

Susceptibility to Astrocytoma and Meningioma: Influence of Allelism at Glutathione S-Transferase (GSTT1 and GSTM1) and Cytochrome P-450 (CYP2D6) Loci¹

Jose Elexpuru-Camiruaga, Neil Buxton, Vishwapathi Kandula, Pilatha S. Dias, Donald Campbell, James McIntosh, John Broome, Peter Jones, Avis Inskip, Julie Aldersea, Anthony A. Fryer, and Richard C. Strange²

Departments of Neurosurgery [J. E.-C., N. B., V. K., P. S. D., D. C., J. M.] and Pathology, Central Pathology Laboratory [J. B.], and Clinical Biochemistry Research Laboratory, School of Postgraduate Medicine, Keele University [A. I., J. A., A. A. F., R. C. S.], North Staffordshire Hospital, Stoke-on-Trent, Staffordshire, United Kingdom ST4 7QB, and Department of Mathematics, Keele University, Staffordshire, United Kingdom [P. J.]

Abstract

We describe a case-control study to identify associations between polymorphism at the cytochrome P-450 (CYP2D6) and glutathione S-transferase (GSTT1 and GSTM1) loci and susceptibility to astrocytoma and meningioma. Accordingly, genotype frequencies in 112 astrocytoma and 50 meningioma patients were compared with frequencies in 577 controls. GSTM1 genotype frequencies in these groups were not different. Logistic regression analysis showed GSTT1 null and CYP2D6 poor metabolizer were risk factors in astrocytoma (odds ratio = 2.67 P = 0.0005 and odds ratio = 4.17 P = 0.0043, respectively) and meningioma (odds ratio = 4.52, P = 0.0001 and odds ratio = 4.90, P = 0.0132, respectively) when corrected for the other variables. No interactive effects between genotypes were identified. The data suggest polymorphism at loci encoding carcinogen-metabolizing enzymes influences susceptibility to astrocytoma and meningioma, possibly by determining effectiveness in the detoxification of environmental carcinogens.

Introduction

Astrocytomas are the most common primary brain tumor, the others include meningiomas and pituitary adenomas. The natural history of astrocytomas is unclear but tumors are believed to progress from relatively benign stage I-II lesions to malignant astrocytomas III-IV. About 70% of patients are first seen with grade III-IV tumors. Meningeal tumors are largely benign and only rarely demonstrate malignant progression. The pathogenesis of brain tumors is unclear, although the importance of environmental factors is suggested by studies showing that some xenobiotics can induce tumors in brain and meninges (1, 2). Thus, intracranial implantation of polycyclic aromatic hydrocarbons results in a variety of neuroectodermal and meningeal tumors. Nitroso compounds such as *N*-methyl-*N*-nitrosourea and *N*-ethyl-*N*-nitrosourea also induce central nervous system tumors after systemic administration (2). Such xenobiotics are present in the diet, cigarette smoke, car exhaust fumes, and other pollutants, as well as the rubber industry where volatile nitroso compounds and methyl halides are used (2, 3). Interestingly, significant associations between intake of processed meats and cheese, occupation, and risk of glioma have been identified (3). Studies on exposure to fire smoke also suggest the importance of combustion-derived chemicals; although there was no excess risk of overall mortality from cancer, firefighters ages <40 years had an excess risk of brain tumors (4). The association between chemical carcinogens and neuroectodermal tumors suggests susceptibility may be related to polymorphism at loci encoding

Phase I CYP³ and Phase II GST as a variety of potential carcinogens, including polycyclic aromatic hydrocarbons, nitrosoureas, and methyl halides are substrates for these enzymes (5, 6). The Phase I CYP2D6 locus is polymorphic with PM and extensive metabolizer genotypes being linked with altered risk of various pathologies (5-9). Of the Phase II, GST supergene family, the μ class GSTM1 locus demonstrates allelism that arises from homo- and heterozygotic combinations of GSTM1*0, GSTM1*A, and GSTM1*B (6). Homozygotes for the deleted GSTM1*0 allele express no protein and are also at altered risk of several pathologies (6, 10). The θ class, GSTT1, is also polymorphic with homozygotes for the deleted GSTT1*0 allele comprising 18% of Caucasians (7, 11, 12). GSTT1 catalyzes the detoxification of oxidized lipid and DNA, epoxides, and methyl halides (11, 12). Individuals null at both GST loci might, therefore, be at a particularly high risk for chemical-induced DNA damage. We describe a case-control study to determine the relevance of GSTM1, GSTT1, and CYP2D6 genotypes in mediating susceptibility to astrocytoma and meningioma.

