland at the Johns Hopkins University. Urine 1-OHPG concentrations were measured using immunoaffinity chromatography and synchronous fluorescence spectroscopy as described previously (3,8). NicAlert Strips (Jant Pharmaceutical, Encino, CA) were used to measure urine cotinine equivalents as directed by the manufacturer. This test produces categorical results ranging from 0 (<10 ng/ml cotinine equivalents) to 6 (>2000 ng/ml). Because only a small number of subjects had urine cotinine results in each of Categories 2, 3 and 4, we collapsed these three groups into a single category. We considered subjects exposed to tobacco smoke if they reported smoking cigarettes or had elevated cotinine.

Table I. Subject characteristics

Age, mean (SD)	46 (14)
Sex, *N* (%)	
Males	98 (49%)
Females	101 (51%)
Residence, *N* (%)	
Rural	35 (18%)
Urban	160 (82%)
Ever drink maté, *N* (%)	
No	45 (23%)
Yes	154 (77%)
Ever drink *cachaça*, *N* (%)	
No	165 (83%)
Yes	34 (17%)
Current tobacco smoker, *N* (%)	
No	103 (52%)
Yes	96 (48%)
Urine cotinine, *N* (%)	
0	49 (25%)
1	41 (21%)
2,3,4	39 (20%)
5	36 (18%)
6	34 (17%)
Smoke exposed[a], *N* (%)	
No	85 (43%)
Yes	114 (57%)
Prepared BBQ in the last week?, *N* (%)	
No	148 (74%)
Yes	51 (26%)

[a]We defined smoke-exposed subjects as subjects who are current tobacco smokers or have a cotinine value greater than Category 1 (>30 ng/ml).

DNA extraction and genotype analysis

DNA extractions and genotyping were completed by BioServe Biotechnologies (Laurel, MD). DNA was isolated from the ISOCODE cards as recommended by the manufacturer. Genetic polymorphisms were assayed using MASSCODE for all single nucleotide polymorphisms (SNPs) except GST M1 and GST T1 deletions, which were analyzed using a duplex PCR-RFLP assay (9), and the UGT1A1*28 repeat polymorphism was assayed by PCR and gel electrophoresis (10). We successfully genotyped 92–98% of samples at each polymorphic site. We tested HWE using the *genhwi* command in Stata with exact *P*-values and used $P < 0.01$ as the cut point for evidence of deviation (11). No deviations were detected.

Statistical analysis

Statistical analyses were carried out using SAS 9.1 for Windows (SAS institute, Cary, NC) and STATA/SE 8.0 for Windows (Stata, College Station, TX). We tabulated the median and interquartile range (IQR) of 1-OHPG by genotypes for comparison. Next we examined the association between individual genetic polymorphisms and urine 1-OHPG concentration using linear regression models. First, we examined 1-OHPG concentrations in a crude model, with no covariates other than indicator variables for genotypes, and then with the addition of personal covariates previously shown to be associated with urine 1-OHPG concentration (6). These covariates included age, sex, rural versus urban residence, consumption of maté, *cachaça*, tobacco smoke exposure and the frequency of preparing barbeque.

Results

We measured urine 1-OHPG in 199 healthy subjects from Rio Grand do Sul, Brazil.

We genotyped subjects for polymorphisms in seven genes potentially involved in B[*a*]P metabolism. We examined the genetic linkage between polymorphisms within and between these genes (Table II). We found good evidence of linkage between some of the polymorphisms within each gene. Several SNP pairs had D's > 0.9 and high r^2. Two genes

