Genetic polymorphisms of CYP3A4, GSTT1, GSTM1, GSTP1 and NQO1 and the risk of acquired idiopathic aplastic anemia in Caucasian patients

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Background and Objectives. Various drugs and xenobiotics are involved in the pathogenesis of acquired aplastic anemia. Their harmful potential depends on the amount of exposure to them and on the detoxifying capacity of the recipient. Genetic polymorphisms of some important detoxifying enzymes are associated with low or absent catalytic activity of the protein. We assessed whether, in a Caucasian population, low or null activity polymorphisms of CYP3A4, GSTT1, GSTM1, GSTP1 and NQO1 were associated with the risk of developing aplastic anemia and with the response to immunosuppressive therapy.

Design and Methods. In 77 Caucasian patients with aplastic anemia and in 156 normal controls we evaluated the distribution of the following polymorphisms which are associated with low or no activity of the corresponding enzyme: (i)-290 A→G of the CYP3A4 gene, deletions of (ii) GSTT1 and (iii) GSTM1 genes, (iv) 313A→G of the GSTP1 gene and (v) 609 C→T of the NQO1 gene.

Results. The distribution of the genotypes of all tested polymorphisms was not different in patients and controls. No differences were seen among the patients when the group was subdivided by age and severity of the disease. Only the GSTM1 null genotype was significantly more frequent in male patients than in male controls. The frequency of all tested polymorphisms did not differ in patients who did or did not respond to immunosuppressive therapy.

Interpretations and Conclusions. The low/null activity polymorphisms of the detoxifying enzymes CYP3A4, GSTT1, GSTM1, GSTP1 and NQO1 are not associated with the risk of developing aplastic anemia or to the response to immunosuppressive therapy in Caucasian patients.

Keywords: CYP3A4, GSTs, NQO1, aplastic anemia.

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A single nucleotide polymorphism (-290 A→G) in the 5'-flanking region of the CYP3A4 gene has been associated with reduced levels of the corresponding protein in Caucasian subjects. GSTT1 and GSTM1 genes have a deletion which, in the homozygous state, leads to the absence of the protein (null genotype). The GSTP1 gene displays a polymor-
phism (313A→G) (NCBI dbSNP: rs 947894) producing an isoleucine to valine substitution at codon 105, this being responsible for a less efficient variant enzyme. The NQO1 point mutation polymorphism (609 C→T) (NCBI dbSNP: rs1800566) produces a proline to serine substitution that destabilizes and inactivates the protein.\(^1\)

We investigated whether null or reduced activity polymorphisms of the enzymes CYP3A4, GSTT1, GSTM1, GSTP1, and NQO1, which metabolize compounds that may induce aplastic anemia, were associated with the risk of developing idiopathic aplastic anemia in a Caucasian population. Since these enzymes may also be involved in the metabolism of drugs used in the immunosuppressive treatment of aplastic anemia, we also assessed whether the polymorphisms might influence the response to this therapy.

### Design and Methods

Seventy-seven Caucasian patients, diagnosed as having idiopathic aplastic anemia according to International criteria,\(^6\) entered the study.

Samples were studied after informed consent had been given by the parents of the patients or, whenever eligible, from the patients themselves. Table 1 shows the characteristics of the patients. Their polymorphisms were compared with those observed in 156 Caucasian children (99 [63\%] males, 57 [37\%] females, median age 4.08, range 0-19.6 years) who were hospitalized in Institutions throughout the Italian territory for conditions (e.g trauma) for which a genetic background can be excluded. Within the population of patients the distribution of the polymorphisms was also analyzed according to response to immunosuppressive treatment.

