

Genetic Polymorphisms in *GSTM1*, *-P1*, *-T1*, and *CYP2E1* and the Risk of Adult Brain Tumors

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Abstract

GST and *CYP2E1* genes are involved in metabolism of several compounds (e.g., solvents) that may play a role in brain cancer etiology. We evaluated associations between polymorphisms in these genes and adult brain tumor incidence. Cases were 782 patients with brain tumors diagnosed from 1994 to 1998 at three United States hospitals. Controls were 799 patients admitted to the same hospitals for nonmalignant conditions. DNA was extracted from blood samples that had been collected from 1277 subjects (80% of all subjects; 604 controls; 422 gliomas, 172 meningiomas, and 79 acoustic neuromas), and genotyping was successfully conducted for *GSTM1* null, *GSTT1* null, *GSTP1* I105V, *GSTP1* A114V, *CYP2E1* RsaI, and *CYP2E1* Ins96. The *GSTP1* 105 Val/Val genotype was associated with increased glioma incidence [odds ratio (OR), 1.8; 95% confidence limits (CLs), 1.2, 2.7], with the estimated effect following a trend of increasing magnitude by number of variant alleles (*Ile/Ile*: OR, 1.0; *Ile/Val*: OR, 1.3; *Val/Val*: OR, 2.1). The *CYP2E1* RsaI variant was weakly associated with glioma (OR, 1.4; 95% CL, 0.9, 2.4) and acoustic neuroma (OR, 2.3; 95% CL, 1.0, 5.3), with some indication of stronger associations among younger subjects. Estimated effects of the gene variants differed by glioma subtype. There was evidence of supermultiplicativity of the joint effect of *GSTP1* I105V and *CYP2E1* RsaI variants on both glioma and acoustic neuroma, even following adjustment of estimates toward a common prior distribution using hierarchical regression models. Previously reported associations between the *GSTT1* null genotype and overall glioma incidence were not replicated, but an association

with meningioma was observed (OR, 1.5; 95% CL, 1.0, 2.3). These findings may provide clues to both genetic and environmental determinants of brain tumors.

Introduction

The causes of brain tumors in adults are poorly understood. High doses of ionizing radiation and certain rare genetic disorders have been consistently associated with increased incidence, although such established risk factors explain only a small proportion of these heterogeneous malignancies (1, 2). There is some evidence that solvents, pesticides, and polycyclic aromatic hydrocarbons may be risk factors for brain tumors (1–9); however, these inferences are generally based on analyses of occupational job title rather than specific chemical exposures. Because individuals experience multiple occupational and environmental exposures, it may be useful to look for patterns of association between brain tumor incidence and genes involved in the metabolism of important categories of chemicals, because such genes can influence the body's ability to interact with multiple chemical substrates.

*GST*² and *CYP* genes encode enzymes involved in the activation and detoxification of a wide variety of chemicals. *GST* genes, including mu (M), pi (P), and theta (T) *GSTs*, produce enzymes that catalyze reduced glutathione-dependent reactions with compounds containing an electrophilic center (10). The range of potential *GST* substrates is very large, including occupational and environmental carcinogens such as solvents, pesticides, and polycyclic aromatic hydrocarbons. Polymorphisms in *GSTM1* and *GSTT1* result in absence of a functional gene product (11, 12). Two polymorphisms in *GSTP1* have been discovered (I105V, A114V) for which effects on function are not known (13), although there is some indication of decreased enzyme activity among individuals with genotypes containing the 105 valine allele (14). Another gene hypothesized to play a role in human brain tumors is *CYP2E1*, which metabolizes and activates solvents that also act to induce its expression, including benzene, styrene, carbon tetrachloride, ethylene glycol, and ethanol (15). The frequencies of *CYP2E1* variant sequences, including RsaI and Ins96, differ considerably between ethnic groups (15, 16).

Persons with variant alleles for *GST* and *CYP2E1* genes may differ in their ability to metabolize carcinogenic compounds and, thus, may have an altered risk of cancer. Associations between *GSTT1* null genotype and increased incidence of meningioma ($n = 50$) and glioma subtype astrocytoma ($n = 112$) were observed in a hospital-based case-control study (17). A second case-control study reported no association between *GSTT1* and glioma ($n = 118$), although an association with the

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² The abbreviations used are: *GST*, glutathione S-transferase; *CYP*, cytochrome P450; NIEHS, National Institute of Environmental Health Sciences; OR, odds ratio; CL, confidence limit.

glioma subtype oligodendroglioma ($n = 16$) was observed (18). There was no difference in the distribution of *GSTT1* genotypes between glioma cases ($n = 90$) and controls in a third study (19). All three studies reported no overall association between *GSTM1* null genotype and glioma (17, 19, 20). Other tumor types, such as acoustic neuroma, have not been extensively studied, nor have associations between brain tumors and *GSTP1* or *CYP2E1* variants. To evaluate relationships between adult brain tumor incidence and genes involved in the metabolism of major categories of chemicals previously associated with the risk of brain tumors in adults, we examined the effects of polymorphisms in *GST* and *CYP2E1* genes in a side-by-side comparison of three major categories of malignant and benign brain tumors, namely the gliomas, meningiomas, and acoustic neuromas.

Patients and Methods

Study Population. The study has been described in detail elsewhere (21). Eligible cases were adult patients with intracranial tumors including glioma, meningioma, or acoustic neuroma (referred to as "brain tumors" here) newly diagnosed from 1994 to 1998, and treated at one of three participating United States hospitals located in Phoenix, Arizona; Boston, Massachusetts; and Pittsburgh, Pennsylvania. We sought approval of physicians to contact newly diagnosed brain tumor cases for recruitment into the study. We enrolled 489 glioma, 197 meningioma, and 96 acoustic neuroma patients, for a total of 782 patients with malignant or benign brain tumors, representing 92% of those contacted. Information on tumor pathology was based on the diagnosis from each hospital.

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, sex, 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 administered a structured, computerized questionnaire that included detailed questions on: lifetime job history; specific occupational exposures, processes, and tasks; cellular telephones and other forms of communication devices; exposure to diagnostic and therapeutic radiation and other aspects of medical history; reproductive characteristics and history; use of hair dyes; and family history of cancer and selected other conditions.

