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Increased Risk for Aplastic Anemia and Myelodysplastic Syndrome in Individuals Lacking Glutathione S-Transferase Genes

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Background. Aplastic anemia (AA) and myelodysplastic syndrome (MDS) are marrow failure states that may be associated with chromosomal instability. An absence of the glutathione S-transferase (GST) enzyme may genetically predispose individuals to AA or MDS. ***Procedure and Results.*** To test this hypothesis, we determined the *GSTM1* and *GSTT1* genotypes in a total of 196 patients using multiplex PCR. The *GSTT1* null genotype was found to be overrepresented in Caucasian, Asian, and Hispanic patients with either AA or MDS. We confirmed a difference in the expected frequency of the *GSTM1* null genotype in Caucasian MDS patients. The double null *GSTM1/GSTT1* genotype was also overrepresented in Caucasian AA and MDS patients. In our population, 26% of AA patients and 40% of MDS patients had a chromosomal abnormality identified by karyotype or FISH analyses for chromosomes 7 and 8. Patients with AA and the *GSTT1* null genotype had an increased frequency of chromosomal abnormalities ($P = 0.003$). ***Conclusion.*** There seems to be an increased risk for AA and MDS in individuals lacking *GSTT1* or both *GSTM1/GSTT1*. Pediatr Blood Cancer 2004;42:122–126.
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Key words: aplastic anemia; chromosomal abnormality; genotype; glutathione S-transferase; myelodysplastic syndrome

BACKGROUND

Acquired aplastic anemia (AA) and myelodysplastic syndrome (MDS) are distinct but related bone marrow failure syndromes, diagnosed by marrow morphology. Approximately 20% of MDS cases present with a hypocellular marrow and, in turn, AA may evolve to MDS or develop cytogenetic abnormalities [1]. For each disorder, the etiology is unknown, however, there may be gene-environment interactions that predispose to disease [1,2]. For example, an increased incidence of AA has been observed in Asia [3]. Glutathione S-transferase (GST) is a key biometabolic enzyme for a number of chemical toxins, including the organophosphates, alkylating agents, epoxides, and polycyclic aromatic hydrocarbons [4]. Low GST activity may genetically predispose some individuals to marrow failure, perhaps as a result of increased susceptibility to endogenous or environmental toxins.

Glutathione S-transferase M1 (*GSTM1*) and T1 (*GSTT1*) genes are polymorphic in humans. It has been well established that the frequencies of *GSTT1* null and *GSTM1* null genotypes in healthy human subjects vary greatly among different ethnic populations and can reach frequencies as high as 0.58 (*GSTT1* null) and 0.67 (*GSTM1* null) in Asians [5–10]. Racial or ethnic variation in bone marrow failure risk may reflect innate biological susceptibility as well as differences in environmental exposures or socioeconomic and demographic factors [1,11].

Recently, increased frequencies of *GSTM1* and *GSTT1* gene deletions were reported in 57 Korean patients with AA [12]. Similarly, a higher risk for MDS was noted in individuals with *GSTT1* gene deletions, although subsequent studies of patients with different ethnicities yielded conflicting results [13–19]. Here, we have determined the frequency of homozygous *GSTM1* and *GSTT1* gene deletions in an ethnically heterogeneous cohort of AA and MDS patients referred to the National Institutes of Health. We also expanded our study to determine if there

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was a difference in the frequency of chromosomal abnormalities in patients with *GSTM1* or *GSTT1* null genotypes compared to those with *GSTM1* or *GSTT1* present.

STUDY DESIGN AND METHODS

The study population included 196 patients, 108 diagnosed with either moderate or severe AA and 88 with MDS, referred to the National Heart Lung and Blood Institute, Hematology Branch between 1997 and 2000 (age range, 3–85 years; median age, 41 years; 112 males and 84 females). Standard diagnostic criteria were utilized, and no patients with congenital bone marrow failure (Fanconi anemia) were included. Race was self-reported by each patient (142 Caucasian, 28 Hispanic, 16 Black, and 10 Asian). To assure patient anonymity, no other patient identifiers were used. Cytogenetic data by conventional karyotype of the bone marrow were available for 185 (94%), and a bone marrow pellet was available for 113 (57%). Control values were based on selecting the median value of previously published gene frequencies of healthy human subjects sorted by race [5–10].

