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Juvenile Hemochromatosis Locus Maps to Chromosome 1q

A. Roetto,[1,*] A. Totaro,[2,*] M. Cazzola,[3] M. Cicilano,[1] S. Bosio,[1] G. D'Ascola,[4] M. Carella,[2] L. Zelante,[2] A. L. Kelly,[5] T. M. Cox,[5] P. Gasparini,[2] and C. Camaschella[1]

[1]Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Torino; [2]IRCCS CSS San Giovanni Rotondo, Foggia, Italy; [3]Dipartimento di Medicina Interna e Oncologia, IRCCS Policlinico San Matteo, Università di Pavia, Pavia, Italy; [4]Centro Microcitemie, Ospedale di Reggio Calabria, Reggio Calabria, Italy; and [5]Department of Medicine, University of Cambridge, Cambridge

Summary

Juvenile hemochromatosis (JH) is an autosomal recessive disorder that leads to severe iron loading in the 2d to 3d decade of life. Affected members in families with JH do not show linkage to chromosome 6p and do not have mutations in the *HFE* gene that lead to the common hereditary hemochromatosis. In this study we performed a genomewide search to map the JH locus in nine families: six consanguineous and three with multiple affected patients. This strategy allowed us to identify the JH locus on the long arm of chromosome 1. A maximum LOD score of 5.75 at a recombination fraction of 0 was detected with marker D1S498, and a LOD score of 5.16 at a recombination fraction of 0 was detected for marker D1S2344. Homozygosity mapping in consanguineous families defined the limits of the candidate region in an ~4-cM interval between markers D1S442 and D1S2347. Analysis of genes mapped in this interval excluded obvious candidates. The JH locus does not correspond to the chromosomal localization of any known gene involved in iron metabolism. These findings provide a means to recognize, at an early age, patients in affected families. They also provide a starting point for the identification of the affected gene by positional cloning.

Introduction

Juvenile hemochromatosis, or hemochromatosis type 2 (JH; MIM 602390), is an inborn error of iron metabolism that leads to severe iron loading and organ failure at age <30 years (Camaschella 1998). JH is a rare disorder, characterized by an autosomal recessive inheritance. The patterns of iron distribution and tissue injury are similar to those in hemochromatosis type 1, or *HFE*-related hereditary hemochromatosis (HH), a common genetic disorder with clinical expression in middle life (Powell et al. 1994; Camaschella and Piperno 1997). In contrast with HH, which predominantly affects males, in JH both sexes are equally affected. The clinical complications of iron overload—liver cirrhosis, cardiac disease, endocrine failure, diabetes, arthropathy, and skin pigmentation—are similar to those of HH, but hypogonadism and cardiopathy are the most common symptoms at presentation. Heart failure and/or major arrhythmias are usually the cause of death in the absence of treatment. Early detection of the disorder is important, because iron depletion by phlebotomy can prevent organ damage and all the disease manifestations (Camaschella 1998). Recent studies have shown that patients with JH (1) do not have mutations in the *HFE* gene that lead to HH (Feder et al. 1996) and (2) do not show linkage to 6p (Camaschella et al. 1997). The biochemical defect of the disorder is unknown; however, iron absorption is greater in JH than in HH (Cazzola et al. 1983), and the estimated rate of iron accumulation, based on phlebotomy requirements for maintenance of normal iron balance, is significantly higher in patients with JH than in patients with HH (Cazzola et al. 1998).

The study of JH may contribute to our understanding of the molecular mechanisms regulating iron absorption. Evidence from in vitro studies (Feder et al. 1998) and studies of placental cells (Pakkila et al. 1997) has shown that the HFE protein may interact with the transferrin receptor, reducing its affinity for transferrin (Lebron et al. 1998). However, whether and how this interaction affects the regulatory cellular iron pool remains uncertain, and the molecular mechanisms that underlie the increased iron absorption in HH, following the lack of the mutant HFE protein on the cell surface, remain unclear. The severity and the early expression of JH suggest a derangement of a gene product that is crucial in the regulation of iron uptake.