Laboratory Analyses. DNA was extracted from peripheral WBCs (buffy coat or granulocytes) from blood samples collected from 1277 subjects [80% of all subjects; 422 gliomas (86%), 172 meningiomas (87%), 79 acoustic neuromas (86%), and 604 controls (76%)], by GenoType, Ltd. in the United Kingdom, using a phenol-chloroform method as described by Daly *et al.* (22). *GSTT1* genotyping was conducted by GenoType, Ltd. Genotyping assays for *GSTM1*, *GSTP1*, and *CYP2E1* polymorphisms were conducted by the NIEHS.

GenoType, Ltd. determined *GSTT1* genotype using an allele-specific PCR-based method described previously (11). At the NIEHS, genotyping was performed using 50 ng of genomic DNA and PCR-based methods. For *GSTM1*, an allele-specific PCR method was used (23). Analysis for *GSTP1* I105V variant genotypes used a restriction fragment length polymorphism-PCR method (14). To detect the *GSTP1* A114V single nucle-

otide polymorphism, the NIEHS laboratory used a melting temperature-shift genotyping method using two allele-specific forward primers of different lengths and melting temperatures and a common reverse primer (24). The NIEHS laboratory developed a novel PCR multiplex method for the *CYP2E1* RsaI (restriction fragment length polymorphism-PCR) and Ins96 (allele specific-PCR) genotypes using published sequence information (16, 25).

Quality control measures included the addition of replicates [68 samples from 3 individuals who were not study subjects (QC-A, $n = 34$; QC-B, $n = 19$; QC-C, $n = 15$), collected and processed in identical fashion as study subjects] interspersed throughout the batches for all six genotyping assays and duplicates (two samples for each of 92 individuals who were study subjects) interspersed throughout the batches for all assays, except *GSTT1*.

Statistical Analyses. SAS software versions 6.12 and 8.0 (SAS Institute Inc., Cary, NC), and Epicure for Windows (1998; Hirosoft International Corporation, Seattle, WA) were used for statistical analyses. We computed Hardy-Weinberg equilibrium for *GSTP1* and *CYP2E1* genotypes among the control group, to determine whether the distribution of alleles was as expected (the *GSTM1* and *-T1* genotypes were coded as wild type or null, making direct calculation of Hardy-Weinberg equilibrium impossible).

The effect of each gene variant on the incidence of each brain tumor type, using the nonvariant genotype as the referent, was estimated by conventional maximum likelihood using unconditional logistic regression to calculate ORs and 95% CLs. All effect estimates for gene variants were adjusted for the study matching factors of age (coded in years: 18–29; 30–39 as the referent; 40–49; 50–59; 60–69; 70–79; 80–99), race (non-Hispanic white as the referent; Hispanic white; black; other), sex (male; female as the referent), hospital (Phoenix, AZ, as the referent; Boston, MA; Pittsburgh, PA), and proximity of patient's residence to the hospital (coded in miles: 0–4 as the referent; 5–14; 15–29; 30–49; 50 or more). We checked the influence of the control series composition on results by examining the main effect of each genotype on the three tumor types, while excluding one major category of control discharge diagnoses at a time.

In addition to models in which each of the variant genotypes was treated separately, one model for each tumor type analyzed all six genotypes simultaneously, using penalized quasi-likelihood hierarchical regression modeling (26, 27) in SAS/GLIMMIX (28). We chose to use hierarchical regression modeling because simulation studies have indicated that estimates from this approach are generally more accurate and stable than those calculated using conventional likelihood methods, especially when considering multiple exposures and sparse data (26). Because previous results on *GSTT1* and *GSTM1* genotypes and brain tumors were few and were conflicting for *GSTT1*, we assumed that we did not have prior knowledge indicating any one variant genotype as more likely to be associated with brain tumors than any other genotype. Therefore, the true effects for the six gene variants were assumed to be exchangeable, random effects, arising from a common prior normal distribution with an unknown mean and a variance of 0.35; thus assuming, with 95% certainty, that the true rate ratio for each gene variant would fall within a 10-fold range (note: for a 10-fold range, residual variance = $(1n(10))/3.92^2 \approx 0.35$) (26). These values for the prior distribution were chosen to insure that the expected value of the betas for the gene variants would be the mean of all the betas, and that large

Table 1 Frequencies of characteristics of brain tumor cases and controls from three United States hospitals (1994–1998)

Characteristic	Controls (n = 799)	Glioma (n = 489)	Meningioma (n = 197)	Acoustic neuroma (n = 96)	All brain tumors (n = 782)
Sex					
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					
White	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 (yr)					
≤30	113 (14.1%)	63 (12.9%)	4 (2%)	4 (4.2%)	71 (9.1%)
31–50	320 (40.1%)	177 (36.2%)	78 (39.6%)	41 (42.7%)	296 (37.9%)
51–70	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					
<HS ^a	105 (13.1%)	64 (13.1%)	24 (12.2%)	5 (5.2%)	93 (11.9%)
HS or GED	234 (29.3%)	122 (24.9%)	57 (28.9%)	28 (29.1%)	207 (26.5%)
1–3 yr college	245 (30.7%)	130 (26.6%)	68 (34.5%)	21 (21.9%)	219 (28.0%)
4 yr 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, AZ	405 (50.7%)	244 (49.9%)	99 (50.3%)	72 (75.0%)	415 (53.0%)
Boston, MA	220 (27.5%)	153 (31.3%)	79 (40.1%)	22 (22.9%)	254 (32.5%)
Pittsburgh, PA	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%)

^a HS, high school; GED, general equivalency diploma.

magnitude values for effects of the gene variants would rarely occur, consistent with a prior assumption of probably modest effects of gene variants in the context of background exposures.

