Histocompatibility antigens as markers of abnormal iron metabolism in idiopathic hemochromatosis

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To determine the frequency of HLA histocompatibility antigens in persons with idiopathic hemochromatosis and their usefulness as genetic markers of the disease, HLA typing for the A, B and C loci was carried out. HLA-A3 was found in 61% of 18 unrelated individuals with idiopathic hemochromatosis compared with 25% of 253 randomly chosen control subjects ($P < 0.001$), and HLA-B7 was found in 50% and 22% respectively ($P < 0.025$). Eighty-six members of seven families with idiopathic hemochromatosis were screened for abnormalities in iron metabolism with tests for serum iron concentration, transferrin saturation, serum ferritin concentration and iron content of the hepatocytes. Of the 14 persons selected for liver biopsy because of abnormalities detected by these tests, 8 had increased amounts of stainable iron in the hepatocytes. Body iron overload was subsequently demonstrated in six of the seven, who had undergone repeated phlebotomy. In sibships having one member with hemochromatosis, only 1 of 22 members had two haplotypes in common with the proband, whereas in sibships having more than 1 member with the disease 4 of 5 affected members had two haplotypes in common. HLA typing in families with hemochromatosis may provide a means of identifying persons at risk of acquiring the disease.

Afin d'établir la fréquence des antigènes d'histocompatibilité HLA chez les personnes ayant une hémochromatose idiopathique et leur utilité en tant qu'indicateurs génétiques pour cette maladie, on a procédé au typage HLA des loci d'histocompatibilité A, B et C. L'antigène HLA-A3 a été retrouvé chez 61% de 18 individus non apparentés et porteurs d'une hémochromatose idiopathique, comparativement à 25% de 253 sujets témoins choisis au hasard ($P < 0.001$), alors que HLA-B7 a été retrouvé chez 50% et 22% respectivement ($P < 0.025$). Quatre-vingt-six membres de sept familles souffrant d'hémochromatose idiopathique ont été soumis au dépistage des anomalies du métabolisme du fer à l'aide des tests de la concentration sérique en fer, de saturation à la transferrine, de la concentration sérique en ferritine et de la teneur en fer des hépatocytes. Sur les 14 personnes choisies pour une biopsie du foie à cause des anomalies décelées lors de ces tests, 8 avaient des quantités accrues de fer colorable dans les hépatocytes. La surcharge corporelle en fer a ensuite été mise en évidence chez six des sept patients, qui avaient subi des phlébotomies répétées. Dans les fratries où un membre était atteint d'hémochromatose, 1 seul membre sur 22 partageait deux haplotypes avec le proposant, alors que chez les fratries ayant plus d'un membre porteur de la maladie, 4 membres affectés sur 5 avaient deux haplotypes en commun. Le typage des antigènes HLA chez les familles atteintes d'hémochromatose peut fournir un moyen d'identifier les personnes susceptibles d'acquérir la maladie.

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Idiopathic hemochromatosis is regarded as a familial disorder caused by an inborn error in metabolism.[1] Support for a genetic basis for the disease is provided by the presence of abnormalities in iron metabolism in relatives[2] and from the discovery of a relation between idiopathic hemochromatosis and the HLA histocompatibility antigens A3 and B14 in France,[3] and A3 and B7 in Great Britain.[4-6] This relation was not observed in a group of patients with alcoholic liver disease and iron overload, which suggests that it is a different disease from idiopathic hemochromatosis.[7] The role of HLA haplotypes in the inheritance of the disease remains controversial.[3,6,8-10] Simon and coworkers[11] reported that among siblings there was a significant association between the presence of hemochromatosis and the possession of the same two HLA haplotypes. Further studies of more families are needed to delineate the part HLA plays in the transmission of the disorder.

The study described in this paper was carried out to determine the frequency of HLA-A, -B and -C antigens in persons with idiopathic hemochromatosis in Ontario, and to assess the relation between HLA haplotypes and abnormalities in iron metabolism in members of seven families with the disease.

