*Am J Hum Genet* 38:805–811, 1986

# Mapping the Locus for Hereditary Hemochromatosis: Localization between HLA-B and HLA-A

CORWIN Q. EDWARDS,[1] LINDA M. GRIFFEN,[2] MARY M. DADONE,[2]
MARK H. SKOLNICK,[3] AND JAMES P. KUSHNER[2]

SUMMARY

We studied a family with HLA-linked hereditary hemochromatosis in which an informative recombination occurred within the HLA region. The father, an obligate heterozygote for hereditary hemochromatosis, had HLA haplotypes *A2,B13* and *A11,B27*. The mother, also an obligate heterozygote, had HLA haplotypes *A29,B44* and *A2,B7*. Three haplotypes were found among three homozygous affected offspring. Two affected siblings were HLA-identical with haplotypes *A2,B13* and *A29,B44*. The proband had HLA haplotypes *A2,B13* and *A2,B44*, the latter a recombinant haplotype inherited from her mother. Since the maternal hemochromatosis allele was linked to the *A29,B44* haplotype, and since the proband has hemochromatosis, the maternal hemochromatosis allele was transmitted to the proband with the B44 antigen. This is the first known example of recombination in an individual with HLA-linked hemochromatosis in whom the hemochromatosis allele appeared to segregate with the HLA-B antigen instead of the -A antigen. The possibility of either a double reciprocal recombination event or a gene conversion event cannot be excluded. Combined with earlier observations of segregation of the hemochromatosis allele with the *A* locus in HLA recombinants, the findings in this pedigree map the hemochromatosis locus between the *HLA-B* and *HLA-A* loci rather than outside the HLA region.

Received September 16, 1985; revised December 12, 1985.

This work was supported by grants AM-20630 and RR-00064 from the National Institutes of Health.

[1] 4C-334, Department of Internal Medicine, LDS Hospital and University Medical Center, 50 North Medical Drive, Salt Lake City, Utah 84132.

[2] 4C-433, University of Utah College of Medicine, 50 North Medical Drive, Salt Lake City, UT 84132.

[3] Medical Department of Biophysics and Computing, University of Utah College of Medicine.

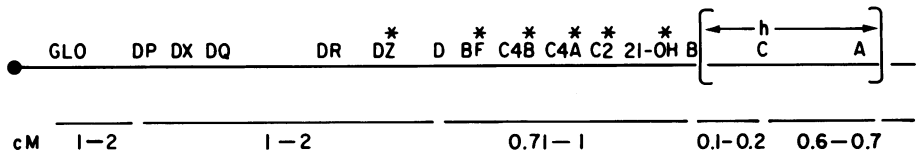


FIG. 1.—Schematic representation of the short arm of chromsome 6 not drawn to scale (7-14): ●, centromere; *cM*, centimorgans; *GLO*, glyoxylase; *DP* (formerly called SB); *DQ* (formerly DC, MB, or DS); *DR*, predominant class II molecule; *BF*, properdin factor B; *C4A* and *C4B*, fourth component of complement, fractions A and B; *C2*, second component of complement; *21-OH*, 21 hydroxylase; *h*, hemochromatosis allele; *D,B,C,* and *A*, HLA loci; * indicates sequence uncertain.

## INTRODUCTION

Hereditary hemochromatosis is inherited as an autosomal recessive disorder [1]. The allele responsible for hereditary hemochromatosis is located on the short arm of chromosome 6 in tight linkage with the *A* locus of the HLA complex [2–6]. Mapping studies have assigned the known loci on the short arm of chromosome 6 in the sequence shown in figure 1 [7–14]. Estimates of the recombination fraction between the hemochromatosis locus and other loci in the HLA region have yielded values of less than .025 [2–4, 15–17].

There have been 11 reports of recombination between the hemochromatosis locus and other loci of the HLA region [8, 17–22]. In the three adequately detailed reports, the hemochromatosis allele always segregated with the *HLA-A* locus [8, 19, 20]. Until now it has not been possible to determine if the position of the hemochromatosis allele is between the *HLA-B* and *-A* loci (distal to the *B* locus and proximal to the *A* locus) or distal to the *HLA-A* locus beyond the HLA region [23].

Here, we describe a family in which three siblings were homozygous for HLA-linked hemochromatosis. In one of the affected individuals, an informative recombination occurred within the HLA region. The abnormal allele at the hemochromatosis locus segregated with the *HLA-B* locus, providing the first evidence that the hemochromatosis allele lies between the *HLA-B* and *-A* loci.

