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

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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.

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Haemochromatosis (HC) is a common autosomal recessive disorder of iron metabolism that affects 1 to 4 per 1000 subjects in white populations.¹ 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.^{2,3} 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.¹ Recently, an HFE candidate gene has been located 4.5 Mb distal to HLA-A.⁴ It is an HLA class I-like gene in which two missense mutations have been identified: (1) a cysteine to tyrosine substitution at position 282 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,^{4,5} Australia,⁶ and Brittany.⁷ 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 hepatosiderosis without sideronecrosis but complicated by extensive fibrosis. Neither hepatocytic degenerative changes, such as Mallory bodies nor steatosis were found. Iron was predominantly deposited in parenchymal cells with a decreasing gradient from the periportal to the centrolobular zone. The hepatic iron index (HII=15) was in the range of HC,⁸ as were the tissular iron score (TIS) of 40 and the TIS/age ratio of 2 (normal range 0 to 0.14).⁹ 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 limit (serum iron 15 mol/l, transferrin saturation 20%, ferritin 59 μ g/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 23 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

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Table 1 Iron status, biochemical parameters, and HLA haplotypes of the two affected sibs (II.1 and II.2) and their parents (I.1 and I.2)

	I.1	I.2	II.1	II.2
Sex	M	F	M	F
Age at first testing (y)	53	49	20	23
Serum iron ($\mu\text{mol/l}$)	11.1	21.4	57.5	50
Transferrin saturation (%)	—	33	100	95
Serum ferritin ($\mu\text{g/l}$)	87	24	1967	2575
AST-ALT (U/l)	—	—	62-115*	34-30†
Haemoglobin (g/dl)	15.3	12.3	14.2	13.6
Mean corpuscular volume (fl)	90	91	92	86
Hepatic iron concentration ($\mu\text{mol/g}$)	—	—	299	Not done
Total body iron stores (g)	—	—	6.5	>4
HLA-A, B haplotypes	A2,B18 A11,B55	A24,B51 A68,B53	A2,B18 A68,B53	A11,B55 A24,B51

Normal values : serum iron <25 $\mu\text{mol/l}$, transferrin saturation <40%, serum ferritin <300 $\mu\text{g/l}$, hepatic iron concentration <36 $\mu\text{mol/g}$ dry weight, serum aspartate transaminase (AST) <35* and <22†, serum alanine transaminase (ALT) <40* and <25†.

15, with subsequent irregularly spaced menses occurring at 40 to 120 day intervals. At the time of study, she had never used oral contraceptives or been pregnant, had no history of oral or parenteral iron intake, and was not overweight (BMI=18.1 kg/m^2). Her haematological parameters were normal (table 1), as were inflammatory markers. Physical examination was unremarkable and she refused any endocrine investigation or liver biopsy. However, she underwent phlebotomy therapy and was still not iron depleted (serum ferritin 518 $\mu\text{g/l}$) after removal of 4 g of iron by weekly venesections. Unlike their offspring, the parents (I.1 and I.2) had a normal iron status (table 1) in the absence of any cause which may have delayed the accumulation of iron (no dietary idiosyncrasy, no blood donation, no pathological blood loss or malabsorption). They traced their origin to central France and had no common ancestor identified in their six generation pedigree.

Genetic analysis of the family showed that both the proband and his sister lacked the C282Y and H63D mutations. Furthermore, they did not share any parental HLA haplotype (table 1). These unexpected findings warrant discussion with respect to the possible aetiology of the disease segregating within the family. Careful re-examination of phenotypic data confirmed the absence of any known cause of secondary iron overload. Iron associated disorders, including hepatitis C and acraeruloplasminaemia, were easily excluded. Steatohepatitis was also ruled out, since neither of the two sibs had the usually associated features (no overweight, no abnormality of glucose and lipid metabolism, no arterial hypertension) and the proband had no liver fat deposition or Mallory bodies. Furthermore, none of the usual causes of increased iron uptake except for haemochromatosis could explain the distribution of hepatic iron in the proband, whose disease fitted the accepted phenotypic criteria for HC (HII >1.9, TIS/age >0.15, and total of body iron excess >5g).⁸⁻¹⁰ The significant increase of body iron stores (>4 g) in the clinically affected sister further supported the diagnosis of HC. This was not incompatible with the observation that both sibs were negative for C282Y and H63D. Indeed, a significant proportion of well defined haemochromatosis patients lack C282Y, some

of whom are also negative for H63D. Such "negative" cases account for 7-9% of patients in the USA^{4,5} and southern France,¹¹ and up to 21% in Italy.¹² Since no additional mutation has been found except in one case,¹³ other gene(s) potentially relevant for haemochromatosis are expected to be discovered within the extensive linkage disequilibrium zone telomeric to HLA-A.^{4,14} 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 penetrance 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.^{4,17} 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.^{3,19} 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

inaccurate for predicting genotypes at risk for JH. On the other hand, the frequency of the disease may have been underestimated, since it may be difficult to distinguish juvenile and typical haemochromatosis on the sole basis of a different rate of iron accumulation.³ In practice, the risk for having unrecognised JH is mainly restricted to the group of patients under 30 years who are negative for C282Y, so that any proband in this group should be considered as a putative juvenile case. Then, until the identification of the underlying molecular defect(s) allows unambiguous distinction from the HLA linked form, his/her first degree relatives should be carefully examined, especially unaffected sibs whose iron status should be periodically tested regardless of the number of 6p parental haplotypes they share with the proband. Together with the recent suggestion that certain C282Y homozygotes may remain unaffected,²⁰ the increasing evidence of locus heterogeneity in primary iron loading disorders will have a critical impact on future diagnosis and screening strategies, especially in ethnically heterogeneous populations.

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