Methods

Selection of subjects

Eighteen unrelated individuals (12 men and 6 women) with idiopathic hemochromatosis were studied. They were questioned about relatives with liver disease or disorders of iron metabolism. Body iron overload was established by the presence of stainable iron in more than 50% of hepatic parenchymal cells in a liver biopsy specimen and the subsequent removal of more than 3 g of iron by phlebotomy.

Investigations were performed in the members of seven families with idiopathic hemochromatosis. Abnormalities in iron metabolism were sought by measuring the serum iron concentration, the transferrin saturation and the serum ferritin concentration. When an increase in either the transferrin saturation or the serum ferritin concentration was found, the hemosiderin content of hepatocytes was estimated in histologic sections of a specimen obtained by percutaneous liver biopsy. The apparent presence of body iron overload in seven family members was confirmed by phlebotomy in the six who had undergone venesections at the time this report was written. An eighth individual (II,14, family D) had body iron stores of 1.4 g even though 50% to 75% of the hepatocytes in the liver biopsy specimen contained hemosiderin.

Serum iron and transferrin saturation

The serum iron concentration and the transferrin saturation were measured with a diagnostic kit obtained from Hoffmann–La Roche Limited, Vaudreuil, PQ. The normal ranges were 65 to 175 μg/dL and 16% to 55% respectively.

Serum ferritin

The radioimmunoassay described by Luxton and associates[12] was used to measure the serum ferritin concentration. Upper limits of normal were based on the 90th percentile for serum ferritin concentrations in Canadians who participated in the Nutrition Canada survey.[13] For men the upper limit varied from 90 ng/mL at age 20 years to 350 ng/mL at age 50 years and older, and for women the limit ranged from 45 ng/mL at age 20 years to 200 ng/mL at age 70 years and older.

Hepatic iron

Sections of liver specimens obtained by percutaneous biopsy were stained for hemosiderin with Prussian blue.[14] The presence of hemosiderin in more than 50% of hepatocytes was considered to indicate hepatic iron overload.

HLA typing

Typing for the A, B and C loci was performed by the complement-dependent cytotoxic technique,[15] and the results were compared with those obtained in 253 randomly chosen, ostensibly healthy control subjects from Ontario.

Statistical analysis of the data was performed by the chi-square test with Yates' correction for continuity; for the population studies a further factor was applied to correct the number of antigenic specificities studied.[16] The Fisher exact test was used in the haplotype studies in the families.

Results

The frequency of HLA-A3 and of HLA-B7 was significantly higher ($P < 0.001$ and < 0.025 respectively) in the unrelated individuals with hemochromatosis than in the controls (Table I). HLA-B14 was also more frequent, but not significantly so, in the individuals with idiopathic hemochromatosis. No significant difference in any of the other haplotypes was found between the two groups.

In family A (Fig. 1) the propositus was homozygous for HLA-A11,B40. None of his brothers or sisters had his diplotype or showed abnormalities in iron metabolism.

Both father and son in family B (Fig. 2) had idiopathic hemochromatosis, and they had one HLA haplotype in common — A3,Bw35,Cw4. One daughter (II,1) also had this haplotype but had no abnormality in iron metabolism. No other tested member of this family had the same diplotype as the affected sibling, and none showed abnormalities in iron metabolism.

Only one member of family C (Fig. 3) appeared to have iron overload. One sister (II,6) with a normal transferrin saturation and a serum

Table I—Frequency of HLA histocompatibility antigen haplotypes in 18 unrelated individuals with idiopathic hemochromatosis and in 253 ostensibly healthy control subjects

	Haplotype frequency (%)		
Subject group	HLA-A3	HLA-B7	HLA-B14
Persons with idiopathic hemochromatosis	61	50	17
Healthy controls	25	22	8
P value	< 0.001	< 0.025	< 0.40

