Variation in genes relevant to aromatic hydrocarbon metabolism and the risk of adult brain tumors

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Genes involved in phase I and phase II regulation of aromatic hydrocarbon–induced effects exhibit sequence variability that may mediate the risk of adult brain tumors. We evaluated associations between gene variants in *CYP1A1*, *CYP1B1*, *GSTM3*, *EPHX1*, and *NQO1* and adult brain tumor incidence. Cases were patients with glioma (n = 489), meningioma (n = 197), or acoustic neuroma (n = 96) diagnosed from 1994 to 1998 at three U.S. hospitals. Controls were 799 patients admitted to the same hospitals for nonmalignant conditions. DNA was extracted from blood samples collected from 1277 subjects, and genotyping was conducted for *CYP1A1* I462V, *CYP1B1* V432L, *EPHX1* Y113H, *GSTM3* *A/*B (intron 6 deletion), and *NQO1* P187S. The *CYP1B1* V432L homozygous variant was associated with decreased risk of meningioma (odds ratio [OR] = 0.6; 95% CI, 0.3–1.0) but not the other tumor types. The *GSTM3* *B/*B genotype was associated with increased risk of glioma (OR = 2.3; 95% CI, 1.0–5.2) and meningioma (OR = 3.6; 95% CI, 1.3–9.8). Increased risks associated with *GSTM3* *B/*B were observed in younger subjects (age < 50) and older subjects (age ≥ 50), in men and women, and within each study site. The magnitude of association for *GSTM3* with glioma and meningioma was greater among ever-smokers than among those who had never smoked. None of the other genotypes showed consistent associations with any tumor type. The association with the *GSTM3* *B allele, while intriguing, requires replication, and additional research is needed to clarify the function of the *GSTM3* alleles studied here. *Neuro-Oncology 8, 145–155, 2006 (Posted to Neuro-Oncology [serial online], Doc. 05-036, January 27, 2006. URL www.dukeupress.edu/neuro-oncology; DOI: 10.1215/15228517-2005-003)*

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[2]Abbreviations used are as follows: CYP, cytochrome P-450; DNA, deoxyribonucleic acid; EPHX, epoxide hydrolase; GST, glutathione S-transferase; NQO, NAD(P)H:quinone oxidoreductase; OR, odds ratio; PAH, polycyclic aromatic hydrocarbon; QC, quality control.

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Both genetic and environmental factors are likely to be important causes of primary brain tumors. The few clues about brain tumor etiology indicate that certain occupations involving exposure to polycyclic aromatic hydrocarbons (PAHs)[2] or other aromatic hydrocarbons may be associated with increased risk (Inskip, P.D., et al., 1995), most notably work in the petroleum industry (Carozza et al., 2000; Demers et al., 1991; Preston-Martin, 1989; Thomas et al., 1986, 1987); however, multiple exposures in implicated occupations limit possible conclusions about aromatic hydrocarbons. Smoking, a major source of aromatic hydrocarbon exposure, has been associated with brain tumor incidence in several studies (Burch et al., 1987; Lee et al., 1997), but not consistently so (Inskip, P.D., et al., 1995), and sometimes only among certain subgroups (Efird et al., 2004; Phillips et al., 2005). It is possible that underlying variability in genes responsible for biotransformation and metabolism of aromatic hydrocarbons could hinder the consistency of studies of chemical exposures. For this reason, it may be illuminating to study the association of variants in genes involved in aromatic hydrocarbon metabolism with the risk of brain tumors.

The conversion of PAHs to DNA-reactive products depends on a complex series of biotransformations. In the case of benzo[*a*]pyrene, transformation events include oxidation by cytochrome P-450 enzymes (such as *CYP1A1*) to create the active benzo[*a*]pyrene epoxide (Pelkonen and Nebert, 1982; Shimada et al., 1996), hydration by microsomal epoxide hydrolase (*EPHX1*) to the less toxic benzo[*a*]pyrene diol, oxidation by P-450 enzymes (such as *CYP1B1*) to the highly carcinogenic benzo[*a*]pyrene diol epoxide, detoxification of benzo[*a*]-pyrene and benzo[*a*]pyrene diol epoxide by glutathione *S*-transferases (such as *GSTM1*, *GSTT1*, and possibly *GSTM3*) by addition of reduced glutathione to electrophilic compounds (Omiecinski et al., 2000; Strange et al., 2001), and reduction of oxidative potential of quinones derived from benzo[*a*]pyrene diol by NAD(P)H:quinone oxidoreductase 1 (*NQO1*) (Palackal et al., 2002; Pastorelli et al., 1998; Ross et al., 2000). There is evidence from animal experiments that NAD(P)H protects from PAH-induced carcinogenicity; this protection is thought to operate through decreases in quinone-induced DNA adduct formation and DNA mutagenicity, including that induced by benzo[*a*]pyrene quinine (Joseph and Jaiswal, 1998; Long et al., 2001).