### Polymorphism analysis

Genotyping was performed on DNA extracted from bone marrow (patients) or peripheral blood (controls) samples. The genotypes for GSTM1 and GSTT1 were determined by polymerase chain reaction (PCR) as previously described by Chen et al.\(^4\) This assay distinguishes two categories for each GSTM1 and GSTT1. One, present, being either homozygous or heterozygous for GSTM1 or GSTT1 and the other, null as having a homozygous deletion of GSTM1 or GSTT1. The GSTP1 genotype was analyzed by PCR/restriction fragment length polymorphism (RFLP) studies of codon 105 according to Harries et al.\(^7\) This assay distinguishes genotypes homozygous for the wild type allele (AA), heterozygous (AG) and homozygous for the variant allele (GG).

The C609T polymorphism of the NQO1 gene was analyzed by standard PCR/RFLP as previously described.\(^8\) Based on genotype analysis, wild type individuals (CC) were assigned to the high NQO1 activity category, while those heterozygous (CT) or homozygous (TT) for the C609T polymorphism, were assigned to the low activity category. Genotyping of CYP3A4 was performed by standard PCR/RFLP as described elsewhere.\(^9\)

### Statistical analysis

Descriptive statistics were reported as absolute frequencies and percentages for qualitative data. Comparisons of genotypes between patients and controls, and between responders and non-responders to immunosuppressive treatment was per-
formed by means of the χ² test or the Fisher’s exact test in the case of expected frequencies less than 5. All the statistical tests were two sided; a p value <0.05 was considered as statistically significant. The statistical package Stata (STATA, release 7, Stata Corporation, College Station, TX, USA) was used to perform all the analyses.

**Results**

The frequencies of CYP3A4, GSTT1, GSTM1, GSTP1 and NQO1 polymorphisms were very similar (Table 2) in the patients and in the controls. Analysis by severity of the disease, age and gender, did not show differences between patients and controls for any of the polymorphisms except for the GSTM1 null genotype that was significantly (p=0.012) more frequent in aplastic males (70.7%) than in controls male (47.4%).

Regarding the response to treatment (Table 3) there was no significant difference in the frequency of any of the analyzed polymorphisms between the group of the patients responding to immunosuppression and the group not responding.

The frequency of CYP3A4, GSTT1, GSTM1, GSTP1 and NQO1 mutated/null genotypes in our control population overlapped those reported for Caucasians in the literature. This was also true for the GSTT1 and GSTM1 null genotypes when the subjects analyzed were divided by gender.

**Discussion**

This study is one of the largest conducted in the setting of idiopathic aplastic anemia in Caucasian populations both regarding the sample size and the number of the tested polymorphisms. Our data indicate that genetic polymorphisms of the detoxifying enzymes GSTT1, GSTM1, GSTP1, CYP3A4 and NQO1 are not associated with the risk of developing idiopathic aplastic anemia, suggesting that these polymorphisms do not play a major role in the pathogenesis of idiopathic aplastic anemia in Caucasian patients.

Whereas two former studies on GSTM1 in Caucasian subjects were in agreement with ours, another study, performed on a Korean population, showed a significant association between GSTM1 and GSTT1 null genotypes and the risk of aplastic anemia. It is noteworthy that the GSTT1 and GSTM1 null genotypes are more frequent in the normal Korean population (GSTT1 45.3 %, GSTM1 60%) than in our Caucasian controls (GSTT1 18%, GSTM1 50%). This reflects genetic variation between the two races and is in keeping with the three-fold increased frequency of aplastic anemia in the Far East.

The greater frequency of the GSTM1 null genotype in male patients with aplastic anemia than in normal male subjects has no obvious explanation. The GSTM1 null genotype is reported not to be influenced by sex in Caucasians and, when analyzed according to gender, the distributions of the GSTM1 genotypes in our controls matched those in other normal Caucasian control groups. We cannot exclude that this difference has occurred by chance.

Our GSTT1 data do not agree with findings in Caucasian North American, German and Korean populations that showed an association between GSTT1 deletion and aplastic anemia. Whereas in the Koreans a genetic difference may account for the discrepancy, there is no obvious explanation for the diversity with other Caucasian patients since the frequency of null GSTT1 genotype in our normal controls overlaps that observed in other Caucasian subjects among whom genetic variations of glutathione S-transferases are reported to be very slight.