The disease, although quite rare, has been reported in different ethnic groups (Lamon et al. 1978; Cazzola et

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Address for correspondence and reprints: Dr. Clara Camaschella, Dipartimento di Scienze Cliniche e Biologiche, Università di Torino, Azienda Ospedaliera San Luigi, 10043-Orbassano, Torino, Italy. E-mail: Camaschella@ope.net

* These authors contributed equally to this work.

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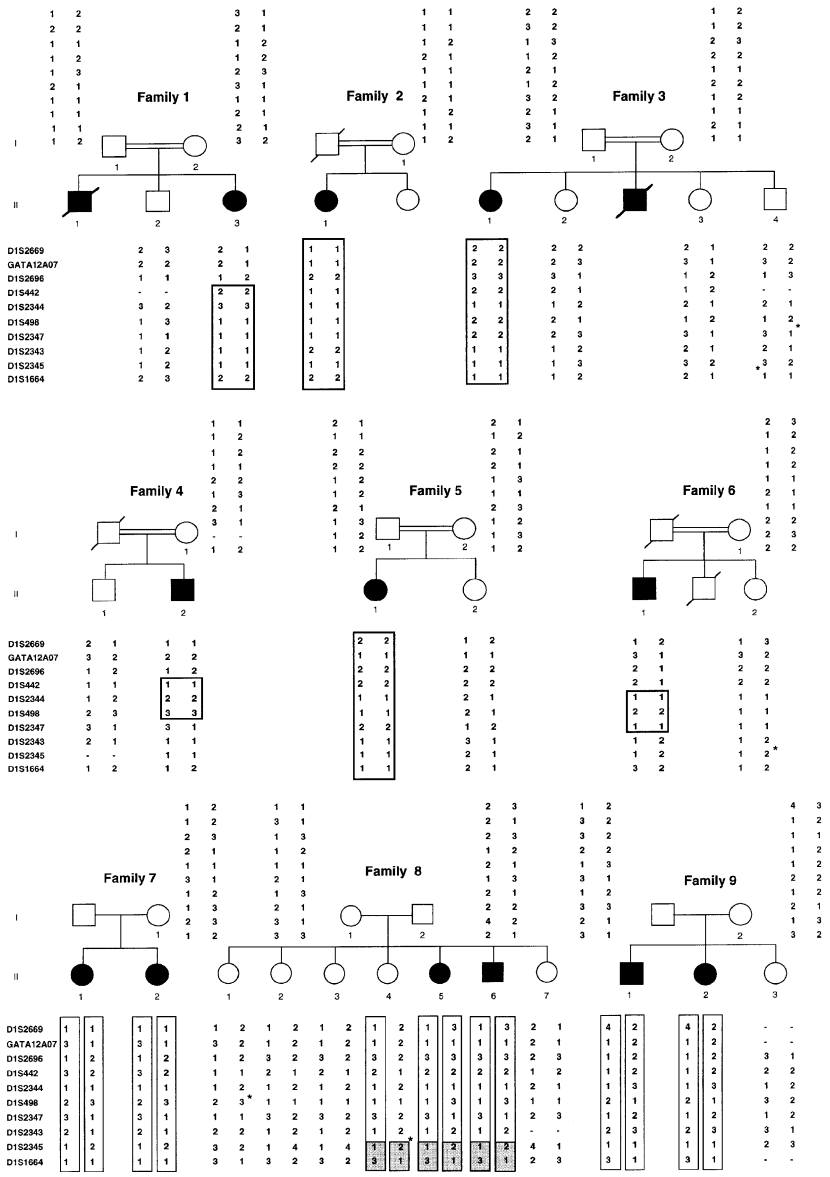

Figure 1 Haplotype analysis of chromosome 1q markers of the JH kindreds. Marker order is according to the available maps. Homozygosity regions are boxed with a thick line in families 1–6. Haplotypes associated with the disease are boxed with a thin line in families 7–9. Recombinations are indicated by an asterisk (*). The shaded areas correspond to segments of haplotypes shared by normal and affected subjects in family 8. Alleles are coded, with "1" indicating the shorter-size allele.

al. 1983; Haddy et al. 1988; Kelly et al. 1998). A few cases with a high level of consanguinity have been described in Italy (Camaschella et al. 1997; Cazzola et al. 1998) that confirm the rarity of the disorder (Camaschella et al. 1997).