Materials and Methods

Patient Samples

Unrelated patients with astrocytoma [n = 112; mean age, 54.3 \pm 14.9 (SD) years; 63% male], meningioma (n = 50; mean age, 57.6 \pm 17.5 years; 38% male) and controls (n = 577; mean age, 58.6 \pm 17.5 years; 48% male) were recruited, with Ethics Committee approval, in the North Staffordshire Hospital. The astrocytoma group comprised 94 patients with high-grade (astrocytoma III-IV, glioblastoma multiforme; mean age, 56.9 \pm 15.1 years; 63% male) and 18 patients with low-grade disease (astrocytoma I-II; mean age, 42.1 \pm 17.4 years; 61% male). No cases of juvenile astrocytoma were included. Only English Caucasians from North Staffordshire who could give informed consent were studied. The area is recognized as having a stable population base with relatively little population movement (13). Each of the cases was interviewed by a clinician (J. E.-C., N. B., and V. P.) on admission. None had a family history of brain tumors, nor had they received radiation, chemotherapy, or suffered a significant trauma to the head. Diagnostic samples were obtained at craniotomy, burr-hole, or stereotactic biopsy, and standard histological criteria (Kernohans grading system) were used by one author (J. B.) to diagnose and grade these lesions. The cases composed over 75% of the patients with astrocytoma and meningioma in North Staffordshire treated during the 2-year study and represented a typical sample of this patient group. The remaining 25% of such patients were inadvertently missed. None of those approached refused to participate. Genotype frequencies in these cases were compared with those in hospitalized, unrelated controls undergoing investigation or surgery for tension headaches (25%), inguinal hernia (~20%), varicose veins (~15%), gall stones (~15%), diverticular disease (~10%), renal stones (~1%), benign breast lumps (~5%), hiatus hernia (~6%), and acute appendicitis (~3%). Patients with diabetes, asthma, any malignancies, or having received blood

Received 6/27/95; accepted 8/18/95.

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¹ We gratefully acknowledge the support of the North Staffordshire Hospital Neurosurgery Trust Fund.

² To whom requests for reprints should be addressed.

³ The abbreviations used are: CYP, cytochrome P-450; GST, glutathione S-transferase; PM, poor metabolizer; CI, confidence interval.

transfusions within 3 months of blood sampling, were excluded. Smoking status was assessed by history. Nonsmokers had never smoked. Current smokers smoked at least 10 cigarettes/day and had done so for at least 1 year. Exsmokers had smoked at least 10 cigarettes/day for up to 1 year before entry into the study. The proportion of never smokers in the case and control groups (49%) was not significantly different.

Identification of GSTT1, GSTM1, and CYP2D6 Genotypes

Blood (3 ml) was taken into EDTA at venepuncture for routine investigation and stored (-40°C) until DNA extraction by phenol-chloroform (7). Two mutant CYP2D6 alleles were identified; the G→A transition using intron 3/exon 4 primers, followed by BstNI digestion, and the exon 5 deletion using primers to exon 5/intron 5, followed by HpaII digestion (6, 7, 9). GSTM1 genotypes were identified by amplification refractory mutation system-based PCR by using a common primer to intron 6 and GSTM1*A- and GSTM1*B-specific primers in exon 7. Primers to exons 4/5 and, as positive control, β-globin were included. The assay identifies GSTM1*0 homozygotes and GSTM1*A/GSTM1*B heterozygotes and the GSTM1 A and GSTM1 B phenotypes. It does not distinguish the GSTM1*0/GSTM1*A and GSTM1*A/GSTM1*A genotypes or the equivalent GSTM1 B genotypes (10). GSTT1 null and expressing subjects were identified by using a PCR approach (7, 12).

Statistical Analysis. χ² tests were used to examine for homogeneity between cases and controls. Because some genotype frequencies were small, the StatXact-Turbo statistical package was used to obtain exact P values. The influence of CYP2D6 PM, GSTT1 null, GSTM1 null, gender, and age on susceptibility were studied by logistic regression analysis. Possible interactions between variables shown to be significantly different in case and control groups were also studied, allowing for identification of those factors (alone and in combination) that contributed most to observed differences. As the proportion of ever/never smokers in the case and control groups were not different, we did not test for interactions between smoking and genotypes.

Results

Astrocytoma. Table 1 shows the frequency of GSTM1, GSTT1, and CYP2D6 genotypes in controls, and patients with astrocytoma. GSTM1 frequencies in these groups were not significantly different. The GSTT1 null and CYP2D6 PM genotypes, however, were significantly increased in both the total and high-grade astrocytoma groups compared with controls. The frequency of CYP2D6 PM was also increased in the low-grade group compared with controls (X²₁ = 6.183; exact P = 0.0442). Inspection of the data suggested the frequencies of GSTT1 null in the high- and low-grade astrocytoma groups were different, although this did not achieve statistical significance possibly because of the small number of low-grade cases.

