Increased frequencies of glutathione S-transferase *(GSTM1* and *GSTT1)* gene deletions in Korean patients with acquired aplastic anemia

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Patients with reduced ability to metabolize environmental carcinogens or toxins may be at risk of developing aplastic anemia. Glutathione S-transferase (GST) has been implicated in detoxifying mutagenic electrophilic compounds. This study asked whether the homozygous gene deletions of *GSTM1* and *GSTT1* affect the likelihood of developing aplastic anemia. The incidence of *GSTM1* and *GSTT1* gene deletions was significantly higher for aplastic anemia patients (odds ratio [OR]: 3.1, *P* = .01 and OR: 3.1, *P* = .004, respectively) than for healthy controls. Among the aplastic anemia patients, 17.5% (10:57) had chromosomal abnormalities at the time of diagnosis, and all aplastic anemia patients with chromosomal abnormalities showed *GSTT1* gene deletions (*P* = .048). Individuals with *GSTM1* and *GSTT1* gene deletions may have greater susceptibility to aplastic anemia. It is possible that genetic instability or chromosomal damage due to abnormal detoxification of environmental toxins might have worked as an important pathophysiologic mechanism of aplastic anemia for patients with *GSTT1* gene deletions. (Blood. 2001;98:3483-3485)

Introduction

Aplastic anemia has an age-adjusted incidence of 11.0 per million population per year in Korea and in Japan, and 2.2 in Europe and in the United States.[1] Many studies have suggested the pathophysiologic role of immunologically mediated bone marrow failure, and in practice, most patients with aplastic anemia respond favorably to immunosuppressive therapies.[2] However, this hypothesis has limitations in explaining the ethnic differences in the prevalence of aplastic anemia and the chromosomal instability associated with aplastic anemia. Toxic environmental factors, such as drugs, chemicals, and infections, and inherited genetic factors have been postulated to contribute to the etiology of aplastic anemia.[2] The exact mechanism of drug-induced aplastic anemia is unknown and may involve specific metabolic pathways as well as aberrant immune responses. A case of anticonvulsant-induced aplastic anemia first provided evidence of the role of drug metabolites in aplastic anemia in humans and suggested that the increased susceptibility to toxicity might be based on an inherited abnormality in metabolite detoxification.[3] It is therefore possible that patients with reduced ability to metabolize environmental carcinogens or toxins are at risk of developing aplastic anemia. An animal study for benzene-induced hematotoxicity conducted according to differences in xenobiotic detoxifying activities of bone marrow stromal cells supported the hypothesis that the inherited absence of a xenobiotic enzyme, especially the glutathione S-transferase (GST) of the detoxification pathway, is an important determinant of aplastic anemia.[4]

The μ (*GSTM1*) and θ (*GSTT1*) members of the *GST* multigene family, which are polymorphic in humans, are involved in detoxifying mutagenic electrophilic compounds, and an increased frequency of these *GST* gene deletions has been associated with several malignancies.[5-7] The present study investigated whether homozygous gene deletions of *GSTM1* and *GSTT1* increase the incidence of aplastic anemia and explored the relationship between the *GST* genotype and the chromosomal abnormalities in aplastic anemia patients to clarify the multistep pathogenesis of aplastic anemia based on this possible genetic predisposition.

Study design

Bone marrow (BM) samples from 57 patients with idiopathic severe aplastic anemia (male-female ratio, 29:28; median age, 31 years; range, 5-84 years) and peripheral blood samples from 75 healthy controls (male-female ratio, 38:37; median age, 38 years; range, 19-62 years) were analyzed. No patients had a clinical history of occupational or drug exposures or of viral infections such as hepatitis.

Chromosome and fluorescence in situ hybridization analysis

Cytogenetic studies on BM samples at the initial diagnosis were performed using the standard G-banding with trypsin-Giemsa staining, and karyotypes were interpreted according to the International System for Cytogenetic Nomenclature.[8] For 18 patients who showed no analyzable mitotic cells or fewer than 5 metaphases in the conventional chromosome analysis, the interphase fluorescence in situ hybridization (FISH) analysis was performed using CEP 8 and 7 (Vysis, Downers Grove, IL) for the detection of trisomy 8 and monosomy 7, the most commonly reported chromosomal abnormalities in patients with aplastic anemia.[9-10] FISH was done according to the protocol supplied by Vysis. The cutoff levels obtained from 15 control samples for trisomy 8 and monosomy 7 were 1.2% and 4.8%, respectively.

