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Diagnosis of juvenile hemochromatosis in an 11-year-old child combining genetic analysis and non-invasive liver iron quantitation

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Abstract Juvenile or type2 hemochromatosis is a rare autosomal recessive disorder which leads to severe iron overload early in life. As in the classic adult form of the disease iron toxicity causes liver cirrhosis, cardiomyopathy, and endocrine complications, but the onset of the disease is anticipated in the second to third decades of life. Experience of this disease in children is limited. Molecular diagnosis is unfeasible because the type2 hemochromatosis gene is still unknown, although it is known that the disease locus maps to chromosome1q. Combining linkage analysis with markers encompassing chromosome1 locus and a non-invasive method for liver iron quantitation we diagnosed juvenile hemochromatosis in a presymptomatic stage in an 11-year-old Italian child. A regular phlebotomy protocol reduced iron overload preventing all the disease complications. *Conclusion:* Juvenile hemochromatosis patients have severe iron overload within the first years of life, strengthening the greater iron absorption that occurs in this as compared to other types of hemochromatosis. Early detection is essential, because treatment in presymptomatic stages prevents organ damage.

Keywords Juvenile hemochromatosis · Iron · HFE · SQUID · Linkage analysis

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Abbreviations *SQUID* Superconducting quantum interference device · *LIC* liver iron concentration

Introduction

Hereditary hemochromatosis is a common autosomal recessive disorder of iron metabolism characterized by increased iron absorption and progressive iron deposition in parenchymal cells, resulting in organ damage and failure. Four types of hemochromatosis have been identified caused by mutations in different genes. The classic form or type1 hemochromatosis (OMIM235200) is associated with mutations in the *HFE* gene on chromosome6p [13]. Clinical symptoms, including liver cirrhosis, diabetes, and cardiac and endocrine complications, usually occur in middle to advanced age males [1, 27]. The ''juvenile form'', now renamed type2 hemochromatosis (OMIM602390), affects both sexes and usually leads to clinical symptoms in the second to third decades of life [5, 8], but biochemical signs of iron loading may be present in infancy [10, 18]. The clinical course is severe with a prevalence of hypogonadism and heart dysfunction, the latter being the main cause of death. Type2 hemochromatosis is rare, unrelated to HFE [6], and the corresponding locus (*HFE2*) maps to 1q21 [29]. Recently two other forms of hemochromatosis have been characterized. Type3 hemochromatosis (OMIM604250), caused by mutations in transferrin receptor2 on chromosome7q [7], has a phenotype similar to the classic form, but with earlier appearance [14, 19, 30]. Finally, type4 hemochromatosis (OMIM606069), characterized by dominant inheritance and atypical features with early iron accumulation in reticuloendothelial cells, is due to mutations of *SLC11A3* gene on 2q32 encoding for ferroportin1 [11, 20, 21, 31]. The expression of the latter two disorders in the pediatric age is unknown.

Here we describe the clinical and the molecular studies in an 11-year-old Italian child, with strikingly

increased serum iron and serum ferritin. Combining genetic analysis and liver iron quantitation by a non-invasive technique (SQUID) the diagnosis of type2, juvenile hemochromatosis was established.

Subject and methods

The patient had a complete clinical and laboratory evaluation. Transferrin saturation, serum ferritin, and liver function tests were measured by standard methods. Liver iron concentration (LIC) was assessed by a non-invasive approach based on SQUID (superconducting quantum interference device) biomagnetometry (model 5700; Tristan Technologies, San Diego, Calif., USA) described in detail elsewhere [3]. This method is based on the specific ferritin iron susceptibility as expressed by the following relationship $\chi_{Ferritin} = 1600 \times 10^{-6}$ [SI units/($\mu gFe/g_{liver}$)].

DNA was prepared from peripheral blood buffy coats by standard phenol-chloroform extraction [32]. The C282Y and H63D mutations in *HFE* were studied on genomic DNA, using PCR-based tests and restriction enzyme digestion, as previously described [9]. Linkage analysis to chromosome1q was investigated by intrafamilial segregation of microsatellites (D1S442, D1S2344, D1S498, D1S1556, and GATA13C08) mapping within the critical region [29, 30]. New microsatellite markers mapping within the same region (D1S2222, D1S2175) were also studied. Haplotypes were constructed manually.