DNA was isolated from bone marrow samples using Qiagen tissue prep kit according to the manufacturer's guidelines (Qiagen, Valencia, CA). Analysis for the *GSTM1* and *GSTT1* genes was performed by multiplex PCR, as previously described [5]. In brief, 30 ng of isolated DNA was analyzed in a single assay for *GSTM1*, *GSTT1*, and *CYP1A1*. All primers were custom synthesized at Life Technologies (Invitrogen, Corp, Rockville, MD). Primers were: GSTM1 (5′-GAACTCCCTGAAAAGCTAAA-GC-3′) and (5′-GTTGGGCTCAAATATACGGTGG-3′); GSTT1 (5′-TTCCTTACTGGTCCTCACATCTC-3′) and (5′-TCACCGGATCATGGCCAGCA-3′); CYP1A1 (5′-GAACTGCCACTTCAGCTGTCT-3′) and (5′-CAGCT-GCATTTGGAAGTGCTC-3′). A 50 µl reaction mixture containing 30 pmol of each primer, 200 µmol dNTPs, 5 µl of 10 × PCR buffer, 2 U Taq DNA polymerase (Qiagen PCR Core Kit) was amplified in a PTC-200 Peltier Thermal Cycler. PCR conditions were as follows: initial melting 94°C × 5 min, followed by 35 cycles of melting 94°C × 2 min, annealing 59°C × 1 min, extension 72°C × 1 min, and a final extension at 72°C × 10 min. The PCR products were analyzed on an ethidium bromide stained 2% agarose gel. The presence or absence of *GSTT1* and *GSTM1* genes was determined by examination for characteristic bands at 480 bp (*GSTT1*) and 215 bp (*GSTM1*). A band at 312 bp corresponding to the internal control (*CYP1A1*) was used to confirm successful PCR amplification.

Fluorescent in situ hybridization (FISH) was performed on 113 patients with available bone marrow pellets and 14 normal bone marrow controls, using alpha satellite DNA probes for chromosomes 7 and 8 (VYSIS, Inc., Downer's Grove, IL). A probe mixture was applied to the target area and codenatured at 75°C for 3 min followed by hybridization at 42°C. Approximately 300 interphase nuclei per slide were scored for monosomy 7 and trisomy 8 as per criteria established by Vysis. Aneuploidy was established for monosomy 7 as >2.91% and for trisomy 8 as >2.8% of cells, based on results obtained in the normal samples.

Descriptive analyses included gene frequencies and contingency tables. Associations between variables were tested with chi-square and Fisher exact test where appropriate. Standard odds ratio and power were computed. Significance was obtained for $P < 0.05$.

RESULTS AND DISCUSSION

Frequency of *GSTM1* and *GSTT1* Null Genotypes in Bone Marrow Failure Patients Stratified by Race

Control values were selected (see ref. [8], expected frequencies, Table I) for each genotype based on the median value of previously published gene frequencies of healthy human subjects sorted by race. A recent study reported metabolic gene polymorphism frequencies for over 15,000 control subjects. Significant differences were observed between races, as expected, but much less heterogeneity was observed between White populations from different countries and no differences were seen by age, sex, or type of controls (hospital patients vs. population controls).

The *GSTT1* null genotype was overrepresented in Caucasian, Hispanic, and Asian patients with either AA or MDS (Table I). In contrast, the *GSTM1* null genotype was significantly overrepresented only in Caucasian MDS patients. No significant association was found for the null genotypes in Black patients. The double null genotype was significantly overrepresented only in Caucasians with either AA or MDS.

Data on the *GSTM1* and *GSTT1* null genotypes in marrow failure patients had previously been reported in relatively homogeneous populations, many of which did not include Black or Hispanic patients. For example, an increased frequency of the *GSTT1* null genotype was reported in White American and Japanese MDS patients [13,19]. Subsequent studies were unable to confirm this result in British, French, and Japanese populations [14–17]. Overrepresentation of the *GSTM1* null genotype was also reported in Greek MDS patients [18]. Acquired AA has not been as extensively examined for GST gene deletions. A study of 57 Korean AA patients reported a significant increase in frequency of the *GSTM1* or *GSTT1* null genotypes [12], however, a Brazilian study of 37 AA patients found no difference [20]. Ethnic variation in frequency of GST polymorphisms and different environmental exposures may account for the lack of concordance in the literature. Our referral population was ethnically and geographically diverse, and we have

TABLE I. Frequency of *GSTM1* and *GSTT1* Null Genotype Stratified by Race and Diagnosis

