Glutathione *S*-Transferase Polymorphisms in Children with Myeloid Leukemia: A Children's Cancer Group Study[1]

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Abstract

***GSTM1* and *GSTT1* are polymorphic genes. Absence of enzyme activity is due to homozygous inherited deletion of the gene, reducing detoxification of carcinogens such as epoxides and alkylating agents and potentially increasing cancer risk. We hypothesized that *GST* null genotype would increase risk of acute myeloid leukemia and myelodysplasia (AML/MDS) in children. DNA was extracted from bone marrow slides of 292 AML/MDS patients. PCR amplification was used to assign *GSTM1* and *GSTT1* genotypes for cases and controls. Given that the frequency of the null genotype varies by ethnicity and that the majority of the cases were Caucasian, analyses were restricted to 232 white (non-Hispanic) cases and 153 Caucasian non cancer controls. The frequency of *GSTM1* null was significantly increased in AML/MDS cases compared with controls {64 *versus* 47%; odds ratio (OR), 2.0 [95% confidence interval (CI), 1.3–3.1]; $P = 0.001$}, whereas the frequency of *GSTT1* null genotype in AML/MDS cases was not statistically different from controls. AML comprises biologically distinct subtypes, and a test for homogeneity revealed a statistically significant difference among subtypes ($P = 0.04$; *df*, 8) for *GSTM1* only. In particular, there was an increased frequency of *GSTM1* null genotypes in French-American-British groups M3 [82%; $n = 22$; OR, 5.1 (95% CI, 1.6–21.3)] and M4 [72%; $n = 53$; OR, 2.9 (95% CI, 1.4–6.0)]. We conclude that the *GSTM1* null genotype is a significant risk factor for childhood AML, particularly French-American-British groups M3 and M4. This may indicate an important role for exogenous carcinogens in the etiology of childhood AML.**

Received 11/18/99; revised 3/16/00; accepted 3/27/00.

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[1] This work was supported by supplemental funds to Grant 5U10-CA13539 to the CCG chairman, Dr. W. A. Bleyer. Tissue samples were supplied by the Cooperative Human Tissue Network pediatric division, which is funded by the National Cancer Institute (Grant CA 54021-09).

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Introduction

Conjugation of electrophilic compounds to glutathione, mediated by the family of GST[3] enzymes, is an important detoxifying pathway for environmental mutagens such as organophosphates (including pesticides), alkylating agents, epoxides, and polycyclic aromatic hydrocarbons (1). The *GSTM1* and the *GSTT1* genes are polymorphic in humans, and the phenotypic absence of enzyme activity is due to a homozygous inherited deletion of the gene (2, 3). In view of the likely importance of environmental carcinogens in the etiology of a range of malignancies, it would be expected that individuals with the null phenotype who are unable to detoxify these agents could be at increased risk of malignancy. The *GSTM1* null genotype has been associated with increased risk of colorectal, cervical, laryngeal, head and neck, pituitary, stomach, and multiple skin cancers, and of smoking-induced lung and bladder cancers in a number of studies (reviewed in Ref. 4). Although these studies report positive associations, other similar studies have not reported an association, which may reflect heterogeneity in etiology among populations or in study methodologies. Fewer studies have examined the role of the *GSTT1* null genotype in susceptibility to malignancy, although associations have been reported with astrocytoma, colorectal cancer, laryngeal cancer, and meningioma (5–7). A study performed by the Cancer and Leukemia Group B has shown an increased risk of myelodysplasia in individuals with the *GSTT1* null genotype, although other reports have not confirmed this observation (8–11).

In this report, we describe results of *GSTM1* and *GSTT1* genotyping in 292 children with AML or MDS. We show that the *GSTM1* null genotype is significantly more frequent in childhood AML, particularly FAB groups M3 and M4.