We conducted a full human-genome search in nine families with JH; three families were multiplex and six were consanguineous. By using homozygosity mapping (Lander and Boistein 1987) and linkage analysis, we found genetic linkage between the disease and several markers in an ~4-cM region of chromosome 1q.

Subjects and Methods

JH Families

Nine families with JH were included in the study. The pedigrees are shown in figure 1. Eight kindreds (families 1–8) were Italian. Families 1, 2, and 5–7 (Camaschella et al. 1997), family 3 (Cazzola et al. 1998), and family 8 (Cazzola et al. 1983) have been reported elsewhere. Family 9 is of British origin and corresponds to pedigree C, reported by Kelly et al. (1998). The parents in families

1–6 were first cousins, and three families (7–9) were multiplex.

Criteria for the diagnosis of JH were based on the age at presentation of the disorder and the clinical evidence of severe iron overload in the absence of mutations in the *HFE* gene. In all patients, clinical symptoms of hypogonadism were present at age <30 years. Cardiac disease, as defined by heart failure and/or left-ventricular dilatation with low ejection fraction at echocardiography or arrythmias requiring medical treatment (Piperno et al. 1996), was present in at least one patient in each family except family 9. The degree of iron overload was determined on the basis of liver biopsy, liver iron concentration, and/or quantitation of iron removed by phlebotomies, as described elsewhere (Piperno et al. 1996). Detailed clinical descriptions of the cases are available (Cazzola et al. 1983, 1998; Camaschella et al. 1997; Kelly et al. 1998). Patient II-2 of family 4 was not reported elsewhere but presents clinical features that fit the JH general criteria described above. The obligate-heterozygous parents examined did not display any clinical symptoms.

Molecular Studies

EDTA blood samples were collected for genetic studies after informed consent was obtained in accordance with the guidelines of the local institutions. DNA was obtained from lymphoblastoid cell lines in patients belonging to families 1, 5, and 6 and from peripheral blood buffy coats in the other patients and healthy relatives. DNA was prepared by standard phenol-chloroform extraction (Sambrook et al. 1989). In total, we had access to DNA samples from 39 subjects (12 patients and 27 healthy relatives).

For the genomewide search (GWS), the ABI PRISM Linkage Mapping Set (Perkin-Elmer) was used. This set is characterized by >375 markers that define a 10-cM–resolution human index map (Gyapay et al. 1994). PCR with fluorescently labeled primers was done under the conditions suggested by the supplier. An aliquot of the PCR mixture was reacted in an ABI PRISM 373 or 377 DNA sequencer, and the results were processed by GENESCAN software. Allele assignment was done with the GENOTYPER™ (Perkin-Elmer) software. To saturate the potentially homozygous regions, especially the chromosome 1 region, additional pairs of fluorescently labeled primers were specifically synthesized for markers proximal and distal to panel markers (Dib et al. 1996). Primer sequences and PCR conditions were obtained from the Whitehead Institute Database. All living individuals of each family were genotyped and thus contributed to linkage calculation. Extended haplotypes were constructed by analysis of the segregation of marker alleles within the families.

Linkage Analysis

Statistical analysis was performed on the basis of an assumption of autosomal recessive disease with complete penetrance. The disease-gene frequency was set to .0001, and all marker alleles were considered to be equally frequent. Two-point linkage analysis was performed by means of the MLINK program, version 5.1, from the LINKAGE computer package (Ott 1992). Loops of consanguinity were accommodated, as suggested by Ott (1992). Values for maximum LOD score (Z_{max}) were calculated with the ILINK program from the same computer package. The ~95% confidence limits for the maximum recombination fraction (θ_{max}) at Z_{max} were calculated by the 1-LOD-down method (Ott 1992). Alleles were coded within each family, without loss of informativeness, to reduce computing time. The HOMOG program was used to test for nonallelic heterogeneity by means of pairwise LOD scores between D1S498 and the disease locus.

