Glutathione-*S*-Transferase Variants and Adult Glioma

Margaret Wrensch,[1] Karl T. Kelsey,[2] Mei Liu,[2] Rei Miike,[1] Michelle Moghadassi,[1] Kenneth Aldape,[3] Alex McMillan,[1] and John K. Wiencke[1]

[1]University of California, San Francisco, CA; [2]Harvard School of Public Health, Boston, MA; and [3]MD Anderson Cancer Center, Houston, TX

Abstract

Introduction: Conflicting findings have been reported for associations of primary brain tumors and constitutive polymorphisms in glutathione-*S*-transferases (*GST*s). Methods: We genotyped population-based cases ascertained through rapid case ascertainment and controls identified through random-digit dialing in the San Francisco Bay Area between 1991–1994 (series 1) and 1997–2000 (series 2) for homozygous deletion or presence of *GSTM1* (μ) and *GSTT1* (θ) genes and for two variants in *GSTP* (π; *i.e.*, I105V and A114V). A single neuropathologist for each series determined histological type. Blood or buccal swabs were obtained from about 53.8% of cases and 64.6% of controls. Case-control genotype frequencies were compared overall and by histological type and by age group (≤40, 41–60, and >60), gender, and series. Results: Among whites, 367 cases (179 glioblastoma, 62 other astrocytoma, 94 oligodendroglioma or oligoastrocytoma, and 32 other histologies) and 428 controls were genotyped for all four polymorphisms. Multivariate logistic models including the four *GST* loci, age, gender (except in gender-specific models), and series showed no significant case-control differences for *GST* genotypes. Among cases over age 60, prevalence of *GSTP* I105V Val/Val was 6.4% of 108 cases *versus* 15% of 176 controls [odds ratio (OR) 0.38; 95% confidence interval (CI) 0.15–0.93; *P* = 0.03]. *GSTT1* deletion was nearly significantly more common among glioblastoma cases with tumor *p53* mutation than for those whose tumors did not have *p53* mutation (OR 2.8; 95% CI 0.93–8.4; *P* = 0.07). Conclusions: There is little evidence for associations of *GST* variants with major glioma histological subtypes, but *GST* polymorphisms might influence certain molecular subtypes or progression. (Cancer Epidemiol Biomarkers Prev 2004; 13(3):461–467)

Introduction

Polymorphic variants in the glutathione-*S*-transferases (*GST*s) μ (*GSTM1*), θ (*GSTT1*), and π (*GSTP*) have been studied extensively in relation to cancer etiology because these enzymes, part of the phase II detoxification processes, are involved in metabolism of many electrophilic compounds including carcinogens, mutagens, cytotoxic drugs, and their metabolites and detoxification of products of reactive oxidation (1, 2). Complete gene deletions in *GSTM1* and *GSTT1* and single nucleotide polymorphisms in *GSTP* may result in a significant change in the function of the enzymes (1, 3, 4). Although there are a few rare single gene alterations known to substantially increase the risk of neuroepithelial tumors and therapeutic radiation is a known strong environmental risk factor, these factors account for a small proportion of cases (5). These observations combined with the temporal and geographic distribution of primary malignant brain tumors suggest that most of these tumors arise from a complex interplay of genes, developmental processes, and exogenous and endogenous environmental stimuli. Five studies have reported on the associations of *GST*s and adult-onset primary brain tumors (6–10) with somewhat inconsistent results that will be discussed in more detail below. We report on *GST* genotypes in population-based adult glioma cases and controls from the San Francisco Bay Area.

Materials and Methods

Study Participants. Details of case-control ascertainment for these two series of subjects have been presented in detail elsewhere (11–13). We ascertained all adults newly diagnosed with glioma (*International Classification of Diseases for Oncology*, morphology codes 9380–9481) in six San Francisco Bay Area counties (Alameda, Contra Costa, Marin, San Mateo, San Francisco, and Santa Clara) from August 1991 to April 1994 (series 1) and from May 1997 to August 1999 (series 2). Cases were ascertained within a median of 7 weeks of diagnosis using Northern California Cancer Center's Rapid Case Ascertainment program as described previously (12, 13). Controls ascertained through random-digit dialing using methods described previously (13) were frequency matched to cases by age, race, and gender. We began collecting blood specimens from willing subjects partway through the first series and asked all participants in series 2 to donate either blood and/or buccal specimen. Our previous reports (6, 10) included about 150 white cases and an equal number of controls.

Genotyping. Constitutive DNA was obtained from subjects' buccal or blood specimens. Genotyping for

Received 9/23/03; revised 10/28/03; accepted 11/13/03.