Case report

An 11-year-old boy, whose family originated from Central Italy, was investigated because of an occasional finding of increased serum iron levels. There was no family history of hereditary anemias or iron overload but the parents were consanguineous. Growth and development were normal. Physical examination revealed regular growth and development, normal skin color, and no liver or spleen enlargement. Blood tests showed increased levels of serum iron parameters. Hemolytic anemia and other causes of secondary iron overload, viral infections, and inflammatory chronic diseases were excluded. Liver function tests revealed increased aspartic and alanine aminotransferases (Table 1). Chronic hepatitis viruses B and C were negative. Liver and spleen echotomography was normal. Echocardiographic evaluation showed no cardiac dysfunction and normal ejection fraction (75%). Glucose tolerance test as well as insulin and peptide C levels were normal. Thyroid hormones and cortisol basal levels were within normal ranges. Liver biopsy did not provide enough material for histological studies. Liver iron concentration, obtained by SQUID, was 1,933μgFe/g liver wet weight (ww; normal values <400μgFe/g liver).

Analysis of C282Y and H63D mutations gave negative results. Linkage analysis using markers encompassing the type2 hemochromatosis locus showed that the proband had inherited different 1q haplotypes as compared to the unaffected siblings. In addition, as expected in a family with consanguineous parents, the proband was homozygous for several markers of the juvenile hemochromatosis locus (Fig. 1). These results are compatible with type2 hemochromatosis.

Weekly phlebotomy (5 ml/kg) was started. During the first 4 months of treatment, the total volume of blood removed was 4,000 ml, corresponding to approximately 2 g iron. After 16 phlebotomies serum ferritin decreased, ALT normalized, and SQUID analysis showed a lower liver iron (Table 1). After removal of 3.1 g iron, serum ferritin is 190μg/l. Phlebotomy treatment continues in order to reach iron depletion.

Discussion

Primary iron overload is unusual in children [12, 15]. More common is secondary iron overload that occurs as a result of chronic blood transfusions in thalassemia major, sickle cell, or other hemoglobinopathies [16] and refractory aplastic anemia [17]. One case of childhood

Table 1 Laboratory data of the proband and his relatives

		Proband (II-2)			Father (I-1)	Mother (I-2)	Sister (II-1)	Brother (II-3)
		At diagnosis	After iron removal					
			2.5g[a]	3.1g[a]				
Age (years)		11			39	35	13	6
Hemoglobin (g/dl)		13.1	12.2	12.0				
MCV (fl)		80.8	85	88				
Serum ferritin (μg/l)		963	246	190	205	5.5	24.4	25
Serum iron (μg/dl)		279	200	194	98	73	119	94
Serum transferrin (mg/dl)		201	250	246	318	352	258	282
Transferrin saturation (%)		100	57	55	22	15	33	24
LIC by SQUID[b]		1,933	1,392					
AST (IU/l)		61	32	28				
ALT (IU/l)		98	60	55				
γ-Glutamyltransferase (IU/l)		19						
Alkaline phosphatase (IU/l)		650						
Bilirubin (mg/dl)		0.64						
HFE mutations	C282Y	–/–			–/–	–/–	–/–	–/–
	H63D	–/–			–/–	–/–	–/–	–/–

[a]Grams of iron removed by phlebotomy
[b]Normalvalues <400μgFe/g liver wet weight