Results

Mapping the JH Locus

The GWS was performed on the DNA of the originally described six patients from five unrelated families (Camaschella et al. 1997). After analyzing data from all markers included in the panel, we found evidence for linkage, by homozygosity mapping of the following chromosomal regions: 18q (D18S64), 1q(D1S498), 7p (D7S493), 12q (D12S324), and 11p (D11S935). Several additional markers from each region were then typed in all individuals of the five families used for the GWS and in an additional four families. This analysis allowed us to exclude all the candidate areas except the pericentromeric region of chromosome 1.

According to available maps (Whitehead Institute Database and Genetic Location Database), the order of the additional markers in this region is D1S2669-GATA12A07-D1S2696-cen-D1S442-D1S2344-D1S498-D1S2347-D1S2343-D1S2345-D1S1664-tel. The markers map to the same Whitehead Institute single-linked YAC contig (WC1.16). The marker position was verified by PCR assays on a YAC contig of the region enriched for all the markers available (data not shown). The relative positions of markers D1S2345 and D1S2343 could not be resolved.

Pairwise LOD scores for the markers studied are shown in table 1. The highest LOD score was obtained with D1S498 ($Z_{max} = 5.75$ at $\theta = .00$), but no recombinants were detected with D1S2344 ($Z_{max} = 5.16$ at $\theta = .00$) and D1S442 ($Z = .30$ at $\theta = .00$). Positive results were also obtained with the remaining markers, at $\theta > 0$. The HOMOG program identified no evidence in support of genetic heterogeneity ($\chi^2 = 26.49$; $\alpha = 1$).

Table 1

Pairwise LOD Scores between JH and Chromosome 1 Markers

	Z AT θ =							
MARKERS	.00	.01	.05	.10	.20	.30	.40	Z_{max} (θ_{max})
D1S2669	−4.11	1.33	2.16	2.08	1.43	.75	.24	2.18 (.060)
GATA12A07	−3.26	1.88	2.63	2.44	1.61	.80	.24	2.63 (.054)
D1S2696	−8.64	−.15	1.36	1.56	1.16	.59	.16	1.57 (.094)
D1S442	.30	2.90	3.03	2.63	1.66	.81	.24	3.09 (.030)
D1S2344	5.16	5.01	4.41	3.67	2.32	1.22	.42	5.16 (.000)
D1S498	5.75	5.56	4.84	3.97	2.39	1.16	.37	5.75 (.000)
D1S2347	−.26	2.53	2.72	2.38	1.49	.73	.23	2.76 (.034)
D1S2343	−.40	2.19	2.41	2.13	1.39	.74	.27	2.43 (.036)
D1S2345	∞	1.87	2.67	2.53	1.72	.91	.31	2.68 (.058)
D1S1664	∞	−.72	.84	1.11	.86	.46	.15	1.11 (.106)

Haplotype Analysis and Recombination Events

Results of the analysis of chromosome 1 markers are shown in figure 1. The results of haplotype analysis support the marker order shown in the figure. Affected siblings in multiplex families (families 7–9) have inherited the same haplotype combination, whereas healthy subjects do not share the same haplotypes with their affected siblings. A recombination event was detected in case II-4 of family 8, occurring between D1S2347 and D1S2345 (fig. 1), which helps in definition of the telomeric limit of the JH critical region.

As expected, regions of homozygosity of variable extent are observed in all affected individuals in kindreds with first-cousin matings (families 1–6). Consistent homozygosity regions in all patients include markers D1S2344 and D1S498. A heterozygous genotype for D1S442 is found in patient II-1 of family 6, thus defining the centromeric limit. In addition, case II-2 in family 4 is heterozygous at D1S2347 marker, which defines the telomeric limit. The region spanning D1S442–D1S2347 is ~4 cM on chromosome 1q.

