Letter to the Editor

Identification of the mutations associated with hereditary hyperferritinemia cataract syndrome and hemochromatosis in a Brazilian family

To the Editor:

The iron storage polypeptide ferritin is a 24-subunit heteropolymer composed of heavy chain (H-ferritin; 21 kDa) and light chain (L-ferritin; 19 kDa) peptide subunits that are encoded by genes on chromosomes 11q13 and 19q13.1, respectively (1). Mutations on the ferritin light subunit (FTL) gene (MIM# 134790) can lead to changes in the iron responsive element (IRE) of the respective mRNA, which can inhibit the binding of the iron regulatory proteins (IRPs) to the IRE. This situation can lead to constant ferritin production independent of the iron levels or other biochemical parameters (2).

In 1995, two Italian families were first described with a new autosomal dominant genetic disorder, the ‘hereditary hyperferritinemia cataract syndrome’ (HHCS) (MIM# 600886) (2). HHCS is a genetic dominant disorder associated with bilateral cataract and increased serum ferritin in the absence of iron overload. Mutations in IRE of the L-ferritin result in constitutive, iron-independent ferritin expression (3, 4). Approximately 30 FTL gene mutations have been identified in about 100 families in Europe (France, Italy, UK, the Netherlands, Belgium and Spain), North America (United States and Canada), Australia and most recently in India (5–9).

Patients with HHCS may be wrongly diagnosed with hereditary hemochromatosis (HH) (MIM# 235200). HH is a genetically heterogeneous disorder of iron metabolism related not only with mutations into the HFE gene, but also in four other genes (TIR2, HAMP, HVJ, and FPN1) and characterized by parenchymal iron overload and elevated serum ferritin (5). Studies have reported cases where the FTL gene mutation is associated with mutations in the HFE gene (7, 10).

In this study, a three-generation family with Spanish ancestry (Fig. 1a), living in southeast Brazil, was well known to ophthalmologists for the segregation of dominantly inherited congenital bilateral cataract. A few years ago, the proband II-7, a 43-year-old female, underwent a biochemical evaluation of iron status because of slight anemia. Serum ferritin was 1350 μg/l whereas serum iron and transferrin saturation were normal (90 μg/ml and 25%, respectively). A myelogram with Perls’ stain was performed to evaluate iron stores. The iron stores were normal in bone marrow cells. To evaluate the possible occurrence of HHCS, a family study of biochemical iron parameters was performed (Table 1). The family members with bilateral cataract also had marked elevation of serum ferritin (>1000 μg/ml), with no other hematological or biochemical abnormalities. A complete family history did not reveal any other clinical symptoms that are apparently linked to the HHCS.

All participants provided written informed consent according to the protocol approved by the Ethics Committee of Federal University of São Paulo prior to the study. Genomic DNAs from family members (I:2, II:1–II:9, III:14, III:17) were isolated from peripheral blood lymphocytes using Wizard® SV Genomic DNA Purification System (Promega, Madison, WI) according to the manufacturer’s instructions. For polymerase chain reaction (PCR), 200 μmol/l of each deoxyribonucleotide (Fermentas, Ontario, CA), 200 nmol/l of each primer, 20 mU/μl of Taq polymerase enzyme (Fermentas) and 3 ng/μl of DNA sample were used. The primers used were synthesized by IDT Inc. (San Jose, CA), with the following sequences: FTL – 5′-TCCTTGCCACCGCAGATTT-3′ (sense) and 5′-TTGGCAAGAAGGAGCTAAC-3′ (antisense); HFE (for H63D mutation) – 5′-ACA TGGTAAAGCCCTGTTGC-3′ (sense) and 5′-GCCACA TCTGGCCTGAAATT-3′ (antisense); HFE (for H63D mutation) – 5′-ACATGTT TAAGGCCTGTGC-3′ (sense) and 5′-GCCACA TCTGGCCTGAAATT-3′ (antisense); HFE (for C282Y mutation) – 5′-TGCAAGGGTAAACAG ATCC-3′ (sense) and 5′-CAGCCCAACCCCCCCCTAC AAA-3′ (antisense). The amplification reaction was developed with an initial denaturation step of 2 min at 94°C, followed by 30 cycles of 30 s at
Table 1. Laboratory parameters of serum ferritin and transferrin saturation