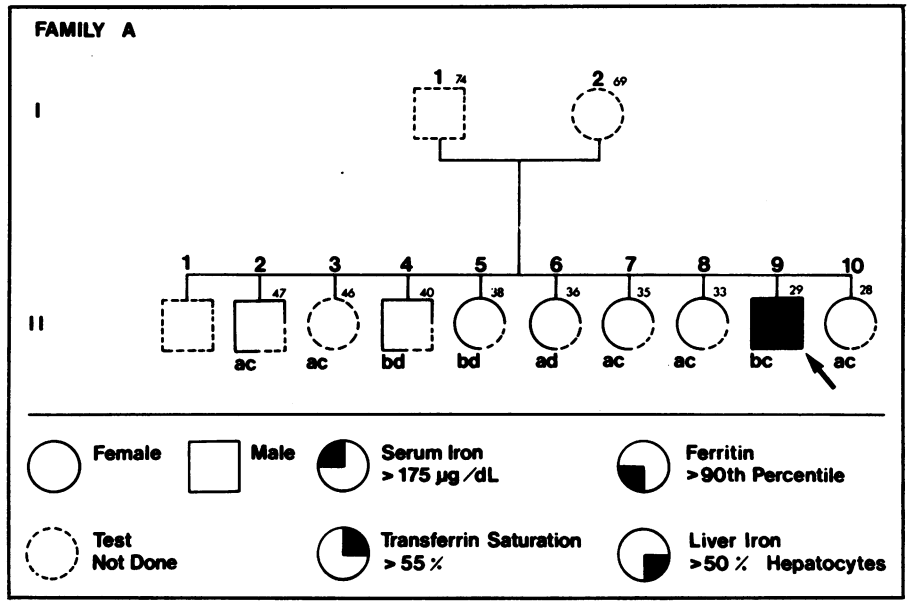

FIG. 1—HLA haplotypes of family A: a = Aw24,B15,Cw3; b = A11,B40,Cw3; c = A11,B40; d = A29,B27,Cw1. Age (years) given on upper side of symbol for each individual. Arrow indicates propositus.

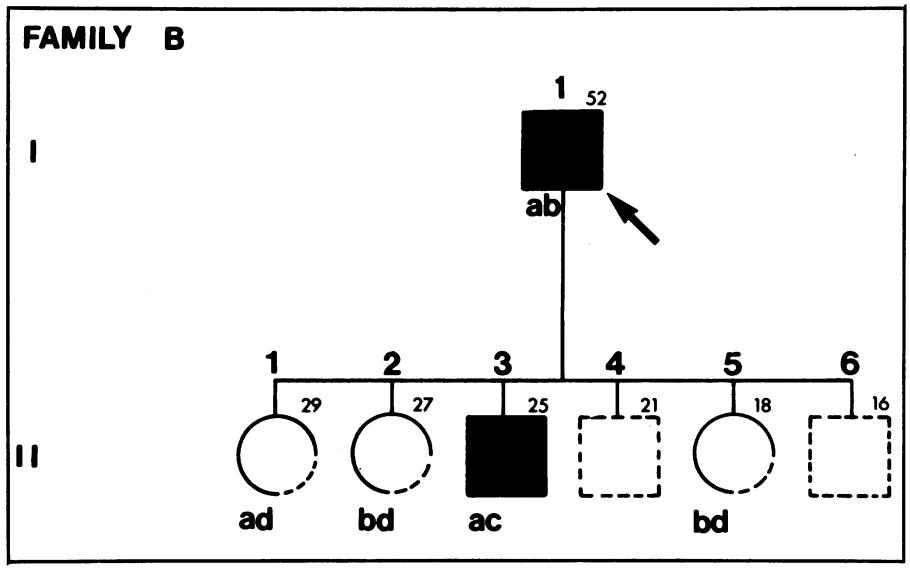

FIG. 2—HLA haplotypes of family B: a = A3,Bw35,Cw4; b = A2,B15,Cw3; c = A3,B40,Cw3; d = A1,B8.

ferritin value (53 ng/mL) between the 90th and 95th percentiles for her age underwent percutaneous liver biopsy, and no increase in hepatic iron content was found. One sibling (II,4) had a diplotype identical to that of the proband but no biochemical evidence of iron overload; this is the only instance of a sibling having a diplotype identical to that of the proband and not having the disease.