In a previous report, we presented case-control study results for some genes known to be involved in metabolism of PAHs or other potential carcinogens, namely, *CYP2E1*, *GSTM1*, and *GSTT1* (De Roos et al., 2003). For the current investigation, we selected several additional candidate genes related to PAHs or other aromatic hydrocarbons; all of the selected metabolic genes exhibit sequence variation that may relate to function. Substitution of valine with isoleucine in exon 7 of *CYP1A1* results in a variant (I462V) with increased arylhydrocarbon hydroxylase activity (Cosma et al., 1993; Crofts et al., 1994; Kiyohara et al., 1996, 1998; Taioli et al., 1995). The functional significance of a *CYP1B1* variant, V432L, is not well known; however, some studies suggest that the valine product results in higher catalytic activity toward some PAH dihydrodiols relative to leucine (Shimada et al., 1999), possibly leading to increased levels of reactive intermediates. The *GSTM3* gene has a three-base-pair deletion in intron 6, and the two alleles are referred to as GSTM3*A and GSTM3*B (Inskip, A., et al., 1995; Strange et al., 2001). This deletion creates a recognition motif (-aagata-) for the YY1 transcription factor which could potentially affect detoxification activity by GSTM3*B (Strange et al., 2001). The *EPHX1* variant Y113H has demonstrated increased activity in vitro but not in vivo (Hassett et al., 1994; Omiecinski et al., 2000). In vitro, *EPHX1* activity is increased (about 40%) when associated with the histidine product, probably because of altered protein stability (Hassett et al., 1994). The *NQO1* P187S variant resulting from C-to-T substitution leads to reduced enzyme function (Moran et al., 1999; Traver et al., 1997) and thus, presumably, less protection against oxidative damage.

We examined the effects of these metabolic gene variants in a parallel comparison of three major categories of malignant and benign brain tumors, namely the gliomas, meningiomas, and acoustic neuromas. Although we selected genes according to their possible relevance to metabolism of PAHs and other aromatic hydrocarbons, the substrate specificity is quite broad, and it is unclear to what extent the selected genes reflect a coherent pathway. Nevertheless, this exploratory approach was considered appropriate, given the dearth of knowledge about causes of brain tumors.

Material and Methods

Study Population

The study has been described in detail elsewhere (Inskip et al., 2001). Eligible cases were adult patients with intracranial tumors including glioma, meningioma, or acoustic neuroma (referred to as *brain tumors* in this article), newly diagnosed from 1994 to 1998 and treated at one of three participating U.S. hospitals located in Phoenix, Ariz., Boston, Mass., and Pittsburgh, Pa. We sought approval of physicians to contact newly diagnosed brain tumor patients for recruitment into the study. We enrolled 489 glioma, 197 meningioma, and 96 acoustic neuroma patients, for a total of 782 cases of malignant or benign brain tumors, representing 92% of those contacted (88% for glioma; 98% for meningioma and acoustic neuroma). Information on tumor pathology was based on the diagnosis from each hospital. Gliomas were classified as low grade or high grade according to Kleihues and Cavenee (2000).

Controls were patients admitted to the same hospitals and treated for a variety of nonneoplastic conditions. They were frequency-matched to the total case series by hospital, age, gender, race, and proximity of residence to hospital. Of the eligible controls identified and asked to participate, 799 control subjects were recruited, representing 86% of those contacted. Discharge diagnoses of the control subjects were trauma, injury, or poisoning

(24.7%), circulatory disease (22.4%), musculoskeletal disease (21.5%), disease of the digestive system (11.5%), and other (19.9%).

Trained nurses conducted a structured, computerized, in-person interview that included detailed questions on the following: lifetime job history; specific occupational exposures, processes, and tasks; hobbies involving solvent exposures; cellular telephones and other forms of communication devices; medical history; exposure to diagnostic and therapeutic radiation; reproductive history and use of exogenous hormones; use of hair dyes; family history of cancer and selected other conditions; and sociodemographic characteristics. A supplemental self-administered questionnaire addressed diet, vitamin supplements, and electric appliances.

Laboratory Analyses

DNA was extracted from peripheral white blood cells (buffy coat or granulocytes) from blood samples collected from 1277 subjects (81% of all subjects; 422 gliomas [86%], 172 meningiomas [87%], 79 acoustic neuromas [82%], and 604 controls [76%]), by GenoType, Ltd. (United Kingdom) using a phenol-chloroform method as described by Daly et al. (1996). The percentage of potentially eligible subjects who provided both interview data and blood samples was 76% for glioma, 85% for meningioma, 80% for acoustic neuroma, and 65% for controls.

Genotyping was conducted by the NCI Core Genotyping Facility (Gaithersburg, Md.). *CYP1A1* I462V was genotyped by using an MGB Eclipse (Epoch Biosciences, Bothell, Wash.) reaction method. For this, 10 ng of lyophilized sample DNA was used to do a 5-μl MGB Eclipse reaction in a 384 (96*4)-well plate format as described elsewhere (http://snp500cancer.nci.nih.gov/epoch_assays.cfm?snp_id=CYP1A1-01), except 10 ng of DNA was used. The SDS software (Applied Biosystems, Foster City, Calif.) displays the results of allelic discrimination run in a dissociation curve format. The dissociation curve is exported in text format for further analysis by using the MGB Eclipse software as described by Belousov et al. (2004).