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**Table 2. Distribution of CYP3A4, NQO1, GSTP1, GSTM1 and GSTT1 genotypes between cases and controls.**

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype</th>
<th>Patients with aplastic anemia N° (%)</th>
<th>Controls N° (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4-290A→G</td>
<td>AA (wild type)</td>
<td>65 (88) 135 (92)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AG (low activity)</td>
<td>6 (8) 5 (8)</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG (low activity)</td>
<td>3 (4) 4 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NQ01 609 C→T</td>
<td>CC (wild type)</td>
<td>49 (64) 90 (62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT (low activity)</td>
<td>25 (33) 48 (33)</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT (low activity)</td>
<td>2 (3) 8 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTP1 313 A→G</td>
<td>AA (wild type)</td>
<td>40 (53) 77 (50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AG (low activity)</td>
<td>33 (44) 63 (41)</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG (low activity)</td>
<td>2 (3) 13 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1</td>
<td>M present</td>
<td>33 (43) 77 (50)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M null</td>
<td>44 (57) 76 (50)</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>GSTT1</td>
<td>T present</td>
<td>64 (83) 118 (82)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T null</td>
<td>13 (17) 26 (18)</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>GSTM1/GSTT1</td>
<td>M null/T null</td>
<td>8 (10) 13 (8)</td>
<td>0.61</td>
<td></td>
</tr>
</tbody>
</table>

For technical reasons it was not possible to analyze all the genotypes in all the eligible patients.
It is worth noting that in the cases of \textit{GSTM1} and \textit{GSTT1}, the null polymorphism is a gene deletion that causes total absence of the enzyme. Thus the lack of correlation of the above polymorphisms with aplastic anemia also indicates that the absence of these detoxifying systems does not affect the risk of developing this disease in Caucasian subjects.

None of the tested polymorphisms was associated with differences in response to immunosuppressive therapy. This suggests that they do not influence the outcome of patients with aplastic anemia receiving such treatment. It is likely that factors other than metabolic genes, such as the number of residual stem cells or the pressure of the activated immune system on the hematopoietic compartment, may have a more important influence on the outcome of the disease after immunosuppression. Since our study focused only on the role of metabolic polymorphisms, toxic exposure was not investigated. Indeed, very scanty data are available in this respect on our patients. However the lack of association between metabolic polymorphisms and aplastic anemia suggests that toxic exposure is one of the etiological factors of this disease which is worth further investigation.

In summary our study is against the tested metabolic polymorphisms having an important role in the risk of developing aplastic anemia. This does not mean that detoxifying systems do not affect the risk of developing this disease at all. Other enzymes interacting with drugs and toxic agents involved in stem cell damage may be involved. Moreover, since \textit{GSTP1}, \textit{CYP3A} and \textit{NQO1} activity may be influenced by substrates, inducers and inhibitors,\textsuperscript{29-32} it is possible that low activity polymorphisms may not always faithfully reflect the true \textit{in vivo} enzymatic effect. Other factors, such as the characteristics of the exposure to toxics, may turn out to play a more relevant role in the multifactorial pathogenesis of aplastic anemia.

CD conceived and designed the study, analyzed and interpreted the data, wrote the manuscript, revised it critically and finally approved it; JS collected the data of the patients, participated to the interpretation of the data, revised the paper critically and finally approved it; AB, participated in the design of the study, revised the paper critically and finally approved it; DL participated in the...
interpretation of the data, revised the paper critically and finally approved it; SV participated in the interpretation of the data, revised the paper critically and finally approved it; AP participated in the design of the study, revised the paper critically and finally approved it; FB carried out the statistical analysis, participated in the interpretation of the data, revised the paper critically and finally approved it; GM participated in the design of the study, revised the paper critically and finally approved it; UR participated in the interpretation of the data, revised the paper critically and finally approved it; ML participated in the interpretation of the data, revised the paper critically and finally approved it.

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