In family D (Fig. 4) the two sisters in the first generation were homozygous for HLA-A3,B7, although they may not have had the same haplotype: one of them (I,1) had an increase in both the transferrin saturation and the serum ferritin concentration as well as excessive hepatic iron, whereas the other (II,2) had an increase in transferrin saturation but no increase in the hepatic iron content. The two sons of the proband (II,1 and II,2) were also homozygous for HLA-A3,B7 and both had hemochromatosis. The daughter of I,2 (II,14) had increased transferrin saturation, a normal serum ferritin concentration and 3+ stainable hepatic iron; after repeated venesection she was found to have iron stores of 1.4 g. Both fathers of the children in generation II must have possessed the A3,B7 haplotype, but they were not available for study. Because the haplotypes could not be identified with certainty this family was excluded from further analysis.

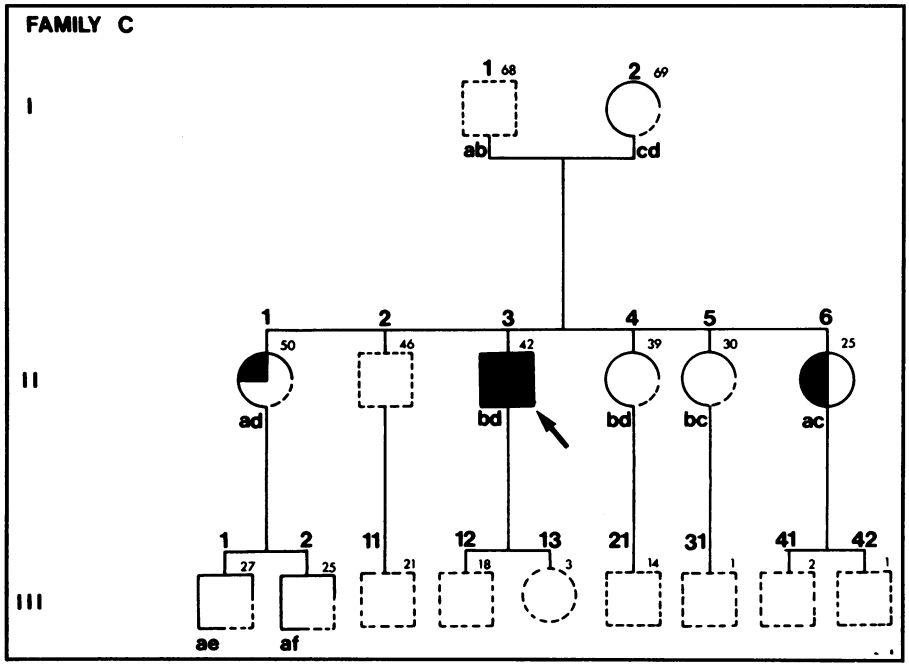

FIG. 3—HLA haplotypes of family C: a = A3,B7; b = A3,B14; c = A3,B27,Cw2; d = A29,B7; e = A2,B7; f = (A3,B7). Haplotype in brackets assumed to be present.

