To the editor:

A valine deletion of ferroportin 1: a common mutation in hemochromatosis type 4?

Hemochromatosis type 4 is an atypical hemochromatosis characterized by dominant inheritance, increased serum ferritin, normal transferrin saturation, and prevalent iron deposition in the reticuloendothelial (RE) cells rather than in hepatocytes. Heterozygous missense mutations of the iron export protein ferroportin 1 (FPN1)³ have been reported in 2 large pedigrees.¹² We have characterized a 3-base pair deletion of FPN1 in 2 related Italians and in 1 unrelated British patient.

The Italian proband (patient 1 in Table 1) was a 26-year-old female presenting with mild anemia and normal erythrocyte indices. Serum iron and transferrin saturation were slightly decreased, and serum ferritin levels, increased. Hemolytic anemia and inflammatory chronic diseases were excluded. Bone marrow aspirate showed normal erythroid maturation. Liver biopsy did not reveal fibrosis. Perl staining was positive especially in Kupffer cells, with few hepatocyte granules. Quantitative liver iron concentration (LIC) was obtained by magnetic susceptometry (SQUID).⁵⁻⁷ A low-intensity venesection protocol reduced ferritin without causing anemia. The proband’s mother (patient 2) had breast cancer 3 years ago. She is in complete remission, having undergone radical mastectomy and chemotherapy and is being treated with tamoxifen. She has had persistent hyperferritinemia over the last 3 years. Liver function tests were normal. LIC was elevated. The British patient (patient 3) was a 30-year-old asymptomatic male whose father had been diagnosed with hemochromatosis. On presentation he had a raised ferritin but normal transferrin saturation. He had a slight degree of hepatomegaly and had normal liver function test results. On venesection this patient’s ferritin concentration returned to the normal range without him becoming anemic. He has had 4.25 g iron removed.

All 3 patients are heterozygotes for the His63Asp mutation in HFE. Sequencing the whole FPN1 gene of the 2 Italian patients identified a heterozygous GTT deletion, corresponding to the loss of a valine in a 3 valine repeat at position 160-162 (Val162del) of the protein, likely due to a slipped strand mispairing. The same deletion in the British patient was first detected by denaturing high performance liquid chromatography (DHPLC) and then confirmed by sequence analysis.

The finding of the same mutation in families of different ethnic origin (see accompanying articles by Devalia et al,⁸ page 695, and Wallace et al,⁹ page 692) suggests that Val162del results from multiple deletion events. Due to the mechanism of this deletion, it is likely to be the most common mutation both in hemochromatosis type 4 and possibly in non-Cys282Tyr hemochromatosis.

Table 1. Results of hematologic and clinical data of the patients studied

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>26</td>
<td>58</td>
</tr>
<tr>
<td>Sex</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Hemoglobin, g/L</td>
<td>115</td>
<td>127</td>
</tr>
<tr>
<td>MCV, fL</td>
<td>91</td>
<td>96</td>
</tr>
<tr>
<td>MCH level, pg</td>
<td>29.33</td>
<td>31.75</td>
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<tr>
<td>Serum ferritin, µg/L</td>
<td>1022</td>
<td>5376</td>
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<tr>
<td>Serum iron, µg/dL</td>
<td>65</td>
<td>100</td>
</tr>
<tr>
<td>Serum transferrin, mg/dL</td>
<td>254</td>
<td>204</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td>18</td>
<td>35</td>
</tr>
</tbody>
</table>

*LIC (SQUID), µg/g tissue ww* 1083 2598 ND

MCHV represents mean corpuscular volume; MCH, mean cell hemoglobin; ww, wet weight; and ND, not determined.

*Mean of 2 determinations.
†Mean of several determinations.
‡Mean of 2 determinations.

References

To the editor:

Leu208Val and Ile181Leu variants of cytochrome P450 CYP2C9 are not related to the acenocoumarol dose requirement in a Spanish population

Cytochrome P450 CYP2C9 is the principal catalyst of warfarin and acenocoumarol hydroxilation reactions in human liver microsomes. There is a growing interest in the identification of genetic variants of cytochrome P450 CYP2C9, which can modify its ability to inactivate warfarin and acenocoumarol, since a reduced CYP2C9 activity on these drugs would put patients at risk of over anticoagulation and subsequent bleeding complications. But the allele frequencies for these variants differ considerably among different ethnic groups: Caucasians carry the 2C9*2 and 2C9*3 variants (8% to 20% and 6% to 10%, respectively) more frequently than Asians do (0% and 2% to 5%, respectively). Recently, Leung et al have identified several genetic polymorphisms of cytochrome P450 CYP2C9 in a Chinese population. Two of them, Leu208Val and Ile181Leu, could have importance in the sensitivity to oral anticoagulant treatment in Chinese patients. Allele 208Val is more frequent than the Caucasian wild-type Leu208 in the Chinese population (75% heterozygotes and 19% homozygotes) and is associated with a lower warfarin dose requirement, which could explain why the Chinese population is more sensitive to warfarin than the Caucasian one. The Ile181Leu allelic variant was present in 9% of studied patients in heterozygous form and was associated with a higher warfarin dose requirement in that population.

We have studied Leu208Val and Ile181Leu variants in 106 Spanish anticoagulated patients with a stable requirement for acenocoumarol to keep the international normalized ratio (INR) between 2 and 3.41 patients required no more than 7 mg/wk acenocoumarol; 44 patients required between 7 mg/wk and 28 mg/wk; finally, 21 patients required more than 28 mg/wk). The population is described in detail elsewhere. Genotyping for Leu208Val and Ile181Leu was done by polymerase chain reaction followed by digestion with restriction enzyme. Primers for genetic analysis were TGTCCTCCCTGAATGTACCTGTC (forward) and GTGCCTACCTGGATCCAGGCCTGGTC (reverse). A forced mismatch was included in position 3 from the 3′ end of forward primer to create in combination with 5′-GAATT-3′ (polymorphism Ile181Leu) a restriction site for NruI. The reverse primer also has a forced mismatch in position 3 from the 3′ end to create in combination with 6′-AGGT-3′ (polymorphism Leu208Val) a restriction site for Tsp45I.

Neither Leu208Val nor the Ile181Leu variants were detected in any of the studied patients, indicating that neither of these genetic variants is involved in the variability of acenocoumarol requirement in this Spanish population: if these polymorphisms played a significant role in determining the acenocoumarol dosage in this population, we should have found some patients carrying the Leu208Val variant in the group with low acenocoumarol requirement and patients carrying the Ile181Leu variant in the high-dose group.

In conclusion, we demonstrate that the Leu208Val and the Ile181Leu polymorphisms of cytochrome P450 CYP2C9 do not seem to play an important role in sensitivity to acenocoumarol in the Spanish population. Factors such as 2C9*2 and 2C9*3 variants of CYP2C9, age, sex, pharmacologic interactions, or associated diseases do not completely account for the interindividual differences in sensitivity to anticoagulant treatment. Therefore, it is probable that unknown genetic variants influencing the coumarin metabolism, which are perhaps different in different populations, will be described in the near future.

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References

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