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Juvenile and Adult Hemochromatosis Are Distinct Genetic Disorders

Abstract

Juvenile Hemochromatosis (JH) is a rare genetic disorder that causes iron overload. JH clinical features are similar to those of hemochromatosis (HFE), but the clinical course is more severe and is characterized by an earlier onset and by a prevalence of cardiac symptoms and endocrine dysfunctions. Here we describe seven Italian patients belonging to five unrelated families with clinical features typical of JH. In four out of five families the parents were consanguineous. Analysis of HFE gene mutations in all the cases and nucleotide sequence of the gene in one case excluded this gene as responsible for JH. Segregation analysis of 6p markers closely associated with HFE in families with consanguineous parents clearly showed that JH is unlinked to 6p and thus genetically distinct from HFE.

Key Words

Iron overload
Hemochromatosis, juvenile
HFE gene
Microsatellites

Introduction

Hemochromatosis (HFE) is a recessive genetic disorder caused by a deregulation of intestinal iron absorption, which leads to excessive iron accumulation and organ damage. This results, in midlife, in a variety of clinical disorders that include liver cirrhosis, heart failure, diabetes, endocrinopathies, arthropathy and susceptibility to liver cancer [1, 2]. HFE is an HLA-A-associated disease [3]. Recently, the HFE gene, an atypical HLA class I-like gene, provisionally designated HLA-H, has been cloned on chromosome 6p [4]. The prevalent mutation, Cys282Tyr (C282Y), accounts for 83–100% of patients of northern European descent [4–7] and for 64–76% of patients from southern Europe [8, 9]. A second mutation His63Asp (H63D) has an undefined role in the pathogenesis of the disease [4–8].

Juvenile hemochromatosis (JH) differs from typical HFE. While HFE has a prevalent male expression, JH affects both sexes. Also, iron accumulation begins early in life and causes clinical symptoms before the age of 30 years. JH is a more severe disease: hypogonadotropic hypogonadism and heart failure and/or arrhythmias are prevalent [10–12]. If untreated, the disease is lethal, because of cardiac complications. Although the organ damage in JH is more severe, parenchymal iron distribution is similar to HFE, as inferred by liver biopsies or autopsy findings [10, 11, 13]. Functional studies on iron metabolism in JH have not been reported. In the only case published, iron absorption was 100%, despite the severe iron overload, a value never reached in HFE. Studies of HLA-A inheritance in the literature are limited and inconclusive [11]. Thus, both the metabolic defect of JH and its relationship with HFE remain uncertain.

Here we report 7 Italian JH patients belonging to five different families. In four families the parents were consanguineous. Molecular analysis provides evidence that the HFE gene is not involved in JH. The analysis of family

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segregation of polymorphic markers mapping on both sides of HFE gene excludes the linkage of JH to the 6p region associated with HFE and thus demonstrates that JH is genetically distinct from HFE.

Patients and Methods

Case 1

A 15-year-old girl was found to have increased serum ferritin during family screening. Liver biopsy, performed during abdominal surgery for acute appendicitis, showed moderate fibrosis and hepatocellular iron storage. The patient, now aged 18, is well on regular phlebotomy treatment.

Case 2

A brother of case 1. At 9 years a liver biopsy was performed for persistent hypertransaminasemia after hepatitis A virus infection. At the age of 23 he experienced heart failure and atrial fibrillation due to severe dilated cardiomyopathy. A second liver biopsy revealed hemochromatosis and cirrhosis. Despite phlebotomy treatment the patient died aged 24 of refractory heart failure.

Their parents are first cousins (fig. 1: family R) of southern Italian descent and have normal iron parameters.

Case 3

A 20-year-old female had secondary amenorrhea due to hypogonadotropic hypogonadism. The patient showed moderate spleen and liver enlargement and hypochromic microcytic anemia due to a concomitant β-thalassemia trait. Liver biopsy revealed a cirrhotic liver with heavy iron deposition both in hepatocytes and in Kupffer cells. The patient was started on regular subcutaneous desferrioxamine and on phlebotomy treatment.

Her parents are first cousins (fig. 1: family M) from southern Italy with normal iron values.