CYP1B1 L432V, *EPHX1* Y113H, *GSTM3* *A or *B, and *NQO1* P187S were genotyped by using TaqMan (Applied Biosystems) methods. In this procedure, 5 ng of lyophilized sample DNA was used to do a 5-μl TaqMan reaction in a 384 (96*4)-well plate format as described elsewhere (see the TaqMan assays at http://snp500cancer.nci.nih.gov).

Quality-control (QC) measures included the addition of replicates (62 samples from three individuals who were not study subjects [QC-A, n = 31; QC-B, n = 18; QC-C, n = 13], collected and processed in identical fashion as samples from study subjects) and duplicates (two samples for each of 87 individuals who were study subjects) interspersed throughout the batches for all assays. Assay-specific positive controls for the three possible genotypes were included on each assay plate.

Statistical Analyses

SAS software version 9.1 (SAS Institute Inc., Cary, N.C.) was used for all statistical analyses. We calculated chi-squared statistics to test Hardy–Weinberg equilibrium of each genotype among the control group to determine whether the distribution of alleles was as expected (Hernandez and Weir, 1989).

The effect of each gene variant on the incidence of each brain tumor type, with the homozygous common genotype as the referent, was estimated by conventional maximum likelihood using unconditional logistic regression to calculate odds ratios (ORs) and 95% confidence limits. All effect estimates for gene variants were adjusted for the study by matching factors of age (coded in years: 18–29, 30–39 as the referent, 40–49, 50–59, 60–69, 70–79, or 80–90), race/ethnicity (non-Hispanic white as the referent, Hispanic white, black, or other), gender (male, female as the referent), hospital (Phoenix as the referent, Boston, or Pittsburgh), and proximity of patient's residence to the hospital (coded in miles: 0–4 as the referent, 5–14, 15–29, 30–49, or ≥50). We checked the influence of the control series composition on results by examining the consistency of the effect of each genotype on each tumor type, while excluding one major category of control discharge diagnoses at a time.

We conducted subgroup analyses to examine whether associations with each gene variant differed by age group (<50 years, ≥50 years), gender, study site (Phoenix, Boston, Pittsburgh), or smoking status (ever, never). Other factors of interest, such as race and family history of nervous system tumors, did not have sufficient numbers within groups to enable subgroup analyses. We also examined the association of each gene variant with high-grade and low-grade glioma, and with specific glioma subtypes including glioblastoma, anaplastic astrocytoma, other astrocytoma, oligodendroglioma, and mixed oligoastrocytoma; these analyses included all controls. Chi-squared statistics and corresponding P values (based on 4 degrees of freedom) were calculated to test whether the distribution of each gene variant differed among the five glioma subtypes. For subgroup analyses and subanalyses by tumor type, we calculated the risk associated with the homozygous variant (where numbers allowed or where an association appeared to occur primarily for the homozygous variant) or the combined heterozygous–homozygous variant genotypes. The homozygous common genotype was always used as the referent.

We tested for interaction between polymorphisms in several putative activating enzymes (phase I) and detoxification enzymes (phase II); in other words, we tested whether the presence of two variant alleles in these genes imparted increased risk that was greater than multiplicative of the risk associated with either variant alone. Interactions between metabolic genes (from this study and our previous report [De Roos et al., 2003]) were examined for specific combinations indicated by function or epidemiologic data as potentially interactive in the context of aromatic hydrocarbon metabolism. We investigated combinations of *CYP1A1* I462V and

GSTM1 null because of reports of interaction between these genotypes in the risk of several types of cancer (Hung et al., 2003; London et al., 2000; Murata et al., 1998, 2001; Nimura et al., 1997; Olshan et al., 2000; Sato et al., 2000; Stucker et al., 2000; Wang et al., 2002), *GSTM3* *A/*B and *GSTM1* null because of possible interaction of these genes reported for various cancers (Loktionov et al., 2001; Yengi et al., 1996), *EPHX1* Y113H, and *GSTM1* null because interaction was previously observed in a study of orolaryngeal cancer (Park et al., 2003), and *NQO1* P187S and *CYP2E1*5* (*RsaI*) because these genes are dually involved in quinone metabolism (Nebert et al., 2002). Each combination was examined by including indicator variables for the two individual-variant effects and one joint-variant effect in a logistic regression model with the matching factor variables, and interaction on the multiplicative scale was tested in a separate logistic regression model using the *P* value for an interaction term.

Results

Demographic characteristics of cases and controls are presented in Table 1. Frequency matching ensured comparability of cases and controls in the study with respect to race. Patients with brain tumors were, on average, older and more highly educated than controls. Meningioma patients were more often female as compared to controls or patients with other tumor types. A greater proportion of acoustic neuroma cases were from the Phoenix study site, as compared to the other tumor types or controls. Controls were more likely than patients with brain tumors to live in closer proximity to the hospital.