## MATERIALS AND METHODS

The eight subjects studied were members of a family in which three young siblings had hereditary hemochromatosis. The proband, a 21-year-old woman, was identified during our prospective study evaluating the accuracy of the percent saturation of transferrin as a phenotypic marker to detect homozygosity for hemochromatosis in 10,000 American Red Cross volunteer blood donors [24]. At the time of blood donation, the proband signed a consent form permitting measurement of the percent saturation of transferrin and authorizing further studies if an elevated value was detected. The procedures used were reviewed and approved by the Clinical Research Center Committee on Human Rights and the Committee on Human Rights of the University of Utah in collaboration with the Salt Lake Area Chapter of the American Red Cross.

### *Assessment of Iron Stores*

Serum iron concentration, percent saturation of transferrin, and serum ferritin concentration were measured in all eight family members. The proband underwent per-

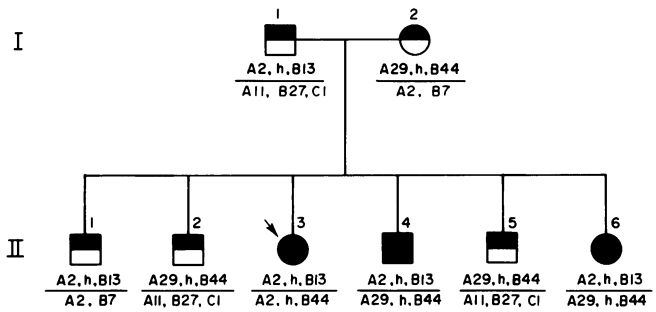


FIG. 2.—Family with hemochromatosis in which an informative recombination occurred within the HLA region. The proband is indicated by *an arrow*. Generations are indicated by *roman numerals;* pedigree numbers appear *above sex symbols;* males represented by *squares;* females by *circles;* hemochromatosis allele by *letter h;* abnormal homozygotes ■ or ●; and heterozygotes by ◨ or ◓. The HLA haplotypes are separated by a line; HLA antigens of each haplotype appear in this order: A-locus antigen, B-locus antigen, and lastly the C-locus antigen.

cutaneous needle biopsy of the liver with assessment of hepatic parenchymal cell stainable iron and hepatic iron concentration [25].

*HLA Typing*

Histocompatibility antigen testing was performed for all eight family members using commercially available trays of antisera [26]. The antisera used were able to identify 18 *HLA-A,* 26 *HLA-B,* and four *HLA-C* locus antigens (C1–C4).

## RESULTS

The family is represented in figure 2, and the results of studies assessing their iron stores are shown in table 1. The proband's transferrin was 80% saturated. She had never donated blood, received blood transfusions, or taken medicinal iron prior to the donation which led to her participation in this study. She

TABLE 1

IRON STATUS OF FAMILY MEMBERS

| INDIVIDUALS | AGE, SEX (YRS) | SERUM IRON (μg/dl) | TRANSFERRIN SATURATION (%) | SERUM FERRITIN (ng/ml) | |
|---|---|---|---|---|---|
| | | | | Observed | Normal mean by age, sex* |
| Normal values | | < 170 | < 50 | | |
| I-1 | 48 M | 117 | 40 | 261 | 92 |
| I-2 | 46 F | 114 | 38 | 115 | 29 |
| II-1 | 24 M | 120 | 42 | 102 | 93 |
| II-2 | 22 M | 135 | 51 | 179 | 93 |
| II-3 | 21 F | 216 | 80 | 234 | 23 |
| II-4 | 18 M | 255 | 100 | 385 | 18 |
| II-5 | 14 M | 120 | 50 | 9 | 18 |
| II-6 | 12 F | 177 | 74 | 110 | 17 |

* Normal values for ferritin concentration by age and sex are from Valberg et al. [27].

TABLE 2

PROBABLE GENOTYPE ASSIGNMENTS FOR EIGHT MEMBERS OF FAMILY BASED ON TRANSFERRIN SATURATION AND PEDIGREE DATA ONLY (HLA DATA NOT USED)

| | PROBABILITY GENOTYPE* | | |
|---|---|---|---|
| INDIVIDUALS | nn | nh | hh |
| I-1 | .000000 | .999994 | .000006 |
| I-2 | .000000 | .99997 | .000003 |
| II-1 | .154493 | .845478 | .000029 |
| II-2 | .067774 | .930450 | .001776 |
| II-3 | .000005 | .001284 | .998712 |
| II-4 | .000000 | .000000 | 1.000000 |
| II-5 | .074571 | .924299 | .001130 |
| II-6 | .000127 | .018267 | .981607 |

NOTE: Probable genotype assignments were calculated by published methods [28, 29].

