GENETIC ABNORMALITIES AND JUVENILE HEMOCHROMATOSIS:

MUTATIONS OF THE HJV GENE ENCODING “HEMOJUVELIN”

Running head: Juvenile Hemochromatosis and Hemojuvelin

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Juvenile hemochromatosis is an early-onset form of iron storage disease characterized by hypogonadotrophic 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, and we suggest that it be designated HJV. We sequenced this gene in members of two previously reported kinships that manifest typical juvenile hemochromatosis. In one kinship, two previously undescribed mutations of HJV were identified, C80R and L101P. In the second kinship, two previously identified mutations, G320V and I222N, were found. These studies confirm that mutations in HJV cause juvenile hemochromatosis.
The first description of what is now designated as juvenile hemochromatosis was published in 1932 by BezanHon et al. Their patient, age 20, was described as having pigmentary cirrhosis with an enlarged liver, infantilism and multiple endocrine insufficiencies. He died of cardiac failure.

These findings correspond closely to the syndrome as described more recently. Hypogonadotrophic 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 letter is recommended for designation of a gene family, but the gene encoding hemojuvelin is not a part of the HFE family. Thus, a more appropriate designation for this gene is HJV, which has been adopted by OMIM.
We previously described seven cases of juvenile hemochromatosis in two kinships, including one that had originally been reported in 1962\(^4\). We have now sequenced the \(HJV\) gene of these patients with early onset iron overload. We confirm that indeed mutations of this gene are responsible for the syndrome of juvenile hemochromatosis in most patients. We report the existence of two mutations of the \(HJV\) gene that have not been previously described and define the haplotypes in which they occur.

**Study Subjects**

We evaluated persons in each of two unrelated kinships from the southeast U.S.; all were white. In kinship A, we previously reported four siblings with a juvenile hemochromatosis clinical phenotype\(^4\) and demonstrated that they were homozygous for the same Ch1q haplotype. The third of the four sibs, an 18 year-old female who had amenorrhea since age 12, was selected for the present genetic analyses. At diagnosis, she had transferrin saturation 92\%, serum ferritin 2003 ng/mL, severe hypogonadotrophic hypogonadism, hepatomegaly, and hyperpigmentation. We also evaluated her father’s cousin, one of the original 1967 probands who presented at age 23 with amenorrhea, severe hypogonadism, and transferrin saturation 96\%, and had phlebotomy-proven iron overload \(^4,10\). This woman had one Ch1q haplotype apparently identical with that of her younger first cousin once removed, and a second, different Ch1q haplotype, and we had presumed her to be a compound heterozygote for the Ch1q-linked gene. In kinship B, we evaluated the parents of a woman who died at age 23 of cardiomyopathy and was discovered at autopsy to have severe, multiorgan iron overload and hepatic cirrhosis. At age 17, she experienced cessation of menses, and later developed other signs of severe hypogonadism and hyperpigmentation\(^4\). The clinical characteristics of these patients are summarized in Table 1.
Methods

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

The primers and conditions for the amplification of the HFE gene and the hepcidin 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. Amplification was performed using the PCR conditions described above.

Amplified DNA products were purified using Qiagen’s Qiaquick PCR purification kits. Genotypes were determined by direct sequencing using the ABI 3100 DNA sequencer.

Results

The pedigrees and the result of mutation analysis of the three kinships are shown in figures 1-3. Subject II-5 from kinship A was a compound heterozygote with C80R and L101P mutations in the hemojuvelin gene. Subject III-2, in kinship A was, as expected from the chromosome 1q haplotype that had been determined earlier, homozygous for a hemojuvelin mutation, L101P. The C80R and L101P mutations had 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 supplementary material. A large number of genes in the region of the *HJV* gene were sequenced in subject II-5 and/or III-3. No mutations were found. The genes that were examined are listed in the supplementary material.

Subjects I-1 and I-2 from kinship B were the parents of a woman who died at age 23 of putative juvenile hemochromatosis. Subject I-1 was a simple heterozygote carrying the G320V mutation in the *HJV* gene. Subject I-2 was a simple heterozygote carrying the 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 two 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 reported \(^4,5,8\) are typical of rare heritable disorders. Most patients with juvenile hemochromatosis are diagnosed under the age of 30 years and have hypogonadism, hepatic fibrosis, or cirrhosis, or cardiomyopathy \(^8,13-15\). 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 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 four sibs are affected, that the penetrance of HJV mutations is very high, in contrast with hemochromatosis associated with HFE mutations.