We examined each gene variant-disease association separately for three age groups (≤40 years, 40–60 years, >60 years), and for each sex. Other factors of interest, such as race or family history of nervous system tumors, did not have sufficient numbers in subgroups to allow stratified analyses. We also examined the association of each gene variant with high- and low-grade tumors, and with specific glioma subtypes including glioblastoma, anaplastic astrocytoma, other astrocytoma, oligodendroglioma, and mixed oligoastrocytoma. χ^2 statistics and corresponding *P*s (based on 4 degrees of freedom) were calculated to test whether the distribution of each gene variant differed between the five glioma subtypes.

We evaluated associations of brain tumor incidence with several combinations of *GSTP1* variants. To evaluate the risk associated with increasing numbers of variant *GSTP1* valine alleles, we estimated the effects of *GSTP1* I105V heterozygous (*Ile/Val*) and homozygous (*Val/Val*) variant genotypes, compared with the wild type (*Ile/Ile*). Because there are demonstrated differences in structure and stability of *GSTP1* proteins expressed from alleles with different combined I104V and A114V variants (13), we estimated effects of *GSTP1* alleles with different variant combinations (*GSTP1**A, wild type for both; *GSTP1**B, 105 *Val/Val* and 114 *Ala/Ala*; *GSTP1**C, 105 *Val/Val* and 114 *Ala/Val* or *Val/Val*). Trend tests were con-

ducted where a monotonic trend by increasing number of variant alleles was observed, by calculating a *P* for the beta coefficient in a logistic regression model with the exposure coded as an ordinal categorical variable.

Data were analyzed for potential interactions of gene variants that were associated with any of the brain tumor types in our study (namely, *CYP2E1* RsaI, *GSTP1* I105V, and *GSTT1* null). Individual and joint effects of each gene variant combination were estimated to assess potential interaction, and likelihood ratio tests were used to test gene-gene interactions on the multiplicative and additive scales using Epicure software. Because estimates of individual and joint effects from logistic regression models were very imprecise due to small numbers of subjects with combined variant genotypes, we also used hierarchical regression models to shrink unstable estimates toward a common mean. These models treated the parameters for the joint exposure and two independent exposures as arising from the same prior distribution with an unknown mean and a variance of 0.35.

Results

Cases and controls in the study were comparable with respect to race (Table 1). Cases, on average, were older and more highly educated than controls. Meningioma cases were more often female compared with controls or the other tumor types. Genotyping was successfully conducted for *GSTMI* null

Table 2 Genotype frequencies among brain tumor cases and controls^a

Genotype	Controls (n = 604) ^b	Glioma (n = 422) ^b	Meningioma (n = 172) ^b	Acoustic neuroma (n = 79) ^b
<i>GSTM1</i>				
Present	254 (44.2%)	191 (47.4%)	85 (50.3%)	34 (46.6%)
Null	321 (55.8%)	212 (52.6%)	84 (49.7%)	39 (53.4%)
<i>GSTP1</i> I105V				
Ile/Ile or Ile/Val	508 (89.6%)	329 (83.1%)	156 (92.9%)	63 (87.5%)
Val/Val	59 (10.4%)	67 (16.9%)	12 (7.1%)	9 (12.5%)
<i>GSTP1</i> A114V				
Ala/Ala	498 (86.5%)	345 (85.4%)	149 (87.6%)	63 (86.3%)
Ala/Val or Val/Val ^c	78 (13.5%)	59 (14.6%)	21 (12.4%)	10 (13.7%)
<i>GSTT1</i>				
Present	445 (81.6%)	309 (80.1%)	121 (76.1%)	59 (84.3%)
Null	100 (18.4%)	77 (19.9%)	38 (23.9%)	11 (15.7%)
<i>CYP2E1</i> RsaI				
<i>CYP2E1</i> *1A/ <i>CYP2E1</i> *1A	540 (94.2%)	367 (91.8%)	155 (92.3%)	65 (87.8%)
<i>CYP2E1</i> *1A/ <i>CYP2E1</i> *5 or <i>CYP2E1</i> *5/ <i>CYP2E1</i> *5 ^c	33 (5.8%)	33 (8.2%)	13 (7.7%)	9 (12.2%)
<i>CYP2E1</i> Ins96				
<i>CYP2E1</i> *1C/ <i>CYP2E1</i> *1C	535 (93.0%)	385 (95.3%)	155 (92.3%)	72 (97.3%)
<i>CYP2E1</i> *1C/ <i>CYP2E1</i> *1D or <i>CYP2E1</i> *1D/ <i>CYP2E1</i> *1D ^c	40 (7.0%)	19 (4.7%)	13 (7.7%)	2 (2.7%)

^aFrequencies are calculated from the total number of samples successfully genotyped for each variant.

^bNumber represents the total with blood samples.

^cVariant includes heterozygous and homozygous genotypes; <1% were homozygous variant.

Table 3 ORs and 95% CLs for the association of each gene variant with brain tumor incidence^a

Gene variant	Glioma (422 cases, 604 controls) ^b		Meningioma (172 cases, 604 controls) ^b		Acoustic neuroma (79 cases, 604 controls) ^b	
	Variant cases/controls [n (%)]	OR (95% CL)	Variant cases/controls [n (%)]	OR (95% CL)	Variant cases/controls [n (%)]	OR (95% CL)
<i>GSTM1</i> null	212 (52.6%)/321 (55.8%)	0.9 (0.7, 1.1)	84 (49.7%)/321 (55.8%)	0.9 (0.6, 1.3)	39 (53.4%)/321 (55.8%)	0.9 (0.6, 1.6)
<i>GSTP1</i> 105 Val/Val	67 (16.9%)/59 (10.4%)	1.8 (1.2, 2.7)	12 (7.1%)/59 (10.4%)	0.7 (0.4, 1.5)	9 (12.5%)/59 (10.4%)	1.3 (0.6, 2.9)
<i>GSTP1</i> 114 Ala/Val or Val/Val	59 (14.6%)/78 (13.5%)	1.0 (0.7, 1.5)	21 (12.4%)/78 (13.5%)	0.9 (0.5, 1.6)	10 (13.7%)/78 (13.5%)	1.2 (0.6, 2.5)
<i>GSTT1</i> null	77 (19.9%)/100 (18.4%)	1.1 (0.8, 1.5)	38 (23.9%)/100 (18.4%)	1.5 (1.0, 2.4)	11 (15.7%)/100 (18.4%)	0.9 (0.4, 1.8)
<i>CYP2E1</i> RsaI						
<i>CYP2E1</i> *1A/ <i>CYP2E1</i> *5 or <i>CYP2E1</i> *5/ <i>CYP2E1</i> *5	33 (8.3%)/33 (5.8%)	1.4 (0.9, 2.4)	13 (7.7%)/33 (5.8%)	1.3 (0.6, 2.6) ^c	9 (12.2%)/33 (5.8%)	2.3 (1.0, 5.3) ^c
<i>CYP2E1</i> Ins96						
<i>CYP2E1</i> *1C/ <i>CYP2E1</i> *1D or <i>CYP2E1</i> *1D/ <i>CYP2E1</i> *1D	19 (4.7%)/40 (7.0%)	0.7 (0.4, 1.2)	13 (7.7%)/40 (7.0%)	0.8 (0.4, 1.7)	2 (2.7%)/40 (7.0%)	0.4 (0.1, 1.7) ^c

