46,XX, inv(6)(p21.1p23) in a pedigree with hereditary haemochromatosis

Charles P Venditti, Nicole K Seese, Glenn S Gerhard, Amy E Ten Elshof, Karen A Chorney, Philip N Mowrey, Paul G Lacey, Joan H M Knoll, Michael J Chorney

Abstract

Hereditary haemochromatosis (HFE) is a recessive genetic disease of iron overload which has been shown by linkage analysis to reside on the short arm of chromosome 6, close to the major histocompatibility complex (MHC). Positional cloning of the putative HFE locus has been hampered, in part, by the lack of a structural alteration on 6p. In this report, we describe a pedigree with HFE which carries a balanced paracentric inversion of chromosome 6, inv(6)(p21.1p23), a rarely reported chromosomal rearrangement in this region. We have determined the inheritance of the chromosome harbouring the inversion, which segregates as an HFE chromosome. Because the HFE locus has been mapped distal to the HLA-F class I locus at 6p21.3, the breakpoints associated with this chromosomal rearrangement may provide a significant genomic landmark for positional cloning of the HFE gene.

(*J Med Genet* 1997;34:24-27)

Keywords: hereditary haemochromatosis; human chromosome 6 inversion; major histocompatibility complex.

Idiopathic or hereditary haemochromatosis (HFE) (McKusick No 235200) is an autosomal recessive disease of progressive iron accumulation which can result in cirrhosis, carbohydrate intolerance, cutaneous hyperpigmentation, cardiomyopathy, arthritis, and hypogonadotrophic hypogonadism.¹ In certain white populations the carrier frequency may approximate 5%.² Because the expression of the HFE defect is partially dependent on dietary factors and gender, the definitive diagnosis of HFE can be clinically challenging in predisposed people. The identification of the mutation(s) causing HFE may allow for the assignment of more certain clinical diagnoses.

The genetic defect in HFE has been linked to the human leucocyte antigen A locus (HLA-A), which resides at the telomeric end of the major histocompatibility complex (MHC) class I region.^{3,4} The precise location of the HFE gene is not known at present, but has been the subject of several recent genetic mapping experiments. The results of a series of linkage⁵ and linkage disequilibrium studies⁶⁻¹⁰ indicate that the HFE gene is associated with polymorphisms defined by the microsatellite D6S105, but, based on defined recombinants,

may reside anywhere between the HLA-F gene¹¹ and the telomeric microsatellite D6S299,⁷ which is approximately 4 cM from the HLA-A locus.¹² Our own data,⁸ based on the study of an American patient collection, support the localisation of the HFE locus between markers D6S464 and D6S1558, but most likely close to D6S1260; this is in general agreement with data obtained by Raha-Chowdhury *et al.*⁷ Aside from an HLA-A locus subregional deletion,¹³ no other structural perturbation of chromosome 6 has been reported in a patient with HFE.

In the present report, we describe a pedigree with HFE which carries an inv(6)(p21.1p23) chromosome. We show by cytogenetic and molecular genetic analysis the inheritance of this chromosome in this family and discuss the relevance of the chromosomal abnormality to the mapping of the HFE disease gene.

Materials and methods

In order to establish cell lines, 20 cc of peripheral blood was obtained from volunteer patients (consent forms, approved by the Institutional Review Board of the Pennsylvania State University College of Medicine (IRB Protocol No 93-140 EP) were reviewed and signed by all participants). Lymphocytes were purified from these samples using Ficoll-Paque Plus (Pharmacia, Uppsala, Sweden) according to the manufacturer's protocol and immortalised with Epstein-Barr virus (EBV). Each line used in this study was then serologically typed for HLA-A and HLA-B antigens in the Milton S Hershey Medical Center's Histocompatibility Laboratory.

Standard PHA stimulated lymphocyte chromosome preparations were made from peripheral blood lymphocytes from the proband and her brother. Karyotypes of the proband's children were performed as part of a conventional amniocentesis protocol. Analysis of prometaphase chromosomes from the proband's established B lymphocyte lines using GTG banding (Giemsa-trypsin) was performed as previously described.¹⁴ DNA was isolated from the proband's cell line by conventional methods.

DNA was also obtained from paraffin embedded tissue sections from archival (1992) necropsy material from the proband's mother and from similar archival (1979) material from a prostate biopsy from the proband's father. Ten µm sections were deparaffinised in xylene followed by proteinase K digestion at 55°C.

Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, The Milton S Hershey Medical Center, Hershey, PA 17033, USA

C P Venditti
N K Seese
A E Ten Elshof
K A Chorney
P G Lacey
M J Chorney

Department of Pathology, The Pennsylvania State University College of Medicine, The Milton S Hershey Medical Center, Hershey, PA 17033, USA
G S Gerhard

Department of Pediatrics, The Pennsylvania State University College of Medicine, The Milton S Hershey Medical Center, Hershey, PA 17033, USA
M J Chorney

Department of Cytogenetics, LabCorp, Nashville, TN 37217, USA
P N Mowrey

Division of Genetics, Children's Hospital and Department of Pathology, Beth Israel Hospital, Harvard Medical School, Boston, MA 02115, USA
J H M Knoll

Correspondence to: Dr M J Chorney.

Received 28 May 1996
Revised version accepted for publication
1 August 1996

Table 1 Response of proband (patient 1.2) to phlebotomy treatment. Following repeated abnormal serum iron indices, 1900 ml of blood were withdrawn over an almost two year period resulting in normalisation of iron parameters

Date	Ferritin (10–100 ng/ml)	Iron (50–125 µg/ml)	TIBC (200–352 µg/ml)	Saturation (20–50%)	Phlebotomy
July 1990	107	199	210	94.8	
January 1991	108	217	219	99.1	
September 1991	114	222	214	100	< – 1000 ml
September 1992	57	163	237	69	< – 900 ml
July 1993	7	83	271	31	

DNA was then purified by standard phenol-chloroform extraction.

Polymerase chain reaction (PCR) analysis of DNA was performed using primers to amplify 12 polymorphic microsatellite markers spanning the MHC to D6S299 interval as described previously.⁸

Case report

The proband (patient 1.2) is a 43 year old, G2 P2002, premenopausal female with a history of infertility and polycystic ovarian disease. Her menstrual history was completely regular (28 day cycle, five day period) until the birth of her children, when the cycle length shortened. Following surgical removal of one ovary and fallopian tube and wedge resection of the other ovary, patient 1.2 became pregnant in 1985 by artificial insemination and a routine amniocentesis performed during this pregnancy showed a fetal 46,XX, inv(6)(p21.1p23) karyotype. Subsequent chromosomal analysis showed that the mother (proband) carried the same inverted chromosome as the fetus. The proband’s second pregnancy was without complications; the healthy infant (born in 1987) also carried the inversion chromosome.

Apart from polycystic ovarian disease, the proband had no other documented or suspected medical problems until 1990. At that time, a routine laboratory panel indicated several abnormal iron indices; these included a raised total iron of 199 µg/dl (range 50–125 µg/dl), total iron binding capacity of 210 µg/dl (range 200–352 µg/dl), a transferrin saturation of 94.8% (range 20–55%), and a ferritin level of 107 ng/ml (range 10–300 ng/ml) in an otherwise asymptomatic, healthy female. Over the following year, these tests were repeated every two months and on all occasions total iron and transferrin saturation remained abnormally high. During this period, the proband’s 82 year old mother (patient 1.1) died and necropsy showed cardiomegaly with underlying cardiac fibrosis and hypertrophy, haemochromatosis with diffuse 4+ hepatocellular iron deposition (evinced through Prussian Blue staining based upon a severity score range of 1–4+), and bronchopneumonia. The cause of death was attributed to dysrhythmia. After considering the maternal necropsy results and available data which suggest that raised transferrin saturation is a sensitive and specific indicator of homozygous haemochromatosis,^{15–17} the diagnosis of idiopathic haemochromatosis in patient 1.2 was assigned. The HFE disease status of the proband’s dead father is not known.

The proband’s 57 year old brother (patient 1.3) was examined and noted to have an in-

creased total serum iron (195 µg/dl), an increased transferrin saturation (77.4%), raised levels of ferritin (484 ng/ml) and transferrin (248 mg/dl), and a total iron binding capacity of 252 µg/dl. Karyotype analysis showed that he also carried the same paracentric inversion as patient 1.2. The proband’s two children, carriers of the inversion chromosome, were also tested and found to be normal with respect to serum iron indices (serum irons of 86 and 85 µg/dl, total iron binding capacities of 294 and 309 µg/dl, transferrin saturations of 29.3 and 27.5%, and ferritin levels of 18 and 9 ng/ml).

Both the proband and her brother are currently being treated by phlebotomy with appropriate restoration of serum iron indices towards normal values (table 1). Liver biopsies have been offered and refused by both patients.