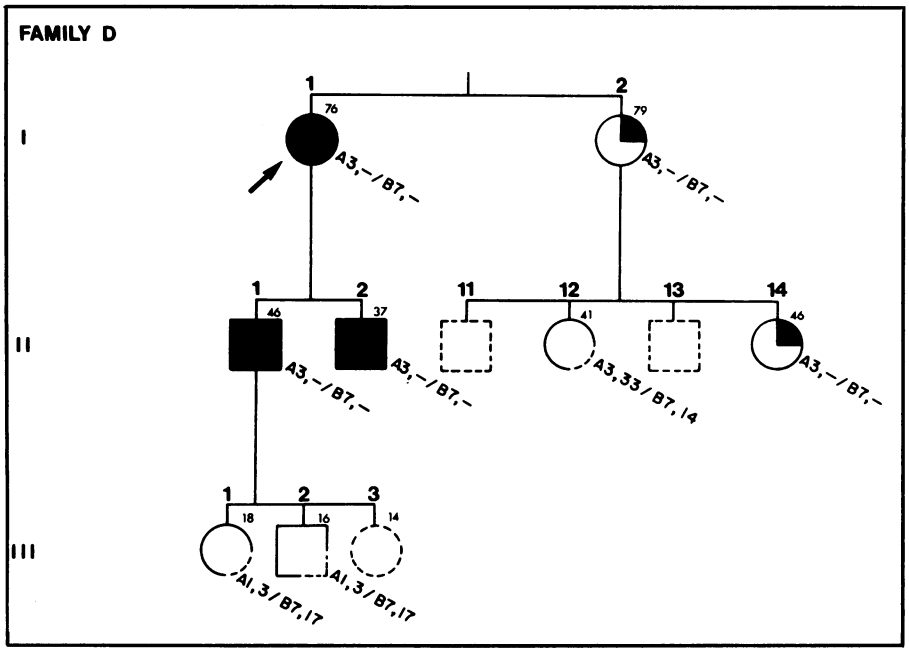

FIG. 4—HLA haplotypes of family D.

In family E (Fig. 5) the two subjects in the first generation with increased hepatic iron content (I,2 and I,4) had identical haplotypes — A1,-B15,Cw3 and A11, Bw35, Cw4. The sister with this haplotype (I,1) had increased transferrin saturation but the serum ferritin concentration was normal. She had no chemical evidence of iron overload and refused to have a liver biopsy because she felt perfectly well at the age of 83 years. The other sister (I,5) had the A1,B15,Cw3 haplotype and increased transferrin saturation; the serum ferritin concentration was normal, the hepatic iron content was not increased, and she was found, by repeated venesection, to have body iron stores of 1 g.

In family F (Fig. 6) the two siblings (II,1 and II,3) with haplotypes identical to that of the propositus had increased serum iron concentration, transferrin saturation and serum ferritin concentration. An increase in body iron stores was demonstrated in the propositus, but her two sisters refused further investigation. The other sister (II,4) with one haplotype in common with the propositus had a transferrin saturation of 53% and a serum ferritin concentration of 422 ng/mL. The brother, who had the A3,B15,Cw3 haplotype in common with the propositus had no abnormality in iron metabolism.

In the largest family we studied, family G (Fig. 7), the HLA-A3,B7 haplotype that was present in the affected member of the second generation (II,14) and two affected members of the third generation (III,32 and III,36) appeared to have originated from the maternal grandmother. The other affected member in the third generation (III,41) had the diplotype HLA-A1,B8/A2,B12. Minor abnormalities were found in the serum iron concentration of three members of the third generation (III,13, III,45 and III,64) and in the serum ferritin concentration of one member (III,64), but none had an increased hepatic iron content.

In the five sibships having one

member with hemochromatosis only 1 person had two haplotypes in common with the proband; the other 21 persons were not haplotype-identical (Table II). The absence of the proband's two haplotypes among the unaffected siblings was significant ($P < 0.045$).

In the four sibships having more than one member with hemochromatosis four siblings with hemochromatosis had both haplotypes in common with the proband, while one affected sibling had only one haplotype in common (Table III). Of the apparently unaffected siblings in these families one had both haplotypes and six had one haplotype in common with the proband. There was a significant association ($P < 0.04$) between the presence of the proband's two haplotypes and hemochromatosis.

Discussion

It is difficult to establish firmly the pattern of inheritance of idiopathic hemochromatosis because it generally takes more than 20 years for unequivocal clinical manifestations to develop. To screen family members for the early stages of the disease we measured both the transferrin saturation and the serum ferritin concentration.[17] In those with an abnormal result of one or other test a liver biopsy was done to detect excessive hepatic parenchymal iron. Of the 14 siblings who underwent