Multiplex polymerase chain reaction for polymorphic analysis of *GSTM1* and *GSTT1*

The genetic polymorphism analysis for the *GSTM1* and *GSTT1* genes was determined using the multiplex polymerase chain reaction (PCR) procedure

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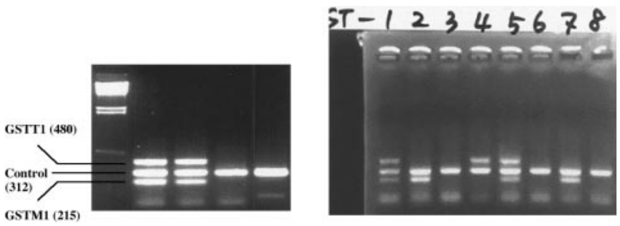

Figure 1. Multiplex PCR products analyzed on 2% agarose gel. The presence or absence of *GSTM1* and *GSTT1* genes was detected by the presence or absence of a band at 480 base pair (bp) (corresponding to *GSTT1*) and a band at 215 bp (corresponding to *GSTM1*). A band at 312 bp (corresponding to *1A1* gene) was always present and was used as an internal control to document successful PCR amplification. Lanes 1,5, individuals with $GSTT1^{+/+}$ and $GSTM1^{+/+}$; lanes 2,7, individuals with $GSTT1^{-/-}$ and $GSTM1^{+/+}$ alleles; lane 4, individuals with $GSTT1^{+/+}$ and $GSTM1^{-/-}$ alleles; lanes 3,6,8, individuals with deletions for both *GSTM1* and *GSTT1*.

Table 1. Frequencies of GSTM1 and GSTT1 gene deletions in aplastic anemia patients and healthy controls

Gene deletions	GSTM1 (%)	GSTT1 (%)	GSTM1 and GSTT1 (%)
AA patients (n = 57)	47 (82.5)	41 (71.9)	35 (61.4)
Odds ratio	3.1*	3.1†	3.6‡
AA patients with CA (n = 10)	8 (80.0)§	10 (100)‖	8 (80.0)§
Controls (n = 75)	45 (60.0)	34 (45.3)	23 (30.7)

AA indicates aplastic anemia; CA, chromosomal abnormalities.
*95% CI, 1.4-7.1 ($P = .01$).
†95% CI, 1.5-6.4 ($P = .004$).
‡95% CI, 1.7-7.4 ($P = .001$).
§$P = .29$.
‖$P = .048$.

of Abdel-Rahman et al.[11] Isolated DNA (50 ng) was amplified in a 50-μL reaction mixture containing 30 pmol of each of the following: *GSTM1* primers of 5′-GAA CTC CCT GAA AAG CTA AAG C-3′, 5′-GTT GGG CTC AAA TAT ACG GTG G-3′ and *GSTT1* primers of 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′, 5′-TCA CCG GAT CAT GGC CAG CA-3′. As an internal control exon, 7 of the *CYP1A1* genes were coamplified using the primers 5′-GAA CTG CCA CTT CAG CTG TCT-3′ and 5′-CAG CTG CAT TTG GAA GTG CTC-3′ in the presence of 200 μmol dNTP (deoxynucleoside triphosphate), 5 μL 10 × PCR buffer, 1.5 mM $MgCl_2$, and 2 U Taq polymerase. The PCR conditions consisted of an initial melting temperature of 94°C (5 minutes) followed by 35 cycles of melting (94°C, 2 minutes) and annealing (59°C, 1 minute), and the extension step (72°C) of 10 minutes terminated the process. The PCR products were then analyzed electrophoretically on an ethidium bromide–stained 2% agarose gel (Figure 1).

