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

Kyung A. Lee, Sun Hee Kim, Hee Yeon Woo, Young Joon Hong, and Hyoun Chan Cho

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, \( P = 0.01 \) and OR: 3.1, \( P = 0.004 \), 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 (\( P = 0.048 \)). 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)

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of Abdel-Rahman et al.\textsuperscript{11} Isolated DNA (50 ng) was amplified in a 50-µL reaction mixture containing 30 pmol of each of the following: \textit{GSTM1} primers of 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and 3'-GGT CCT CAC A TC TC-3', \textit{GSTT1} primers of 5'-GAA CTC CCT GAA AAG CTA AAG C-3' and 3'-GTT GGG CAC A TC TC-3', and \textit{CYP1A1} in the presence of 200 µM dNTP (deoxynucleoside triphosphate), 1.5 mM MgCl\textsubscript{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), annealing (59°C, 1 minute), and 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).

**Figure 1.** Multiplex PCR products analyzed on 2% agarose gel.

The presence or absence of \textit{GSTM1} and \textit{GSTT1} genes was detected by the presence or absence of a band at 215 bp (corresponding to \textit{GSTT1}). A band at 480 base pair (bp) (corresponding to \textit{GSTM1}). A band at 312 bp (corresponding to 1A1 gene) was found in 45.3% of 75 controls. Most aplastic anemia patients showed deletions were found in 41 (71.9%) of 57 patients and in 34 (61.4%) of 55 controls.

### Results and discussion

The \textit{GSTM1} gene deletions were found in 47 (82.5%) of 57 aplastic anemia patients and in 45 (60.0%) of 75 controls. The \textit{GSTT1} gene deletions were found in 41 (71.9%) of 57 patients and in 34 (54.6%) of 57 controls. Most aplastic anemia patients showed \textit{GSTM1} gene deletions (odds ratio [OR]: 3.1, 95% confidence interval [CI]: 1.4-7.1, \(P = .01\)), but the incidence of \textit{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 \textit{GSTM1} and \textit{GSTT1} gene deletions (Table 1). Because some environmental exposures involve multiple chemical substrates of both \textit{GSTM1} and \textit{GSTT1}, the possibility should be considered that combined deletions of \textit{GSTM1} and \textit{GSTT1} interact to produce a higher risk of aplastic anemia.\textsuperscript{12} Our results also showed a higher odds ratio in patients with combined deletions of both \textit{GSTs} than in those with a single isoform.

The incidence of the \textit{GSTM1} and \textit{GSTT1} gene deletions differs among ethnic groups, and it is higher in Koreans. In our study with Korean subjects, the incidence of \textit{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 \textit{GSTM1} gene deletion was also higher (60%) in Koreans than in whites (30%) and African Americans (33%).\textsuperscript{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 \textit{GSTM1} and \textit{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 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 \textit{GSTT1} gene deletions (\(P = .048\)). The \textit{GSTT1} gene deletion has been associated with carcinogen-induced chromosomal changes in lymphocytes, with diepoxibutane being one such carcinogen.\textsuperscript{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.\textsuperscript{14} Therefore, chromosomal damage due to abnormal detoxification of environmental toxins might be an important pathophysiological mechanism

### Table 1. Characteristics of 10 aplastic anemia patients with chromosomal abnormalities

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, y/sex</th>
<th>Karyotype at Dx</th>
<th>Tx</th>
<th>F/U cytogentic</th>
<th>Time from Dx (mo)</th>
<th>Evolution to MDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>37/F</td>
<td>48,XX,+8,+9[20]</td>
<td>BMT</td>
<td>48,XX,+8,+9[20], 46,XY[20] (BMT)</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>15/F</td>
<td>47,XX,+8[10/46,XX][1]</td>
<td>IST</td>
<td>47,XX,+8[15/46,XX][5]</td>
<td>53</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>65/F</td>
<td>47,XX,+8.22psd[20]</td>
<td>CON</td>
<td>ND</td>
<td>16</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>41/M</td>
<td>46,XY,t(8;21)[q22;q22][1/46,XY][10]</td>
<td>CON</td>
<td>ND</td>
<td>NA</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>12/M</td>
<td>46,XY,t(16)[p13.1;q12.2][2/46,XY][3]</td>
<td>CON</td>
<td>46,XY[20]</td>
<td>33</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>29/F</td>
<td>46,XX,t(19)[p11.2:q11][7]</td>
<td>CON</td>
<td>ND</td>
<td>18</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>15/F</td>
<td>46,XX,t(4;14)[p10:q10][4/46,XX][16]</td>
<td>CON</td>
<td>46,XY[20] (BMT)</td>
<td>35</td>
<td>—</td>
</tr>
<tr>
<td>8</td>
<td>71/F</td>
<td>46,XX,del(10)[p13][4/46,XX][18]</td>
<td>IST</td>
<td>ND</td>
<td>41</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>26/F</td>
<td>2.5% Trisomy 8 (FISH)</td>
<td>BMT</td>
<td>46,XX[1/46,XY][19] (BMT)</td>
<td>27</td>
<td>—</td>
</tr>
</tbody>
</table>

\*Bone marrow findings: some clusters of megakaryocytes with nuclear atypism and immature granulocytic cells.
of aplastic anemia for patients with \textit{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.

References

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