HLA, cytogenetic, and molecular genetic results

Fourteen metaphase cells from the proband’s immortalised lymphocytes were re-examined. Representative chromosome 6 pairs are shown in fig 1 and the paracentric inversion of chro-

Figure 1 Representative G banded chromosomes 6 from the proband. Pairs of G banded chromosomes are shown on the left; the chromosome carrying the paracentric inversion (inv(6)(p21.1p23)) is marked by an asterisk. An ideogram of chromosome 6 is shown. The breakpoints are indicated by arrows.

Patient	HLA type	Chromosome	Age	Fe Sat
1.1	NA	NA	82	100%
1.2	A3,B18 A24,B51	46,XX,inv(6)(p21.1p23)	42	95%
1.3	A3,B7 A24,B51	46,XY,inv(6)(p21.1p23)	57	77%
1.4	A24,B48 A2,B38	46,XY	39	NA
1.5	A24,B48 A24,B51	46,XX,inv(6)(p21.1p23)	7	27%
1.6	A24,B48 A24,B51	46,XY,inv(6)(p21.1p23)	6	29%

Figure 2 Pedigree of the family with *inv*(6). The proband is indicated with an arrow. Shadowing refers to the haemochromatotic state (questionable in the proband's father, although he must be considered to be at least an obligate carrier), with half shading illustrating carriers in the pedigree. *Inv* indicates that the subject harbours the *inv*(6) chromosome. Iron saturation in the table on the right indicates the percentage of transferrin bound with iron; the normal range of this value is 20–55%. NA = not available for analysis.

Table 2 Results of PCR analysis of polymorphic microsatellite loci in DNA from the proband and her parents. VNDR allele assignments and PCR conditions have been previously described.⁸ The configuration of alleles the proband inherited from her mother are consistent with an HFE associated HLA-A3 carrying chromosome. The inversion chromosome is thus inferred to have been transmitted by the father

Locus	Marker name	Type	Allele sizes (bp)	Mother	Proband	Father
D6S265	AFM101XA1	(CA) _n	122–144	1,1	1,3	3,3
D6S306	AFM248XH1	(CA) _n	236–250	4,4	4,4	4,5
D6S105	MFD61	(CA) _n	116–138	5,5	5,10	10,5
D6S464	AFM323VB5	(CA) _n	202–230	3,3	3,3	3,8
D6S1260	CS-5	(CA) _n	144–166	4,4	4,4	4,3
D6S1558	AFMA192WG9	(CA) _n	251–271	4,5	5,5	5,5
D6S1016	GGAA10G12	Tetra	236–268	1,1	1,6	6,5
D6S1281	GATA89B07	Tetra	176–208	7,5	5,5	5,4
D6S1545	AFMA11ZE1	(CA) _n	225–233	2,2	2,2	2,4
D6S1554	AFMA183WB5	(CA) _n	162–176	2,1	1,1	1,2
D6S276	158.3/158.5	(CA) _n	198–228	8,9	9,9	9,8
D6S299	AFM217XG7	(CA) _n	210–234	10,10	10,7	7,1

mosome 6 (*inv*(6)(p21.1p23)) was confirmed. A pedigree is presented in fig 2 along with HLA typing, karyotype, and transferrin saturation results. By combining the results of HLA haplotype and karyotype studies (fig 2), we determined that the inversion chromosome possesses the HLA-A24 and HLA-B51 MHC class I alleles.

The results of PCR microsatellite analysis of the proband and her parents are shown in table 2. Marker D6S265, located 70 kb centromeric to HLA-A18, is tightly linked to various HLA-A alleles.⁹ The occurrence of allele 1 in marker D6S265, in addition to other markers through D6S1260 (700 kb telomeric to D6S105), indicates that the proband inherited a “common” HFE HLA-A3 associated chromosome from her mother. The atypical HLA-A24 associated HFE inversion chromosome appears to be of paternal origin (fig 3).

Discussion

Mapping and positional cloning efforts to identify the HFE gene have recently accelerated. Several linkage disequilibrium studies and recombinant subjects have narrowed the HFE critical region to the genomic expanse between the HLA-F locus (centromeric) and the microsatellite marker D6S299 (telomeric). Results using geographically and ethnically different control and patient populations point towards different areas within this region.^{5–10} Whether the breakpoints of the inversion chromosome reported here fall within this region is of prime interest.