In addition to univariate analysis, multivariate analysis using logistic regression was used to determine which of the variables (gender, age, GSTM1, GSTT1, and CYP2D6), in the presence of the others, continued to demonstrate significant differences between the total and high-grade astrocytoma cases and controls. Analysis of data from the low-grade group was not undertaken because of the small number of cases. In the total astrocytoma group, GSTT1 null and CYP2D6 PM, corrected for the other variables, were significantly different (odds ratio = 2.67; 95% CI = 1.53–4.65; P = 0.0005 and odds ratio = 4.17; 95% CI = 1.57–11.09; P = 0.0043, respectively). Corresponding data for the high-grade astrocytoma group were similar for GSTT1 null (odds ratio = 3.02; 95% CI = 1.70–5.39; P = 0.0002) and CYP2D6 PM (odds ratio = 3.33; 95% CI = 1.17–9.44; P = 0.0236). The other variables (gender, GSTM1, and age) were not significantly different in either the total or high-grade astrocytoma groups and controls. As GSTT1 null and CYP2D6 PM were important variables, we considered a model with these two main effects and their interaction. The frequencies of individuals with both GSTT1 null and CYP2D6 PM in the control (4 of 408) and total astrocytoma groups (4 of 114) were almost significantly different (exact P = 0.0732; odds

Table 1 CYP2D6, GSTM1, and GSTT1 genotype frequencies in patients with astrocytoma and meningioma

	GSTM1 genotypes			
	GSTM1 null	GSTM1 A	GSTM1 B	GSTM1 A/B
Astrocytoma				
Total group (n = 109)	65 (59.6%)	24 (22.0%)	15 (13.8%)	5 (4.6%)
High grade (n = 92)	55 (59.8%)	20 (21.7%)	13 (14.1%)	4 (4.4%)
Low grade (n = 17)	10 (58.8%)	4 (23.5%)	2 (11.8%)	1 (5.9%)
Meningioma (n = 49)	27 (55.1%)	13 (26.5%)	9 (18.4%)	0 (0%)
Controls (n = 577)	315 (54.6%)	163 (28.2%)	76 (13.2%)	23 (4.0%)
	GSTT1 genotypes			
	GSTT1 null	GSTT1 positive		
Astrocytoma				
Total group (n = 109)	35 (32.1%) ^a	74 (67.9%)		
High grade (n = 92)	32 (34.8%) ^b	60 (65.2%)		
Low grade (n = 17)	3 (17.6%)	14 (82.4%)		
Meningioma (n = 47)	21 (44.7%) ^c	26 (55.3%)		
Controls (n = 494)	91 (18.4%)	403 (81.6%)		
	CYP2D6 genotypes			
	EM	HET	PM	
Astrocytoma				
Total group (n = 109)	64 (58.7%)	32 (29.4%)	13 (11.9%) ^d	
High grade (n = 92)	56 (60.9%)	26 (28.2%)	10 (10.9%) ^e	
Low grade (n = 17)	8 (47.1%)	6 (35.3%)	3 (17.6%) ^f	
Meningioma (n = 48)	22 (45.8%)	20 (41.7%)	6 (12.5%) ^f	
Controls (n = 412)	261 (63.3%)	133 (32.3%)	18 (4.4%)	

^a GSTT1 null in total astrocytoma group versus controls. Odds ratio = 2.09 (95% CI 1.28–3.39); P = 0.0032.

^b GSTT1 null in high-grade astrocytoma versus controls. Odds ratio = 2.36 (95% CI 1.41–3.94); P = 0.0007.

^c GSTT1 null in meningioma versus controls. Odds ratio = 3.57 (95% CI 1.82–6.92); P = 0.0002.

^d CYP2D6 PM in total astrocytoma group versus controls. Odds ratio = 2.96 (95% CI 1.28–6.64); P = 0.0102.

^e CYP2D6 PM in high-grade astrocytoma versus controls. Odds ratio = 2.61 (95% CI 1.10–6.37); P = 0.027.

^f CYP2D6 PM in meningioma versus controls. Odds ratio = 3.12 (95% CI 0.96–8.78); P = 0.0587.

ratio = 3.70; 95% CI 0.71–20.0). It is unclear whether these findings resulted from the strength of the main effects or from their interaction.

Meningioma. Table 1 shows the frequency of GSTM1, GSTT1, and CYP2D6 genotypes in controls and patients with meningioma. No significant differences in GSTM1 frequencies were detected. The frequency of GSTT1 null, however, was significantly increased in meningioma (odds ratio = 3.57; exact P = 0.0002), and the frequency of CYP2D6 PM approached significance (odds ratio = 3.13; exact P = 0.0587).

