Short Communication

Genetic Polymorphisms in Glutathione *S*-Transferase μ and θ, *N*-Acetyltransferase, and *CYP1A1* and Risk of Gliomas[1]

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Abstract

The role of genetic polymorphisms in modulating susceptibility to carcinogenic exposures has been well explored for tobacco-related neoplasms but not for other neoplasms including gliomas. It is relevant to explore these polymorphisms because certain carcinogenic exposures such as nitrosamines are implicated in the risk of gliomas. We therefore conducted a pilot case-control study to examine the role of polymorphisms in *GSTM1*, *GSTT1*, *NAT2* (rapid, intermediate, and slow acetylation), and *CYP1A1* and risk of glioma. Ninety patients diagnosed with glioma were ascertained as part of an ongoing genetic epidemiological study and were age, gender, and race matched with 90 healthy controls. We used PCR based methodology to determine the prevalence of the above genetic polymorphisms using sequences and PCR conditions directly adapted from studies reported previously. We calculated univariate odds ratios and performed multiple logistic regression to assess interactions between polymorphisms. We found no statistically significant associations between the null genotypes of *GSTM1* and *GSTT1*, and *CYP1A1* and risk of gliomas. However, there was an intriguing pattern with *NAT2* acetylation status (odds ratios, 1.81, 1.34, and 0.61 for rapid, intermediate, and slow acetylation, respectively; $P = 0.10$ for trend). It is unlikely that any single polymorphism is sufficiently predictive of risk, and a panel of markers integrated with epidemiological data should be conducted on a large number of study subjects to fully understand the role of genetic polymorphisms and brain tumor risk.

Introduction

Most cancers arise from gene-environment interactions where susceptible individuals develop cancer after exposure to toxic or mutagenic environmental agents. Intracranial implantation of polycyclic aromatic hydrocarbons, combustion-derived chemicals and nitroso compounds, and dietary nitrosamines and occupational exposures to various chemicals are also associated with brain tumors (1–3). These substances are usually metabolized by enzymes that respond to environmental challenges, such as CYP,[3] *GST*, and *NAT*. If an individual is unable to detoxify these xenobiotic toxins because of an altered expression of these enzymes, cancer risk may be increased. Interactions of environmental factors and genetic variants of *GSTT* and *GSTM* and the CYP enzyme *CYP2D6* may be important risk factors for brain tumors (1, 4–8).

GSTM1 and *GSTT1* are involved in metabolizing a wide range of xenobiotics, including environmental carcinogens, chemotherapeutic agents, and reactive oxygen species (9–14). *GST* deficiencies may result from a somatic or inherited mutation, which can lead to tumor formation (12). If an individual has inherited the homozygous form of the null polymorphisms of the *GSTM1* or *GSTT1* gene, they are less able to conjugate and detoxify specific substrate intermediates (12). Absent or deficient *GST* enzyme activity may result in poorer elimination of electrophilic carcinogens, particularly in the presence of phase I enzymes such as the CYP enzymes. The frequency of a homozygous deletion in the *GSTM1* genotype ranges from 38–67% in Caucasians, from 33–63% in East Asians, and from 22–35% in African-Americans (12). Approximately 20% of Caucasians have the *GSTT1* null genotype, but the frequency in African and Asian populations may be more similar to *GSTM1*. The estimates for *GSTT1* were derived from control populations of case-control studies (12).

Another putative risk factor is the *Val/Val* homozygous genotype of the *CYP1A1* gene, a CYP enzyme, which codes for aryl hydrocarbon hydroxylase. The population prevalence of this genotype has been estimated at 2–5% (15, 16). Aryl hydrocarbon hydrolase is involved in the biotransformation of polycyclic aromatic hydrocarbons into reactive diol epoxide metabolites with carcinogenic potential (17).

Mutations in the *NAT2* isoenzyme, which is found in 34–78% of the general population, are also thought to increase the risk of cancer. *NAT2* metabolizes primary aromatic amines and hydrazine groups and some drugs (isoniazid, sulfonamides, and caffeine), dyes, pesticides, and cooking-generated carcinogens (18–20). *NAT2* mutations have thus been associated with increased drug toxicity and an elevated risk of developing certain cancers (21).