Grant support: National Cancer Institute RO1CA52689 and P50CA 097257.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Margaret Wrensch, Department of Epidemiology and Biostatistics, University of California, 44 Page Street, Suite 503, San Francisco, CA 94143-1215. Phone: (415) 476-1979; Fax: (415) 502-1787. E-mail: wrensch@itsa.ucsf.edu

homozygous deletion of *GSTM1* and *GSTT1* has been described previously (6, 10, 14). For *GSTP* A114V, the PCR was done with primers CAAGCAGAGGAGA-ATCTGGG and AATGAAGGTCTTGCCTCCCT. Cycle condition is 94C for 5 min followed by 36 cycles of 94C for 30 min, 60C for 30 min, and 72C for 30 min. Fifteen microliters of the PCR product were then digested overnight with *Cac*8I at 37C. Genotype is determined on 4% agarose gel. For *GSTP* I105V, the PCR is done with primers GTAGTTTGCCCAAGGTCGAG and AGC-CACCTGAGGGGTAAG. Cycle condition is 94C for 5 min followed by 32–40 cycles of 94C for 30 min, 58C for 30 min, and 72C for 60 min. Fifteen microliters of the PCR product were then digested with *Bsm*A1 at 55C for at least 2 h. Genotype is determined on 2.5% agarose gel.

As described previously (15), we determined *p53* mutation status of astrocytic tumors using single-strand conformation polymorphism followed by sequencing to classify tumors as containing or not containing *p53* mutation.

Statistical Analysis. We began by examining genotype frequencies for cases (all, glioblastoma, and nonglioblastoma) and controls by series, by gender within series, and for whites within series. For all ethnicities and whites separately, we also examined genotype frequencies by age group ≤40, 41–60, and >60 for controls, all cases, and cases who either had glioblastoma, other astrocytic tumors (anaplastic astrocytoma, astrocytoma, and astrocytoma not otherwise specified), tumors with an oligodendroglial component (oligodendroglioma or oligoastrocytoma), or other (ependymoma, medulloblastoma, juvenile pilocytic astrocytoma, ganglioblastoma, and other). We also compared genotype frequencies by *p53* mutation status of the tumors.

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using logistic regression. Models were run separately for each type of gene adjusting for matching factors, age, gender, and ethnicity. Final models for whites included adjustment for age, gender, series, and all four genes (*GSTM1* and *GSTT1* null *versus* present, *GSTP* I105V Val/Val *versus* Ile/Val or Ile/Ile, and *GSTP* A114V Ala/Val or Val/Val *versus* Ala/Ala). SAS software (16) was used for data management and PROC LOGISTIC was used to estimate all ORs and 95% CIs. We also examined cases, histological subtypes, and controls for distributions for various combinations of presence or absence *GST* variants. We also summed the number of *GST* variants that each individual carried, counting one for deletion of *GSTM1* or *GSTT1* and one for each variant allele of *GSTP* I105V and A114V.

Results

Cases and Controls. As described in detail elsewhere (11–13), there were 1129 eligible cases from both series 1 and 2. Of these, full interview was obtained for 81% (n = 896) and 2% (n = 23) completed only a telephone interview. Only 11% (n = 128) refused to enter the study, 1% (n = 22) were too ill or had a language problem, 4% (n = 47) could not be located, and 1% (n = 13) did not get a doctor's consent to contact. Of those cases with completed interview, 2% (n = 17) had to be dropped from the study because a neuropathology review indicated that the subject either did not have glioma or medulloblastoma (n = 4), permission for review was not obtained (n = 4), tumor specimens were unavailable (n = 4), or tumor specimens were insufficient for diagnosis (n = 5). Of the 879 case participants, 461 (52%) provided blood or buccal specimen, and *GST* genotyping was conducted for 458 subjects (52% of total) with 187 of 476 (39%) from series 1 and 271 of 403 (67%) from series 2.

Of the 15,894 phone numbers in both series contacted through random-digit dialing, 8% (n = 1316) yielded eligible controls. Of the total phone numbers contacted, 19% (n = 3086) were not in service, 14% (n = 2242) were businesses, 7% (n = 1047) were faxes or modems, 12% (n = 1964) had no response after 10 calls, 12% (n = 1824) were refusals, 5% (n = 827) had language or health problems, 20% (n = 3158) were eligible but the quota for their age/race/gender had been filled, and 3% (n = 430) were either too young, had multiple lines, lived out of the area, or closed out. Of the eligible controls, 66% (n = 864) completed a full interview, 13% (n = 169) completed an abbreviated telephone interview only, 14% (n = 184) refused to enter the study, 1% (n = 17) either had a language problem or were too ill to interview, 3% (n = 43) were not located, 3% (n = 39) were either related to cases, out of the area, good matches but the study closed, or otherwise could not be used. Of the 864 controls from series 1 and 2, 558 (65%) provided blood or buccal specimens and 487 (56%) were genotyped for *GST*s with 152 of 462 (33%) from series 1 and 335 of 402 (83%) from series 2.