While the appearance of the disease phenotype in two successive generations might have suggested dominant inheritance with low penetrance, genetic analysis makes it clear that the inheritance is autosomal recessive, and that the pattern observed represents pseudodominance. Such pseudodominant inheritance is common when the disease gene frequencies are high. For example, it was once believed that Gaucher disease was a dominant disorder \(^16\), but it is now clear that the apparent dominance was due to the mating of heterozygotes with a homozygotes in a population in which about 7% were heterozygous. Pseudodominance must be much less common in the case of rare mutations such as those of hemojuvelin.
Acknowledgments

The technical assistance of Mrs. Terri Gelbart and Mrs. Carol Halloran is gratefully acknowledged.
Reference List


Table 1. Characteristics at Diagnosis of Patients with Juvenile Hemochromatosis*  

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<td>*HJV genotype</td>
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<td>I222N/G320V</td>
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<td>21 F</td>
<td>23 F</td>
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<td>15</td>
<td>12</td>
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<td>92</td>
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<td>4425</td>
<td>1047</td>
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*Based on an earlier publication*
Table 2. Primers used for amplification of the *HJV* coding region

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FIGURE LEGENDS

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 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 = serum transferrin saturation; Ftn = serum ferritin concentration. Determined alleles of HFE and HJUV are displayed without parentheses, deduced genotypes within parentheses.

Figure 2. Pedigree of Alabama kinship B with juvenile hemochromatosis (JH). Arrow designates JH index case. Ages 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 = serum transferrin saturation; Ftn = serum ferritin concentration. Determined alleles of HFE and HJUV are displayed without parentheses, deduced genotypes within parentheses.
Figure 1

II-7: Age 53
Tf sat 36%; Ftn 265 ng/mL
HFE
wt/wt
LOC148738
L101P/wt

II-8: Age 50
Tf sat 8%; Ftn 265 ng/mL
HFE
H63D/wt
LOC148738
L101P/wt

III-1: Age 26
Tf sat 90%; Ftn 2647 ng/mL
HFE
wt/wt
LOC148738
L101P/L101P
Hashimoto thyroiditis

II-5: Age 59
Tf sat 96%; Ftn 70 ng/mL
HFE
wt/wt
LOC148738
C80R/L101P

II-6: Age 21
Tf sat 98%; Ftn 265 ng/mL
HFE
wt/wt
LOC148738
C80R/L101P

II-7: Age 53
Tf sat 36%; Ftn 265 ng/mL
HFE
wt/wt
LOC148738
L101P/wt

II-8: Age 50
Tf sat 26%; Ftn 70 ng/mL
HFE
H63D/wt
LOC148738
L101P/wt

I-2: Age 82
Tf sat 45%; Ftn 265 ng/mL
HFE
wt/wt
LOC148738
L101P/wt

II-1
LOC148738
C80R/wt

II-2

II-3

II-4

II-5

II-6

II-7

II-8

I-3: Age 50
Tf sat 8%; Ftn 265 ng/mL
HFE
H63D/wt
LOC148738
L101P/L101P

III-2: Age 24
Tf sat 97%; Ftn 2003 ng/mL
HFE
H63D/wt
LOC148738
L101P/L101P

III-3: Age 21
Tf sat 92%; Ftn 2003 ng/mL
HFE
H63D/wt
LOC148738
L101P/L101P

III-4: Age 19
Tf sat 44%; Ftn 30 ng/mL
HFE
wt/wt
LOC148738
L101P/L101P

III-5: Age 16
Tf sat 37%; Ftn 32 ng/mL
HFE
wt/wt
LOC148738
L101P/L101P

III-6: Age 11
Tf sat 90%; Ftn 1047 ng/mL
HFE
wt/wt
LOC148738
L101P/L101P
I-1: Age 71
Tf sat 30%; Ftn 279 ng/mL
HFE wt/wt
LOC148738 G320V/wt

II-1: Age 23
Tf sat 85%
(HFE wt/wt)
(LOC148738 G320V/I222N)

II-2: Age 41
Tf sat 28%; Ftn 37 ng/mL
HFE wt/wt
Sarcoidosis

I-2: Age 68
Tf sat 18%; Ftn 279 ng/mL
HFE wt/wt
Rheumatoid arthritis, diabetes mellitus
LOC148738 I222N/wt
Genetic abnormalities and juvenile hemochromatosis: mutations of the HJV gene encoding hemojuvelin

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