Few studies have investigated the potential relationships

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[3] The abbreviations used are: CYP, cytochrome P450; GST, glutathione-*S*-transferase; *GSTT* and *GSTM*, GST θ and μ, respectively; NAT, *N*-acetyltransferase; CI, confidence interval; OR, odds ratio.

Table 1 Univariate analysis of genetic polymorphisms in cases and controls

Genotype	Cases		Controls		OR	95% CI
	n	%	*n*	%		
GSTM1						
Null	47	52.2	39	43.3	1.43	0.79–2.57
Present	43	47.8	51	56.7		
GSTT1						
Null	25	27.8	27	30.0	0.90	0.47–1.43
Present	65	72.2	63	70.0		
NAT 2						
Rapid or intermediate	69	76.7	60	66.7	1.64	0.85–3.16
Slow acetylator	21	23.3	30	33.3		
CYP1A1[a]						
wt/mut + mut/mut	18	20.0	13	14.4	1.48	0.68–3.2
wt/wt	72	80.0	77	85.6		

[a] wt, wild type; mut, mutant.

between genetic polymorphisms and brain tumor risk (4, 7, 8). Therefore, we performed this pilot study of the role of *GSTM1*, *GSTT1*, *NAT2*, and *CYP1A1* polymorphisms as risk factors for gliomas.

Materials and Methods

Patients. The 90 cases included in this study were diagnosed at The University of Texas M. D. Anderson Cancer Center during the years 1991 and 1994 with a histologically confirmed glioma: 49 had glioblastoma multiforme (high grade); 34 had anaplastic astrocytoma (medium grade); and 7 had a low-grade glioma. The patients, 53 men and 37 women, averaged 43.2 years at diagnosis, and 87% were non-Hispanic whites, 13% were African-American, and 4% were Hispanic. We obtained blood samples from the patients when they initially registered at M. D. Anderson and prior to treatment. These glioma cases were a subset of unselected patients participating in a larger ongoing family study. We frequency matched the cases with 90 healthy individuals who were blood donors at the M. D. Anderson blood bank or at work-site blood drives by sex, age (±5 years), and race.

Laboratory Methods. DNA was extracted from the patient's sera and from fresh blood of the controls with a commercially available kit (Qiagen, Chatsworth, CA). We used 50–100 ng of DNA for PCR with custom-synthesized primers (Genosys, Kingwood, TX). PCR and restriction enzyme digestions were performed as reported previously for the primers for *GSTM1* (9), *GSTT1-1* (14), *NAT2* (20), and exon 7 of *CYP1A1* (21). β-Globin was coamplified as an internal positive control for the *GSTM1* and *GSTT1* reactions. We ensured reproducibility by performing duplicate reactions on all samples.

Statistical Methods. For statistical analysis, we computed univariate ORs and 95% CIs and assessed interactions between polymorphisms by multivariate logistic regression (adjusted for age, gender, and ethnicity). Because the number of subjects with rapid acetylator genotypes was small, we combined rapid and intermediate acetylators into one group for the multivariate analyses and assigned the slow acetylators as the referent group.

Results

We found no statistically significant associations by univariate analyses between the individual genetic polymorphisms and risk of glioma (Table 1) but observed an interesting trend in rapid, intermediate, and slow acetylation of *NAT2*, with ORs of 1.81, 1.34, and 0.61, respectively ($P = 0.10$ for trend). We included all of the genetic polymorphisms studied in the multiple logistic model, controlling for age, ethnicity, and gender (Table 2). When we included all of the markers into the multivariate model, we found evidence of a synergistic effect between rapid and intermediate acetylation and *GSTT1* null genotype (OR, 7.16; $P = 0.04$). This risk estimate, however, is based on small numbers: only 2 cases and 10 controls were both *GSTT1* null and intermediate/rapid acetylators.