Patients and Methods

Study Population. The study population included 292 incident cases of childhood AML or MDS treated in CCG therapeutic studies between 1988 and 1994. Eligible patients included those participating in an epidemiological study of AML and included infants <1 year of age. Comparison of outcomes showed no difference in survival between those with and without *GST* genotype analysis. Clinical data, including age, gender, immunophenotype, WBCs at diagnosis, presence of chloroma, presence of central nervous system disease, and presence of Down syndrome, were collected prospectively. Cytogenetic data were available for 130 cases (44%). Histological subgroups were classified based on criteria established and revised by the French American British Cooperative Study Group (12). Of the 292 cases, 42 were M0/M1, 74 were M2, 27 were M3,

[3] The abbreviations used are: GST, glutathione *S*-transferase; AML, acute myeloid leukemia; MDS, myelodysplasia; FAB, French-American-British; CCG, Children's Cancer Group; OR, odds ratio; CI, confidence interval; ALL, acute lymphoblastic leukemia.

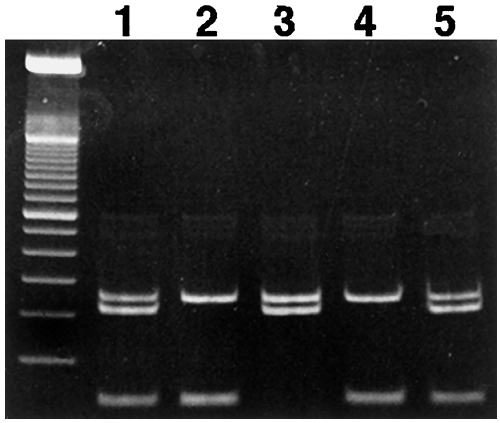

Fig. 1. PCR of genomic DNA samples for GSTM1 (151 bp) and GSTT1 (70 bp), and internal control band K-RAS (164 bp), which is present in all samples. *Lanes 1* and *5* shows individuals with GSTM1 and GSTT1; *Lanes 2* and *4* show GSTM1-null individuals; *Lane 3* shows a GSTT1 GSTM1-positive individual. A 50-bp size marker is present to the *left* of *Lane 1*.

64 were M4, 45 were M5, 6 were M6, 17 were M7, 8 were RAEB, and 9 were RAEBt. Given that the frequency of *GST* null genotype varies by ethnicity, analyses were restricted to 232 white (non-Hispanic) cases. Cases were drawn from the geographic areas served by CCG (13). Controls were 153 white (non-Hispanic) non-cancer adult individuals derived from a number of sources including blood donors and participants in a pilot epidemiological study within Minnesota. Proportions of the null genotype in this population were comparable to reported frequencies (14–19).

Genotyping. For cases, DNA was extracted from archival bone marrow slides or from cryopreserved marrow samples as described previously (20). Briefly, cells were scraped from the slide with a scalpel, using sterile technique to prevent DNA contamination. Cells were suspended in PCR buffer [50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1% Triton X-100], boiled for 10 min, extracted twice with phenol, and precipitated with ethanol. DNA was washed once with 70% ethanol, resuspended in Tris-EDTA buffer, and amplified. For controls, DNA was extracted from a small aliquot of a blood donation using standard techniques. Triplex amplification was performed using primers for *GSTM1* (5′-GAGATGAAGTCCTTCAGA-3′ and 5′-GCTTCACGTGTTATGGAGGTT-3′; band size, 151 bp), *GSTT1* (5′-ATGTGACCCTGCAGTTGC-3′ and 5′-GAGATGTGAGCACCAGTAAGGAA-3′; band size, 70 bp) and as an internal control for DNA degradation, primers that amplify *K-RAS* (5′-GTACTGGTGGAGTATTTGATAGTG-3′ and 5′-TAGCTGTATCGTCAAGGCAC-3′; band size, 164 bp). Primers for *GSTM1* and *GSTT1* were designed to be shorter than the control primer to ensure that false-negative genotypes were not reported because of failure of amplification of a longer PCR band from these archival DNA samples. Results of amplifications using these primer sets were compared with results obtained with published primer sets in control samples, and identical results were obtained (8). In addition, the identity of the *GSTT1* PCR product was verified by sequencing prior to initiation of the study. Each 50-μl PCR reaction contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, and 100 μM deoxynucleotide triphosphates. Forty cycles of amplification were performed at an annealing temperature of 58°C, and products were visualized by agarose gel electrophoresis (Fig. 1). A negative control containing no DNA template was included in each experiment and showed no amplified products. The χ^2 test and Fisher's exact test were used to determine the differences in distribution of genotypes between cases and controls. ORs and 95% CIs using exact methods were calculated. A test for homogeneity was performed across morphological subtypes (21).