Comparing Series 1 and 2. For all participants and those subjects genotyped, Table 1 shows the numbers of subjects, basic demographic characteristics (age, gender, and ethnicity), and histological distribution of cases. There were some differences between the series; most notably, a higher proportion of subjects were genotyped from series 2 than series 1 because funding for collecting specimens was first obtained partway through series 1. The percentage of ethnicities categorized as ''other,'' which consists of all ethnicities other than white or African American, increased from 11% to 15% of study participants. Series 2 participants were on average, slightly older than series 1. The mean age was 54 years for series 1 cases and 56 years for series 2. These changes may reflect the changing demographics of the Bay Area over the period of 5 years between the studies. In addition, note that although the ages of cases and controls were matched for the overall group, genotyped cases are on average younger than genotyped controls, primarily because case survival (and therefore ability to obtain constitutive material for genotyping) decreases with age. The histological distributions of cases in the two series were similar, except that series 2 had a higher percentage of cases with oligodendroglioma and lower percentage with oligoastrocytoma. Because the proportion of oligodendroglioma and oligoastrocytoma changed substantially between series 1 and 2, we combined these two histologies in subsequent analyses as tumors with an oligodendroglial component.

No noteworthy or significant age, gender, and ethnicity adjusted case-control differences are apparent for the various *GST* genotypes in either series (Table 2);

Table 1. Characteristics of cases and controls in two series, the San Francisco Bay Area Adult Glioma Study 1991–2000

Variables	Both series		Series 1 (1991–1995)		Series 2 (1997–2000)	
	Cases	Controls	Cases	Controls	Cases	Controls
Total no. participants	879	864	476	462	403	402
No. participants with *GST* genotype data (%)	458 (52)	505 (58)	187 (39)	167 (36)	271 (67)	338 (84)
Average age (SE)						
Total participants	55 (0.55)	55 (0.56)	54 (0.77)	54 (0.78)	56 (0.78)	56 (0.79)
Genotyped participants	51 (0.68)	56 (0.70)	48 (1.1)	53 (1.2)	52 (0.88)	57 (0.83)
% Female						
Total participants	44	46	43	45	45	46
Genotyped participants	41	46	37	47	44	46
Ethnic group						
Total participants						
% White	83	84	84	86	81	83
% Black	4	4	5	3	4	4
% Other	13	12	11	11	15	14
Genotyped participants						
% White	83	87	86	96	81	84
% Black	4	4	4	0	3	4
% Other	13	10	10	4	15	12
Histological distribution[a]						
Total no. participants (%)						
Glioblastoma	522 (59)		281 (59)		241 (60)	
Anaplastic astrocytoma and astrocytoma	151 (17)		89 (19)		62 (15)	
Oligoastrocytoma (mixed)	57 (6)		47 (10)		10 (2)	
Oligodendroglioma	92 (11)		34 (7)		58 (14)	
Other[b]	57 (6)		25 (5)		32 (8)	
No. genotyped participants (%)						
Glioblastoma	223 (49)		82 (44)		141 (52)	
Anaplastic astrocytoma and astrocytoma	85 (18)		39 (21)		46 (17)	
Oligoastrocytoma (mixed)	40 (9)		31 (16)		9 (3)	
Oligodendroglioma	70 (15)		19 (10)		51 (19)	
Other	40 (9)		16 (9)		24 (9)	

[a]Histological diagnosis based on uniform neuropathology review (except for two glioblastoma patients in series 2).
[b]Other includes ependymoma (8), juvenile pilocytic astrocytoma (16), medulloblastoma (10), ganglioblastoma (2), and other (21).

in addition, there were no notable differences in genotype frequencies for glioblastoma or cases with other histologies. Table 3 shows that there were, however, some very large differences in genotype frequencies by ethnic group within both cases and controls, which were highly statistically significant among cases. Because we lack sufficient numbers of subjects among the non-white ethnicities to fully explore the basis for these differences, we limited our subsequent analyses to whites.