Case 4

A 20-year-old woman developed amenorrhea due to hypogonadotropic hypogonadism. She had a history of hepatitis A infection in infancy and persistent hypertransaminasemia. Liver biopsy showed coarse granular iron in hepatocytes, fibrosis and hepatocyte regeneration. A diagnosis of cirrhotic hemochromatosis was subsequently confirmed in two different institutions in Italy and the United Kingdom. The patient now aged 38 is well on phlebotomy treatment.

Her parents are first cousins of southern Italian origin (fig. 1: family T).

Case 5

A 30-year-old male had a history of hypogonadism with a testis biopsy showing tubular sclerosis and interstitial fibrosis. At that time he had severe heart failure which was symptomatically treated. Liver biopsy revealed fibrosis and massive iron deposition in the hepatocytes and Kupffer cells. Plasma testosterone and gonadotropins were remarkably reduced and a gonadotropin response to LH-RH was absent. Phlebotomies were started with an improvement of the clinical conditions.

His parents are first cousins of northern Italian origin (fig. 1: family F).

Case 6

A 21-year-old woman developed amenhorrea and was treated with estrogen replacement therapy. Ten years later a pregnancy was induced by gonadotropin therapy. The patient had severe heart failure during the 7th month of pregnancy. Liver biopsy, performed after delivery because of persistently abnormal liver function tests, revealed iron-loaded hepatocytes and fibrosis. Phlebotomies were started and the patient is now well aged 46.

Case 7

The sister of case 6 developed amenorrhea at the age of 26. Liver biopsy performed because of high transaminase values and the positive family history showed parenchymal iron overload and portal fibrosis. She is now well on phlebotomies aged 39.

The pedigree of family L. is reported in figure 1.

In none of the patients was there a history of excessive dietary or medicinal iron intake or of blood transfusions. There was a searching for evidence of HBV and HCV infections, but it was not found in all cases.

Clinical and Hematological Studies

Serum iron, transferrin saturation and serum ferritin were determined by standard methods. HLA typing was performed by the conventional microlymphocyte cytotoxicity test. Liver iron was evaluated at biopsy after Prussian blue staining and graded on a scale of 1–4 depending on the extent of hepatocyte involvement. Liver iron concentration (LIC) was determined by atomic absorption spectrophotometry and expressed as μmol/g dry tissue and the hepatic iron index = LIC/age (years) was calculated [1, 14]. Liver biopsies performed in cases 1 and 2 (two distinct samples) were reevaluated.

Molecular Studies

Informed consent was obtained in all cases for the genetic studies. DNA was isolated from peripheral blood buffy coats. PCR amplification of genomic DNA was performed on 250 ng DNA in an automated Thermal Cycler (Perkin Elmer, Norvalk, Conn.) using conditions previously described [8, 15]. Oligonucleotide primers and analysis of D6S105, D6S265 and D6S1281 microsatellites were as described [15]. Haplotypes were defined by using a combination of three 6p markers and assigned on the basis of allele segregation within the family. Analysis of C282Y and H63D mutations in HFE gene and direct sequencing of the exons and exon-intron boundaries were as described [8].

Linkage Analysis

Pairwise lod scores (Z) were calculated using the computer program MLINK from the LINKAGE package.

Results

Clinical Studies

Consanguinity in four families and the presence of two affected siblings in the fifth family confirm that JH is a recessive condition. The patients had typical features of JH (table 1). Five out of 7 were females. Transferrin saturation and serum ferritin were strikingly elevated in all