Table 2 Multivariate analysis of genetic risk and risk of glioma

Genetic polymorphism	OR[a]	95% CI	*P*
NAT2 acetylator	1.64	0.84–3.17	0.14
GSTT1 null	0.87	0.45–1.67	0.67
GSTM1 null	1.39	0.77–2.52	0.28
CYP1A1 wt/mut + mut/mut	1.47	0.66–3.24	0.35

[a] Adjusted by age, sex, and ethnicity.

Discussion

Elexpuru-Camiruaga *et al.* (4) reported on a case-control study of 109 astrocytoma patients and 577 controls who were *GSTT1* null had a 2.7-fold risk of cancer; however, we could not confirm these findings. Interestingly, in their study, only 18.4% of the controls exhibited the *GSTT1* null genotype, considerably less than the 30% found in our study and the 38% rate reported in the literature (12). Elexpuru-Camiruaga *et al.* (4) had more than 500 control subjects, and possibly their estimate is more stable, or this British population does not possess the higher risk allele. The prevalence of the *GSTM1* reported in the control population (54.6%) of this same study was more similar to ours (43.4%). The study by Wiencke *et al.* (7) reported a similar proportion of the controls with the null genotype (50%).

The relationship between acetylation and susceptibility to glioma is also unclear. Our study showed a nonsignificant trend in rapid (OR, 1.81), intermediate (OR, 1.34), and slow (OR, 0.61) acetylator forms of the *NAT2* polymorphism similar to the colon cancer studies (22). On the other hand, the slow acetylator phenotype has been associated with increased susceptibility in studies of occupational bladder cancer (22).

Although the gene-gene interaction is based on very few subjects and must be interpreted with utmost caution, there is biological plausibility for synergism between the two risk genotypes. Chance is the most likely explanation for the observed interaction between *GSTT1* and *NAT2* fast acetylator, especially because the numbers in the cells are small. The results of this pilot study indicate the need for a more in-depth study of genetic polymorphisms, environmental factors, and brain tumor risk in larger, well-designed case-control studies.

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References

1. Preston-Martin, S., and Mack, T. Gliomas and meningiomas in men in Los Angeles County: Investigation of exposures to *N*-nitroso compounds. IARC Sci. Publ., *105:* 197–203, 1991.

2. Giles, G. G., McNeil, J. J., Donnan, G., Webley, C., Staples, M. P., Ireland, P. D., Hurley, S. F., and Salzberg, M. Dietary factors and the risk of glioma in

adults: results of a case-control study in Melbourne, Australia. Int. J. Cancer, *59:* 357–362, 1994.

3. Hochberg, F., Toniolo, P., Cole, P., and Salcman, M. Nonoccupational risk indicators of glioblastoma in adults [published erratum appears in J. Neuro-Oncol., *9:* 90, 1990]. J. Neuro-Oncol., *8:* 55–60, 1990.

4. Elexpuru-Camiruaga, J., Buxton, N., Kandula, V., Dias, P. S., Campbell, D., McIntosh, J., Broome, J., Jones, P., Inskip, A., Alldersea, J., Fryer, A. A., and Strange, R. C. Susceptibility to astrocytoma and meningioma: Influence of allelism at glutathione *S*-transferase (*GSTT1* and *GSTM1*) and cytochrome P-450 (*CYP2D6*) loci. Cancer Res., *55:* 4237–4239, 1995.

5. Lasker, S. E., Iatropoulos, M. J., Hecht, S. S., Misra, B., Aamin, S., Zang, E., and Williams, G. M. *N*-Ethyl-*N*-nitrosourea induced brain tumors in rats monitored by nuclear magnetic resonance imaging, plasma proton nuclear magnetic resonance spectroscopy and microscopy. Cancer Lett., *67:* 125–131, 1992.

6. Demers, P. A., Heyer, N. J., and Rosenstock L. Mortality among firefighters from three northwestern United States cities. Br. J. Indust. Med., *49:* 664–670, 1992.

7. Wiencke, J. K., Wrensch, M. R., Miike, R., Zuo, Z., and Kelsey, K. T. Population-based study of glutathione *S*-transferase μ gene deletion in adult glioma cases and controls. Carcinogenesis (Lond.), *18:* 1431–1433, 1997.