Genotyping was successfully conducted for *CYP1A1* I462V (98.1% of all study samples analyzed), *CYP1B1* V432L (95.9%), *EPHX1* Y113H (97.3%), *GSTM3* *A/*B (97.0%), and *NQO1* P187S (91.7%), and genotyping of all five variants was successful for 76.9% of the samples analyzed for all five genotypes. Missing values, primarily the result of insufficient quantity of high-quality DNA or poor amplification for a specific locus, were equally likely to be from case or control samples. We achieved 97% to 100% agreement among replicates for all assays and 93% to 98% agreement between duplicate samples for all assays according to the kappa statistic. The frequencies of the rare alleles for *CYP1A1* I462V, *CYP1B1* V432L, *EPHX1* Y113H, *GSTM3* *A/*B, and *NQO1* P187S (Table 2) were similar to those in other study populations (Chang et al., 2003; Garte et al., 2001; Kelsey et al., 1997; Strange et al., 2001). There was no evidence of departure from Hardy–Weinberg equilibrium for any genotype.

GSTM3 *B/*B genotype was associated with increased risk of both glioma and meningioma, with moderate associations for the *B/*B genotype versus *A/*A (glioma: OR = 2.3; 95% CI, 1.0–5.2; and meningioma: OR = 3.6; 95% CI, 1.3–9.8) (Table 2). None of the acoustic neuroma cases were *GSTM3* *B/*B genotype; however, the heterozygous genotype gave no indication of any association between *GSTM3* and acoustic neuroma (OR = 1.1; 95% CI, 0.6–1.9). *CYP1B1* 432 Val/Val was inversely associated with meningioma (OR = 0.6; 95% CI, 0.3–1.0), although there was no dose–response relationship according to the number of variant alleles. *EPHX1* 113 His/His was associated with slightly increased risks of all three tumor types (ORs = 1.5) that were not statistically significant. Neither the *CYP1A1* I462V nor the *NQO1* P187S variant was associated with the risk of any brain tumor type.

Although data were sparse in subgroup analyses (Table 3), the positive association of the *GSTM3* *B/*B genotype with glioma and meningioma was present within different age groups and in both genders. The magnitude of association of *GSTM3* *B/*B with glioma and meningioma was greater among ever-smokers than never-smokers, and the interaction between *GSTM3* and smoking was statistically significant for meningioma; nevertheless, ORs for the variant genotype were elevated among nonsmokers as well. The *GSTM3* *B/*B genotype was associated with both low-grade and high-grade tumors (results not shown in the tables, ORs = 1.8 and 2.3, respectively), and with all subtypes of glioma except anaplastic astrocytoma (ORs range from 1.8 to 3.8, none statistically significant; not shown in the tables). Increased risk associated with *GSTM3* *B/*B was similar across the three study sites, as well as in all subanalyses testing the sensitivity of results to exclusions from the control series (results not shown). The subgroup analyses of *CYP1B1* V432L with meningioma did not reveal any particular group with decreased risk associated with the homozygous variant genotype, as results were fairly uniform across groups of age, gender, and smoking status. For glioma, *EPHX1* 113 His/His was associated with increased risk primarily among subjects 50 years or older, women, and ever-smokers. These patterns were less clear for meningioma and acoustic neuroma. Although no overall association was observed with the variant P187S variant, the subgroup analysis suggested that variant genotype might be associated with increased glioma and acoustic neuroma risk among men and among those who had ever smoked.

There was some indication of positive interaction between *CYP2E1*5* (*RsaI*) and *NQO1* P187S variants in the risk of glioma or acoustic neuroma (Table 4). Individuals with the combination of increased *CYP2E1* activity (*CYP2E1*1A*/*CYP2E1*5* or *CYP2E1*5*/*CYP2E1*5*) and reduced *NQO1* activity (carriers of *NQO1* 187 Ser) were at approximately threefold increased risk of glioma and fourfold increased risk of acoustic neuroma. This interaction was of borderline statistical significance ($P = 0.05$) for glioma, but not for acoustic neuroma because of small numbers. This potential interaction was not observed for meningioma. There was no evidence of interactions between *CYP1A1* I462V and *GSTM1* null, between *GSTM3* *A/*B and *GSTM1* null, or between *EPHX1* Y113H and *GSTM1* null for any tumor type (results not shown).

Table 1. Frequencies of characteristics of brain tumor cases and controls from three U.S. hospitals (1994–1998)