Table II. Polymorphism names, RS numbers, location and disequilibrium

Gene and polymorphism	RS	Chromosome	Linkage disequilibrium matrix D′ (r^2)					
AhR G2290A	rs4986826	7 p21						
AhR A43188G	rs2066853	7 p21						
			Cyp1A1 C461A	Cyp1A1 T1101C	Cyp1A1 A5360C	Cyp1A2 G8066A	Cyp1A2 A3860G	Cyp1A2 C5734A
Cyp1A1 C461A	rs1799814	15 q24	X	0.22 (0.011)	−0.98 (0.042)	−0.99 (0.045)	−0.47 (−0.0021)	−1.00 (0.018)
Cyp1A1 T1101C	rs1048943	15 q24		X	−1.00 (0.18)	0.36 (0.022)	0.71 (0.45)	−1.00 (0.018)
Cyp1A1 T881C[a]	rs1800031	15 q24		X	X	X	X	X
Cyp1A1 A5360C	rs2606345	15 q24			X	−0.47 (0.22)	−0.95 (0.18)	0.026 (0.00027)
Cyp1A2 G8066A	rs2472304	15 q24				X	1.00 (0.20)	0.97 (0.37)
Cyp1A2 A3860G	rs2069514	15 q24					X	−1.00 (0.077)
Cyp1A2 C5734A	rs762551	15 q24						X
			CYP1B1 C7431A	CYP1B1 G3457C	CYP1B1 G7644C			
CYP1B1 C7431A	rs1056827	2 p22	X	−0.76 (0.17)	0.98 (0.97)			
CYP1B1 G3457C	rs1800440	2 p22		X	−0.79 (0.18)			
CYP1B1 G7644C	rs10012	2 p22			X			
GSTM1 deletion		1 p13						
GSTT1 deletion		22 q11						
UGT1A1*28		2 q37						

[a]There was no variation in Cyp1A1 T881C; therefore, no D′ values are included.

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Table III. Median (25th–75th percentile) urine 1-OHPG concentration and regression coefficients and *P*-values for crude and adjusted models[a] by genotype

Polymorphic site	Genotype	*N* (%)	Median (IQR)	Crude model		Adjusted model[a]	
				β	*P*-value	β	*P*-value
AhR G2290A	GG	139 (73%)	2.16 (0.57, 5.20)	REF		REF	
	GA	41 (22%)	2.22 (0.63, 10.0)	0.16	0.66	−1.08	0.67
	AA	10 (5%)	0.41 (0.051, 1.90)	−1.34	0.040	−1.08	0.089
AhR A43188G	AA	169 (92%)	2.03 (0.33, 5.18)	REF		REF	
	AG	14 (8%)	2.32 (1.40, 7.30)	0.47	0.40	0.31	0.56
	GG	0 (0%)	—	—	—	—	—
Cyp1A1 C461A	CC	174 (92%)	2.03 (0.33, 5.90)	REF		REF	
	CA	15 (8%)	3.05 (1.59, 7.55)	0.91	0.39	1.01	0.040
	AA[b]	1 (<1%)	0.46	—	—	—	—
Cyp1A1 T1101C	TT	140 (74%)	2.03 (0.32, 5.60)	REF		REF	
	TC	39 (21%)	2.73 (0.51, 6.92)	0.32	0.39	0.13	0.72
	CC	10 (5%)	1.81 (1.59, 7.55)	0.66	0.32	0.57	0.37
Cyp1A1 T881C	TT	195 (100%)	2.16 (0.57, 5.90)	—	—	—	—
Cyp1A1 A5360C	AA	50 (27%)	2.32 (1.02, 5.01)	REF		REF	
	AC	90 (48%)	2.00 (0.10, 5.20)	−0.49	0.16	−0.61	0.077
	CC	47 (25%)	2.66 (1.03, 7.23)	0.04	0.92	−0.10	0.80
Cyp1A2 G8066A	GG	46 (25%)	2.79 (0.63, 6.92)	REF		REF	
	GA	94 (51%)	2.09 (0.32, 6.60)	−0.12	0.75	−0.18	0.61
	AA	46 (25%)	2.03 (0.38, 5.01)	−0.36	0.40	−0.32	0.44
Cyp1A2 A3860G	AA	138 (70%)	2.19 (0.57, 5.84)	REF		REF	
	AG	49 (25%)	2.03 (0.14, 5.39)	−0.17	0.61	−0.44	0.16
	GG	9 (5%)	2.79 (1.52, 5.90)	0.75	0.28	1.34	0.038
Cyp1A2 C5734A	CC	101 (53%)	2.22 (0.57, 6.54)	REF		REF	
	CA	73 (38%)	2.03 (0.15, 4.63)	−0.28	0.36	−0.11	0.71
	AA	16 (8%)	2.22 (1.33, 5.60)	0.35	0.52	0.40	0.44
CYP1B1 C7431A	CC	94 (49%)	2.00 (0.38, 5.84)	REF		REF	
	CA	82 (43%)	2.54 (0.82, 5.61)	0.14	0.64	−0.17	0.55
	AA	14 (7%)	0.56 (0.051, 2.79)	−1.17	0.041	−1.56	0.0044
CYP1B1 G3457C	GG	63 (33%)	1.52 (0.051, 5.14)	REF		REF	
	GC	95 (49%)	2.66 (0.95, 5.90)	0.76	0.019	0.73	0.018
	CC	34 (18%)	2.44 (0.63, 10.98)	0.94	0.027	1.08	0.0063
CYP1B1 G7644C	GG	95 (50%)	2.16 (0.51, 6.54)	REF		REF	
	GC	82 (43%)	2.54 (0.82, 6.73)	0.04	0.90	−0.21	0.47
	CC	13 (7%)	0.10 (0.051, 2.79)	−1.54	0.0099	−1.86	0.0010
GSTM1	Present	104 (55%)	2.28 (0.31, 6.41)	REF		REF	
	Deleted	85 (45%)	1.65 (0.63, 5.01)	−0.03	0.91	0.01	0.96
GSTT1	Present	162 (85%)	2.16 (0.57, 5.84)	REF		REF	
	Deleted	28 (15%)	1.78 (0.18, 4.12)	−0.49	0.23	−0.52	0.17
UGT1A1*28	UGT6	101 (57%)	1.65 (0.33, 5.61)	REF		REF	
	UGT6/7	56 (32%)	2.44 (1.08, 7.73)	0.35	0.28	0.38	0.23
	UGT7	17 (10%)	0.63 (0.05, 3.05)	−0.60	0.24	−0.57	0.24
	UGT other[c]	3 (1%)	4.25 (0.95, 25.95)	1.32	0.25	1.22	0.26