* nn = normal, nh = heterozygote, hh = abnormal homozygote.

had never menstruated because of ovarian agenesis, and, at age 18, she underwent hysterectomy and bilateral salpingo-oophorectomy (hypoplastic, streak ovaries). Liver biopsy revealed an increase in hepatic parenchymal cell stainable iron (grade 2), and the hepatic iron concentration was elevated at 140 μg/100 mg liver wet weight (normal females < 19). There was no histologic evidence of cirrhosis, periportal fibrosis, chronic hepatitis, or hepatoma. Among her five siblings, both her 18-year-old brother (II-4) and 12-year-old sister (II-6) had markedly elevated values of the percent saturation of transferrin (table 1). These values (100% and 74%) strongly suggest homozygosity for hereditary hemochromatosis [28].

The paternal *A2,B13* HLA haplotype and the maternal *A29,B44* haplotypes were considered linked to hemochromatosis alleles as they were present in two individuals in generation II (II-4 and II-6, fig. 2) considered homozygous for hereditary hemochromatosis (based on the percent saturation of transferrin). The proband aside, no individuals in generation II possessing only the *A2,B13* or the *A29,B44* haplotype (but not both) had a value for percent saturation of transferrin that indicated homozygosity for hereditary hemochromatosis. Any alternate explanation of the observed HLA haplotypes in this pedigree (compatible with the probable genotype assignments based on the percent saturation of transferrin alone, not using HLA data, table 2) would require recombinations in at least four of the other siblings.

The proband inherited the paternal hemochromatosis-linked *A2,B13* HLA haplotype. She also inherited a recombinant maternal haplotype, *A2,B44*, apparently resulting from crossing over within the HLA region between the maternal *A2,B7* and *A29,B44* haplotypes (fig. 2). The proband's percent saturation of transferrin, elevated serum ferritin concentration and the findings on liver biopsy indicate that she is homozygous for HLA-linked hemochromatosis. As the proband inherited the maternal hemochromatosis allele with the HLA-B44

alloantigen from the hemochromatosis-linked maternal *A29,B44* haplotype, the hemochromatosis allele must have segregated with the *HLA-B* locus at the time of recombination rather than with the *-A* locus.

## DISCUSSION

Discriminant analysis has demonstrated that individuals with a percent saturation of transferrin greater than 62 have a 92% likelihood of being homozygous for hereditary hemochromatosis [28]. The proband and two of her siblings are presumably homozygous for hereditary hemochromatosis as they all had a percent saturation of transferrin of 74% or greater, and therefore under the model presented previously [28, 29], they all had probabilities greater than 98% of being homozygous (table 2). The proband's amenorrhea likely contributed to signs of hepatic iron loading at age 21. Her younger brother, age 18 (II-4, table 1), has a serum ferritin concentration that is higher than normal. It has not yet been possible to perform a liver biopsy on him. Her younger sister, age 12 (II-6, table 1), had no laboratory evidence of iron loading other than the percent saturation of 74, but some young homozygotes for hemochromatosis are not significantly iron loaded when they are detected as part of a family study [30–33].

Because the hemochromatosis allele is linked to the HLA complex, and because of the recessive mode of inheritance of the disease, homozygous affected siblings in a pedigree are generally HLA identical. One mechanism responsible for the occasional occurrence of HLA nonidentity in homozygous siblings is the mating of a homozygous affected parent with a heterozygous partner [4, 6, 34–38]. This is not the case in this pedigree as neither parent was homozygous for the hemochromatosis gene (table 2). The one HLA haplotype difference between the homozygous affected proband and her two homozygous affected siblings appears to be due to a single reciprocal recombination between the maternal haplotypes.

This recombinant is a particularly informative one as it is the first to permit probable assignment of the hemochromatosis allele to the region between the *HLA-B* and *HLA-A* loci. *HLA-C* locus typing was not helpful in this pedigree (only one paternal *C*-locus alloantigen could be detected), and, thus, it is not possible to determine if the hemochromatosis allele lies between *HLA-B* and *-C* or between *HLA-C* and *-A*. In all prior reports of recombination between the hemochromatosis allele and the HLA region, the hemochromatosis allele segregated with the *HLA-A* locus antigen. This, plus allelic association data, suggests that the hemochromatosis locus is located closer to the *HLA-A* locus than to the *HLA-B* locus.