Logistic regression was used to determine which of the variables in the presence of the others continued to demonstrate significant differences between cases and controls. Thus, gender (odds ratio = 2.24; 95% CI = 1.09–4.61; P = 0.0289), GSTT1 null (odds ratio = 4.52; 95% CI = 2.18–9.34; P = 0.0001) and CYP2D6 PM (odds ratio = 4.90; 95% CI = 1.39–17.26; P = 0.0132), corrected for the other variables, were significantly different. GSTM1 and age were not significantly different in cases and controls. The frequencies of individuals with both the GSTT1 null and CYP2D6 PM genotypes in the controls (4 of 408) and meningioma (3 of 48) were significantly different (exact P = 0.0283; exact odds ratio = 6.67; 95% CI = 0.95–41.7), although it is unclear if these findings resulted from the strength of the main effects or their interaction.

Discussion

Although the pathogenesis of neuroectodermal tumors is unclear, the association with polycyclic aromatic hydrocarbons, nitroso compounds, and methyl halides (1–4) suggests allelism at loci encoding detoxifying enzymes will influence susceptibility to these tumors.

Accordingly, genotype frequencies were studied in meningioma and in patients with low- and high-grade astrocytomas. Data from these cases were studied together and separately because it is unclear whether these tumors represent a continuum or are heterogeneous with histological grades describing different rates of disease progression.

We have shown that both GSTT1 null and CYP2D6 PM are associated with an altered risk for meningioma and astrocytoma, presumably because these genotypes confer an impaired ability to catalyze the metabolism of endogenous and exogenous carcinogens (5, 6, 8). Thus, occupational exposure to dichloromethane, a GSTT1 substrate (12), is associated with an increased risk of astrocytoma. Interestingly, although dichloromethane can be metabolized by two pathways, oxidation by CYP and conjugation with glutathione, it is the latter pathway that shows the best correlation with tumor incidence in exposed mice (11, 12). A variety of *CYP* genes are expressed in brain, indicating that the tissue is an important site for the metabolism of exogenous xenobiotics and endogenous compounds, such as neurotransmitters, steroids, and catechols (8). CYP2D6 has attracted interest because the PM genotype is associated with a 2–3-fold increased risk of Parkinson's disease (9). Although the mechanism for this effect is unclear, the CYP2D6 enzyme can utilize the potent neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, as well as various drugs that act on the central nervous system, suggesting the enzyme participates in the local generation of toxic, reactive intermediates (8). At least 16 *CYP2D6* alleles have been identified, many of which confer impaired activity (6, 8). It is noteworthy, therefore, that significant differences in the frequency of PM genotypes in cases and controls were found, although we only identified the mutation at the intron 3/exon 4 boundary and the deletion in exon 5.

Although the frequency of individuals with both the GSTT1 null and CYP2D6 PM genotypes appeared to be higher in the total astrocytoma and meningioma groups than in controls, it is not clear whether the data indicate significant interaction between these genes. Similarly, no interactions were identified between these genes and gender, although as expected, females were shown to be at higher risk of meningioma (14). Whereas the GSTT1 null was significantly associated with risk of both astrocytoma and meningioma, no significant effect of GSTM1 genotypes on susceptibility was identified. Wiencke *et al.* (15) has reported that GSTM1 null frequencies are higher in women with early onset astrocytoma; we did not detect such differences (50% GSTM1 null in cases *versus* 52% in controls), although it is noteworthy that sample sizes were relatively small in both studies. Both GSTM1 and GSTT1 protect against epoxide-induced sister chromatid exchange (11, 16, 17). Thus, individuals null at both loci would be expected to be at greater risk than those lacking only one gene. Logistic regression analysis, however, did not demonstrate an interactive effect, suggesting GSTM1 and GSTT1 do not use the same epoxide substrates. Support for this view comes from studies described by Norppa *et al.* (17) showing that lymphocytes from individuals with GSTT1 null, but not GSTM1 null, suffer an increased frequency of sister chromatid exchange after exposure to diepoxybutane. Furthermore, other μ class GST, including GSTM1 and GSTM3 are expressed in human brain (18, 19). Activity measurements indicate GSTM3 is the major μ isoform in brain and, unlike GSTM1, is expressed in most, if not all, individuals (19). This isoform may, therefore, protect GSTM1 null individuals from damage induced by nitrosoureas and other μ class substrates.

This study identifies a genetic predisposition to glioma that provides a link with the epidemiological association with chemical carcinogens. A better understanding of factors that predispose to these tumors will enable identification of causative factors and development of prevention strategies. The significance of GSTT1 and CYP2D6 indicates that other polymorphic genes involved in the metabolism of carcinogenic compounds (CYP2E1 and CYP1A1) and/or DNA repair may also be promising candidates.

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