Candidate Gene Analysis

The *CD1D* gene was evaluated as a potential candidate gene in the defined interval. To assess its candidacy, the polymorphic tetranucleotide CHLC.GATA3B11 was analyzed. This marker maps to the same well-characterized YAC 748F9 as does *CD1D*. The results obtained show that three of six patients expected to be homozygous (II-1 in family 2, II-2 in family 4, and II-1 in family 6) are heterozygous at this locus (data not shown). In addition, both CHLC.GATA3B11 and D1S3312, a sequence-tagged site (STS) corresponding to the *CD1D* fifth exon, do not map to the YAC contig covering the defined critical region (data not shown).

Discussion

This study provides evidence that the JH locus maps to chromosome 1q, in an ~4-cM interval defined by markers D1S442 and D1S2347. Given the small number of families evaluated, this study emphasizes the power that homozygosity mapping, combined with the availability of a dense microsatellite map, has in the localization of genes that cause rare recessive diseases.

In the defined interval on chromosome 1q, several known genes, as well as multiple unidentified transcripts, are mapped. Known genes include genes with immunological functions, such as T-complex γ-subunit, a high-affinity γ-Fc receptor, and CD2. The CD1 cluster, which consists of five atypical HLA class I genes (*CD1A, CD1B, CD1C, CD1D,* and *CD1E*), maps in the proximity of the critical region, to the same single-linked YAC contig WC1.16. This is an interesting fact, considering that the *HFE* gene responsible for HH is an atypical HLA class I gene (Feder et al. 1996). *CD1A, CD1B,* and *CD1* are involved in glycolipid-antigen presentation (Porcelli et al. 1998). *CD1D* is highly conserved in evolution, has an unusual structure with a narrow binding grove, similar to other class I genes such as FcnR and *HFE* (Wilson and Bjorkman 1998), and is highly expressed in intestinal epithelial cells (Kasai et al. 1997). The *CD1D* structural features, its pattern of expression, and its map position prompted us to evaluate this gene as a potential candidate for JH. However, the candidacy of *CD1D* was excluded on the basis of both homozygosity and physical mapping. Preliminary results show that patients expected to be homozygous are instead heterozygous at a polymorphic marker closely associated with this locus. In addition, both the *CD1D*-associated marker and the STS corresponding to the *CD1D* fifth exon map outside the minimal critical region. No other genes in the defined interval are obvious candidates for JH.

Our future effort will be to enrich the identified in-

terval of polymorphic markers. No common haplotype has been observed among patients with the studied markers, likely because of the large extension of the region. If a shared haplotype is found inside the 4-cM region, this will be used to restrict the candidate area.

The definition of the JH locus is a starting point for the identification of the JH gene by positional cloning. The immediate benefit of our results is to provide markers that allow the early detection of the disease in affected families. The need for family counseling for hemochromatosis is usually based on *HFE* mutations or HLA-A–haplotype segregation, which is of no value in JH. Localization of the JH locus to chromosome 1q allows analysis for linkage in other patients with atypical forms of hemochromatosis without mutations in the *HFE* gene (Carella et al. 1997) and/or without classical juvenile expression (Camaschella et al., in press). From a biological point of view, the results obtained in this study indicate that the JH gene does not correspond to any of the known genes involved in iron metabolism whose chromosomal localization is defined. For this reason, we expect that cloning the JH gene will identify a new iron protein with a crucial role in iron metabolism.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for JH [MIM 602390])

Genetic Location Database, http://cedar.genetics.soton.ac.uk/ (for order of markers)

Whitehead Institute Database, http://www-genome.wi.mit.edu (for primer sequences and PCR conditions and for order of markers)

References

Camaschella C (1998) Juvenile haemochromatosis. Baillieres Clin Gastroenterol 12:227–235

Camaschella C, Fargion S, Sampietro M, Roetto A, Bosio S, Garozzo G, Arosio C, et al. Inherited HFE-unrelated hemochromatosis in Italian families. Hepatology (in press)