^aEstimates within each cell are from individual unconditional logistic regression models for each gene variant; all estimates adjusted for matching factors including age, sex, race, hospital and distance of residence from hospital.

^bNumbers represent the total with blood samples; the number included in each model may differ depending on the number of samples successfully genotyped for each variant.

^cEstimate from hierarchical regression model differed >10% from maximum likelihood estimate (meningioma: *CYP2E1* RsaI, OR = 1.0; acoustic neuroma: *CYP2E1* RsaI, OR = 1.3; acoustic neuroma *CYP2E1* Ins96, OR = 0.7).

(97.5% of all samples analyzed), *GSTP1* I105V (96.5%), *GSTP1* A114V (97.9%), *GSTT1* null (90.8%), *CYP2E1* RsaI (97.3%), and *CYP2E1* Ins96 (97.8%), and genotyping of all six variants was successful for 89% of the samples analyzed for all six genotypes. Missing values, primarily the result of insufficient quantity of DNA or poor amplification for a specific locus or overall, were equally likely to be from case or control samples. We achieved 99–100% agreement between duplicate samples, and among replicates for *GSTM1*, *GSTP1*, and *CYP2E1* assays, and 95% agreement among replicate samples analyzed for *GSTT1*.

Prevalences of variant genotypes in the control group (Table 2) were *GSTM1* null (55.8%), *GSTP1* 105 Val/Val (10.4%), *GSTP1* 114 Ala/Val and Val/Val (13.5%), *GSTT1* null (18.4%), *CYP2E1* RsaI *CYP2E1**1A/*CYP2E1**5 and *CYP2E1**5/*CYP2E1**5 (5.8%), and *CYP2E1* Ins96

*CYP2E1**1C/*CYP2E1**1D and *CYP2E1**1D/*CYP2E1**1D (7.0%), similar to published values (10, 15, 16, 29). There was no evidence of departure from Hardy-Weinberg equilibrium for the *GSTP1* or *CYP2E1* genotypes. There was significant linkage disequilibrium between the *GSTP1* I105V and A114V genotypes among controls ($\chi^2 = 159.2$, $P = 0.001$), largely caused by the absence of the combined 105 (Ile/Ile)/114 (Ala/Val or Val/Val) genotypes. *GSTP1* I105V and *CYP2E1* Ins96 genotypes were also statistically associated among controls ($\chi^2 = 5.0$, $P = 0.02$).

ORs for associations between gene variants and the risk of each brain tumor type are shown in Table 3. The *GSTP1* 105 Val/Val genotype was associated with an 80% increased glioma incidence. Meningioma was not strongly associated with any of the genotypes examined, but there was a weak association with *GSTT1* (Table 3; OR, 1.5; 95% CL, 1.0, 2.3). The *CYP2E1* RsaI

Table 4 ORs and 95% CLs for the association of each gene variant with brain tumor incidence, stratified by age^{a,b}