As shown in Table 4, among whites, cases and controls had very similar genotype frequencies for the

Table 2. *GST* genotype frequencies of controls and cases overall and by histological group, the San Francisco Bay Area Adult Glioma Study 1991–2000

	Controls		All cases				Glioblastoma				Other histologies			
	n/*N*	%	*n*/*N*	%	OR	95% CI	*n*/*N*	%	OR	95% CI	*n*/*N*	%	OR	95% CI
GSTM1 null														
Series 1	84/166	51	93/184	51	1.0	0.7–1.5	36/80	45	0.8	0.5–1.3	57/104	55	1.2	0.7–2.0
Series 2	177/337	53	140/264	53	1.0	0.7–1.4	74/137	54	1.1	0.7–1.6	66/127	52	1.0	0.7–1.5
GSTT1 null														
Series 1	35/167	21	36/183	20	0.9	0.5–1.6	17/79	22	1.0	0.5–2.0	19/104	18	0.8	0.5–1.6
Series 2	74/337	22	54/264	20	0.9	0.6–1.4	24/137	18	0.8	0.5–1.3	30/127	24	1.1	0.7–1.8
GSTP I105V Val/Val														
Series 1	23/159	14	21/180	12	0.8	0.4–1.5	6/80	8	0.5	0.2–1.2	15/100	15	1.0	0.5–2.1
Series 2	40/337	12	28/269	10	0.9	0.5–1.4	17/140	12	1.0	0.6–1.9	11/129	9	0.7	0.3–1.4
GSTP A114V Ala/Val or Val/Val														
Series 1	26/154	17	28/181	15	0.9	0.5–1.6	10/81	12	0.7	0.3–1.5	18/100	18	1.1	0.6–2.1
Series 2	45/337	13	43/271	16	1.2	0.8–1.9	25/141	18	1.4	0.8–2.4	18/130	14	1.0	0.6–1.9

Note: *n*, number of subjects with particular genotype; *N*, total number of subjects. ORs are crude ORs; age, gender, and ethnicity adjusted ORs were the same as those shown to within one decimal point. Series 1 and 2 were subjects participating in 1991–1994 and 1997–2000, respectively.

Table 3. *GST* genotype frequencies by ethnic group, the San Francisco Bay Area Adult Glioma Study 1991–2000

	No.	*GSTT1* null [no. (%)]	*GSTM1* null [no. (%)]	*GSTP* I105V Val/Val [no. (%)]	*GSTP* A114V Ala/Val or Val/Val [no. (%)]
Controls					
Asian	19	7 (37)	10 (53)	0	1 (5)
Black	14	3 (23)	5 (38)	1 (8)	0
Latino	25	6 (24)	12 (48)	4 (16)	1 (4)
White	442	92 (21)	231 (52)	58 (13)	67 (16)
Other	5	1 (20)	3 (60)	0	0
P		0.58	0.87	0.38	0.12
Cases					
Asian	27	14 (52)	15 (56)	1 (4)	0
Black	17	4 (27)	5 (31)	4 (25)	2 (12)
Latino	25	2 (8)	14 (58)	7 (28)	0
White	380	69 (19)	196 (53)	35 (9)	65 (17)
Other	9	1 (13)	3 (38)	2 (22)	0
P		0.0004	0.41	0.007	0.01

Note: *P* values compare genotype frequencies by ethnic group separately within cases and within controls.

four *GST* genotypes studied; multivariate adjustment for all four genes as well as age, gender, and series supported this similarity. There were no marked or significant differences in genotype frequencies among cases with different histological types (Table 5); however, *GSTT1* deletion was somewhat less common among patients with astrocytoma or anaplastic astrocytoma and *GSTP* A114V Ala/Val or Val/Val was more common among patients categorized with ''other'' histologies.

There appeared to be an increasing trend with age for the association of *GSTT1* null genotype and oligodendroglial tumors, with 10%, 25%, or 40% of cases aged ≤40, 41–60, and >60 having this genotype while 20%, 18%, and 23% of controls in these ages had this genotype; however, the interaction of *GSTT1* null genotype and age was not significant ($P = 0.27$ for the test of equal slopes of age with case-control status for *GSTT1* null and *GSTT1* present genotypes).

The following significant or nearly significant differences were noted in analyses that stratified subjects by histological type and/or age group. Among subjects over age 60, *GSTP* I105V Val/Val was less common in cases (6.4% of 108) than controls (15% of 176; OR 0.38; 95% CI 0.15–0.93; $P = 0.03$); a similar result obtained for glioblastoma cases *versus* controls. Over all age groups and among those under age 40, *GSTP* A114V Ala/Val or Val/Val was more common in cases with ''other'' histologies than controls [28% of 32 other histology cases and 16% of 428 controls had these genotypes (OR 2.5; 95% CI 1.0–6.0; $P = 0.05$); among those under age 40, 35% of 17 other histology cases and 16% of 69 controls had these genotypes (OR 3.2; 95% CI 0.9–12.1; $P = 0.08$)].