Camaschella C, Piperno A (1997) Hereditary hemochromatosis: recent advances in molecular genetics and clinical management. Haematologica 82:77–84

Camaschella C, Roetto A, Cicilano M, Pasquero P, Bosio S, Gubetta L, Di Vito F, et al (1997) Juvenile and adult hemochromatosis are distinct genetic disorders. Eur J Hum Genet 5:371–375

Carella M, D'Ambrosio L, Totaro A, Grifa A, Valentino MA, Piperno A, Girelli D, et al (1997) Mutation analysis of the HLA-H gene in Italian hemochromatosis patients. Am J Hum Genet 60:828–832

Cazzola M, Ascari E, Barosi G, Claudiani G, Dacco M, Kaltwasser JP, Panaiotopoulos N, et al (1983) Juvenile idiopathic haemochromatosis: a life-threatening disorder presenting as hypogonadotropic hypogonadism. Hum Genet 65:149–154

Cazzola M, Cerani P, Rovati A, Iannone A, Claudiani G, Bergamaschi G (1998) Juvenile genetic hemochromatosis is clinically and genetically distinct from the classical HLA-related disorder. Blood 92:2979–2981

Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154

Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, et al (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 13:399–408

Feder JN, Penny DM, Irrinki A, Lee VK, Lebron JA, Watson N, Tsuchihashi Z, et al (1998) The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. Proc Natl Acad Sci USA 95: 1472–1477

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, et al (1994) The 1993–1994 Genethon human genetic linkage map. Nat Genet 7:246–339

Haddy TB, Castro OL, Rana SR (1988) Hereditary hemochromatosis in children, adolescents, and young adults. Am J Pediatr Hematol Oncol 10:23–34

Kasai K, Matsuura A, Kikuchi K, Hashimoto Y, Ichimiya S (1997) Localization of rat CD1 transcripts and protein in rat tissues—an analysis of rat CD1 expression by in situ hybridization and immunohistochemistry. Clin Exp Immunol 109:317–322

Kelly AL, Rhodes DA, Roland JM, Schofield P, Cox TM (1998) Hereditary juvenile haemochromatosis: a genetically heterogeneous life-threatening iron storage disease. QJM 91: 607–618

Lamon JM, Marynick SP, Rosenblatt R, Donnelly S (1979) Idiopathic hemochromatosis in a young female: a case study and review of the syndrome in young people. Gastroenterology 76:178–183

Lander ES, Boistein D (1987) Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. Science 236:1567–1570

Lebron JA, Bennett MJ, Vaughn DE, Chirino AJ, Snow PM, Mintier GA, Feder JN, et al (1998) Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. Cell 93:111–123

Ott J (1992) Human genetic linkage, 2d ed. Johns Hopkins University Press, Baltimore, pp 13–32

Parkkila S, Waheed A, Britton RS, Bacon BR, Zhou XY, Tomatsu S, Fleming RE, et al (1997) Association of the trans-

ferrin receptor in human placenta with HFE, the protein defective in hereditary hemochromatosis. Proc Natl Acad Sci USA 94:13198–13202

Piperno A, Arosio C, Fargion S, Roetto A, Nicoli C, Girelli D, Sbaiz L, et al (1996) The ancestral hemochromatosis haplotype is associated with a severe phenotype expression in Italian patients. Hepatology 24:43–46

Porcelli SA, Segelke BW, Sugita M, Wilson IA, Brenner MB (1998) The CD1 family of lipid antigen-presenting molecules. Immunol Today 19:362–368

Powell LP, Jazwinska E, Halliday JW (1994) Primary iron overload. In: Brock H, Halliday JW, Pippard MJ, Powell LP (eds) Iron metabolism in health and disease. WB Saunders, London, pp 227–270

Sambrook J, Frisch E, Maniatis T (1989) Molecular cloning: a laboratory manual. Vol 1–3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Wilson IA, Bjorkman PJ (1998) Unusual MHC-like molecules: CD1, Fc receptor, the hemochromatosis gene product, and viral homologs. Curr Opin Immunol 10:67–73