Background and Objectives. Iron status has implications for normal erythrocyte and leukocyte function and for platelet count, size, and activation. Increased storage of iron is considered a potential risk factor participating in the pathogenesis of malignant diseases. Since HFE gene mutations have recently been implicated in unbalanced iron homeostasis, we set out to examine the prevalence of these mutations in patients with hematologic disorders.

Design and Methods. C282Y and H63D mutations were determined in 232 patients with various hematologic disorders treated at Oulu University Hospital between 1987 and 2000. DNA samples extracted from either the peripheral blood or bone marrow of these patients were amplified by a polymerase chain reaction (PCR) method using sequence-specific primers, and the products were analyzed on agarose gels.

Results. There was a slight tendency towards lower frequencies of the C282Y allele in patients with acute myeloid leukemia (AML) (3.8%, n=53) and higher frequencies in those with essential thrombocythemia (ET) (16.2%, n=37). Contrary to some expectations, however, the frequency of the C282Y allele in acute lymphoblastic leukemia turned out to be normal (7.0%, n=43). Our data showed no significant deviations in H63D mutation frequency in any of the categories of patients.

Interpretation and Conclusions. Our results do not show any significant association between HFE gene mutations and hematologic malignancies. The divergent frequencies observed for the C282Y mutation in patients with AML and ET highlight the need for larger population studies of HFE mutations in patients with hematologic diseases.

Key words: hematologic disorder, hereditary hemochromatosis, HFE, iron.
leukemia (AML) and the HFE gene mutations C282Y and H63D. Similarly, the C282Y mutation alone showed no association with the incidence of multiple myeloma (MM), even though patients with the HFE Tyr 282 allele together with TfR Ser 142 homozygosity appeared to have an increased risk of MM.

The present study was designed to analyze HFE genotypes in patients with various hematologic disorders. The divergent frequencies observed for the C282Y mutation in the group of patients with AML and those with essential thrombocythemia (ET) provide a foundation for larger population studies of HFE mutations in patients with hematologic diseases.

**Design and Methods**

**Patients**

Peripheral blood or bone marrow samples were collected from 232 patients (106 men and 126 women) with various hematologic disorders; ALL (n=43), AML (n=53), chronic myeloid leukemia (CML, n=16), ET (n=37), myelodysplastic syndromes (MDS, n=35), MM (n=33), and polycythemia vera (PV, n=15). The samples were taken from patients during routine diagnostic and treatment evaluation at Oulu University Hospital between 1987 and 2000. ALL and AML were classified on the basis of their lymphoid or myeloid morphologic appearance, respectively, according to the French American British (FAB) criteria. In addition, diagnoses of acute leukemia were based on the immunophenotype as assessed by flow cytometry using standard lineage immunophenotype markers. The diagnosis of MDS was also based on the FAB criteria, while that of PV was based on the criteria of the PV Study Group.

**HFE genotyping**

DNA was extracted either from peripheral blood or from bone marrow using a blood kit (Nucleospin, Macherey-Nagel, Düren, Germany). A polymerase chain reaction (PCR) to amplify the extracted DNA was run using the sequence specific primers (SSP) described by Smillie. Both the C282Y and the H63D mutations were tested. The control primers designed by Steffensen et al. were used to ensure that the PCR method was working properly. The PCR products were analyzed by electrophoresis on a 1.5% agarose gel containing ethidium bromide and visualized by ultraviolet light stimulation. Positive and negative controls were included in every group of samples analyzed.

All the statistical differences between the prevalences of HFE genotypes in the various hematologic disorders and in the control group were assessed using Fisher’s exact probability test. p values of < 0.05 were considered statistically significant.

**Results**

The frequencies of the C282Y mutations in the various hematologic diseases and in the control group are summarized in Table 1. The frequencies of the H63D mutations are given in Table 2.

**Discussion**

One of the most interesting observations of our study was that the frequency of the C282Y mutation was markedly low in patients with AML (3.8%, n=53), as previously published data for a control group from the same hospital district (n=128) had indicated frequencies of 10.2% for the C282Y mutation and 20.3% for the H63D mutation. The difference between the frequencies in the AML group and that in the present control group did not, however, reach statistical significance (p=0.237). The low frequency of the C282Y mutation in the AML patients was in contradiction to the previous assumption that the gene mutation responsible for HH would increase the risk of hematologic malignancies. It should not be forgotten, either, that our results did not provide any evidence to support the recently published data showing that the C282Y mutation is a male-specific risk factor for childhood ALL. Dorak et al. analyzed C282Y genotypes in two independent groups of children with ALL from South Wales and the West of Scotland. They reported C282Y mutation frequencies of 23.4% and 37.4% in the two groups of male childhood ALL patients, respectively. In our study, both the ALL and childhood ALL patients (43 ALL patients, of whom 32 were aged 16 years or less at the time of diagnosis) had normal C282Y mutation frequencies and none of the male childhood ALL patients (n=14) carried the C282Y mutation. Whether the divergent mutation frequencies observed in the present and previous studies reflect a genetic heterogeneity in two distant populations remains to be elucidated.

Iron overload is relatively common in patients...
with AML, due to multiple RBC transfusions. Hence, AML patients need a functional machinery to handle excessive iron. It is known that macrophages normally play a central role in these processes and cellular iron uptake by macrophages is defective in homozygous C282Y patients because of HFE protein dysfunction. Based on these facts, we propose that the C282Y mutation in conjunction with multiple RBC transfusions could cause severe iron overload in AML patients, which could in turn lead to serious complications. Higher mortality among C282Y mutant AML patients due to iron toxicity could theoretically cause a low C282Y mutation frequency. However, this still does not apparently explain the low mutation frequency observed in our AML patients.