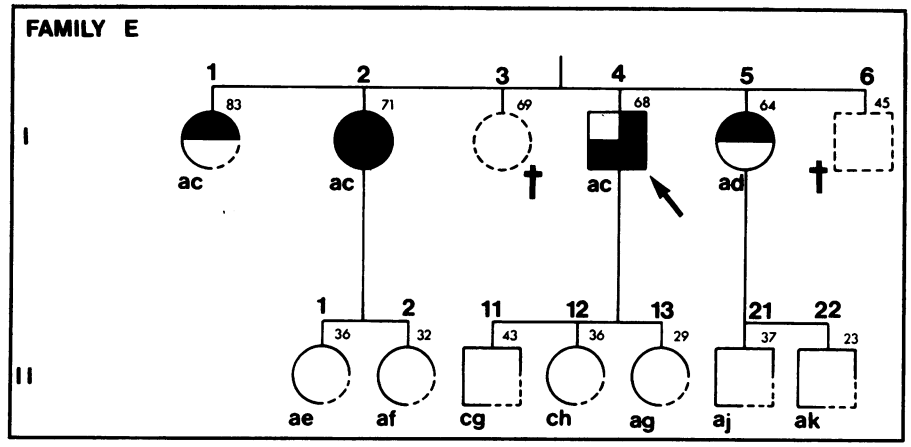

FIG. 5—HLA haplotypes of family E: a = A1,B15,(Cw3); c = A11,Bw35,Cw4; d = A28,B7; e = A1,B8; f = A24,B8; g = A1,B17; h = A2(Bw35); j = A3,(Bw35); k = A28,B7.

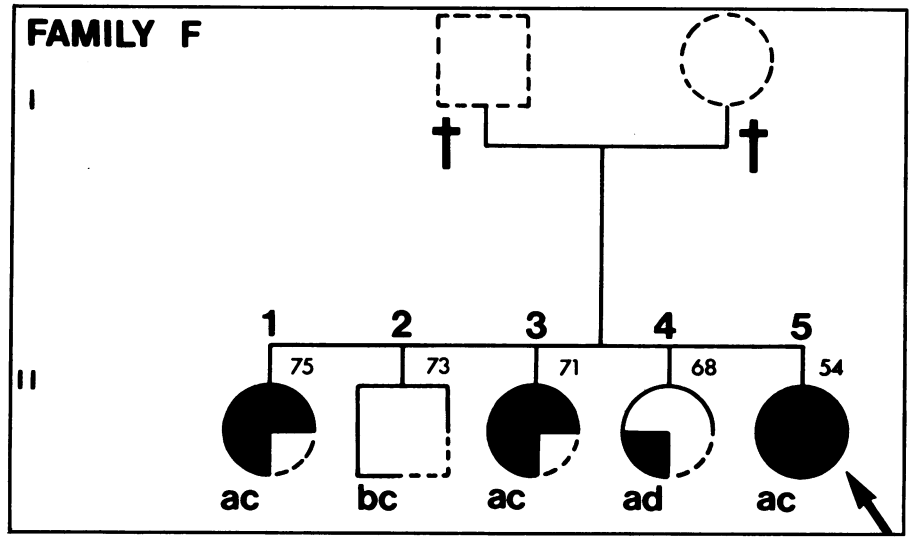

FIG. 6—HLA haplotypes of family F: a = A1,B7; b = A2,B8; c = A3,B15,Cw3; d = A1,B8.