Gene variant	Glioma (422 cases, 604 controls) ^c		Meningioma (172 cases, 604 controls) ^c		Acoustic neuroma (79 cases, 604 controls) ^c	
	Variant cases/controls [n (%)]	OR (95% CL)	Variant cases/controls [n (%)]	OR (95% CL)	Variant cases/controls [n (%)]	OR (95% CL)
<i>GSTP1</i> 105 Val/Val		1.8 (1.2, 2.7)		0.7 (0.4, 1.5)		1.3 (0.6, 2.9)
Age ≤40	23 (19.8%)/19 (10.3%)	2.2 (1.1, 4.3)	3 (10.7%)/19 (10.3%)	1.0 (0.2, 4.9)	3 (15.8%)/19 (10.3%)	1.6 (0.3, 7.2)
Age 40–60	29 (19.9%)/27 (11.8%)	1.9 (1.1, 3.4)	6 (7.4%)/27 (11.8%)	0.7 (0.3, 1.9)	5 (13.9%)/27 (11.8%)	1.4 (0.5, 4.2)
Age >60	15 (11.2%)/13 (8.4%)	1.3 (0.6, 3.0)	3 (5.1%)/13 (8.4%)	0.5 (0.1, 2.2)	1 (5.9%)/13 (8.4%)	0.5 (0.1, 2.3)
<i>GSTT1</i> null		1.1 (0.8, 1.5)		1.5 (1.0, 2.4)		0.9 (0.4, 1.8)
Age ≤40	29 (25.4%)/35 (19.2%)	1.3 (0.8, 2.4)	10 (35.7%)/35 (19.2%)	2.1 (0.8, 5.5)	2 (11.1%)/35 (19.2%)	0.6 (0.1, 3.1)
Age 40–60	23 (15.8%)/41 (18.8%)	0.8 (0.4, 1.4)	18 (23.4%)/41 (18.8%)	1.4 (0.7, 2.8)	3 (8.3%)/41 (18.8%)	0.4 (0.1, 1.6)
Age >60	25 (19.8%)/24 (16.6%)	1.3 (0.7, 2.4)	10 (18.5%)/24 (16.6%)	1.4 (0.6, 3.3)	6 (37.5%)/24 (16.6%)	2.5 (0.7, 8.5)
<i>CYP2E1</i> RsaI						
<i>CYP2E1</i> *1A/ <i>CYP2E1</i> *5 or <i>CYP2E1</i> *5/ <i>CYP2E1</i> *5		1.4 (0.9, 2.4)		1.3 (0.6, 2.6)		2.3 (1.0, 5.3)
Age ≤40	14 (11.9%)/8 (4.3%)	3.1 (1.2, 7.9)	2 (6.7%)/8 (4.3%)	1.8 (0.3, 11.3)	4 (19.0%)/8 (4.3%)	8.1 (1.7, 38.9)
Age 40–60	10 (6.9%)/14 (6.0%)	1.2 (0.5, 2.9)	8 (10.1%)/14 (6.0%)	1.5 (0.5, 4.2)	4 (11.1%)/14 (6.0%)	2.0 (0.6, 6.8)
Age >60	9 (6.6%)/11 (7.2%)	0.9 (0.4, 2.4)	3 (5.1%)/11 (7.2%)	0.7 (0.2, 2.7)	1 (5.9%)/11 (7.2%)	0.9 (0.1, 8.4)
<i>CYP2E1</i> Ins96						
<i>CYP2E1</i> *1C/ <i>CYP2E1</i> *1D or <i>CYP2E1</i> *1D/ <i>CYP2E1</i> *1D		0.7 (0.4, 1.2)		0.8 (0.4, 1.7)		0.4 (0.1, 1.7)
Age ≤40	7 (5.9%)/20 (10.7%)	0.5 (0.2, 1.2)	5 (16.7%)/20 (10.7%)	0.5 (0.1, 2.2)	0/20 (10.7%)	0.0 (0.0–∞)
Age 40–60	5 (3.4%)/13 (5.6%)	0.6 (0.2, 1.8)	4 (5.1%)/13 (5.6%)	0.7 (0.2, 2.4)	2 (5.6%)/13 (5.6%)	0.8 (0.2, 4.0)
Age >60	7 (5.1%)/7 (4.6%)	1.3 (0.4, 3.8)	4 (6.8%)/7 (4.6%)	1.4 (0.3, 5.4)	0/7 (4.6%)	0.0 (0.0–∞)

^a All estimates are adjusted for matching factors including age, sex, race, hospital, and distance of residence from hospital.

^b Within each age category, analyses of the association between each gene variant on the risk of each tumor type used the nonvariant genotype as the referent.

^c Numbers represent the total with blood samples; the number included in each model may differ depending on the number of samples successfully genotyped for each variant.

variant was weakly associated with increased incidence of all three tumor types, whereas *CYP2E1* Ins96 showed inverse associations; however, the small numbers of subjects with *CYP2E1* variant genotypes made estimates imprecise. There was no association of the *GSTM1* or *GSTP1* I114V variants with the risk of any tumor type. The results of conventional maximum likelihood estimation including each gene variant in a separate model were generally similar to those calculated using hierarchical regression models adjusting for all genotypes simultaneously, indicating little confounding between different genotypes. Differences in results between the two modeling strategies occurred where data were sparse; for example, the association between *CYP2E1* RsaI and acoustic neuroma appeared moderate in logistic regression modeling (OR, 2.3), but was shrunk considerably in hierarchical modeling (OR, 1.3), indicating a potentially weak effect. Analyses to check the sensitivity of our results to the control series composition did not indicate any major bias resulting from inclusion of any of the control discharge diagnoses. One exception was noted for the association between meningioma and *GSTT1* null genotype, for which the OR dropped from 1.5 to 1.2 when subjects with circulatory disease were excluded from the control group.

For those genotypes that showed associations with brain tumors, there was some indication of stronger associations among younger subjects (Table 4). Although the number of subjects in each age group did not support a formal assessment of heterogeneity of effect, the positive association of the *GSTP1* 105 Val/Val genotype with glioma decreased progressively, albeit modestly, across increasing age groups (age, ≤40 years; OR, 2.2; age, >60; OR, 1.3), as did associations for *GSTT1* null with meningioma (age, ≤40 years; OR, 2.1; age, >60; OR, 1.4). Whereas *GSTP1* 105 Val/Val was not strongly associated with acoustic neuroma overall incidence, there was an almost

3-fold increased risk among younger subjects (age, <50 years; OR, 2.9; 95% CL, 1.1, 7.8; results not shown in Table 4). Associations between the *CYP2E1* RsaI variant with all three tumor types followed the same pattern, with the strongest associations among those less than age 40. Similarly, inverse associations of the *CYP2E1* Ins96 variant were only observed among those aged 60 years or younger. There were no meaningful differences in associations between gene variants and brain tumors by sex (results not shown).

Although several glioma subtypes were positively associated with *GSTP1* I105V and *CYP2E1* RsaI variants, only anaplastic astrocytoma was moderately associated with both variants (Table 5). χ^2 tests indicated that the distribution of some genotypes differed between glioma subtypes, namely, *GSTM1*, *GSTP1* A114V, and *CYP2E1* RsaI. Some differences were observed for associations of several gene variants with oligodendroglioma, compared with the other subtypes, including an inverse association with *GSTM1* null, no association with *GSTP1* 105 Val/Val, and a positive association with *GSTP1* 114 Ala/Val or Val/Val. No apparent confounding factor accounted for these findings. There were no important differences between associations of gene variants with high- versus low-grade tumors (results not shown).

Association of the homozygous variant *GSTP1* 105 Val/Val genotype with glioma incidence (OR, 2.1; 95% CL, 1.4, 3.1) was stronger than that of the heterozygous Ile/Val genotype (OR, 1.3; 95% CL, 1.0, 1.7), demonstrating a trend of increasing magnitude of association by the number of variant alleles (Table 6). However, there was little evidence that the combined effect of both *GSTP1* I105V and A114V variants on the risk of any brain tumor type differed from that for the *GSTP1* I105V variant alone (Table 6).