Another interesting finding was that the patients with ET appeared to have a slightly higher tendency for having the C282Y mutation (16.2%, n=37). Although this allele may represent a passenger mutation frequently associated with ET, we cannot exclude the possibility that it may be more directly involved in the pathogenic mechanisms of the disease. The fact that iron has effects on platelet count, the size of platelets and their activation points to a possibility that this mutation, even in heterozygous form, could alter the platelet count and/or platelet function in some individuals. Our unpublished data have shown that HFE protein is expressed in platelets, which makes the present finding even more attractive.

We also determined the prevalence of another typical HFE mutation, H63D. Since the relevance of this mutation has remained questionable, even in HH patients, the significance of its slightly elevated frequency in our MDS patients naturally remains open. The percentage of H63D mutations in any of the patient categories did not show any statistically significant difference from that recorded for a control population from the same hospital district.

In summary, we found slightly divergent frequencies of the C282Y mutation in patients with AML (3.8%, n=53) and ET (16.2%, n=37). Even though these frequencies did not differ significantly from those observed in the control population (10.2%), the present results suggest that HFE mutations may play a role in the pathogenesis of certain hematologic disorders. However, a larger study population would be required to show or exclude a significant association between HFE gene mutations and the prevalence of hematologic disorders.

### Contributions and Acknowledgments

JH wrote the paper, processed the data and assessed the statistical analyses. SP, ERS and PK assisted with writing and interpretations of the data with JH. ERS and PK were responsible for patient referral to our laboratory. All authors were equally responsible for conception and design of the study and approved the final version of the paper. The order of names takes into account the time, work and scientific contribution given by all authors. SP as a senior author, is cited last. We thank Kirsi Kvist-Mäkelä for the help with HFE genotyping.

### Table 1. Presence of the C282Y mutation in patients with hematologic disorders.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>HET</th>
<th>Count</th>
<th>%</th>
<th>WT</th>
<th>Count</th>
<th>%</th>
<th>Total count</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALL</td>
<td>3</td>
<td>7.0</td>
<td>40</td>
<td>93.0</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>2</td>
<td>3.8</td>
<td>51</td>
<td>96.2</td>
<td>53</td>
<td></td>
<td></td>
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<tr>
<td>CML</td>
<td>1</td>
<td>6.3</td>
<td>15</td>
<td>93.8</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET</td>
<td>6</td>
<td>16.2</td>
<td>31</td>
<td>83.8</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDS</td>
<td>4</td>
<td>11.4</td>
<td>31</td>
<td>88.6</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>2</td>
<td>6.1</td>
<td>31</td>
<td>93.9</td>
<td>33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PV</td>
<td>1</td>
<td>6.7</td>
<td>14</td>
<td>93.3</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>8.2</td>
<td>213</td>
<td>91.8</td>
<td>232</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The frequency of the C282Y mutation for a control group (n=128) from the same hospital district was 10.2%.

### Table 2. Presence of the H63D mutation in patients with hematologic disorders.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>HET</th>
<th>Count</th>
<th>%</th>
<th>HOMOZ</th>
<th>Count</th>
<th>%</th>
<th>WT</th>
<th>Count</th>
<th>%</th>
<th>Total count</th>
</tr>
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<tbody>
<tr>
<td>ALL</td>
<td>6</td>
<td>14.0</td>
<td>37</td>
<td>86.0</td>
<td>43</td>
<td></td>
<td></td>
<td>86.0</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>8</td>
<td>15.1</td>
<td>1</td>
<td>94.9</td>
<td>44</td>
<td></td>
<td></td>
<td>83.0</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>4</td>
<td>25.0</td>
<td>12</td>
<td>75.0</td>
<td>16</td>
<td></td>
<td></td>
<td>75.0</td>
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<tr>
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<td>83.8</td>
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<tr>
<td>MDS</td>
<td>9</td>
<td>25.7</td>
<td>25</td>
<td>74.3</td>
<td>35</td>
<td></td>
<td></td>
<td>74.3</td>
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<tr>
<td>MM</td>
<td>4</td>
<td>12.1</td>
<td>29</td>
<td>87.9</td>
<td>33</td>
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<td></td>
<td>87.9</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>PV</td>
<td>3</td>
<td>20.0</td>
<td>12</td>
<td>80.0</td>
<td>15</td>
<td></td>
<td></td>
<td>80.0</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>17.2</td>
<td>2</td>
<td>82.8</td>
<td>232</td>
<td></td>
<td></td>
<td>82.8</td>
<td>232</td>
<td></td>
</tr>
</tbody>
</table>

The frequency of the H63D mutation for a control group (n=128) from the same hospital district was 20.3%.


33. Steffensen R, Varming K, Jersild C. Determination of gene frequencies for two common haemochromatosis muta-


Peer Review Outcomes

What is already known on this topic
Iron overload is typically found in patients with HFE-related genetic hemochromatosis, and individuals who are heterozygous for the HFE C282Y mutation may have marginally elevated iron stores. Some reports have associated excess iron with cancer risk, particularly of the colorectum and of the liver. Little is known about any association between increased iron stores or HFE mutations and risk of hematologic malignancies.

What this study adds
This study excludes any significant association between HFE gene mutations and hematologic malignancies. Although this was expected, the present study provides a formal demonstration.

Manuscript processing
This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Prof. Cazzola and the Editors. Manuscript received September 12, 2001; accepted November 21, 2001.

Potential implications for clinical practice
This study demonstrates that gene mutations responsible for HH do not increase the risk of hematologic malignancies. The results imply that HFE genotyping is not necessary in patients with hematologic malignancies treated with multiple RBC transfusions.

Mario Cazzola, Editor-in-Chief