Genetic abnormalities and juvenile hemochromatosis: mutations of the HJV gene encoding hemojuvelin

Pauline L. Lee, Ernest Beutler, Sreenivas V. Rao, and James C. Barton

Juvenile hemochromatosis is an early-onset form of iron storage disease characterized by hypogonadotropic hypogonadism and cardiomyopathy. Recently, the putative causative gene (LOC148738) encoding a protein designated hemojuvelin was cloned. The previously proposed designation of this gene as HFE2 is contrary to established convention, because it is not a member of the HFE family. We suggest that it be designated HJV. We sequenced this gene in members of 2 previously reported kinships that manifest typical juvenile hemochromatosis. In one kinship, 2 previously undescribed mutations of HJV were identified, c.238T>C (C80R) and c.302T>C (L101P). In the second kinship, 2 previously identified mutations, G320V and I222N, were found. These studies confirm that mutations in HJV cause juvenile hemochromatosis. (Blood. 2004;103:4669-4671)

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Introduction

The first description of what is now designated as juvenile hemochromatosis was published in 1932 by Bezançon et al. Their patient, age 20 years, was described as having pigmentary cirrhosis with an enlarged liver, infantilism, and multiple endocrine insufficiencies. He died of cardiac failure.

Those findings correspond closely to the syndrome as described more recently. Hypogonadotropic hypogonadism and cardiac failure, not only an early age of onset of iron overload, distinguish this disorder clinically from the much more common type of hemochromatosis that results from mutations of the HFE gene. Most cases of juvenile hemochromatosis were found to be genetically linked to chromosome 1q. However, a few patients with the same syndrome did not have this linkage and were shown to have mutations of the HAMP gene encoding hepcidin.

Because no gene known to regulate iron homeostasis was known to exist on chromosome 1q, this putative juvenile hemochromatosis gene became a prime target for positional cloning. Very recently, the putative gene responsible for the Ch1q-linked form of the disorder was cloned. It encodes a transcription unit of previously unknown function (LOC148738) that has been designated hemojuvelin. The suggested designation of the gene encoding hemojuvelin, namely HFE2, is inappropriate because it is contrary to the accepted guidelines for gene nomenclature. The use of an Arabic numeral is recommended for designation of a gene family. Because no gene known to regulate iron homeostasis was known to exist on chromosome 1q, this putative juvenile hemochromatosis gene became a prime target for positional cloning. Very recently, the putative gene responsible for the Ch1q-linked form of the disorder was cloned. It encodes a transcription unit of previously unknown function (LOC148738) that has been designated hemojuvelin.

The online version of the article contains a data supplement.

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Center Institutional Review Board for these studies. Informed consent was provided according to the Declaration of Helsinki.

Methods

The primers used to amplify the HJV gene are shown in Table 2. Amplification was performed in a 50-µL reaction mix containing 50 to 200 ng genomic DNA, 150 ng of each primer, 33.5 mM Tris (tris(hydroxymethyl)aminomethane) HCl pH 8.8, 8.3 mM (NH₄)₂SO₄, 3.35 mM MgCl₂, 85 µg/mL bovine serum albumin, and 1 U Taq polymerase. After initial denaturation at 95°C for 4 minutes, amplification was performed for 30 cycles at 95°C for 1 minute, 60°C for 30 seconds, and 72°C for 30 to 50 seconds.

The primers and conditions for the amplification of the HFE gene were as described previously. The region around the HJV gene contained single nucleotide polymorphisms and microsatellites that were useful for haplotyping the subjects. The primers and annealing temperatures for haplotype analysis are described in the supplementary material available at the Blood website (see the Supplemental Tables link at the top of the online article). Amplification was performed using the polymerase chain reaction (PCR) conditions described earlier.

Amplified DNA products were purified by using Qiaquick PCR purification kits (Qiagen, Valencia, CA). Genotypes were determined by direct sequencing using the ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA).

Results

The pedigrees and the result of mutation analysis of the 2 kinships and in those previously reported 4,5,8 are typical of rare heritable disorders. Most patients with juvenile hemochromatosis have compound heterozygosity for a hemojuvelin mutation, L101P. The C80R and L101P mutations have not been reported previously. The haplotypes in which they were found are defined as a series of single nucleotide polymorphisms (SNPs) in the region surrounding the HJV gene and are summarized in the Supplemental Tables. A large number of genes in the region of the HJV gene were sequenced in subject II-5 and/or subject III-3. No mutations were found. The genes that were examined are listed in the Supplemental Tables.