Characteristic	Controls (N = 799) n (%)	Glioma (N = 489) n (%)	Meningioma (N = 197) n (%)	Acoustic Neuroma (N = 96) n (%)	All Brain Tumors (N = 782) n (%)
Gender					
Female	436 (54.6)	212 (43.4)	151 (76.6)	60 (62.5)	423 (54.1)
Male	363 (45.4)	277 (56.6)	46 (23.4)	36 (37.5)	359 (45.9)
Race/ethnicity					
White (non-Hispanic)	715 (89.5)	444 (90.8)	163 (82.7)	89 (92.7)	696 (89.0)
Hispanic	54 (6.8)	26 (5.3)	14 (7.1)	6 (6.3)	46 (5.9)
Black	19 (2.4)	10 (2.0)	9 (4.6)	0	19 (2.4)
Other	9 (1.3)	11 (1.9)	11 (5.6)	1 (1.0)	23 (2.9)
Age (years)					
⩽30	113 (14.1)	63 (12.9)	4 (2)	4 (4.2)	71 (9.1)
31 to 50	320 (40.1)	177 (36.2)	78 (39.6)	41 (42.7)	296 (37.9)
51 to 70	270 (33.8)	174 (35.6)	79 (40.1)	41 (42.7)	294 (37.6)
>70	96 (12.0)	75 (15.3)	36 (18.3)	10 (10.4)	121 (15.5)
Educational level					
Less than high school graduate	105 (13.1)	64 (13.1)	24 (12.2)	5 (5.2)	93 (11.9)
High school graduate or equivalent	234 (29.3)	122 (24.9)	57 (28.9)	28 (29.1)	207 (26.5)
1–3 years of college	245 (30.7)	130 (26.6)	68 (34.5)	21 (21.9)	219 (28.0)
4 years of college	105 (13.1)	89 (18.2)	23 (11.7)	23 (24.0)	135 (17.3)
Graduate or professional school	89 (11.1)	68 (13.9)	24 (12.2)	18 (18.8)	110 (14.1)
Missing	21 (2.7)	16 (3.3)	1 (0.5)	1 (1.0)	18 (2.2)
Hospital site					
Phoenix	405 (50.7)	244 (49.9)	99 (50.3)	72 (75.0)	415 (53.0)
Boston	220 (27.5)	153 (31.3)	79 (40.1)	22 (22.9)	254 (32.5)
Pittsburgh	174 (21.8)	92 (18.8)	19 (9.6)	2 (2.1)	113 (14.5)
Proximity of residence to hospital (miles)					
0–5	262 (32.8)	125 (25.6)	59 (29.9)	22 (22.9)	206 (26.3)
5–15	229 (28.7)	155 (31.7)	56 (28.4)	30 (31.3)	241 (30.8)
15–30	163 (20.4)	116 (26.6)	43 (21.8)	17 (17.7)	176 (22.5)
30–50	59 (7.4)	42 (8.6)	17 (8.6)	3 (3.1)	62 (7.9)
⩾50	86 (10.8)	51 (10.4)	22 (11.2)	24 (25.0)	97 (12.4)
Blood sample					
Yes	604 (75.6)	422 (86.3)	172 (87.3)	79 (82.3)	673 (86.1)
No	195 (24.4)	67 (13.7)	25 (12.7)	17 (17.7)	109 (13.9)
DNA sample sent to lab for genotyping					
CYP1A1 I462V	556 (69.6)	388 (79.4)	161 (81.7)	72 (75.0)	621 (79.4)
CYP1B1 V432L					
EPHX1 Y113H					
NQO1 P187S	543 (68.0)	382 (78.1)	158 (80.2)	70 (72.9)	610 (78.0)
GSTM3 *A/*B	542 (67.8)	382 (78.1)	158 (80.2)	70 (72.9)	610 (78.0)

Table 2. Gene variant associations with brain tumor incidence (OR and 95% CI)[a]

Genotype	Amino Acid	Controls	Glioma		Meningioma		Acoustic Neuroma	
		n (%)[b]	n (%)	OR (95% CI)	n (%)	OR (95% CI)	n (%)	OR (95% CI)
CYP1A1 I462V								
AA	Ile/Ile	491 (90.3)	343 (89.6)	1.0	139 (88.5)	1.0	67 (94.4)	1.0
AG or GG	Ile/Val or Val/Val	53 (9.7)	40 (10.4)	1.2 (0.8–1.9)	18 (11.5)	1.1 (0.6–2.1)	4 (5.6)	0.5 (0.1–1.4)
CYP1B1 V432L								
CC	Leu/Leu	178 (34.4)	109 (29.6)	1.0	58 (38.2)	1.0	23 (33.8)	1.0
CG	Leu/Val	241 (46.5)	191 (51.9)	1.3 (0.9–1.7)	75 (49.3)	1.0 (0.7–1.6)	34 (50.0)	1.2 (0.7–2.3)
GG	Val/Val	99 (19.1)	68 (18.5)	1.1 (0.7–1.6)	19 (12.5)	0.6 (0.3–1.0)	11 (16.2)	1.0 (0.4–2.2)
EPHX1 Y113H								
TT	Tyr/Tyr	268 (50.9)	194 (52.2)	1.0	83 (53.9)	1.0	36 (52.2)	1.0
TC	Tyr/His	216 (41.0)	134 (36.0)	0.9 (0.7–1.2)	54 (35.1)	0.7 (0.5–1.1)	25 (36.2)	0.8 (0.4–1.3)
CC	His/His	43 (8.2)	44 (11.8)	1.5 (0.9–2.3)	17 (11.0)	1.5 (0.8–2.9)	8 (11.6)	1.5 (0.6–3.6)
GSTM3 *A/*B								
*A/*A	-/-	382 (72.4)	252 (67.7)	1.0	96 (64.0)	1.0	49 (73.1)	1.0
*A/*B	-/AAG	134 (25.4)	106 (28.5)	1.2 (0.9–1.6)	44 (29.3)	1.2 (0.8–1.9)	18 (26.9)	1.1 (0.6–1.9)
*B/*B	AAG/AAG	12 (2.3)	14 (3.8)	2.3 (1.0–5.2)	10 (6.7)	3.6 (1.3–9.8)	0 (0.0)	0.0 (0.0–∞)
NQO1 P187S								
CC	Pro/Pro	346 (69.9)	230 (65.5)	1.0	106 (71.6)	1.0	41 (65.1)	1.0
CT	Pro/Ser	131 (26.5)	107 (30.5)	1.3 (0.9–1.7)	37 (25.0)	0.8 (0.5–1.2)	20 (31.8)	1.3 (0.7–2.5)
TT	Ser/Ser	18 (3.6)	14 (4.0)	1.2 (0.6–2.5)	5 (3.4)	0.6 (0.2–2.0)	2 (3.2)	1.2 (0.2–5.5)

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Estimates within each cell are from individual unconditional logistic regression models for each gene variant; all estimates adjusted for matching factors including age, gender, race, hospital, and distance of residence from hospital.