[a]Adjusted model includes variables for age, sex, place of residence, maté, cachaça, tobacco smoke exposure and frequency of preparing barbeque.
[b]Because there was only a single subject AA for Cyp1A1 C461A we combined the heterozygotes and the homozygous variant subjects into a single category for the logistic regression models.
[c]The three subjects included in the other category had alleles 5 (2) and 7/8 (1).

located in tandem on chromosome 15, *Cyp1A1* and *Cyp1A2*, also showed linkage between some of the polymorphisms.

Table III presents the median (IQR) urinary 1-OHPG concentration for each of the genetic polymorphisms examined in this study. Several of the polymorphisms show strong differences in the median. To better show the crude associations, we plotted the individual urine 1-OHPG values by genotype for selected variants in *Ahr*, *CYP1A1*, *CYP1A2* and *CYP1B1* (Figure 1).

We tested the association between each polymorphism and the measured concentration of urinary 1-OHPG (Table III) and found significant crude associations for each of the polymorphisms in *Cyp1B1* and one of the SNPs in *Ahr*. Adjustment for factors known to influence urine 1-OHPG concentration did not alter the associations with *Cyp1B1* polymorphisms, while the *Ahr* association was substantially attenuated. The adjusted model also showed significant associations for one SNP in *Cyp1A1* and one SNP in *Cyp1A2*. These two SNPs are not linked ($D' = -0.47$, $r^2 = -0.0021$). The Cyp1A1 C461A association appeared to be slightly confounded by smoking. Subjects carrying the variant allele were non-significantly less likely to smoke than those with homozygous CC. The Cyp1A2 A3860G association showed no single strong confounder; the association became stronger overall after the addition of all adjusting variables.

To examine the joint effects of different polymorphisms we selected the single SNPs from *Ahr*, *Cyp1A1* and *Cyp1A2* that were associated with urine 1-OHPG concentration and the *Cyp1B1* SNP with the strongest association. Using an indicator variable for the homozygous variant genotype we fit models for each variant alone, for comparison, and all four in a single model (Table IV). The combined model had no effect on the *Cyp1B1* polymorphism association, reduced the