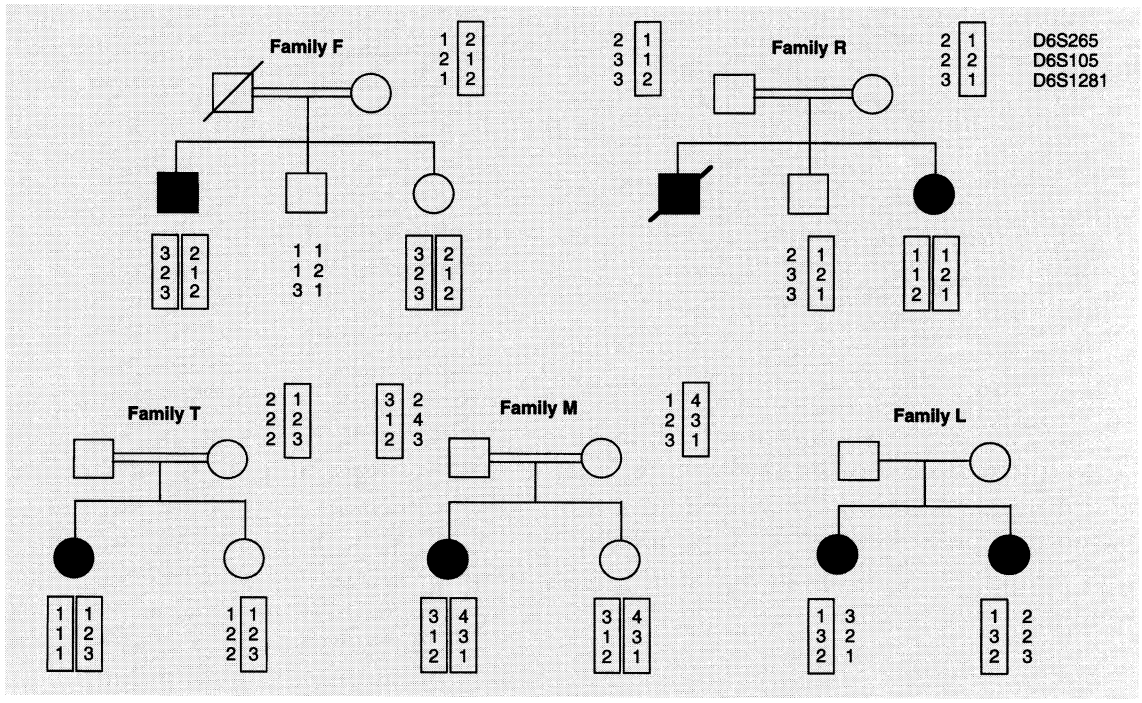

Fig. 1. Pedigrees of the families studied. Chromosomes 6 haplotypes are shown in the pedigrees. Alleles for the markers analyzed are coded, 1 indicating the shorter size allele.

Table 1. Clinical data and iron parameters of the patients studied

Case No.	Age years	Sex	TS %	SF µg/l	Hb g/dl	Liver biopsy		Cardio-pathy	Carbo-hydrate tolerance	Hypo-gonadism	Skin pigment-ation	HLA-A
						histo-logy	side-rosis[a]					
1	15	F	88	615	14.7	F	3	–	Impaired	–	+	ND
2	23[b]	M	100	4,585	15.8	C	4	+	Impaired	+	+	ND
3	20	F	98	3,768	10.7[c]	C	4	+	Impaired	+	+	A29/A30
4	20	F	82	1,400	12.9	C	4	–	N	+	+	A26/A30
5	30	M	77	3,800	13.6	F	4	+	Impaired	+	+	A2/A19
6	21	F	75	2,300	13.0	F	3	+	Impaired	+	+	A3/A2
7	26	F	98	2,000	13.1	F	4	–	N	+	+	A3/A28

TS = Transferrin saturation; SF = serum ferritin; F = fibrosis; C = cirrhosis; N = normal; ND = not determined.

[a] Grading for siderosis was 1–4.

[b] Hepatic iron overload already present at 9 years.

[c] β-Thalassemia trait.

cases. Heart failure and arrhythmias were documented in 4 patients and were fatal in case 2. Hypogonadism was observed in 6 cases and impaired glucose tolerance in 4. Liver function tests were abnormal, except in case 1, the youngest subject (data not shown). Liver biopsies showed iron overload involving most hepatocytes with coalescent hemosiderin granules in periportal areas. Liver fibrosis or cirrhosis was present in all patients. In 2 cases the hepatic iron index was in the range of HFE (4.1 and 14.2 in case 3 and 5, respectively). A previously unnoticed iron overload was observed in the liver biopsy of case 2 performed at 9 years of age.

Table 2. Two-point lod scores of JH with chromosome 6p markers

Markers	Lod scores at recombination fraction						
	0.0	0.01	0.05	0.1	0.2	0.3	0.4
D6S265	∞	−7.06	−3.18	−1.76	−0.67	−0.27	−0.09
D6S105	∞	−5.60	−2.40	−1.27	−.042	−0.13	−0.03
D6S1281	∞	−7.93	−3.90	−2.31	−0.98	−0.41	−0.14

Case 3 had interacting β-thalassemia heterozygosity, a condition that may produce mild iron overload in the advanced age. Other family members affected with the same condition had normal iron parameters. The patient's parents, obligate carriers of JH, have normal iron parameters (data not shown).