Results and discussion

The *GSTM1* gene deletions were found in 47 (82.5%) of 57 aplastic anemia patients and in 45 (60.0%) of 75 controls. The *GSTT1* gene deletions were found in 41 (71.9%) of 57 patients and in 34 (45.3%) of 75 controls. Most aplastic anemia patients showed *GSTM1* gene deletions (odds ratio [OR]: 3.1, 95% confidence interval [CI], 1.4-7.1, $P = .01$), but the incidence of *GSTT1* gene deletions was also significantly higher (OR: 3.1, 95% CI, 1.5-6.4, $P = .004$) for aplastic anemia patients. These results revealed a significantly elevated risk of developing aplastic anemia in individuals with the *GSTM1* and *GSTT1* gene deletions (Table 1). Because some environmental exposures involve multiple chemical substrates of both GSTs, the possibility should be considered that combined deletions of *GSTM1* and *GSTT1* interact to produce a higher risk of aplastic anemia.[12] Our results also showed a higher odds ratio in patients with combined deletions of both *GSTs* than in those with a single isoform.

The incidence of the *GSTM1* and *GSTT1* gene deletions differs among ethnic groups, and it is higher in Koreans. In our study with Korean subjects, the incidence of *GSTT1* deletion in healthy controls was significantly higher (45.3%) compared to those of white Americans (20.4%), African Americans (21.8%), and Mexican Americans (9.7%). The frequency of *GSTM1* gene deletion was also higher (60%) in Koreans than in whites (50%) and African Americans (33%).[13] We consider that the relatively high incidence of aplastic anemia in Koreans could be explained by the ethnic difference shown in the prevalence of the homozygous deleted genotypes of *GSTM1* and *GSTT1*.

Of the 57 aplastic anemia patients, 10 patients (17.5%) had chromosomal abnormalities at the time of diagnosis. The chromosomal abnormalities were as follows: 3 cases of trisomy 8 and 1 case each of trisomy 8 and 9, t(8;21), inv(16), t(4;14), t(X;19), del(10), and monosomy 10 (Table 2). All aplastic anemia patients with chromosomal abnormalities showed *GSTT1* gene deletions ($P = .048$). The *GSTT1* gene deletion has been associated with carcinogen-induced chromosomal changes in lymphocytes, with diepoxibutane being one such carcinogen.[12] Recent data have also pointed to the interactions of the Fanconi anemia phenotype and GST, and especially the diepoxibutane-induced glutathione depletion and GST inhibition, as playing an important role in the oxidative stress in the Fanconi anemia phenotype.[14] Therefore, chromosomal damage due to abnormal detoxification of environmental toxins might be an important pathophysiologic mechanism

Table 2. Characteristics of 10 aplastic anemia patients with chromosomal abnormalities

Patient	Age, y/Sex	Karyotype at Dx	Tx	F/U cytogenetics	Time from Dx (mo)	Evolution to MDS
1	37/F	48,XX,+8,+9[20]	BMT	48,XX,+8,+9[20], 46,XY[20] (BMT)	5	—
2	15/F	47,XX,+8[10]/46,XX[1]	IST	47,XX,+8[15]/46,XX[5]	53	+*
3	65/F	47,XX,+8,22pss[20]	CON	ND	16	—
4	41/M	46,XY,t(8;21)(q22;q22)[1]/46,XY[10]	CON	ND	NA	—
5	12/M	46,XY,inv(16)(p13.1q22)[2]/46,XY[3]	CON	46,XY[20]	33	—
6	29/F	46,XX,t(X;19)(p11.2;q11)[7]	CON	ND	18	—
7	15/F	46,XX,t(4;14)(p10;p10)[4]/46,XX[16]	BMT	46,XY[20] (BMT)	35	—
8	71/F	46,XX,del(10)(p13)[4]/46,XX[18]	IST	ND	41	—
9	55/M	45,X,−Y[6]/45,XY,−10[3]/46,XY[11]	IST	ND	26	—
10	26/F	2.5% Trisomy 8 (FISH)	BMT	46,XX[1]/46,XY[19] (BMT)	27	—

Dx indicates diagnosis; Tx, treatment; F/U, follow-up; MDS, myelodysplastic syndrome; F, female; BMT, bone marrow transplantation; AA, aplastic anemia; IST, immunosuppressive therapy; CON, conservative therapy; ND, not done; M, male.

*Bone marrow findings: some clusters of megakaryocytes with nuclear atypism and immature granulocytic cells.

of aplastic anemia for patients with *GSTT1* gene deletion, although the numbers are too small to draw a concrete conclusion.

We believe that further studies to define both the mechanism of GSTs leading to the development of aplastic anemia and specific substrates for GST-related aplastic anemia will be an important approach in understanding the pathophysiology of aplastic anemia.

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