There was some evidence for positive interaction of *GSTP1* I105V and *CYP2E1* RsaI variants on the risk of both

Table 5 ORs and 95% CLs for associations of gene variants with the incidence of glioma subtypes^a and χ^2 for the distribution of gene variants between glioma subtypes^b

Gene variant and glioma subtype	Variant cases [n (%)]	χ^2 statistic (P)	OR (95% CL)
<i>GSTM1</i> null			
All glioma combined	212 (52.6%)	10.4 (0.03)	0.9 (0.7, 1.1)
Glioblastoma	110 (57.0%)		0.9 (0.7, 1.3)
Anaplastic astrocytoma	30 (54.6%)		0.9 (0.5, 1.6)
Other astrocytoma	9 (40.9%)		0.4 (0.2, 1.1)
Oligodendroglioma	15 (32.6%)		0.4 (0.2, 0.7)
Mixed oligoastrocytoma	12 (46.2%)		0.6 (0.3, 1.4)
<i>GSTP1</i> 105 Val/Val			
All glioma combined	67 (16.9%)	5.8 (0.22)	1.8 (1.2, 2.7)
Glioblastoma	34 (17.8%)		2.2 (1.4, 3.7)
Anaplastic astrocytoma	12 (22.2%)		2.5 (1.2, 5.1)
Other astrocytoma	1 (4.6%)		0.4 (0.04, 3.2)
Oligodendroglioma	4 (8.9%)		0.7 (0.2, 2.1)
Mixed oligoastrocytoma	4 (15.4%)		1.8 (0.6, 5.6)
<i>GSTP1</i> 114 Ala/Val or Val/Val			
All glioma combined	59 (14.6%)	9.0 (0.06)	1.0 (0.7, 1.5)
Glioblastoma	27 (14.0%)		0.8 (0.5, 1.4)
Anaplastic astrocytoma	9 (16.4%)		1.3 (0.6, 2.8)
Other astrocytoma	4 (18.2%)		0.4 (0.4, 4.6)
Oligodendroglioma	12 (25.5%)		2.6 (1.2, 5.5)
Mixed oligoastrocytoma	0		0.0 (0.0, ∞)
<i>GSTT1</i> null			
All glioma combined	77 (19.9%)	1.8 (0.77)	1.1 (0.8, 1.5)
Glioblastoma	34 (18.1%)		1.0 (0.6, 1.6)
Anaplastic astrocytoma	10 (19.6%)		1.0 (0.5, 2.2)
Other astrocytoma	4 (20.0%)		1.0 (0.3, 3.2)
Oligodendroglioma	12 (26.7%)		1.5 (0.7, 3.0)
Mixed oligoastrocytoma	4 (16.7%)		0.8 (0.3, 2.4)
<i>CYP2E1</i> RsaI <i>CYP2E1</i> *1A/ <i>CYP2E1</i> *5 or <i>CYP2E1</i> *5/ <i>CYP2E1</i> *5			
All glioma combined	33 (8.3%)	10.3 (0.04)	1.4 (0.9, 2.4)
Glioblastoma	10 (5.2%)		0.9 (0.4, 1.9)
Anaplastic astrocytoma	10 (18.2%)		2.8 (1.2, 6.5)
Other astrocytoma	1 (4.4%)		0.6 (0.1, 4.8)
Oligodendroglioma	4 (8.9%)		2.0 (0.6, 6.2)
Mixed oligoastrocytoma	2 (7.7%)		1.6 (0.3, 7.8)
<i>CYP2E1</i> Ins96 <i>CYP2E1</i> *1C/ <i>CYP2E1</i> *1D or <i>CYP2E1</i> *1D/ <i>CYP2E1</i> *1D			
All glioma combined	19 (4.7%)	1.8 (0.77)	0.7 (0.4, 1.2)
Glioblastoma	9 (4.6%)		0.8 (0.4, 1.9)
Anaplastic astrocytoma	3 (5.5%)		0.8 (0.2, 2.7)
Other astrocytoma	0		0.0 (0.0, ∞)
Oligodendroglioma	1 (2.2%)		0.3 (0.04, 2.4)
Mixed oligoastrocytoma	1 (3.9%)		0.7 (0.1, 5.4)

^a All estimates are adjusted for matching factors including age, sex, race, hospital, and distance of residence from hospital.

^b χ^2 statistic and P for the distribution of each gene variant between glioma subtypes, calculated based on 4 degrees of freedom.

glioma and acoustic neuroma, for which the estimated joint effects were statistically greater than would be expected under either an additive or multiplicative null (Table 7). Even in hierarchical regression analyses in which estimates were shrunk toward a common prior mean, the joint associations were greater than multiplicative. Other combinations of *GSTT1*, *GSTP1* I105V, and *CYP2E1* RsaI genotypes demonstrated no suggestion of interaction.

Discussion

In this study, we present evidence of associations between *GSTP1* I105V and *CYP2E1* variant genotypes and the risk of glioma. *GSTP1* I105V and *CYP2E1* RsaI variants were each positively associated, whereas the *CYP2E1* Ins96 variant was inversely associated with glioma incidence. The joint association of variant *GSTP1* I105V and *CYP2E1* RsaI genotypes with

glioma was greater than multiplicative, indicating effect modification. A general similar pattern was observed between the results for glioma and acoustic neuroma, although the small number of acoustic neuroma cases made estimates imprecise and limited our ability to examine trends. Whereas the *GSTT1* genotype was not associated with glioma or acoustic neuroma, we observed an association between *GSTT1* null and increased meningioma incidence. Although some estimates were imprecise, where hierarchical regression modeling was used for the purpose of obtaining more reasonable and stable estimates for the multiple exposures, our interpretation did not change.

