Disorders of Iron Metabolism

Hemojuvelin (*HJV*)-associated hemochromatosis: analysis of *HJV* and *HFE* mutations and iron overload in three families

Juvenile hemochromatosis is a severe form of hereditary iron overload. It can be caused by mutations in either hepcidin or hemojuvelin genes. In this study we identified the molecular basis of juvenile hemochromatosis in three Australian families and assessed the role of potential modifying genes in individuals carrying *HFE* mutations.

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Juvenile hemochromatosis (JH) is an autosomal recessive iron overload disorder. It has an earlier onset and more severe clinical course than *HFE*-associated hemochromatosis. Frequent clinical findings include cardiomyopathy and hypogonadism.[1] JH can be caused by mutations in the hepcidin gene (*HAMP*) on chromosome 19[2] or the recently identified hemojuvelin gene (*HJV*) on chromosome 1.[3] Both *HAMP* and *HJV* have been proposed as potential modifiers in HFE-associated hemochromatosis. In one study, 5 of 392 subjects homozygous for C282Y who carried an additional *HAMP* mutation had more severe iron overload phenotypes.[4] In the same study, 4 individuals with digenic inheritance of either *C282Y*, *H63D* or *S65C* and a *HAMP* mutation had iron overload.[4] In another study digenic inheritance of C282Y and *HAMP* mutations was associated with iron overload in 2 patients.[5] These studies suggest that *HAMP* can have a modifying effect in subjects who are either homozygous or heterozygous for mutations in *HFE*. Two recent reports studied the potential modifying effect of *HJV* with conflicting results. In one study of 310 *C282Y* homozygotes, 8 of 9 who also carried a *HJV* mutation appeared to have a more severe phenotype.[6] In the second study no *HJV* mutations were detected in a group of 49 *C282Y* homozygotes; the authors concluded that in the subjects studied *HJV* mutations were not associated with disease penetrance.[7] In these two studies individuals with digenic inheritance of *HJV* and *HFE* mutations were not identified,[6,7] hence, the effect of digenic inheritance of *HJV* and *HFE* on phenotype has not been determined.

In this study we identified three female patients with severe iron overload and features typical of JH. Patient #1 presented at the age of 12 years with severe congestive cardiomyopathy; she also had hypopituitarism, short stature and delayed puberty. Patient #2 presented, aged 18 years, with lethargy and arthralgia. Patient #3 presented at the age of 32 years with lethargy and hypogonadism; she also had osteoarthritis and osteoporosis. All had elevated serum iron indices, abnormal liver function tests and substantial hepatic iron loading with either liver fibrosis or cirrhosis. Transferrin saturation, serum ferritin concentrations, *HFE* and *HJV* mutation status are shown for each patient and their family members in Figure 1.

DNA from the 3 patients was isolated and the coding region and splice sites of *HAMP* and *HJV* were PCR amplified by polymerase chain reaction (PCR) and sequenced. Primer sequences and PCR conditions are

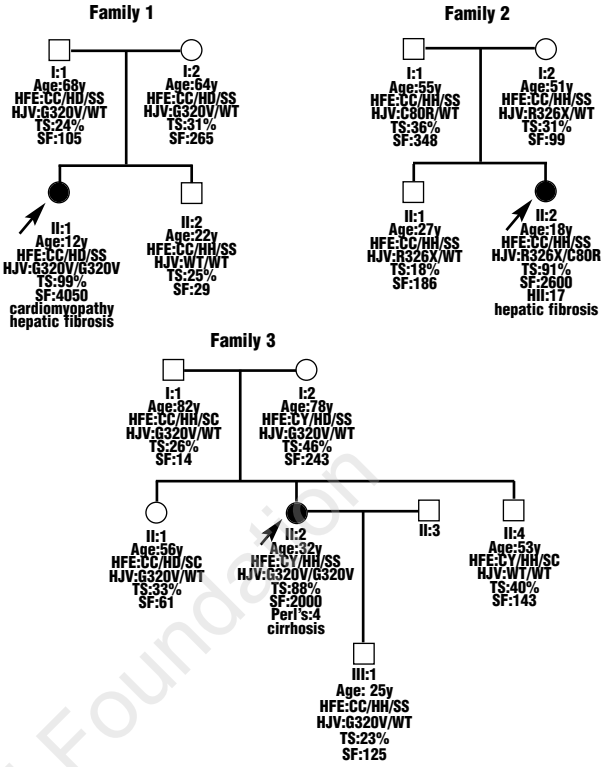

Figure 1. Pedigrees of three families affected by JH. Arrows and filled circles indicate affected probands. Genotype status for the *HFE* mutations *C282Y/H63D/S65C* and mutations in the *HJV* gene are shown. Transferrin saturation (TS) and serum ferritin (SF) were measured at diagnosis for patients 1 and 2; values at diagnosis are not available for patient 3 and the values given are those measured once treatment had commenced. For all other family members the age at which TS and SF were measured is shown. HII, hepatic iron index; Perls', Perls' stain grade.

available upon request. The presence of the *HJV G320V* mutation in family members was determined using an *Nla*IV restriction endonuclease digestion assay of exon 4a PCR products. The presence of *R326X* and *C80R* mutations in family members was determined by direct sequence analysis.