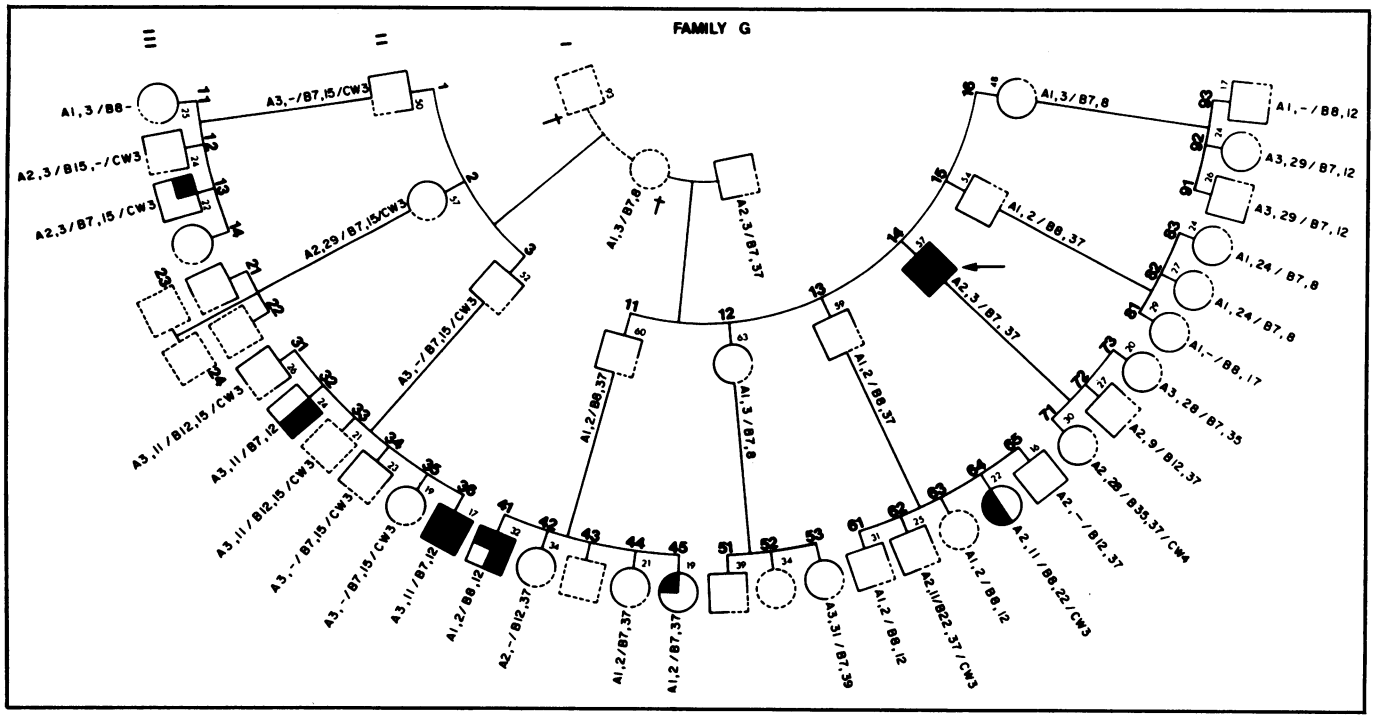

FIG. 7—HLA haplotypes of family G.

liver biopsy, hemochromatosis was deemed to be absent in the 7 who had a grade of hemosiderin of zero to 2+. (Some of the young people in this group may exhibit manifestations of iron overload later.) Of the eight persons with 3+ to 4+ hepatic iron, iron overload was confirmed in the seven who underwent venesections, and it was assumed to be present in the remaining subject (III,41; family G), in whom venesections are pending. In the four women who refused liver biopsy, iron overload was considered to be absent in the one with a serum ferritin concentration of 60 ng/mL (I,1; Fig. 5), and to be present in the three (II,1, II,3 and II,4; Fig. 6) with serum ferritin values of 334, 675 and 422 ng/mL respectively. None of these patients had disorders known to produce an increase in the serum ferritin concentration disproportionate to the size of the body iron stores.[18]

Our results in Canadians have confirmed the association between HLA-A3 and idiopathic hemochromatosis observed previously in France[3] and in Great Britain.[4-6] In contrast to the results from France, we did not find an increased frequency of HLA-B14. The high frequency of HLA-B7 in our patients with hemochromatosis is explained by linkage dysequilibrium between this antigen and HLA-A3. The HLA-A3 antigen was not absolutely associated with the disease process: 25% of our healthy control subjects had this antigen, and many of the relatives of our subjects with idiopathic hemochromatosis had this antigen but no obvious abnormality in the transferrin saturation, the serum ferritin concentration or the hepatic iron content. In family C one subject (III,2) appeared to be homozygous for HLA-A3,B7, yet no abnormalities in transferrin saturation or serum ferritin concentration were present, and in family D two elderly sisters (I,1 and I,2) were apparently homozygous, yet only one had evidence of iron overload. Nevertheless, HLA-A and -B haplotypes appear to be important for the development of hemochromatosis.