The paucity of reported inversions associated with the short arm of chromosome 6 suggests an enhanced stability of such rearrangements within this region.²⁰ The location of the MHC and the lack of meiotic recombination associated with certain HLA haplotypes supports this observation.⁸ Recombination in paracentric inversion heterozygotes is also reduced because acentric and deletion products resulting from crossing over within the inversion loop of meiotically paired homologous chromosomes are usually lost. The occurrence of this inversion in two sibs and in the children of the proband suggests that this particular chromosomal rearrangement has been stably transmitted through at least two generations.

The serological markers encoded by the polymorphic HLA genes could not conclusively predict the parental origin of the chromosome

Figure 3 Scale map of the MHC class I region and distal markers⁸ used to determine the inheritance of the inversion chromosome. The order and relative positions of the markers are approximate but based on available mapping data. Previous linkage disequilibrium mapping analysis showed the probable position for the haemochromatosis disease gene to be close to marker D6S1260.⁸

in the proband. PCR based typing studies with 12 informative VNDR loci dispersed over 4 cM on the short arm of chromosome 6, using DNA derived from archival material from both of the proband's dead parents, indicate that the inversion chromosome was derived from the paternal lineage. Thus, it is most likely that the proband's mother carried the HLA-A3/HLA-B7 and HLA-A3/HLA-B18 chromosomes, while the father contributed the HLA-A24/HLA-B51 inv(6)(p21.1p23) chromosome to both HFE affected offspring. The presence of the inversion chromosome in the father is inferred by its occurrence in both the proband and her brother, although the direct confirmation of this hypothesis must await the cloning of the breakpoints.

The clinical diagnosis of haemochromatosis in the proband and her brother was based upon repeated abnormal serum iron indices, the presence of a firmly established, posthumous diagnosis of HFE in their mother, and an appropriate response to phlebotomy therapy. Despite the absence of liver biopsy data, the young age and gender of the proband, the more severe biochemical abnormalities in her brother, and the established genetic susceptibility they both possess, the simplest explanation for this scenario in these otherwise healthy subjects is homozygosity for the HFE disease gene. Several large and independent studies, which have looked at the biochemical expression of haemochromatosis in heterozygous members of HFE pedigrees, have generally concluded that the disease is completely recessive with respect to both clinical manifestations and serum iron abnormalities.^{15-17,21} Thus the proband's children will probably be normal with respect to iron status.

The disease gene mapping efforts of many researchers have been aided by the identification of a chromosomal rearrangement²² like the structural anomaly described in this report. Based on the identification of this paracentric inversion and its association with HFE, we are currently attempting to ascertain the precise location of the distal breakpoint by identifying bacterial artificial chromosome clones (BACs) telomeric to the D6S105 locus for use in FISH studies, although a complete physical map of this region is not yet available and current cytogenetic marker assignments are at low resolution.^{23,24} This approach should enable us to characterise molecularly the chromosomal rearrangement and allow for the isolation of genes in the proximity of this genomic landmark. Additionally, this chromosome may prove to be useful in the future endeavours which seek to correlate cytogenetic and physical maps of the short arm of chromosome 6. Although the possibility exists that the cosegregation of this chromosomal abnormality is coincidental to the occurrence of HFE in this pedigree, the inversion may play a more complex role in the aetiology of the disease, such as exhibiting a position effect.²⁵ The eventual cloning and analysis of the HFE gene may resolve this issue.

This work was supported by grants from the Hackett Foundation (MJC), the March of Dimes (Basic Research Award) (MJC), the NIH (NIDDKD No 1 R01 DK43734-01A2 (MJC); HD 18568 (JHMK)), a Penn State College of Medicine Clinical Research Grant No 95-43 (GSG), and the Beth Israel Pathology Foundation, Inc (JHMK). The authors would like to thank Dr Jean-Yves Le Gall, Dr Roger Ladda, and Ms Padmanee Sharma for their helpful suggestions. The authors also thank the Departments of Pathology of Queen's Medical Center of Honolulu (HA) and Prince George's Hospital Center (Cheverly, MD) for archival material. Dr Margrit Kriker (President of the Hemochromatosis Foundation) and Ms Roberta Crawford (President of the Iron Overload Diseases Association, Inc) are also acknowledged for their continued interest and support.