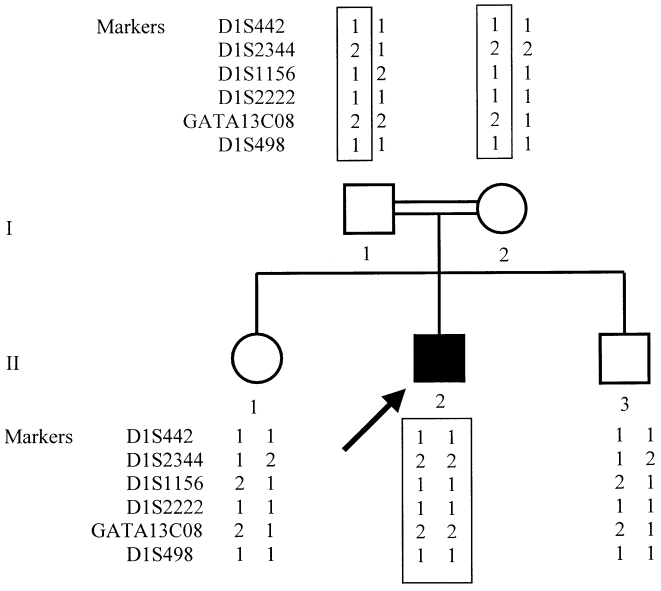

Fig. 1 Pedigree of the family studied. *Black box* indicates the proband. Order of markers is according to the available maps. Affected haplotypes are *boxed*. The homozygosity region extends through all the examined markers in the proband

hemosiderosis is reported caused by increased iron absorption following inappropriate oral iron treatment [24].

Diagnosis of hemochromatosis in children is difficult because of the absence of clinical complications. Since tests for HFE mutations are available, most children with a positive HFE genotype are diagnosed because of family screening, following identification of an affected relative. In this age group an increased transferrin saturation or even increased serum ferritin may be present, but to the best of our knowledge, HFE hemochromatosis has never been reported to be symptomatic in childhood. Even children with HFE mutations associated to beta-thalassemia trait, two conditions that cooperate to lead to early onset of iron overload [25], have been reported to be symptomless with only a slight serum ferritin increase.

Although rare, juvenile hemochromatosis shows clinical complications in the second decade of life, and thus it is expected to lead to earlier iron overload. Three children with severe iron overload in liver biopsies at the ages of 7, 6, and 4 years, respectively, have been described [18] and found to have 1q-associated juvenile hemochromatosis by linkage studies [10].

Early detection of juvenile hemochromatosis is essential, because treatment in presymptomatic stages prevents organ damage. The most common clinical manifestations, hypogonadism and cardiac failure, are not reversed by phlebotomy, making early diagnosis essential to avoid irreversible complications. The most precise test to diagnose all forms of HFE-unrelated hemochromatosis remains liver biopsy [26], which allows to demonstrate and quantitate tissue iron, to assess liver damage, and to show whether iron storage occurs in parenchymal and/or Kupffer cells.

In our patient the early symptoms of iron overload, such as weakness, dark skin pigmentation, or abdominal pain and the classic clinical manifestations, were absent. C282Y and H63D mutations were negative. However, in the absence of secondary iron overload, juvenile hemochromatosis was suspected in this case on the basis of strikingly elevated serum iron and serum ferritin levels. We measured LIC with a non-invasive superconducting (SQUID) susceptometer that values the magnetic susceptibility of liver nonheme iron. This technique has been validated in comparison to chemical measurements of iron obtained from liver biopsy specimens [4] and can be used as an alternative to liver biopsy for iron quantitation. The iron load is remarkably severe when data are corrected for age or body weight (total body iron stores = 68.2 mg/kg body weight) [2]. Since such a degree of iron overload may be associated with the early development of liver fibrosis, phlebotomy was immediately started, which resulted in a rapid decrease of both serum ferritin and LIC (Table 1). After 16 phlebotomies LIC decreased from 1,933 to 1,392μg Fe/g ww (corresponding to 4.6mgFe/g liver dry weight and 49.1mg/kg body weight). No adverse effects were observed and hemoglobin levels remained within normal range.

Definition of hemochromatosis type requires genetic studies. Linkage analysis may help to establish the correct diagnosis of type 2 and to exclude other hemochromatosis types. Linkage to 1q is present in almost all juvenile families so far described in Italy [10], Greece [22], and Canada [28]. A 1q unlinked disorder was found only in a single case of juvenile hemochromatosis from Greece [23].

Finally our study provides evidence that juvenile hemochromatosis patients have severe iron overload within the first years of life, documenting the greater iron absorption that occurs in this as compared to other types of hemochromatosis.

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