Results

The frequency of the *GSTM1* null genotype among the white AML patients as a whole was significantly increased compared with the controls [64 *versus* 47%; OR, 2.0 (95% CI, 1.3–3.1); $P = 0.001$; Table 1]. In contrast, the frequency of the *GSTT1* null genotype was not significantly different from controls [22 *versus* 15%; OR, 1.6 (95% CI, 0.9–2.9); $P = 0.12$]. Given the biologically distinct morphological subtypes of AML, a test for homogeneity was conducted across strata for both *GSTM1* and *GSTT1*. There was a statistically significant difference among AML subtypes for *GSTM1* ($P = 0.04$; *df*, 8); there was no difference for *GSTT1* ($P = 0.84$; *df*, 8). The frequency of the *GSTM1* null genotype was elevated significantly among M3 [82%; OR, 5.1 (95% CI, 1.6–21.3); $P = 0.003$] and M4 subtypes [72%; OR, 2.9 (95% CI, 1.4–6.0); $P = 0.003$] compared with controls. Frequencies were also nonsignificantly elevated among the M2 subtype [61 *versus* 47%; OR, 1.7 (95% CI, 0.9–3.4); $P = 0.11$] and M5 [63 *versus* 47%; OR, 1.9 (95% CI, 0.9–4.1); $P = 0.12$]. Although all cases with RAEBt were *GSTM1* null, this result should be interpreted with caution because only seven cases were studied. Frequencies of the *GSTM1* null genotype were not elevated in subtypes M0/M1, M6, and M7. Examination of age at diagnosis, gender, immunophenotype, WBC count at diagnosis, cytogenetic abnormality, presence of central nervous system disease at diagnosis, presence or absence of chloroma, and presence or absence of Down syndrome showed no statistically significant association with *GSTM1* or *GSTT1* null genotype. Of note, cytogenetic data were available for only a minority of cases in this study.

Discussion

In this study we show an association between the *GSTM1* null genotype and childhood AML. Among controls, null genotype frequencies observed were similar to those reported in previous United States and European studies (14–19). Among cases, the association with *GSTM1* null genotype was most marked in FAB types M3 and M4. These data indicate that these subtypes of AML may be particularly influenced by exposure to environmental agents detoxified by GSTM1. GSTM1 detoxifies a variety of environmental carcinogens, including tobacco smoke, gas emissions, polycyclic aromatic hydrocarbons such as benzo[*a*]pyrene derived from coal combustion, pesticides, and other environmental pollutants such as styrene oxide and *trans*-stilbene oxide (reviewed in Ref. 1). Moreover, results from epidemiological studies have shown associations between M2, M4, and M5 AML and maternal exposure to marijuana and alcohol, and maternal and paternal exposures to pesticides, supporting a potential role for exogenous exposures in the etiology of these subgroups (22–25). Taken together, the data suggest a potentially important role for gene-environment interactions in the etiology of some subtypes of childhood AML.