Molecular Studies

All the patients examined were negative for both C282Y and H63D substitutions in the HFE gene. HLA-A haplotypes were unusual for HFE, except in cases 6 and 7, who shared an HLA A3-related haplotype. Results of the analysis of 6p markers are illustrated in the pedigrees in figure 1. D6S265 is close and D6S105 is about 3 Mb telomeric to HLA-A. D6S1281 is telomeric to the HFE gene [4]. JH patients in families with consanguineous parents were not homozygous for haplotypes of the markers studied. Two-point lod scores were negative at different recombination fractions (table 2). The two affected siblings (cases 6 and 7) shared a single chromosome 6p haplotype. Since their parents were not examined, they still could have a 6p-linked disorder, in the case of a homozygote-heterozygote mating. However, no causal mutations were found in the regions of the HFE gene explored in case 6. Taken together this information excludes the fact that JH is associated with HFE and with the genomic region defined by the 6p markers studied.

Discussion

The patients described had a severe iron overload with clinical symptoms appearing in the second to third decade of life, fulfilling the criteria for JH [10, 11]. β-Thalassemia heterozygosity was present in case 3. This interacting factor has a minor, if any, role in iron overload, as shown by the absence of iron-related symptoms in relatives affected with the same condition.

It has not yet been clarified whether JH is caused by a different genetic defect or whether it is a more severe form of the adult disease. Molecular investigations of the HFE gene in JH are not reported in the literature. To directly rule out HFE gene involvement we have excluded the presence of HFE known mutations in 6 JH patients. Then, we analyzed linkage to other markers mapped on both sides of the HFE gene, exploiting the consanguinity in four families and the presence of two affected subjects in the fifth. Homozygosity mapping is a convenient tool to localize genes in consanguineous families [16]. Affected offspring of consanguineous parents are expected to be homozygous not only at the disease locus, but also at close polymorphic markers. This provides the opportunity of validating or excluding a candidate gene, studying associated markers in informative families. The absence of homozygosity for 6p haplotypes in patients of families M, R, T and F excludes the fact that these loci and the close HFE gene are associated with the disease. This is in agreement with the finding of two haplotype-identical, phenotipically discordant siblings in family M and F and with the lack of identity of the marker alleles in cases 6 and 7, who shared a single haplotype 6. The absence of linkage was confirmed by negative two-point lod scores.

The identification of the HFE gene has not yet clarified the mechanism of iron absorption by the enterocyte. The prevalent mutation inactivates the interaction with β_2-microglobulin [17] and β_2-microglobulin knockout mice develop iron overload [18, 19]. The HFE protein is ubiquitously expressed. However, using immunohistochemical techniques, an intracellular perinuclear staining was observed in the small intestine crypts and a basolateral staining in stomach, colon and biliary tract epithelial cells [20]. Thus, the HFE gene is not expected to be directly involved in mucosal iron uptake. Tentatively the HFE gene might have a regulatory role. It is likely that other proteins contribute to the process of iron uptake [20].

Other genetic disorders causing iron overload are systemic siderosis due to ceruloplasmin deficiency [21, 22] described in Japan and Bantu hemosiderosis [23], the latter results from interaction of an HLA independent genetic component and environmental factors [23]. A similar disorder has been described in African Americans [24]. Since the juvenile expression is not reported in these conditions it is unlikely that JH is related to them. The existence of distinct genetic disorders able to cause iron overload strengthens the idea that different proteins are involved in iron absorption and metabolism. The families described here may be useful to isolate the gene responsible for JH through a 'genome wide search' approach,

exploiting the homozygosity mapping in the affected offspring of consanguineous partners [16].

Finally, most Italian patients with JH originated from central southern Italy (11 and present paper). The possibility that this type of iron overload explains the reduced frequency of HFE mutations found in central southern as compared to northern Italy [Piperno and Camaschella, unpubl. results] is under evaluation.

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