We found no notable or significant differences among histological subgroups, cases, or controls in the distributions of combinations or numbers of variants in *GSTM1*, *GSTT1*, *GSTP* I105V, or *GSTP* A114V.

Among white glioblastoma multiforme (GBM) cases, 13% of 116 subjects with tumors lacking *p53* mutation and 30% of 23 subjects whose tumors had *p53* mutation had *GSTT1* null genotype (OR 2.8; 95% CI 0.93–8.4; $P = 0.07$). For the other *GST* variants, differences in genotype frequencies by tumor *p53* status did not approach statistical significance.

Discussion

Five previous studies (two from the first series of subjects included here) reported on associations of *GST*s with adult glioma (6–10).

***GSTT1* and *GSTM1*.** In a hospital-based study of 109 glioma cases and 577 controls, Caucasians from North Staffordshire, United Kingdom, Elexpuru-Camiruaga (7) *et al.* first showed that *GSTT1* null genotypes were significantly associated with astrocytoma with 32% of cases *versus* 18% of controls having this genotype. About 60% of the cases and 54% of controls were null for *GSTM1* gene, a nonsignificant difference. Respective proportions in the healthy Caucasians have been reported to be 42–60% for *GSTM1* homozygous deletion and 13–26% for *GSTT1* homozygous deletion (17). In a hospital-based study of 90 malignant glioma cases (49 with GBM) and 90 blood donor healthy controls, Trizna *et al.* (9) found no statistically significant associations between *GSTM1* and *GSTT1* polymorphisms and risk of gliomas in adults; 52% of cases and 48% of controls were *GSTM1* null and 28% of cases and 30% of controls were *GSTT1* null. Recently, De Roos *et al.* (8) reported on deletions in *GSTM1* and *GSTT1* in 422 adults with glioma and 604 controls who were part of the National Cancer Institute's hospital-based study of brain tumors in three hospitals in Phoenix, AZ, Pittsburgh, PA, and Boston, MA. They found no significant difference between cases overall and controls for *GSTM1* or *GSTT1* null genotypes; 53% of cases and 56% of controls were *GSTM1* null and 20% of cases and 18% of controls were *GSTT1* null. Among cases of different histological types (glioblastoma, anaplastic astrocytoma, other astrocytoma, oligodendroglioma, and oligoastrocytoma), significant heterogeneity in genotype frequencies was noted for *GSTM1* ($P = 0.03$); subjects with oligodendrogliomas had lower than expected percentage of *GSTM1* null genotype (33%). In this current study, we found no case-control differences overall for *GSTM1* or *GSTT1* deleted *versus* nondeleted genotypes and no indication of differences by histological types. In the combined series, we noted a nonsignifcant deficit of *GSTT1* deleted cases who had

Table 4. *GST* genotypes in adult whites with glioma and controls, the San Francisco Bay Area Adult Glioma Study 1991–2000

Genotype	Controls ($n = 428$)	Cases ($n = 367$)	OR	95% CI	*P*
	% with genotype	% with genotype			
GSTM1 null	53	53	1.01	0.76–1.3	0.94
GSTT1 null	21	18	0.89	0.62–1.3	0.53
GSTP I105V Val/Val	13	10	0.68	0.43–1.1	0.11
GSTP A114V Ala/Val or Val/Val	16	17	1.24	0.84–1.8	0.29

Note: The model contains all four *GST* genes, age, gender, and series.

Table 5. Genotype frequencies of four *GST*s by histological types among white adults with glioma, the San Francisco Bay Area Adult Glioma Study

	Total no.	*GSTM1* null (%)	*GSTT1* null (%)	*GSTP* I105V Val/Val (%)	*GSTP* A114V Ala/Val or Val/Val (%)
All	367	53	18	10	17
Glioblastoma	179	53	18	9	16
Astrocytoma and anaplastic astrocytoma	62	55	11	8	19
Oligodendroglioma and oligoastrocytoma	94	52	20	12	15
Other	32	50	25	9	28
P		0.97	0.36	0.86	0.35

glioblastoma and a significant excess of cases who were *GSTT1* deleted whose glioblastoma tumors contained *p53* mutation compared with those whose glioblastoma tumors did not contain *p53* mutation.