Subjects I-1 and II-6 from kinship B were the parents of a woman who died at age 23 of putative juvenile hemochromatosis.4 Subject I-1 was a simple heterozygote carrying the c.959G>T (G320V) mutation in the HJV gene. Subject II-6 was a simple heterozygote carrying the c.665T>C (I222N) mutation in the HJV gene. Both the G320V and the I222N mutations have been described previously. The deceased subject II-1 from kinship B is, therefore, presumed to be a compound heterozygote carrying both mutations in the HJV gene.

Discussion

The results of these investigations confirm the assignment of the gene causing chromosome 1q-linked juvenile hemochromatosis. In 2 families manifesting the typical clinical picture of this disorder, mutations were found in the HJV gene. The heterogeneity of the HJV mutations and Ch1q haplotypes demonstrated in the present kinships and in those previously reported4,5,8 are typical of rare heritable disorders. Most patients with juvenile hemochromatosis

Figure 1. Abbreviated pedigree of Carolinas kinship A with juvenile hemochromatosis (JH). JH patients II-5 and II-6 were described in a previous report. Arrows designate JH index cases in respective sibships. Ages [in years] are those at time of manuscript preparation (or death); clinical and laboratory findings reflect observations at the time of diagnosis of JH patients or evaluation of corresponding family members. Tf sat indicates serum transferrin saturation; Fin, serum ferritin concentration. Determined alleles of HFE and HJV are displayed without parentheses, deduced genotypes within parentheses. The arrows designate the subjects used for DNA analysis.

Table 1. Characteristics at diagnosis of patients with juvenile hemochromatosis

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>HJV genotype</td>
<td>C80R/L101P</td>
<td>C80R/L101P</td>
<td>L101P/L101P</td>
<td>L101P/L101P</td>
<td>L101P/L101P</td>
<td>L101P/L101P</td>
<td>I222NG320V</td>
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<td>Age at diagnosis, y/sex</td>
<td>23/F</td>
<td>21/F</td>
<td>23/F</td>
<td>21/M</td>
<td>18/F</td>
<td>8/F</td>
<td>23/F</td>
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<td>Age at first symptomatic, y</td>
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<td>17</td>
<td>13</td>
<td>15</td>
<td>12</td>
<td>—</td>
<td>17</td>
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<tr>
<td>Serum iron, µM</td>
<td>49.4</td>
<td>58.2</td>
<td>47.1</td>
<td>42.1</td>
<td>46</td>
<td>49.6</td>
<td>39.0</td>
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<td>Transferrin saturation, %</td>
<td>96</td>
<td>98</td>
<td>90</td>
<td>97</td>
<td>92</td>
<td>90</td>
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<tr>
<td>Serum ferritin, µg/L</td>
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<td>ND</td>
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<td>4425</td>
<td>2003</td>
<td>1047</td>
<td>ND</td>
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<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>Hepatomegaly</td>
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<tr>
<td>Hepatic cirrhosis on biopsy</td>
<td>No</td>
<td>No</td>
<td>ND</td>
<td>Yes</td>
<td>ND</td>
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</tbody>
</table>

The characteristics are based on an earlier publication. — indicates not known; and ND, not done.
are diagnosed younger than age 30 years and have hypogonadism, hepatic fibrosis, or cirrhosis, or cardiomyopathy. However, some patients, their siblings, or other affected members with juvenile hemochromatosis have fewer complications of iron overload at diagnosis. This observation suggests that, although age of onset may influence the phenotype, juvenile hemochromatosis appears to have unique features, especially endocrinopathies (hypogonadotrophic hypogonadism) and cardiomyopathy. These complications may be the result of age-dependent organ susceptibility to iron, unusually high levels of non-transferrin-bound iron, tissue-specific iron deposition, transport or avidity, or other peculiarities of the mutations themselves. It is clear from kinship A, in which 4 siblings are affected, that the penetrance of these mutations is very high, in contrast with hemochromatosis associated with HFE mutations.

References


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