A double reciprocal recombination event or a gene conversion event might also explain the findings in this pedigree. Indirect evidence has been invoked supporting the role of gene conversion in the generation of polymorphism at the HLA loci in man [39, 40]. We cannot exclude the possibility that during meiosis the maternal A29 alloantigen was replaced by the maternal *A2* allele through a gene conversion event or through a double recombination. The development of cloned probes coding for class I antigens mapped within the HLA region should

permit further localization of the hemochromatosis allele and determination of the molecular mechanism responsible for the findings in this pedigree.

## REFERENCES


1. Saddi R, Feingold J: Idiopathic haemochromatosis: an autosomal recessive disease. *Clin Genet* 5:234–241, 1974
2. Simon M, Bourel M, Genetet B, Fauchet R: Idiopathic hemochromatosis. Demonstration of recessive transmission and early detection by family HLA typing. *N Engl J Med* 297:1017–1021, 1977
3. Cartwright GE, Skolnick M, Amos DB, Edwards CQ, Kravitz K, Johnson A: Inheritance of hemochromatosis: linkage to HLA. *Trans Assoc Am Physicians* 91:273–281, 1978
4. Lipinski M, Hors J, Saleun JP, et al.: Idiopathic hemochromatosis: linkage with HLA. *Tissue Antigens* 11:471–474, 1978
5. Kravitz K, Skolnick M, Edwards CQ, et al.: Pedigree analysis of the linkage between HLA and hemochromatosis, in *Genetic Epidemiology,* edited by Morton NE, Chung CS, New York, Academic Press, pp 241–246, 1978
6. Edwards CQ, Skolnick MH, Kushner JP: Hereditary hemochromatosis: contributions of genetic analyses. *Prog Hematol* 12:43–71, 1981
7. Bakker E, Pearson PL, Meera Khan P, Schreuder GMT, Madan K: Orientation of major histocompatibility (MHC) genes relative to the centromere of human chromosome 6. *Clin Genet* 15:198–202, 1979
8. Edwards CQ, Cartwright GE, Skolnick MH, Amos DB: Genetic mapping of the hemochromatosis locus on chromosome six. *Hum Immunol* 1:19–22, 1980
9. Weitkamp LR: Linkage of GLO with HLA and Bf. Effect of population and sex on recombination frequency. *Tissue Antigens* 7:273–279, 1976
10. Polacek LA, Phillips RB, Hackbarth SA, Duquesnoy RJ: A linkage study of the HLA region using C-band heteromorphisms. *Clin Genet* 23:177–185, 1983
11. Olaisen B, Teisberg P, Jonassen R, Thorsby E, Gedde-Dahl T: Gene order and gene distances in the HLA region studied by the haplotype method. *Ann Hum Genet* 47:285–292, 1983
12. Dausset J, Cohen D: HLA at the gene level, in *Histocompatibility Testing 1984,* edited by Albert ED, Bauer MP, Mayr WR, Report on the Ninth International Histocompatibility Workshop and Conference, Berlin, Springer-Verlag, 1984, pp 22–28
13. Rittner C, Mauff G: C4 polymorphism, in ibid., pp 318–324
14. Baur MP, Sigmund S, Sigmund M, Rittner C: Analysis of MHC recombinant families, in ibid., pp 324–325
15. Kravitz K, Skolnick M, Cannings C, et al.: Genetic linkage between hereditary hemochromatosis and HLA. *Am J Hum Genet* 31:601–619, 1979
16. Cartwright GE, Edwards CQ, Kravitz K, et al.: Hereditary hemochromatosis. Phenotypic expression of the disease. *N Engl J Med* 301:175–179, 1979
17. Ritter B, Safwenberg J, Olsson KS: HLA as a marker of the hemochromatosis gene in Sweden. *Hum Genet* 68:62–66, 1984
18. Lipinski M, Hors J, Saleun JP, et al.: Hemochromatose idiopathique: liaison avec le systeme HLA. *Diabete Metab* 4:109–115, 1978
19. Lloyd DA, Adams P, Sinclair NR, Stiller CR, Valberg LS: Histocompatibility antigens as markers of abnormal iron metabolism in idiopathic hemochromatosis. *Can Med Assoc J* 119:1051–1056, 1978
20. Edwards CQ, Skolnick MH, Dadone MM, Kushner JP: Iron overload in hereditary spherocytosis: association with HLA-linked hemochromatosis. *Am J Hematol* 13:101–109, 1982
21. Basset ML: Haemochromatosis: diagnostic and metabolic studies. Thesis, Brisbane, Australia, Univ. of Queensland, 1983