- Bothwell TH, Charlton RW, Motulsky AG. Hemochromatosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D eds. *The metabolic and molecular bases of inherited disease*. New York: McGraw-Hill, 1995:2237-70.
- Edwards CQ, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP. Prevalence of hemochromatosis among 11,065 presumably healthy blood donors. *N Engl J Med* 1988;318:1355-62.
- Simon M, Bourel M, Fauchet R, Genetet B. Association of HLA-A3 and HLA-B14 antigens with idiopathic haemochromatosis. *Gut* 1976;17:332-4.
- Cartwright GE, Skolnick M, Amos DB, Edwards CQ, Kravitz K, Johnson A. Inheritance of hemochromatosis: linkage to HLA. *Trans Assoc Am Physicians* 1978;91:273-81.
- Jazwinska EC, Lee SC, Webb SI, Halliday JW, Powell LW. Localization of the hemochromatosis gene close to D6S105. *Am J Hum Genet* 1993;53:347-52.
- Yaouanq J, Perichon M, Chorney MJ, et al. Anonymous marker loci within 400 kb of HLA-A generate haplotypes in linkage disequilibrium with the hemochromatosis gene (HFE). *Am J Hum Genet* 1994;54:252-63.
- Raha-Chowdhury R, Bowen DJ, Stone C, et al. New polymorphic microsatellite markers place the hemochromatosis gene telomeric to D6S105. *Hum Mol Genet* 1995;4:1869-74.
- Seese N, Venditti CP, Chorney KA, et al. Localization of the hemochromatosis disease gene: linkage disequilibrium analysis using an American patient collection. *BCMD* 1996;22:36-46.
- Gandon G, Jouanolle AM, Chavel B, et al. Linkage disequilibrium and extended haplotypes in the HLA-A to D6S105 region: implications for mapping the hemochromatosis gene (HFE). *Hum Genet* 1996;97:103-13.
- Camaschella C, Roetto A, Gasparini P, et al. Allelic association of microsatellites of 6p in Italian hemochromatosis patients. *Hum Genet* 1996;97:476-81.
- Calandro LM, Baer DM, Sensabaugh GF. Characterization of a recombinant that locates the hereditary hemochromatosis gene telomeric to HLA-F. *Hum Genet* 1995;96:339-42.
- NIH/CEPH Collaborative Mapping Group. A comprehensive genetic linkage map of the human genome. *Science* 1992;258:67-86.
- Venditti CP, Chorney MJ. Class I gene contraction within the HLA-A subregion of the human MHC. *Genomics* 1992;14:1003-9.
- Mowrey PN, Chorney MJ, Venditti CP, et al. Clinical and molecular analyses of deletion 3p25-pter syndrome. *Am J Med Genet* 1993;46:623-9.
- Dadone MM, Kushner JP, Edwards CQ, Bishop DT, Skolnick MH. Hereditary hemochromatosis: analysis of laboratory expression of the disease by genotype in 18 pedigrees. *Am J Clin Pathol* 1982;78:196-207.
- Borecki IB, Rao DC, Yaouanq J, Lalouel JM. Percent transferrin saturation in segregating hemochromatosis. *Am J Med Genet* 1990;36:301-5.
- Adams PC, Gregor JC, Kertesz AE, Valberg LS. Screening blood donors for hereditary hemochromatosis: decision analysis model based on a 30-year database. *Gastroenterology* 1995;109:177-88.
- Burt MJ, Smit DJ, Pyper WR, Powell LW, Jazwinska EC. A 4.5-megabase YAC contig and physical map over the hemochromatosis gene region. *Genomics* 1996;33:153-8.
- Worwood M, Raha-Chowdhury R, Darke C. Distribution of alleles at D6S105 and D6S265 with possible HLA haplotype associations. *Tissue Antigens* 1994;44:322-5.
- Madan K. Paracentric inversions: a review. *Hum Genet* 1995;96:503-15.
- Borecki IB, Lathrop GM, Bonney GE, Yaouanq J, Rao DC. Combined segregation and linkage analysis of genetic hemochromatosis using affection status, serum iron, and HLA. *Am J Hum Genet* 1990;47:542-50.
- Collins FS. Positional cloning moves from perdictional to traditional. *Nat Genet* 1995;9:347-50.
- Bray-Ward P, Menninger J, Leiman J, et al. Integration of the cytogenetic, genetic, and physical maps of the human genome by FISH mapping of CEPH YAC clones. *Genomics* 1996;32:1-14.
- Hudson TJ, Stein LD, Gerety SS, et al. An STS-based map of the human genome. *Science* 1995;270:1945-54.
- Milot E, Fraser P, Frosveld F. Position effects and genetic disease. *Trends Genet* 1996;12:123-6.