[b]The number of subjects included in each model may differ depending on the number of samples successfully genotyped for each variant.

Discussion

Of the gene variants we studied, only *GSTM3* *A/*B (intron 6 deletion) showed noteworthy patterns of association with adult brain tumors, specifically glioma and meningioma. *GSTM3* is expressed in astrocytes, and strong expression has been observed at the boundary between astrocytes and tumor cells (Hand et al., 1996). The associations we observed were quite consistent across different age groups, genders, and study sites. Both low-grade and high-grade gliomas were associated with the *GSTM3* *B allele, as were all glioma subtypes except anaplastic astrocytoma. The fact that we observed a positive association for both glioma and meningioma suggests a common risk factor for the two tumor types that could be metabolized by *GSTM3*. The observation of a stronger magnitude of association among those who had ever smoked suggests a biologic pathway relevant to metabolism of components of cigarette smoke, such as PAHs. However, our results differ from what has been reported in the literature. A previous study found no association between *GSTM3* polymorphism and the risk of astrocytoma (Hand et al., 1996). Initial suppositions about the functional activity of the *GSTM3* *A and *B alleles suggest that increased risk should be associated with the *A/*A genotype rather than the *B/*B genotype (Inskip, A., et al., 1995). In support of this, epidemiologic studies have found that the *B/*B genotype was inversely associated with cancer risk, including basal cell carcinoma (Yengi et al., 1996), laryngeal cancer (Matthias et al., 1998), and oral and pharyngeal cancers (Jahnke et al., 1997; Park et al., 2000). Nevertheless, some researchers have observed increased cancer risk associated with the *GSTM3* *B allele for bladder cancer (Schnakenberg et al., 2000), colorectal cancer (Loktionov et al., 2001), and nonmelanoma skin cancer in renal transplant patients (Ramsay et al., 2001). Unfortunately, little is known about chemical substrates that might be specific to *GSTM3* and not *GSTM1*. The *GSTM3* *B allele creates a new YY1 binding site, and this ubiquitous transcriptional regulator can act as a negative-acting or positive-acting factor, according to the system, which could potentially explain differing directions of association among cancer sites. Beuckmann et al. (2000) report that human *GSTM3* may act as a prostaglandin E_2 synthase in the brain, and if true, this suggests a possible mechanism involving growth regulation. However, because of the relatively low frequency of the *B/*B genotype, findings in epidemiologic studies may be due to chance.

There have been very few studies of the other gene variants that we examined in relation to adult brain tumors. Null results similar to ours have previously been reported for associations of glioma incidence with *CYP1A1* I462V (Trizna et al., 1998) and *NQO1* P187S (Peters et al., 2001). Our data indicate that these geno-

Table 3. Gene variant associations with brain tumor incidence, subgroup analyses (OR and 95% CI)[a,b]

