Non-C282Y familial iron overload: evidence for locus heterogeneity in haemochromatosis

S Pinson, J Yaouanq, A M Jouanolle, B Turlin, H Plauchu

Abstract
Haemochromatosis (HC) is an autosomal recessive disease with progressive iron overload leading to midlife onset of clinical complications. The causal gene (HFE) maps to 6p, in close linkage with the HLA class I genes. An HFE candidate gene recently identified has two missense mutations (C282Y and H63D) associated with the disease. Here we document the phenotypic and genetic analysis of a nuclear family comprising two sibs with symptomatic and massive iron overload before the age of 25. The disease seemed to be recessively transmitted and fitted the agreed criteria for haemochromatosis, but was neither associated with the C282Y and H63D mutations nor linked with HLA markers. Our data strongly support locus heterogeneity in haemochromatosis by showing evidence that the gene responsible for juvenile haemochromatosis (JH) does not map to 6p. In the absence of clear cut phenotypic distinction from typical haemochromatosis, patients below 30 years of age and C282Y negative should be considered as putative juvenile cases. This has practical implications in genetic counselling and family management.

Keywords: juvenile haemochromatosis; HFE mutations; HLA; locus heterogeneity

Haemochromatosis (HC) is a common autosomal recessive disorder of iron metabolism that affects 1 to 4 per 1000 subjects in various populations. It is characterised by increased duodenal absorption of iron and progressive parenchymal iron loading typically resulting in the midlife onset of clinical complications, including cirrhosis, diabetes mellitus, myocardiopathy, endocrine dysfunction, and arthropathy. Some patients below 30 years presenting with symptomatic iron overload have also been reported. Most were isolated cases and whether the juvenile form reflects a more severe expression of the common disease or is a distinct entity has remained elusive. The gene (HFE) responsible for typical haemochromatosis has confidently been mapped to the short arm of chromosome 6 (6p) through its association and linkage with HLA markers. Recently, an HFE candidate gene has been located 4.5 Mb distal to HLA-A. It is an HLA class I-like gene in which two missense mutations have been identified: (1) a cysteine to tyrosine substitution at position 82 of the final protein (C282Y), and (2) a histidine to aspartic acid substitution at position 63 (H63D). The C282Y mutation is strongly associated with the disease. The C282Y homozygous genotype is observed in more than 82% of white patients from the USA, Australia, and Britain. The H63D mutation accounts for a further 5-10% of patients, who are generally C282Y/H63D compound heterozygotes.

Here we document a family comprising two sibs clinically affected with massive iron overload before the age of 25. The proband (II.1) was a 20 year old man who complained of chronic fatigue and presented with generalised skin pigmentation. He had no hepatomegaly or sign of liver failure, no clinical or radiological arthropathy, and no endocrine or heart failure. On biochemical evaluation he had markedly raised serum iron indices and serum amino transferase levels (table 1), which were not alcohol related (no alcohol consumption, normal γ-glutamyl transferase). His body mass index (BMI) was 17.7 kg/m2 and his glucose metabolism was normal with blood glucose levels in the fasting state and two hours after 75 g oral glucose loading at 4.9 and 5.7 mmol/l respectively. Serological markers for hepatitis A, B, and C were negative. Other liver tests were normal, as were haematological parameters (table 1). Liver biopsy showed grade 3-4 hepato- siderosis without sideronecrosis but complicated by extensive fibrosis. Neither hepatic cytotoxic degenerative changes, such as Mallory bodies, nor steatosis were found. Iron was predominantly deposited in parenchymal cells with a decreasing gradient from the perportal to the centrolobular zone. The hepatic iron index (HII=15) was in the range of HC, as were the tissular iron score (TIS) of 40 and the TIS/age ratio of 2 (normal range 0 to 0.14). The diagnosis of HC was based upon (1) the above quantitative criteria, (2) the characteristic pattern of hepatic iron distribution, and (3) the absence of any known cause that might confer similar histological features, such as chronic oral intake of iron or ascorbic acid and ineffective erythropoiesis. The patient was treated by weekly phlebotomies until his serum iron parameters dropped to the low normal limits (serum iron 15 μmol/l, transferrin saturation 20%, ferritin 59 μg/l), reflecting iron depletion which was achieved after removal of 13 litres of blood (that is, 6.5 g of iron).

Family testing showed significantly raised serum iron indices (table 1) in the proband’s 2 year old sister (II.2), whose iron status had never been investigated despite the presence of chronic fatigue and oligomenorrhoea of 8 years’ duration. She had menarche at the age of
of whom are also negative for H63D. Such “negative” cases account for 7-9% of patients in the USA1 and southern France,1 and up to 21% in Italy.10 Since no additional mutation has been found except in one case,11 other gene(s) potentially relevant for haemochromatosis are expected to be discovered within the extensive linkage disequilibrium zone telomeric to HLA-A.14 However, the present pedigree further complicates the problem since the affected sibs were totally discordant for HLA-A and -B alleles.

Despite recessive inheritance and HLA linkage, not all haemochromatosis sib pairs are identical by descent for two HLA parental haplotypes.15 This is usually taken to indicate more than two HFE alleles in the parental gene pool, a situation which frequently arises in populations with a high gene frequency. Crossover events between HFE and HLA-A, although very rare, could account for some additional cases. All these possibilities were ruled out in our family. First, HLA typing of the two parents allowed the unambiguous definition of the four HLA parental haplotypes. Secondly, homozygosity for HC of the father was unlikely, because within affected families this genotype has an estimated frequency of 100% in males over 40.16 Then, by postulating heterozygosity for HC in both parents, one would suppose that two independent crossover events separating HFE and HLA-A have occurred (one in each meiosis, or two in only one meiosis), which is incompatible with the extreme rarity of recombination in the genomic region considered.17 Alternatively, if the father is a heterozygote and the mother a non-expressing homozygote, a crossover event would have occurred in the paternal meiosis leading to II.1 or II.2. This possibility is weakened by the fact that the HLA-A11 paternal haplotype belongs to a category of “stable” haplotypes, whose high degree of conservation is probably the result of a mechanism of recombination suppression.18 Finally, rejection of the classical (HLA linked) form of haemochromatosis is by far the most likely explanation. The early onset of significant iron accumulation in the two sibs, and dysmenorrhoea in the sister, are consistent with juvenile haemochromatosis (JH), despite the absence of cardiac dysfunction and the degree of iron overload which might appear lower than in some of the rare reported cases.19 On the whole, the present pedigree data indicate that the disease might be inherited as an autosomal recessive trait but does not segregate with markers on 6p. This provides evidence that the molecular defect(s) responsible for JH must be searched for outside the 6p chromosomal region, as recently stated by the Italian group.19 Collaborative studies should indicate whether our two cases and those born from consanguineous Italian parents19 are genetically different.

The clear demonstration that at least one gene unlinked to 6p causes a haemochromatosis phenotype in the young has important practical implications in genetic counselling. Obviously, screening for the HFE mutations and family gene tracking with 6p markers are
We are grateful to the members of the family for their cooperation. We thank Professor J C Chuat for carefully reading the manuscript.