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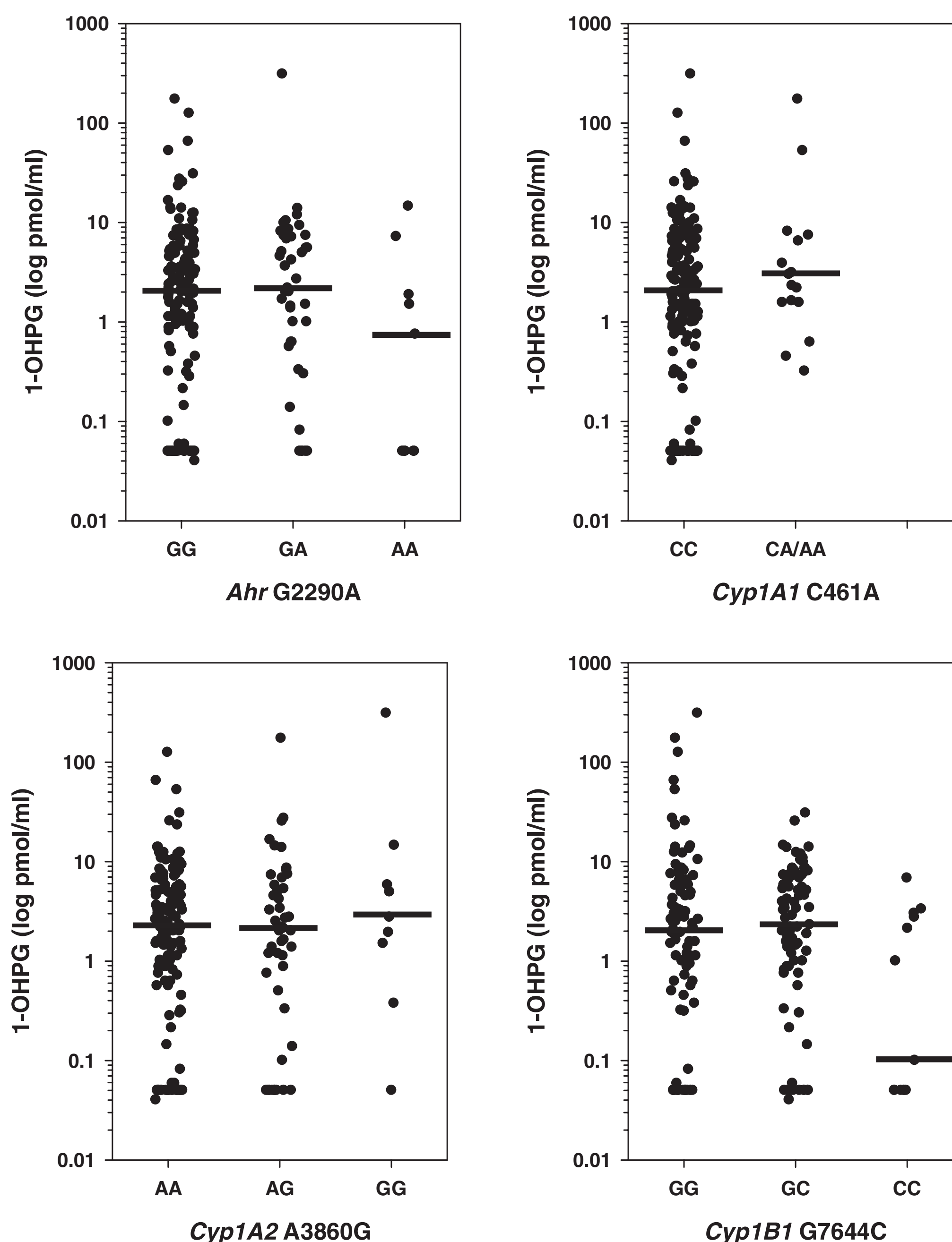

Fig. 1. Dot plots of urine 1-OHPG concentrations for four genetic variants in healthy subjects from Rio Grande do Sul, Brazil. For each polymorphism the individual concentrations were plotted by genotype and the data were jittered to improve clarity. Only one subject was AA for *Cyp1A1* C461A so that subject's data point was plotted with the heterozygotes. Horizontal bars indicate the median concentration.

Table IV. Adjusted single and multi-gene regression models[a] for the association between selected homozygous variant genotypes and urine 1-OHPG concentration

Polymorphic site	Genotype	Single gene model		Multi-gene model[b]	
		β	*P*-value	β	*P*-value
AhR G2290A	AA	−1.12	0.074	−1.44	0.020
Cyp1A1 C461A[c]	*A	1.01	0.040	0.79	0.12
Cyp1A2 A3860G	GG	1.45	0.025	2.18	0.0014
CYP1B1 G7644C	CC	−1.77	0.0013	−1.74	0.0015

[a]Adjusted model includes variables for age, sex, place of residence, maté, cachaça, tobacco smoke exposure and frequency of preparing barbeque.
[b]The multi-gene model includes each of the four genotype variables in a single model with the adjusting variables.
[c]Because there was only a single subject AA for Cyp1A1 C461A, we combined the heterozygotes and the homozygous variant subjects into a single category.

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modeled effect of the *Cyp1A1* polymorphism and strengthened the effect and significance of the other two polymorphisms. A model containing just the exposure variables had a total r^2 of 0.21. Adding the four genotype variables improved the total r^2 to 0.33. Therefore, adding these four genetic variants explained 12% more of the total variation in urine 1-OHPG concentrations, which is a 57% increase in the explanatory power of the model.