Gene Variant and Subgroup	Controls Variant n (%)[c]	Glioma		Meningioma		Acoustic Neuroma	
		Variant n (%)	OR (95% CI)	Variant n (%)	OR (95% CI)	Variant n (%)	OR (95% CI)
CYP1A1 I462V AG or GG							
Age < 50	32 (11.0)	23 (12.6)	1.4 (0.7–2.5)	8 (13.6)	0.8 (0.3–2.0)	2 (6.1)	0.4 (0.1–1.8)
Age ⩾ 50	21 (8.3)	17 (8.5)	1.1 (0.5–2.1)	10 (10.2)	1.2 (0.5–2.8)	2 (5.3)	0.7 (0.1–3.5)
Male	17 (6.7)	18 (10.4)	1.7 (0.8–3.4)	5 (14.7)	1.5 (0.4–6.1)	0	0.0 (0.0–∞)
Female	36 (12.4)	22 (10.5)	0.9 (0.5–1.8)	13 (10.6)	0.9 (0.4–1.9)	4 (8.9)	0.7 (0.2–2.5)
Never smoked	26 (13.5)	16 (9.2)	0.8 (0.4–1.6)	10 (15.4)	1.2 (0.5–2.9)	2 (5.3)	0.3 (0.1–1.5)
Ever smoked	27 (7.9)	23 (11.6)	1.6 (0.9–2.9)	8 (8.9)	1.0 (0.4–2.7)	2 (6.7)	0.8 (0.2–4.2)
CYP1B1 V432L GG							
Age < 50	46 (16.5)	35 (19.9)	1.0 (0.6–1.9)	4 (7.0)	0.3 (0.1–1.2)	6 (20.0)	1.4 (0.4–4.7)
Age ⩾ 50	53 (22.2)	33 (17.2)	1.1 (0.6–1.9)	15 (15.8)	0.6 (0.3–1.4)	5 (13.2)	0.7 (0.2–2.2)
Male	49 (20.8)	33 (16.1)	0.8 (0.5–1.5)	6 (18.8)	0.5 (0.1–2.0)	5 (20.0)	1.2 (0.3–4.6)
Female	50 (17.7)	35 (21.5)	1.5 (0.8–2.7)	13 (10.8)	0.5 (0.2–1.2)	6 (14.0)	0.7 (0.2–2.3)
Never smoked	33 (17.8)	34 (20.2)	1.0 (0.5–2.0)	8 (12.5)	0.4 (0.1–1.4)	6 (15.8)	0.9 (0.2–3.2)
Ever smoked	65 (20.0)	32 (16.8)	1.2 (0.7–2.2)	9 (10.6)	0.4 (0.2–1.0)	5 (18.5)	1.8 (0.4–7.0)
EPHX1 Y113H CC							
Age < 50	25 (8.8)	15 (8.3)	0.9 (0.4–1.8)	5 (8.6)	1.2 (0.4–3.7)	5 (16.1)	2.2 (0.6–8.3)
Age ⩾ 50	18 (7.4)	29 (15.1)	2.0 (1.0–3.9)	12 (12.5)	1.8 (0.8–4.1)	3 (7.9)	1.5 (0.3–6.2)
Male	24 (9.9)	23 (13.8)	1.1 (0.6–2.3)	2 (6.3)	0.5 (0.1–3.7)	4 (15.4)	1.2 (0.3–5.2)
Female	19 (6.7)	21 (10.2)	2.2 (1.1–4.6)	15 (12.3)	1.9 (0.9–4.4)	4 (9.3)	1.6 (0.4–6.1)
Never smoked	20 (10.6)	16 (9.5)	1.2 (0.5–2.7)	10 (15.2)	1.3 (0.5–3.6)	3 (7.9)	0.5 (0.1–2.7)
Ever smoked	22 (6.7)	27 (14.0)	2.2 (1.2–4.2)	6 (7.0)	1.2 (0.4–3.6)	5 (17.9)	4.0 (0.9–18.0)
GSTM3 *B/*B							
Age < 50	6 (2.1)	8 (4.6)	2.6 (0.8–8.8)	4 (7.1)	3.7 (0.7–18.6)	0	0.0 (0.0–∞)
Age ⩾ 50	6 (2.4)	6 (3.1)	2.1 (0.6–7.8)	6 (6.4)	4.2 (1.0–17.1)	0	0.0 (0.0–∞)
Male	5 (2.1)	6 (2.9)	2.2 (0.5–10.1)	5 (16.1)	8.8 (1.6–49.2)	0	0.0 (0.0–∞)
Female	7 (2.5)	8 (4.9)	3.2 (1.0–10.0)	5 (4.2)	1.9 (0.5–7.2)	0	0.0 (0.0–∞)
Never smoked	6 (3.2)	6 (3.9)	2.0 (0.4–9.7)	7 (8.2)	1.8 (0.2–16.8)	0	0.0 (0.0–∞)
Ever smoked	6 (1.8)	8 (4.3)	4.1 (1.3–12.6)	3 (4.8)	8.6 (2.3–32.5)	0	0.0 (0.0–∞)
NQO1 P187S CT or TT							
Age < 50	73 (27.7)	61 (35.9)	1.5 (1.0–2.3)	16 (28.6)	1.0 (0.5–1.9)	12 (41.4)	1.7 (0.7–4.0)
Age ⩾ 50	76 (32.9)	60 (33.2)	1.0 (0.6–1.5)	26 (28.3)	0.6 (0.3–1.1)	10 (29.4)	1.0 (0.4–2.3)
Male	63 (28.5)	52 (33.6)	1.4 (0.9–2.1)	7 (21.9)	0.6 (0.2–1.7)	14 (60.9)	4.8 (1.8–12.8)
Female	86 (31.4)	69 (35.2)	1.1 (0.7–1.8)	35 (30.2)	0.8 (0.5–1.3)	8 (20.0)	0.6 (0.2–1.4)
Never smoked	62 (34.4)	54 (34.8)	1.0 (0.6–1.7)	16 (24.6)	0.4 (0.2–0.9)	10 (30.3)	0.9 (0.4–2.1)
Ever smoked	85 (27.7)	63 (34.1)	1.4 (0.9–2.1)	26 (32.1)	1.2 (0.7–2.1)	11 (40.7)	2.1 (0.8–5.4)

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]All estimates adjusted for matching factors including age, gender, race, hospital, and distance of residence from hospital.

[b]Within each subgroup, the more common homozygous genotype was used as the referent.

[c]The number of subjects included in each model may differ depending on the number of samples successfully genotyped for each variant.

types are also not associated with the risk of meningioma or acoustic neuroma. However, there was some indication that *NQO1* might interact with other genes that are involved in quinone metabolism, namely, *CYP2E1 RsaI*. In our previous research on metabolic gene variants and the risk of adult brain tumors (De Roos et al., 2003), there were modest associations of *CYP2E1 RsaI* and *GSTP1* I105V variant genotypes with increased glioma and acoustic neuroma incidence, and there was some indication of a positive interaction between the gene variants for those tumor types. Results from the current study further underscore the potential importance of multiple gene variants of various biologic pathways in carcinogenesis.