22. BORWEIN ST, GHENT CN, FLANAGAN PR, CHAMBERLAIN MJ, VALBERG LS: Genetic and phenotypic expression of hemochromatosis in Canadians. *Clin Invest Med* 6:171–179, 1983
23. EDWARDS CQ, DADONE MM, SKOLNICK MH, KUSHNER JP: Hereditary haemochromatosis. *Clin Haematol* 11:411–435, 1982
24. KUSHNER J, EDWARDS C, GRIFFEN L, DADONE M, SKOLNICK M: Incidence of homozygosity for HLA-linked hemochromatosis in healthy young blood donors. *Blood* 64(Suppl 1)(5):40a, no. 70, 1984
25. EDWARDS CQ, CARROLL M, BRAY P, CARTWRIGHT GE: Hereditary hemochromatosis. Diagnosis in siblings and children. *N Engl J Med* 297:7–13, 1977
26. RAY JG, HARE DB, PEDERSEN PD, MULLALLY DI, EDS: NIH lymphocyte microcytotoxicity technique, in *NIAID Manual of Tissue Typing Techniques,* Bethesda, Md., National Institute of Allergy and Infectious Diseases, DHEW Publication No. (NIH) 76-545, 1976, pp 22–24
27. VALBERG LS, SORBIE J, LUDWIG J, PELLETIER O: Serum ferritin and the iron status of Canadians. *Can Med Assoc J* 114:417–421, 1976
28. 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* 78:196–207, 1982
29. KUSHNER JP, EDWARDS CQ, DADONE MM, SKOLNICK MH: Heterozygosity for HLA-linked hemochromatosis as a likely cause of the hepatic siderosis associated with sporadic porphyria cutanea tarda. *Gastroenterology* 88:1232–1238, 1985
30. BEAUMONT C, SIMON M, FAUCHET R, ET AL.: Serum ferritin as a possible marker of the hemochromatosis allele. *N Engl J Med* 301:169–174, 1979
31. EDWARDS CQ, CARTWRIGHT GE, SKOLNICK MH, AMOS DB: Homozygosity for hemochromatosis: clinical manifestations. *Ann Intern Med* 93:519–525, 1980
32. VALBERG LS, LLOYD DA, GHENT CN, ET AL.: Clinical and biochemical expression of the genetic abnormality in idiopathic hemochromatosis. *Gastroenterology* 79:884–892, 1980
33. BASSETT ML, HALLIDAY JW, FERRIS RA, POWELL LW: Diagnosis of hemochromatosis in young subjects: predictive accuracy of biochemical screening tests. *Gastroenterology* 87:628–633, 1984
34. SIMON M, HESPEL J-P, FAUCHET R, ET AL.: Heredité recessive de l'hemochromatose idiopathique: deux observations de transmission pseudo—dominante reconnue comme recessive par étude de la surcharge en fer et des genotype, HLA dans les familles. *Nouv Presse Med* 8:421–424, 1979
35. BASSETT ML, HALLIDAY JW, POWELL LW, DORAN T, BASHIR H: Early detection of idiopathic haemochromatosis: relative value of serum-ferritin and HLA typing. *Lancet* 2:4–7, 1979
36. SIMON M, ALEXANDRE J-L, FAUCHET R, GENETET B, BOUREL M: The genetics of hemochomatosis, in *Progress in Medical Genetics,* edited by STEINBERG AG, BEARN AG, MOTULSKY AG, CHILDS B, New Series, vol 4, Genetics of Gastrointestinal Disease, Philadelphia, W.B. Saunders, 1980, pp 135–168
37. BASSETT ML, DORAN TJ, HALLIDAY JW, BASHIR HV, POWELL LW: Idiopathic hemochromatosis: demonstration of homozygous-heterozygous mating by HLA typing of families. *Hum Genet* 60:352–356, 1982
38. OLSSON KS, RITTER B, ROSEN U, HEEDMAN PA, STAUGARD F: Prevalence of iron overload in Central Sweden. *Acta Med Scand* 213:145–150, 1983
39. LÓPEZ DE CASTRO JA, STROMINGER JL, STRONG DM, ORR HT: Structure of crossreactive human histocompatibility antigens HLA-A28 and HLA-A2: possible implications for the generation of HLA polymorphism. *Proc Natl Acad Sci USA* 79:3813–3817, 1982
40. EVANS GA, MARGULIES DH, CAMERINI-OTERO RD, OZATO K, SEIDMAN JG: Structure and expression of a mouse major histocompatibility antigen gene, H-2L$^d$. *Proc Natl Acad Sci USA* 79:1994–1998, 1982