Discussion

Using data collected in this study, we previously demonstrated that healthy subjects, even non-smokers, from Rio Grande do Sul were exposed to moderately elevated levels of PAHs, as measured by urine 1-OHPG concentration, and that smoking tobacco and drinking maté were important predictors of exposure (6). To further investigate the variation of PAH metabolism in these subjects, we examined whether genetic variation in PAH-metabolizing enzyme genes demonstrated phenotypic changes in 1-OHPG excretion.

We found strong associations between three different *Cyp1B1* polymorphisms and 1-OHPG concentration. Two of these polymorphisms were in very strong linkage disequilibrium and therefore produced very similar results. The third polymorphism showed some linkage disequilibrium with the other two ($D' = -0.76$ and $r^2 = 0.18$). We also found modest but significant associations with one variant in each of the *Ahr*, *Cyp1A1* and *Cyp1A2* genes. In general, adjustment for variables associated with higher urine 1-OHPG concentration (age, smoking, maté, etc) had modest effects on the regression coefficients.

The biological differences for the tightly linked Cyp1B1 polymorphisms have been examined *in vitro* and were found to show no major differences in the catalytic activity for two important substrates, ethoxyresorufin and 17β-estradiaol, nor did the variants produce proteins with different stabilities when expressed in COS-1 cells (12). Despite this lack of difference in cell culture, we have found statistically significant differences in urine 1-OHPG concentrations in humans. The polymorphisms are non-synonymous and are thought to occur in areas of the protein that may be critical to enzyme activity (12). The polymorphism in *Cyp1A2* that we found to be significantly associated with urine 1-OHPG is in the gene promoter and is thought to effect enzyme activity by reducing the inducibility of the gene. An early study reported that subjects with the polymorphism have decreased P4501A2 activity in caffeine metabolism (13), but other studies have failed to confirm this result (14,15).

Previous studies of genetic variation in PAH-metabolizing genes that have examined urine 1-OHPG or 1-hydroxypyrene concentrations have also produced inconsistent results for most of the genes studied, including *Cyp1A1* (16,17), *Cyp1A2* (18,19), *GSTM1* (20,21) and *GSTT1* (20,22). The strongest evidence of a phenotypic effect in our study was for polymorphisms in *Cyp1B1*. Two other studies that examined the association between *Cyp1B1* variants and urinary 1-hydroxypyrene did not find an association (16,18). Population- and exposure-specific effects may help explain the inconsistencies. For example, the cited studies have included occupationally and non-occupationally exposed subjects. The exposures to B[*a*]P and co-occurring PAHs are probably very different in coke-oven workers versus subjects exposed to B[*a*]P through tobacco smoke and food. Also, the different ethnic background of the subjects under study may result in other genetic variants that impact PAH metabolism, which have not been measured in the relatively small number of polymorphisms studied to date. On the other hand, inconsistencies in results among free-living individuals from different ethnic backgrounds with different sources of exposure may not be surprising considering the inconsistencies cited using controlled experiments in the metabolism of caffeine or other probes.

Our study has several strengths and weaknesses. Since our study used free-living healthy volunteers, our results may accurately reflect the general population of Southern Brazil. We found that modeling the effect of polymorphisms using a multi-gene model sharpened the associations between genetic polymorphisms and urine 1-OHPG concentrations. And this procedure might improve reproducibility between studies. We demonstrated that the contributions to inter-individual variability in urine 1-OHPG concentrations from lifestyle and genetic sources are essentially independent and additive.

Our study only examined the association between genotypes and urine 1-OHPG at a single point in time for only one level of exposure, which may limit the understanding of the association. That is, the association between certain genotypes and urine 1-OHPG concentration may be different if the same individuals had substantially higher or lower PAH exposure or other unknown co-exposures. Also, we picked a small number of polymorphism in each gene rather than using a comprehensive approach taking into account the linkage disequilibrium blocks for each gene. Essentially all published studies share this limitation and this may contribute to the overall inconsistency in the literature.

In conclusion, we found evidence that *Cyp1B1* genotypes were strongly associated with urine 1-OHPG concentrations. We also found some evidence that genotypes of Ahr, Cyp1A1 and Cyp1A2 were associated with 1-OHPG concentration. Finally, we showed that models including information on personal characteristics, sources of PAH exposure and genetic variation explained considerably more of the variation in urinary 1-OHPG concentrations than models that include only one of these types of data.

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