No sequence changes in the *HAMP* gene were detected. Sequencing of the *HJV* gene showed that all three patients were homozygous or compound heterozygous for mutations in this gene. Patients 1 and 3 were homozygous for the common G320V mutation,[3,8,9] patient 2 was compound heterozygous for the *R326X*[3] and *C80R* mutations.[9] Two of the patients had coinheritance of *HFE* mutations, *H63D* in patient 1 and *C282Y* in patient 3. This led us to analyze the potential effect of digenic inheritance of *HJV* and *HFE* mutations by studying the family members. Five of eleven family members carried at least one of the three most common *HFE* mutations and were heterozygous for *G320V* (Figure 1). None of these five individuals had any clear evidence of iron loading (Figure 1). In one (family 3, I:2) there was a slight increase in transferrin saturation (46%) with normal

serum ferritin concentration (243 μg/L). However, this was in a 78-year old *C282Y/H63D* female and in an earlier measurement at 72 years, the transferrin saturation had been found to be in the normal range (32%). This is the first phenotypic analysis in individuals with digenic inheritance of *HJV* and *HFE* mutations and suggests that at least in these individuals there is no increased risk of iron loading.

In conclusion, we report three cases of JH in Australia caused by *HJV* mutations. In two of the families digenic inheritance of *HJV* and *HFE* mutations did not cause iron loading. Although this is a small study, given the rarity of *HJV* and *HAMP* mutations in the general population it may be difficult to identify sufficient individuals to study the effect of these genes as modifiers of *HFE*. The analysis of family members of JH patients may yield more information on the effect of digenic inheritance of *HFE* and either *HJV* or *HAMP* on iron loading.

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Red Cell Disorders

HbO-Arab mutation originated in the Pomak population of Greek Thrace

HbO-Arab emerged about 2,000 years on a rare haplotype, characteristic of the Greek Pomaks. Its frequency increased as a consequence of high genetic drift within this population, and it was dispersed throughout the Mediterranean basin and Middle East with minor variations of its haplotypic pattern.

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The non-randomness of some restriction fragment length polymorphisms (RFLP) across the *HBB* cluster, was recognized two decades ago.[1] These RFLP segregates, known as *HBB* cluster haplotypes, are associated with certain mutations and populations, thus constituting a link between a mutation and its possible origin.[2] Moreover, a pattern of five polymorphic sites within the *HBB* gene itself, known as the *HBB* gene framework, has been proved to represent archaic gene patterns.[3]

HbO-Arab (*HBB, E121K*) is most frequent worldwide in Greek Pomaks, in whom the frequency of this mutant allele has been calculated to be 0.027.[4] To investigate its origin, we determined 20 single nucleotide polymorphisms (SNP) flanking *HBB* in 52 Pomak (21 HbO-Arab carrying and 31 normal) and 7 non-Pomak (2 HbO-Arab carrying and 5 normal) chromosomes. Fifteen of them alter restriction sites, are dispersed across the *HBB* cluster and constitute the *HBB* cluster haplotype (Figure 1) and 5 are not linked with restriction sites, segregate within *HBB* and form the *HBB* framework (Table 1). All polymorphisms and the mutation site were confirmed by DNA sequencing. DNAsp 3.99 software was used for recombination analysis.[5] Genetree software was used for *time to most recent common ancestor* (TMRCA) analysis.[4]

A summary of the results is presented in Table 1. Almost half of the normal Pomak chromosomes (13/31) are connected with Greek haplotype VI and Pomak haplotype I; these two haplotypes are most unusual (in the case of Greek haplotype VI) or even absent (in the case of Pomak haplotype I) from the normal Greek population. The remaining 18 normal chromosomes revealed the universal haplotypes I, II and III, which are common among the normal Greek population.[6-8] All but three (18/21) HbO-Arab genes in the Pomaks and both HbO-Arab genes of non-Pomak origin are carried over the haplotype Greek VIa. The remaining three HbO-Arab carrying Pomak chromosomes display a variety of the universal haplotype I (Id). It is notable that the 3' subhaplotype is the same [(-) + (+ - - +) +] in all Pomak and non-Pomak chromosomes carrying the HbO-Arab mutation.

Of the 12 normal chromosomes of Pomak origin examined, 8 displayed the CCGCT framework pattern and 4 the CCTCT pattern. In contrast, all the HbO-Arab carrying chromosomes examined (12 of Pomak and 2 of non-Pomak origin) displayed the CCTCT framework pattern. Possible recombination sites in the Pomak population have been detected between (i) *Hind*III/HBG1 and *Hinc*II/HBBP1 and (ii) between 3' and 5' subhaplotypes. These data explain both Greek VI and Pomak I haplotypes. Figure 1 depicts the mechanism of recombination