There have been no previous studies of the *CYP1B1* V432L variant in association with brain tumors; our data showed an inverse association of the variant with meningioma only. Given the increased catalytic activity of the valine product, which produces a toxic intermediate, we would have expected increased risk asso-

Table 4. Combined gene variant associations with brain tumor incidence (OR and 95% CI)[a]

Gene Variant[b]	Controls	Glioma		Meningioma		Acoustic Neuroma	
		Cases	OR (95% CI)	Cases	OR (95% CI)	Cases	OR (95% CI)
NQO1 P187S and *CYP2E1 RsaI*							
Neither	318 (58.6)	211 (55.2)	1.0	95 (60.1)	1.0	35 (50.0)	1.0
NQO1 variant only	141 (26.0)	106 (27.8)	1.1 (0.8–1.5)	38 (24.1)	0.8 (0.5–1.2)	18 (25.7)	1.2 (0.7–2.2)
CYP2E1 variant only	20 (3.7)	13 (3.4)	0.8 (0.4–1.6)	8 (5.1)	1.4 (0.6–3.6)	5 (7.1)	2.3 (0.8–6.9)
Both variants	7 (1.3)	12 (3.1)	3.0 (1.1–8.0)[c]	4 (2.5)	0.9 (0.2–3.6)	4 (5.7)	4.1 (1.0–16.9)

Abbreviations: CI, confidence interval; OR, odds ratio.

[a]Estimates are adjusted for matching factors including age, gender, race, hospital, and distance of residence from hospital.

[b]Variant alleles for each gene are as follows: *NQO1* P187S CT or TT and *CYP2E1 RsaI*, *CYP2E1*1A/CYP2E1*5*, or *CYP2E1*5/CYP2E1*5*

[c]*P* value for multiplicative interaction term = 0.05.

ciated with the variant for a biologic pathway relevant to aromatic hydrocarbons. *CYP1B1* is also involved in steroid hormone metabolism, however, and lower levels of estradiol have been observed among women with the valine product (De Vivo et al., 2002; Garcia-Closas et al., 2002). The valine product has been associated with increased risks of ovarian cancer (Goodman et al., 2001) and estrogen-receptor-positive breast cancer (Bailey et al., 1998) and, conversely, with decreased risk of breast cancer in a study of Asian women (Zheng et al., 2000). Risk factors for meningioma include various hormone-related factors such as gender and parity (Inskip, P.D., et al., 1995), and it is possible that the relevance of the observed association with *CYP1B1* V432L is through an effect on endogenous hormones, although the mechanisms are unclear. Nevertheless, the association between the *CYP1B1* variant and meningioma was similar in men and women, which detracts from the evidence for a hormone-related pathway explaining the gene variant's association with brain tumors.

The results for *EPHX1* Y113H were suggestive of associations within certain subgroups. A slightly increased risk associated with *EPHX1* 113 His/His for glioma was predominated by elevated risks among older subjects (age > 50), women, and ever-smokers. The patterns were less clear for meningioma and acoustic neuroma. The fact that a slight increased risk appears for all three tumor types suggests the possibility of an unusual genotype distribution among the control group. However, we found similar results upon examination of differing compositions of the control group (ORs ranging from 1.2 to 1.7 for glioma in analyses excluding one control discharge diagnosis group at a time). In the context of PAH exposure, we would hypothesize increased risk of cancer associated with the lower predicted activity of the His/His protein product; nevertheless, *EPHX1* high-activity alleles have been associated in some studies with increased risk for various types of cancer (Lancaster et al., 1996; Lee et al., 2002; Park et al., 2003; Wang et al., 2003), which suggests that these gene variants may play a more complex role in human carcinogenesis. The coding region substitution we studied accounts for only a fraction of all variation in human microsomal epoxide hydrolase activity (Hassett et al., 1997; Raaka et al., 1998). Another known variant at codon 139 (H139R) has been observed in vitro to increase *EPHX1* enzyme activity by approximately 25% (for the 139 arginine protein), and additional genetic variants, possibly in regulatory regions of the *EPHX1* gene, may also play a role (Hassett et al., 1997; Omiecinski et al., 2000).

These results add to the literature about the contribution of variation in metabolic genes to adult brain tumor incidence. Overall, these data do not provide strong evidence for the consistent importance of genes involved in biotransformation of PAHs and their metabolites for brain carcinogenesis; however, specific genes may play a role in the context of both PAHs and other exposures. Our observation of a positive association of *GSTM3* *B/*B genotype with increased risk of glioma and meningioma, while intriguing, requires replication. Null results for other genotypes do not rule out possible interactions of the gene variants with relevant substrates, or with other genes involved in brain carcinogenesis. For example, our data indicate that *NQO1* might interact with other genes that are involved in benzene metabolism, such as *CYP2E1*. Future analyses in our study will focus on detailed examination of genotype–exposure interactions for targeted occupational exposures of interest, including PAHs and other aromatic hydrocarbons.

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