8. Hand, P. A., Inskip, A., Gilford, J., Alldersea, J., Elexpuru-Camiruaga, J., Hayes, J. D., Jones, P. W., and Strange, R. C. Allelism at the glutathione *S*-transferase *GSTM3* locus: interactions with *GSTM1* and *GSTT1* as risk factors for astrocytoma. Carcinogenesis (Lond.), *17:* 1919–1922, 1996.

9. Bell, D. A., Taylor, J. A., Paulson, D. F., Robertson, C. N., Mohler, J. L., and Lucier, G. W. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione *S*-transferase M1 (*GSTM1*) that increases susceptibility to bladder cancer. J. Natl. Cancer Inst., *85:* 1159–1164, 1993.

10. Seidegard, J., Vorachek, W. R., Pero, R. W., and Pearson, W. R. Hereditary differences in the expression of the human glutathione transferase active on *trans*-stilbene oxide are due to a gene deletion. Proc. Natl. Acad. Sci. USA, *85:* 7293–7297, 1988.

11. Heckbert, S. R., Weiss, N. S., Hornung, S. K., Eaton, D. L., and Motulsky, A. G. Glutathione *S*-transferase and epoxide hydrolase activity in human leukocytes in relation to risk of lung cancer and other smoking-related cancers. J. Natl. Cancer Inst., *84:* 414–422, 1992.

12. Rebbeck, T. Molecular epidemiology of the human glutathione *S*-transferase genotypes *GSTM1* and *GSTT1* in cancer susceptibility. Cancer Epidemiol. Biomark. Prev., *6:* 733–743, 1997.

13. Zhong, S., Wyllie, A. H., Barnes, D., Wolf, C. R., and Spurr, N. K. Relationship between the *GSTM1* genetic polymorphism and susceptibility to bladder, breast and colon cancer. Carcinogenesis (Lond.), *14:* 1821–1824, 1993.

14. Pemble, S., Schroeder, K. R., Spencer, S. R., Meyer, D. J., Hallier, E., Bolt, H. M., Ketterer, B., and Taylor, J. B. Human glutathione *S*-transferase θ (*GSTT1*): cDNA cloning and the characterization of a genetic polymorphism. Biochem. J., *300:* 271–276, 1994.

15. Wheeler, C. W., and Guenthner, T. M. Cytochrome P-450-dependent metabolism of xenobiotics in human lung. J. Biochem. Toxicol., *6:* 163–169, 1991.

16. Hayashi, S., Watanabe, J., and Kawajiri, K. High susceptibility to lung cancer analyzed in terms of combined genotypes of *P450IA1* and μ-class glutathione *S*-transferase genes. Jpn. J. Cancer Res., *83:* 866–870, 1992.

17. Lin, H. J., Han, C. Y., Lin, B. K., and Hardy, S. Slow acetylator mutations in the human polymorphic *N*-acetyltransferase gene in 786 Asians, blacks, Hispanics, and whites: application to metabolic epidemiology. Am. J. Hum. Genet., *52:* 827–834, 1993.

18. Blum, M., Demierre, A., Grant, D. M., Heim, M., and Meyer, U. A. Molecular mechanism of slow acetylation of drugs and carcinogens in humans. Proc. Natl. Acad. Sci. USA, *88:* 5237–5241, 1991.

19. Deguchi, T. Sequences and expression of alleles of polymorphic arylamine *N*-acetyltransferase of human liver. J. Biol. Chem., *267:* 18140–18147, 1992.

20. Abe, M., Suzuki, T., and Deguchi, T. An improved method for genotyping of *N*-acetyltransferase polymorphism by polymerase chain reaction. Jpn. J. Hum. Genet., *38:* 163–168, 1993.

21. Shields, P. G., Bowman, E. D., Harrington, A. M., Doan, V. T., and Weston, A. Polycyclic aromatic hydrocarbon-DNA adducts in human lung and cancer susceptibility genes. Cancer Res., *53:* 3486–3492, 1993.

22. Smith, G., Stanley, L. A., Sim, E., Strange, R. C., and Wolf, C. R. Metabolic polymorphisms and cancer susceptibility. Cancer Surv., *25:* 27–65, 1995.

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