		GSTM1 null						*GSTT1* null						*GSTM1*/*GSTT1* null					
	Total	No.	Odds ratio	Exp freq	Freq	*P*-value	Power	No.	Odds ratio	Exp freq	Freq	*P*-value	Power	No.	Odds ratio	Exp freq	Freq	*P*-value	Power
Caucasian																			
Total	142	91	1.78	0.52	0.64	<0.003	0.095	47	0.49	0.15	0.33	<0.001	0.789	34	0.32	0.07	0.24	<0.001	0.998
AA	69	40	1.38	0.52	0.58	0.28		21	0.44	0.15	0.30	<0.001	0.265	15	0.28	0.07	0.22	<0.001	0.724
MDS	73	51	2.32	0.52	0.70	0.002	0.163	26	0.55	0.15	0.36	<0.001	0.723	19	0.35	0.07	0.26	<0.001	0.995
Black																			
Total	16	4	0.33	0.32	0.25	0.58		6	0.60	0.24	0.38	0.21		2	0.14	0.04	0.13		
AA	13	2	0.18	0.32	0.15	0.21		5	0.63	0.24	0.39	0.22		1	0.08	0.04	0.076		
MDS	3[a]	2	2.00	0.32	0.66	0.19		1	0.50	0.24	0.33	0.051		1	0.50	0.04	0.33	0.008	0.957
Hispanic																			
Total	28	13	0.87	0.51	0.46	0.63		8	0.40	0.1	0.29	<0.001	0.473	4[a]	0.17	NA	0.14		
AA	18	8	0.80	0.51	0.44	0.57		5	0.38	0.1	0.28	0.01	0.274	3[a]	0.20	NA	0.17		
MDS	10	5	1.00	0.51	0.50	0.95		3	0.43	0.1	0.30	0.03	0.246	1[a]		NA	0.1		
Asian																			
Total	10	6	1.50	0.55	0.60	0.75		8	4.00	0.48	0.80	0.043	0.195	4	0.67	0.25	0.4	0.27	
AA	8	5	1.67	0.55	0.63	0.67		6	3.00	0.48	0.75	0.13		3	0.60	0.25	0.38	0.41	
MDS	2[a]	1	1.00	0.55	0.50	NA		2		0.48	1.0	NA		1		0.25	0.5		

AA, aplastic anemia; MDS, myelodysplastic syndrome; Exp freq, expected frequency; NA, not available.
[a]Number of patients too low to determine statistical significance.

confirmed a difference in expected frequency of the *GSTT1* null genotype and AA or MDS in White and Hispanic patients. However, unlike the Korean study, we found no significant difference in frequency of the *GSTM1* null genotype and AA.

A striking difference in frequency of the double null *GSTM1/GSTT1* genotype in Caucasian AA and MDS patients was also observed. The double null genotype is an important variable to investigate because nullizygosity for *GSTM1* and *GSTT1* has been linked independently to various cancers [4], and deletion of both genes may have different significance from that of either gene alone.

High Frequency of Chromosomal Abnormalities Seen in AA Patients With *GSTT1* Null Genotype

Of the 187 patients with cytogenetics available, 26 of 102 (26%) patients with AA and 34 of 85 (40%) patients with MDS had a chromosomal abnormality identified by either conventional karyotype or FISH (Table II). Of those patients with bone marrow pellets available, FISH analyses identified monosomy 7 or trisomy 8 in 26 (44%) AA patients, of whom 18 had no chromosomal abnormalities identified by conventional karyotype. FISH analyses identified 27 (50%) MDS patients with either monosomy 7 or trisomy 8, of whom 8 had a normal conventional karyotype. The karyotype of each patient was not known at the time that FISH was performed, in order to prevent bias in scoring.

FISH analysis is known to be more sensitive than conventional cytogenetics. In this setting, FISH was limited to determining aneuploidy of chromosomes 7 and 8, which are two frequent chromosomal abnormalities identified in MDS and AA [1,2]. Among the MDS patients, there was no significant correlation between the *GSTM1* null and *GSTT1* null genotypes and chromosomal abnormalities. However, a significant difference in frequency of the *GSTT1* null genotype was observed in AA patients with chromosomal abnormalities, consistent with findings from a Korean study [12]. The etiology of chromosomal aberrations in MDS cannot be correlated to a deletion of either the *GSTT1* or *GSTM1* gene. However, it is possible that the *GSTT1* null genotype decreases an individual's ability to biometabolize toxins, which may lead to an increased risk for chromosomal aberrations in AA.

TABLE II. Patient Karyotype Stratified by Genotype and Diagnosis

	GSTM1 null	*GSTT1* null	*GSTM1*/*T1* null	*GSTM1*/*T1* present
AA				
NL karyotype[a]	38	22	14	32
Abn karyotype[b]	13	13	7	7
P-value (power)	0.160	0.003 (0.243)	0.066	
MDS				
NL karyotype[a]	36	17	11	12
Abn karyotype[b]	23	15	11	7
P-value	0.733	0.239	0.200	

AA, aplastic anemia; MDS, myelodysplastic syndrome; NL, normal karyotype; Abn, abnormal karyotype.
[a]Karyotype from conventional and FISH analysis on bone marrow cells.
[b]Includes aneuploidy and structural defects.

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