**Disorders of Iron Metabolism**

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

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 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.2 In the same study, 4 individuals with digenic inheritance of either C282Y, H63D or S65C and a HAMP mutation had iron overload.1 In another study digenic inheritance of C282Y and HAMP mutations was associated with iron overload in 2 patients.3 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.5 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 individual with digenic inheritance of HJV and HFE mutations were not identified,8 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 available upon request. The presence of the HJV G320V mutation in family members was determined using an NlaIV 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 R326X3 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

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**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.
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.

The non-randomness of some restriction fragment length polymorphisms (RFLP) across the HBB cluster, was recognized two decades ago. 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. 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.

HbO-Arab (HBB, E124K) is most frequent worldwide in Greek Pomaks, in whom the frequency of this mutant allele has been calculated to be 0.027. 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 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. Genetree software was used for time to most recent common ancestor (TMRCA) analysis.

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. 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 Vla.

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) HindIII/HBG1 and HindIII/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.