Previously reported associations between *GSTT1* null genotype and the risk of overall glioma incidence (17) were not observed in this study population, nor in an earlier study (19). In our analyses of glioma subtypes, a positive association was observed with oligodendroglioma, similar to a previous report

Table 6 ORs and 95% CLs for the association of increasing numbers of GSTP1 variant alleles with brain tumor incidence^a

	Glioma (422 cases, 604 controls) ^b		Meningioma (172 cases, 604 controls) ^b		Acoustic neuroma (79 cases, 604 controls) ^b	
	Cases	OR (95% CL)	Cases	OR (95% CL)	Cases	OR (95% CL)
<i>GSTP1</i> I105V genotype						
<i>Ile/Ile</i>	156	1.0	77	1.0	28	1.0
<i>Ile/Val</i>	173	1.3 (1.0, 1.7)	79	1.0 (0.7, 1.5)	35	1.2 (0.7, 2.2)
<i>Val/Val</i>	67	2.1 (1.4, 3.1)	12	0.8 (0.4, 1.5)	9	1.5 (0.6, 3.4)
		<i>P</i> for trend = 0.001				<i>P</i> for trend = 0.31
<i>GSTP1</i> I105V/A114V genotype combinations ^c						
<i>GSTP1</i> *A (105 <i>Ile/Ile</i> ; 114 <i>Ala/Ala</i>)	155	1.0	77	1.0	28	1.0
<i>GSTP1</i> *B (105 <i>Val/Val</i> ; 114 <i>Ala/Ala</i>)	45	2.1 (1.3, 3.4)	8	0.8 (0.3, 1.9)	5	1.2 (0.4, 3.6)
<i>GSTP1</i> *C (105 <i>Val/Val</i> ; 114 <i>Ala/Val</i> or <i>Val/Val</i>)	21	2.0 (1.0, 3.9)	4	0.9 (0.3, 3.0)	4	2.9 (0.8, 10.0)
						<i>P</i> for trend = 0.13

^a All estimates are adjusted for matching factors including age, sex, race, hospital, and distance of residence from hospital.

^b Numbers represent the total with blood samples; the number included in each model may differ depending on the number of samples successfully genotyped for each variant.

^c Categorized to represent three different structural types of GSTP1 enzymes expressed, per Ali-Osman *et al.* (13).

Table 7 ORs and 95% CLs for the association between combined gene variants and brain tumor incidence^a

Gene variants ^c	Glioma (422 cases, 604 controls) ^b			Meningioma (172 cases, 604 controls) ^b			Acoustic neuroma (79 cases, 604 controls) ^b		
	Cases	Logistic regression ^d OR (95% CL)	Hierarchical regression ^e OR (95% CL)	Cases	Logistic regression ^d OR (95% CL)	Hierarchical regression ^e OR (95% CL)	Cases	Logistic regression ^d OR (95% CL)	Hierarchical regression ^{e,f} OR (95% CL)
<i>GSTP1</i> I105V and <i>CYP2E1</i> RsaI									
Neither	303	1.0	1.0	141	1.0	1.0	56	1.0	1.0
<i>GSTP1</i> variant only	56	1.6 (1.1, 2.4)	1.6 (1.1, 2.4)	12	0.8 (0.4, 1.6)	0.8 (0.4, 1.6)	7	1.1 (0.5, 2.7)	1.4 (0.7, 2.9)
<i>CYP2E1</i> variant only	24	1.1 (0.6, 2.0)	1.2 (0.7, 2.1)	13	1.3 (0.6, 2.6)	1.2 (0.6, 2.4)	7	1.8 (0.7, 4.5)	1.9 (0.9, 4.3)
Both variants	7	14.3 (1.7, 122) ^g	3.5 (1.2, 10.1)	0	0.0 (0.0, ∞)	0.8 (0.2, 4.0)	2	32.7 (2.6, 419) ^g	3.6 (0.9, 14.3)
<i>GSTP1</i> I105V and <i>GSTT1</i>									
Neither	252	1.0	1.0	113	1.0	1.0	52	1.0	1.0
<i>GSTP1</i> variant only	48	1.8 (1.2, 2.9)	1.8 (1.1, 2.8)	7	0.7 (0.3, 1.6)	0.7 (0.4, 1.5)	5	1.0 (0.4, 2.7)	0.9 (0.4, 2.2)
<i>GSTT1</i> variant only	61	1.1 (0.7, 1.5)	1.1 (0.8, 1.6)	33	1.4 (0.9, 2.3)	1.3 (0.9, 2.1)	7	0.6 (0.3, 1.4)	0.7 (0.3, 1.4)
Both variants	13	1.8 (0.8, 4.1)	1.7 (0.8, 3.5)	4	1.9 (0.5, 6.9)	1.4 (0.5, 3.9)	2	1.8 (0.4, 8.8)	1.2 (0.4, 3.7)
<i>GSTT1</i> and <i>CYP2E1</i> RsaI									
Neither	281	1.0	1.0	113	1.0	1.0	52	1.0	1.0
<i>GSTT1</i> variant only	67	1.0 (0.7, 1.5)	1.0 (0.7, 1.5)	31	1.4 (0.8, 2.2)	1.4 (0.9, 2.2)	9	0.8 (0.4, 1.7)	0.9 (0.4, 1.7)
<i>CYP2E1</i> variant only	23	1.2 (0.7, 2.2)	1.2 (0.7, 2.1)	6	0.9 (0.3, 2.3)	1.0 (0.5, 2.4)	5	1.5 (0.5, 4.3)	1.3 (0.5, 3.2)
Both variants	7	2.0 (0.6, 6.5)	1.6 (0.6, 4.0)	5	2.4 (0.6, 10.1)	1.7 (0.6, 4.9)	1	1.5 (0.2, 14.5)	1.2 (0.3, 4.3)

^a Unless otherwise stated, estimates are adjusted for matching factors including age, sex, race, hospital, and distance of residence from hospital.

^b Numbers represent the total with blood samples; the number included in each model may differ depending on the number of samples successfully genotyped for each variant.

^c Variant alleles for each gene are: *GSTP1* 105 *Val/Val*, *CYP2E1* RsaI *CYP2E1**1A/*CYP2E1**5 or *CYP2E1**5/*CYP2E1**5, and *GSTT1* null.

^d Maximum likelihood estimates for each pair of genotypes within each tumor type are from unconditional logistic regression models including an indicator term for the joint exposure and two indicator terms for the individual exposures to the two gene variants.

^e Penalized quasi-likelihood estimates for each pair of genotypes within each tumor type are from a hierarchical regression model including indicator terms for the joint exposure and two individual exposures to the two gene variants; these three parameters were assumed to arise from a common distribution with an unknown mean and variance of 0.35.