In our previous reports that included most of the same subjects included here in series 1, we also reported no significant relationship of either the *GSTT1* or the *GSTM1* null genotypes with occurrence of glioma overall in about 160 cases and 160 controls; 53% of cases and 50% of controls were *GSTM1* null and 19% of cases and 20% of controls were *GSTT1* null (6, 10). We reported that 44% of 16 patients with oligodendroglioma were *GSTT1* null genotype yielding a 3-fold OR of 3.2 (95% CI 1.1–9.2) (6). There were some differences in the histopathology categorization in the two earlier reports compared with this current paper because the uniform neuropathology review had not yet been completed. In the finalized uniform review used in this paper, 41% (7 of 17) of white patients from series 1 with oligodendroglioma had *GSTT1* null genotype; the equivalent number for series 2 is 19% (8 of 43) patients (*i.e.*, the second series of subjects failed to replicate the finding in the first series). As mentioned above, there was a rather substantial difference in subjects categorized as oligodendroglioma alone *versus* oligoastrocytoma in series 1 and 2, so we combined these two diagnoses for the present paper; however, to try to understand whether the differences in *GSTT1* null genotypes among oligodendroglioma cases between series 1 and 2 might be due to differential classification, we also examined the frequency of *GSTT1* null genotype in whites with oligoastrocytoma; these were 11% (3 of 27) in series 1 and 22% (2 of 9) in series 2. In the earlier report on *GSTM1* using series 1 cases, we found that *GSTM1* null genotype was related to earlier onset of glioma among the female cases but not in male cases (10); in that report, among white women with glioma, the mean age at diagnosis was 43.9 years for 32 *GSTM1* deleted women and 52.4 years for 29 *GSTM1* nondeleted women; the means were very similar among the women with glioma from series 1 included in this current paper: 34 *GSTM1* deleted women (43.4) and 29 *GSTM1* nondeleted women (52.4). However, in series 2, there was no similar difference in the age at diagnosis by *GSTM1* genotype (numbers of women and mean age at diagnosis for those with and without *GSTM1* deletion were 46 women with average age of 55.5 years and 51 women with average age of 52.9 years, respectively).

This is the first study to evaluate *GST* genotype frequencies by tumor type categorized according to molecular alteration. The finding of a higher rate of *GSTT1* deletion among subjects with glioblastoma tumors with *p53* mutation might be expected based on the idea that such tumors might be initiated by mutations caused by endogenous or exogenous substances, the dose of which might be influenced by the presence or absence of *GSTT1*. It is also possible that the finding was due to chance, given the many comparisons performed in these analyses.

***GSTP* I105V and A114V.** De Roos *et al.* (8) also examined two variants in *GSTP*, I105V and A114V. They found no case-control differences in *GSTP* A114V variants; 15% of cases and 14% of controls were Ala/Val or Val/Val. However, for *GSTP* I105V, 17% of cases *versus* 10% of controls were Val/Val yielding an age, gender, race, and other factor adjusted OR of 1.8 (95% CI 1.2–2.7). In addition, notable was that an even higher percentage of cases age 60 and younger had Val/Val genotype than those over age 60 (20% *versus* 11%), but no similar trend with age was seen in controls. We found no overall case-control differences in percentages with *GSTP* I105V Val/Val genotypes (9% of cases and 13% of controls). However, there were some case-control differences with age in the present study (*i.e.*, cases over age 60 were significantly less likely than controls to be Val/Val genotype). De Roos *et al.* (8) reported nearly significant heterogeneity in *GSTP* A114V genotype frequencies ($P = 0.06$) with higher than expected *GSTP* A114V genotypes of Ala/Val or Val/Val (25%) among subjects with oligodendroglioma. We did not observe this heterogeneity but did find a nearly significant higher occurrence of these genotypes among those with ''other'' histologies (a category that includes a very heterogeneous collection of tumors including juvenile pilocytic astrocytoma, medulloblastoma, ependymoma, ganglioglioma, and other).