This study was performed using DNA extracted from diagnostic bone marrow slides, which contains a mixture of germline and somatic DNA. Previous studies in our laboratory have reported the accuracy of molecular genetic analyses using this DNA source (20). The use of a DNA source that contains

Table 1 *GSTM1* and *GSTT1* genotypes in children with AML/MDS

	n	*GSTM1*			*GSTT1*		
		Null (%)	OR[a] (95% CI)	*P*[b]	Null (%)	OR[a] (95% CI)	*P*[b]
Controls	153	47	2.0 (1.3–3.1)	0.001	15	1.6 (0.9–2.9)	0.12
AML/MDS	232	64			22		
FAB type[c]							
M0/M1	31	58	1.6 (0.7–3.7)	0.33	16	1.1 (0.3–3.3)	0.79
M2	56	61	1.7 (0.9–3.4)	0.11	21	1.5 (0.6–3.5)	0.37
M3	22	82	5.1 (1.6–21.3)	0.003	32	2.6 (0.8–7.8)	0.07
M4	53	72	2.9 (1.4–6.0)	0.003	21	1.5 (0.6–3.5)	0.45
M5	40	63	1.9 (0.9–4.1)	0.12	25	1.9 (0.7–4.6)	0.16
M6[d]	4	25		0.17	0		1.00
M7	13	46	1.0 (0.3–3.5)	1.00	31	2.5 (0.5–9.9)	0.23
RAEB[d]	6	33		0.69	17		1.00
RAEBt[d]	7	100		0.006	14		1.00

[a] OR and 95% CI using exact methods.
[b] Using Fisher's exact test.
[c] Test for homogeneity (GSTM1, $P = 0.04$; GSTT1, $P = 0.84$; *df*, 8).
[d] ORs and 95% CIs were not calculated because of zero cells and/or a very small number of total cases.

a mixture of somatic and germline tissue raises the possibility of reporting an acquired loss of a GST gene, instead of the constitutional genotype. This is unlikely for several reasons: (*a*) the loss of GST genes as an acquired abnormality has not been reported in AML; (*b*) no loss of the control *k-RAS* gene was observed; (*c*) our results were not different from the control population with respect to *GSTT1*; and (*d*) the presence of germline tissue in a constitutionally positive individual should ensure amplification of the gene.

Although there are no available previous studies of the *GST* genotype in childhood AML or MDS, prior studies of adult MDS and AML have provided contradictory results. Chen *et al.* (8) described a 4.3-fold increased risk of MDS associated with the *GSTT1* null genotype in a study of 96 United States Cancer and Leukemia Group B patients. However, subsequent reports from Basu *et al.*(9), Atoyebi *et al.* (10), describing 200 and 166 British MDS cases, respectively, failed to verify this finding, as did a report of 174 French cases (11). These results may indicate heterogeneity in etiology of MDS in different populations, or they may suggest that the initial observation was a chance finding. A recent study by Rollinson *et al.* (26) reported nonsignificant associations between adult AML and *GSTM1* and *GSTT1* null genotypes.

Studies of the *GST* genotype in childhood ALL have also provided varying results. An analysis of 197 cases treated at St Jude's Children's Research Hospital showed an association between the *GSTT1*/*GSTM1* double null genotype and ALL in black cases only (27). In contrast, an analysis of 177 French-Canadian cases showed an overall 1.8-fold increased risk associated with the *GSTM1* null genotype (28). Further investigations are needed to determine whether these studies reflect true differences in genetic susceptibility and etiology of ALL in different racial groups and geographic areas. Previous epidemiological studies suggested that the environmental exposures influencing ALL and AML risk are different, so it might be expected that genetic susceptibility will also differ. The association of the *GSTM1* null genotype but not the *GSTT1* genotype with AML may be an indication of substrate specificity of GSTM1 in metabolism of agents involved in the etiology of M3 and M4 AML.

In conclusion, this study has shown an increased risk of childhood AML associated with the *GSTM1* null genotype, particularly in subtypes M3 and M4. This study emphasizes the need for careful and accurate definition of categories of childhood leukemia in studies of etiology and genetic susceptibility to ensure as homogenous a grouping as possible. Improvements in cytogenetic techniques and development of molecular analyses to categorize leukemias will be valuable in future studies.

Acknowledgments

We thank A. Peterson, L. Svenningson, and T. Tjoa for expert technical assistance.

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Cancer Epidemiol Biomarkers Prev 2000;9:563-566.