<table>
<thead>
<tr>
<th>Individuals</th>
<th>Ferritin (μ/l)</th>
<th>Transferrin saturation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I:2</td>
<td>1,470</td>
<td>39</td>
</tr>
<tr>
<td>II:1</td>
<td>1,350</td>
<td>39</td>
</tr>
<tr>
<td>II:2</td>
<td>135</td>
<td>31</td>
</tr>
<tr>
<td>II:3</td>
<td>29</td>
<td>25</td>
</tr>
<tr>
<td>II:4</td>
<td>1,050</td>
<td>32</td>
</tr>
<tr>
<td>II:5</td>
<td>25</td>
<td>37</td>
</tr>
<tr>
<td>II:6</td>
<td>1,264</td>
<td>28</td>
</tr>
<tr>
<td>II:7</td>
<td>1,240</td>
<td>25</td>
</tr>
<tr>
<td>II:8</td>
<td>50</td>
<td>29</td>
</tr>
<tr>
<td>II:9</td>
<td>65</td>
<td>27</td>
</tr>
<tr>
<td>III:14</td>
<td>1,220</td>
<td>31</td>
</tr>
<tr>
<td>III:17</td>
<td>82</td>
<td>35</td>
</tr>
</tbody>
</table>

I:2, II:1, II:4, II:6, II:7, III:14 – Individuals with mutation in the FTL gene (41G>C) and compound heterozygous HFE genotype (C282Y/H63D); II:2, II:3, II:5, II:8, II:9, III:17 – Individuals with compound heterozygous HFE genotype (C28Y/H63D).

94°C, 30 s at 58°C, 1 min at 72°C and a final stage of 7 min at 72°C. Amplified fragments were then sequenced in an automatic sequencer ABI Prism 377 (Applied Biosystems, Foster City, CA), using the DYEnamese ET Terminator Cycle Sequencing Kit® (Amersham Biosciences, Alameda, CA), according to manufacturer’s protocol.

The presence of H63D and C282Y mutations in HFE gene was investigated separately by PCR and subsequent analysis of restriction fragments. The PCR was performed under the same conditions used to investigate mutations in the FTL gene. Digestion of the PCR products was performed at 37°C for 1 h with Bcl I (Fermentas) in final concentration of 0.27 U/μl for H63D, and Rsa I (Fermentas) in final concentration of 0.23 U/μl for C282Y. The resultant products were visualized by agarose gel electrophoresis.

The affected individuals showed a heterozygous change G>C (Fig. 1b) at position 41 from the transcription start site, in the third residue of the

![Fig. 1](image-url). Brazilian family carrying the 41G>C mutation in the FTL gene. (a) Family pedigree with hereditary hyperferritinemia cataract syndrome (HHCS). The affected marked individuals were already clinically diagnosed with bilateral cataracts and increase serum ferritin. The healthy marked individuals have not yet presented relevant HHCS symptoms. *Individuals with compound heterozygous HFE genotype (C282Y/H63D). (b) DNA automatic sequence of the L-ferritin gene in affected (I:2 and II:7) and unaffected individuals (II:9). (c) Particular of the iron responsive element (IRE) in the 5′-UTR of ferritin mRNA. *The G to C mutation involves one of the five highly conserved nucleotides that characterize the CAGUG loop sequence of IRE in the 5′-UTR of ferritin mRNA in subjects affected by HHCS.
5-base sequence (CAGUG) that characterizes the loop structure of the IRE (Fig. 1c). This position belongs to the IRE in the 5'-UTR of FTL. All affected family members who were tested showed the same nucleotide change. This alteration was not seen in any unaffected members of the family.

L-ferritin is a widely used marker of body iron load because its rate of synthesis is closely regulated by the availability of iron in the tissues through the IRP–IRE system. Therefore, increased serum L-ferritin concentration usually indicates iron overload. In HHCS there is no accumulation of iron in tissues (2). The observation that our probands showed congenital cataracts and increased serum L-ferritin but no iron overload supports this model of pathogenesis of HHCS.

In order to determine whether there was a relationship between inheritance of HHCS and HH, the HFE gene was studied to identify whether the C282Y or H63D substitutions were present in the HHCS probands. Compound heterozygosity for the C282Y or H63D substitutions were present in Spanish (7) and British (14) families with HHCS were observed heterozygous for the H63D mutation (11, 12). Approximately 1 in 10 people are heterozygous carriers and 0.44% is homozygous for the C282Y mutation (13). In Spanish (7) and British (14) families with HHCS were observed heterozygous for the H63D mutation in the HFE gene. Heterozygosis for these polymorphisms was not associated with an iron overload phenotype in a large population study (12) and inheritance of these alleles in our HHCS probands did not alter their HHCS phenotype.

We believe this is the first described case of a Brazilian family with HHCS determined by 41G>C mutation in the FTL gene known as ‘Verona mutation’, first described in an Italian family (15), and H63D and C282Y mutations in the HFE gene, the two genetic disorders related to iron regulation. It is unknown how the mutations may interact, so continued clinical observation over coming years will be important. The most common problem with HHCS is misdiagnosis as HH. With these findings we hope to prevent the unnecessary, and sometimes harmful, phlebotomies in patients with HHCS.

Acknowledgements

We thank the patients for agreeing to participate in the study. This work was supported by grants from Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal do Ensino Superior (CAPES), Brasil.

References


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