Because there is no consistent overall association of the *GST* variants studied with adult glioma in the two series of this current study or in the other reported studies, we conclude that there are no strong effects of *GST* variants on glioma development. However, results of all the studies published thus far include too few subjects in various subsets of glioma to reach definitive conclusions about the associations of *GST* variants with the non-GBM histologies or molecularly defined subsets of glioma. Our study showed that there can be very

substantial heterogeneity of these variants among the cases within series (*e.g.*, *GSTM1* genotypes in GBM cases was 45% in series 1 and 54% in series 2). This suggests that *GST* genotypes might influence the survival rate for glioma. Evidence both for and against associations of *GST* genotypes with prognosis for several cancer sites has been reported (for recent examples, see 18–26). As noted previously, specimen collection began partway through series 1 and overall specimen collection rates and percentages of subjects' genotypes are substantially higher for series 2 than series 1. Consequently, selection bias in specimen collection (and genotyping results) related to survival is more likely in series 1 than in series 2. A detailed analysis of *GST* genotypes related to survival is beyond the scope of this paper, as survival follow-up and collection of pertinent treatment data are not yet complete. However, such an association is possible given that *GSTM1* null genotype in GBM cases was associated with days between diagnosis and specimen collection (OR 1.007; 95% CI 1.003–1.012 for series 1; OR 1.003; 95% CI 1.00–1.01 for series 2) controlling for age, gender, and ethnic group. In addition, there was significant heterogeneity in some *GST* genotypes by ethnic group that was stronger in cases than controls (see Table 3). This points both to the need for very careful control of case-control differences in ethnicity in evaluating possible associations between genotypes and disease status and to consider the effects of these genotypes on survival. It also suggests the benefits of different study groups employing different case-control designs in trying to understand whether true relationships exist between genotypes and disease. For example, the De Roos *et al.* (8) study had the advantage that hospital-based cases would minimize the loss of subjects due to gene survival associations; however, their control group was also hospital based, potentially obscuring associations if gene variants were associated with the other diseases. This present study has the advantage that both cases and controls were population based but has the disadvantage that blood or buccal specimens could not be obtained from cases with poorest survival. Similarities of genotype frequencies in the two different types of studies lend support to validity of consistent findings.

Although there are no overall main effects of these *GST* variants with glioma etiology, these genes might be found to be important in conjunction with appropriate environmental exposures. Unfortunately, very few strong environmental risk factors have been identified for adult glioma. Perhaps, combining environmental and genetic data from these relatively large series of subjects from several study sources, along with uniform histological and molecular categorization of tumors, will eventually lead to a better understanding of risk factors for adult glioma.

Acknowledgments

We thank Dr. Richard Davis for pathology review of series 1 cases; the pathology departments of Alexian Hospital, Alta Bates Medical Center, Brookside, California Pacific Med Center, DR Pinole, Eden Hospital, El Camino Hospital, Good Samaritan, Highland Hospital, John Muir, Kaiser Redwood City, Kaiser San Francisco, Kaiser Santa Teresa, Los Gatos Hospital, Los Medano Hospital, Marin General, Merrithew, Mills Peninsula Hospital, Mt. Diablo Hospital, Mt. Zion Medical Center, Naval Hospital, O'Connor Hospital, Ralph K. Davies Medical Center, Saint Louise, San Francisco General, San Jose, San Leandro, San Mateo County, San Ramon Valley, Santa Clara Valley, Sequoia, Seton Medical Center, St. Francis, St. Luke's, St. Rose, Stanford, Summit, UC San Francisco, Valley Livermore, Veterans Palo Alto, Veterans SF, and Washington Hospital for providing tumor specimens for review and molecular analyses; and Joe Patoka and Pengchin Chen for *p53* mutation analyses of tumors.