^f Models for acoustic neuroma did not include covariates with zero cells (indicator variables for age >70 and black race).

^g Estimated joint effect is statistically greater than expected under an additive or multiplicative null (*P* for likelihood ratio tests <0.05).

(18). We identified some problems with the *GSTT1* null genotyping assay conducted at Genotype, Ltd., including failure of about 9% of samples to amplify. The frequency of *GSTT1* null genotype among our controls was very similar to other populations (10), providing some indication that failure to amplify was not associated with *GSTT1* null genotype. To fill in some missing data on *GSTT1* genotype, the samples that did not amplify were sent to additional laboratories (NIEHS and Thetagen, Inc.) for further attempts. However, statistical analyses based on *GSTT1* null genotyping results only from Genotype, Ltd. did not differ meaningfully from the more full dataset from the three laboratories combined. Another concern relates to the somewhat lower reproducibility of the *GSTT1* assay compared with other genotyping assays conducted in this study. To ex-

plore the possibility that a positive association was masked by nondifferential misclassification of the genotype (30), we conducted simple sensitivity analyses (31) assuming imperfect sensitivity (*e.g.*, 94%) and specificity (*e.g.*, 97%) of the genotyping assay (sensitivity and specificity were estimated from the results of replicate samples). Under these assumptions, the most likely "corrected" OR for *GSTT1* null and glioma would still be negligible (corrected OR, 1.1). However, even a small amount of misclassification could have importantly affected results for glioma subtypes or brain tumor types in which *GSTT1* null genotype was more prevalent; for example, such sensitivity analyses indicated that the most likely corrected OR for oligodendroglioma would be further elevated (observed OR, 1.5; corrected OR, 1.8).

The observed positive association in our study of the *GSTP1* I105V variant and glioma incidence has not been reported previously. *GSTP1* is thought to be the most strongly expressed of the *GST* isoenzymes in the human brain (32, 33), with increased expression in tumors (13, 33, 34). We observed a moderate association between glioma incidence and the I105V variant that followed a trend of increasing magnitude by number of variant alleles. Immunohistochemical screening has shown that expression of *GSTP1* in the adult brain is high in astrocytes and consistently absent in oligodendrocytes (32); these observations would support our results in which the *GSTP1* I105V variant was positively associated with glioblastoma, astrocytoma, and mixed oligoastrocytoma incidence, but not with oligodendroglioma. Analysis of *GSTP1* proteins has shown that three active, structurally different encoded proteins are expressed from three possible combinations of wild-type and variant *GSTP1* I105V or A114V alleles, resulting in functional differences in stability and half-life (13, 35). It is reasonable to hypothesize that such functional differences between *GSTP1* proteins may result in differing capabilities for metabolizing carcinogens. Furthermore, combined *GSTP1* I105V/A114V variants have been observed to be 4-fold more prevalent in malignant gliomas than in normal brain tissues (13). These *GSTP1* variants were in linkage disequilibrium in our study population, similar to previous reports (29), and the A114V variant did not occur independently of the I105V variant. However, no association between brain tumors and the *GSTP1* A114V variant genotype were observed in these data, nor was there evidence that any effect of combined *GSTP1* I105V/A114V variants differed from the effect of the I105V variant alone.

CYP2E1 RsaI and *CYP2E1* Ins96 polymorphisms may result in differing functional activity that could potentially explain the divergent results we observed. In oral clearance studies of chlorzoxazone, the RsaI variant has been associated with decreased metabolic activity (36), whereas Ins96 variant was associated with increased activity (37). However, increased activity of the Ins96 variant has primarily been observed among the obese or those who have recently consumed alcohol (37). Further research into the expression of these two variant *CYP2E1* genotypes in the human brain and among different population subgroups may clarify interpretation of the associations we observed in our study.

Where genotypes were associated with brain tumor incidence, we observed stronger associations among younger age groups. Because the OR is a relative measure, these differences are due, in part, to the higher incidence of brain tumors at older rather than younger adult ages. Thus, even when the absolute magnitude of effect is similar in both age groups, the relative measure can appear stronger in the stratum with lower incidence. For comparison of an absolute measure of effect across age strata, we used incidence rates from the Surveillance, Epidemiology, and End Results program of the National Cancer Institute (<http://seer.cancer.gov/>) to estimate the rate difference associated with the gene variant in each age category, using the formula $RD = (RR-1) \times R_0$ [where RD = rate difference, RR = relative rate as estimated by the OR, R_0 = underlying incidence in the unexposed population (from Surveillance, Epidemiology, and End Results program data for brain and other nervous system tumors, all races, 1994–1998)]. Estimation of the absolute effect of gene variants on glioma incidence indicates that differences in effect by age are supported for the *CYP2E1* RsaI variant, but not for the *GSTP1* I105V variant for which the estimated absolute effect was actually higher for the older age group than for the younger age group (results not shown).

It is possible that selection bias could have produced spurious associations in our hospital-based case-control study, because controls were selected from patients with differing discharge diagnoses. One or more of the gene variants evaluated could be associated with one of the diseases constituting the control series, thereby creating a control group that is not representative of the general source population. Some reassurance was provided by the similarity of genotype frequencies of our controls with those described in the literature. *GSTT1* null genotype has been associated with an increased risk of coronary heart disease in a recent study (38). If this relationship were true, then inclusion of circulatory disease patients in the control group could be masking any potentially real associations between brain tumors and *GSTT1*; however, removal of circulatory disease patients did not change most ORs and changed the OR for meningioma in the opposite direction than would be expected. Because there is not a clear association between *GSTT1* and circulatory disease, we can only speculate on the possibility that selection bias affected our results for *GSTT1*, although it seems unlikely based on these results.

Potential effects of *GST* and *CYP2E1* gene variants on risk of three major categories of adult brain tumors as shown in our data merit further investigation. Replication of these analyses in other study populations will help to resolve the possibility of chance findings. If these findings are confirmed, then pooling of multiple epidemiological studies will provide the statistical power necessary to examine interactions of genotypes with specific occupational and environmental exposures.

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