References

1. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. Science, 1999;286:487–91.
2. Yuspa SH, Shields PG. Etiology of cancer: chemical factors. In: De Vita J, Hellman VTS, Rosenberg SA, editors. Cancer: principles and practice of oncology. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 179–93.
3. Harris MJ, Coggan M, Langton L, Wilson SR, Board PG. Polymorphism of the class glutathione *S*-transferase in normal populations and cancer patients. Pharmacogenetics, 1998;8:27–31.
4. Ishimoto TM, Ali-Osman F. Allelic variants of the human glutathione *S*-transferase P1 gene confer differential cytoprotection against anticancer agents in *Escherichia coli*. Pharmacogenetics, 2002;12:543–53.
5. Wrensch M, Minn Y, Chew T, Bondy M, Berger MS. Epidemiology of primary brain tumors: current concepts and review of the literature. Neuro-oncol, 2002;4:278–99.
6. Kelsey KT, Wrensch M, Zuo ZF, Miike R, Wiencke JK. A population-based case-control study of the CYP2D6 and GSTT1 polymorphisms and malignant brain tumors. Pharmacogenetics, 1997;7:463–8.
7. Elexpuru-Camiruaga J, Buxton N, Kandula V, et al. Susceptibility to astrocytoma and meningioma: influence of allelism at glutathione *S*-transferase (GSTT1 and GSTM1) and cytochrome *P*-450 (CYP2D6) loci. Cancer Res, 1995;55:4237–9.
8. De Roos AJ, Rothman N, Inskip PD, et al. Genetic polymorphisms in GSTM1, -P1, -T1, and CYP2E1 and the risk of adult brain tumors. Cancer Epidemiol Biomarkers & Prev, 2003;12:14–22.
9. Trizna Z, de Andrade M, Kyritsis AP, et al. Genetic polymorphisms in glutathione *S*-transferase θ, *N*-acetyltransferase, and CYP1A1 and risk of gliomas. Cancer Epidemiol Biomarkers & Prev, 1998;7:553–5.
10. Wiencke JK, Wrensch MR, Miike R, Zuo Z, Kelsey KT. Population-based study of glutathione *S*-transferase gene deletion in adult glioma cases and controls. Carcinogenesis, 1997;18:1431–3.
11. Krishnan G, Felini M, Carozza SE, Miike R, Chew T, Wrensch M. Occupation and adult gliomas in the San Francisco Bay Area. J Occup Environ Med, 2003;45:639–47.
12. Wiemels JL, Wiencke JK, Sison JD, Miike R, McMillan A, Wrensch M. History of allergies among adults with glioma and controls. Int J Cancer, 2002;98:609–15.
13. Wrensch M, Lee M, Miike R, et al. Familial and personal medical history of cancer and nervous system conditions among adults with glioma and controls. Am J Epidemiol, 1997;145:581–93.
14. Smith CM, Kelsey KT, Wiencke JK, Leyden K, Levin S, Christiani DC. Inherited glutathione-*S*-transferase deficiency is a risk factor for pulmonary asbestosis. Cancer Epidemiol Biomarkers & Prev, 1994;3:471–7.
15. Chen P, Aldape K, Wiencke JK, et al. Ethnicity delineates different genetic pathways in malignant glioma. Cancer Res, 2001;61:3949–54.
16. SAS/STAT user's guide, version 6. Version 4. Cary (NC): SAS Institute; Inc., 1989
17. Garte S, Gaspari L, Alexandrie AK, et al. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers & Prev, 2001;10:1239–48.
18. Davies SM, Bhatia S, Ross JA, et al. Glutathione *S*-transferase genotypes, genetic susceptibility, and outcome of therapy in childhood acute lymphoblastic leukemia. Blood, 2002;100:67–71.
19. Stoehlmacher J, Park DJ, Zhang W, et al. Association between glutathione *S*-transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. J Natl Cancer Inst, 2002;94:936–42.
20. Voso MT, D'Alo F, Putzulu R, Mele L, et al. Negative prognostic value of glutathione *S*-transferase (GSTM1 and GSTT1) deletions in adult acute myeloid leukemia. Blood, 2002;100:2703–7.

21. Sweeney C, Ambrosone CB, Joseph L, et al. Association between a glutathione *S*-transferase A1 promoter polymorphism and survival after breast cancer treatment. Int J Cancer, 2003;103:810–4.
22. Anda T, Shabani HK, Tsunoda K, et al. Relationship between expression of O^6-methylguanine-DNA methyltransferase, glutathione-*S*-transferase in glioblastoma and the survival of the patients treated with nimustine hydrochloride: an immunohistochemical analysis. Neurol Res, 2003;25:241–8.
23. Sala A, Lanciotti M, Valsecchi MG, et al. Genotypes of the glutathione *S*-transferase superfamily do not correlate with outcome of childhood acute lymphoblastic leukemia. Leukemia, 2003;17:981–3.
24. Choi SC, Yun KJ, Kim TH, et al. T. Prognostic potential of glutathione *S*-transferase M1 and T1 null genotypes for gastric cancer progression. Cancer Lett, 2003;195:169–75.
25. Medeiros R, Pereira D, Afonso N, et al. Platinum/paclitaxel-based chemotherapy in advanced ovarian carcinoma: glutathione *S*-transferase genetic polymorphisms as predictive biomarkers of disease outcome. Int J Clin Oncol, 2003;8:156–61.
26. Miyatake K, Gemba K, Ueoka H, et al. Prognostic significance of mutant *p53* protein, P-glycoprotein and glutathione *S*-transferase- in patients with unresectable non-small cell lung cancer. Anticancer Res, 2003;23:2829–36.

Glutathione-*S*-Transferase Variants and Adult Glioma

Margaret Wrensch, Karl T. Kelsey, Mei Liu, et al.

Cancer Epidemiol Biomarkers Prev 2004;13:461-467.