Our finding that siblings with both haplotypes identical to those of the proband were significantly more likely to have hemochromatosis than siblings with one haplotype in common with the proband confirms the recent observation of Simon and colleagues.[11] It suggests that there are at least two genes predisposing to the development of hemochromatosis, at loci closely linked to the major histocompatibility complex. The requirement for two haplotypes implies either a recessive pattern of inheritance or an oligogenic pattern, with two complementing dominant genes. A recessive mode of transmission of idiopathic hemochromatosis has been proposed for many of the affected families described in the literature.[8,19-23] It would explain the absence of the disease in parents and children in four of our families; however, it would only account for the disease in the succeeding generations of families B and D if the spouses of the probands were both carriers. There have been other reports in which the disease occurred in successive generations.[8,9,24,25] The difficulty in deriving a simple genetic explanation for the inheritance of hemochromatosis may be due to etiologic heterogeneity. Idiopathic hemochromatosis is probably a group of diseases in which the gut is unable to keep out unneeded iron, each of which has a different genetic lesion to account for the inappropriate iron absorption.[26]

Our finding in some family members (I,1 and II,14 of family D and I,5 of family E) of abnormalities in transferrin saturation in the absence of iron accumulation in the body is consistent with previous reports of minor abnormalities in heterozygotes.[8,17,27] Bomford and colleagues[6] postulated that there may be two genes, both carried on chromosome 6, one controlling iron absorption and the other controlling the exchange of iron between plasma and tissue stores. Our results do not support that theory, but this may represent a failing in the families available to us. Goossens, Schreuder and Went[9] also described two families with hemochromatosis in which persons with abnormalities in the serum iron concentration and in iron absorption were not segregated according to this hypothesis. The number of cases in which various minor abnormalities in iron metabolism have not been associated with the inheritance of a particular haplotype is sufficiently large to suggest that only certain families fall into the category described by Bomford and colleagues.[6]

From a practical point of view the association between hemochromatosis and HLA antigens may be helpful in identifying family members at risk before clinical signs of iron overload develop.[11] Siblings having two haplotypes in common with the proband would be at high risk, whereas those having only one haplotype in common would be at low risk and could be followed up less closely; children of an affected parent would not be at risk unless a similar haplotype was

Table II—Data for sibships having one member with hemochromatosis

Family, generation and sibling no.	No. of siblings: Haplotype-identical to proband	No. of siblings: Not haplotype-identical to proband
A: II	0	7
B: II	0	3
C: II	1	3
D: I	—	—
G: II,11 to II,16	0	5
G: III,41 to III,45	0	3
Observed no.	1	21
Expected no.	5.5	16.5

$\chi^2 = 4.02$; $P < 0.045$

Table III—Data for sibships having more than one member with hemochromatosis

Family, generation and no. of designated proband	No. of siblings; haplotype(s) in common with proband*: With hemochromatosis: No haplotype	With hemochromatosis: One haplotype	With hemochromatosis: Two haplotypes	Without hemochromatosis: No haplotype	Without hemochromatosis: One haplotype	Without hemochromatosis: Two haplotypes
D: II,1	—	—	—	—	—	—
E: I,4	0	0	1	0	1	1
F: II,5	0	1	2	0	1	0
G: III,32	0	0	1	0	4	0
Total	0	1	4	0	6	1

*Absence of proband's two haplotypes among unaffected siblings was significant ($P < 0.04$).

received from the other parent. HLA typing appears to be less helpful in families in which hemochromatosis is present in successive generations because members having only one haplotype in common with the proband may be affected. Longitudinal studies of more families with idiopathic hemochromatosis are required to establish the exact place of HLA typing for identifying individuals with an increased likelihood of the disease.

We thank Don MacKinnon, John Ninness and James Haist for technical assistance in determining the serum iron concentration, transferrin saturation and serum ferritin concentration for the subjects, Drs. M.A.M. Ali, K. Smilie, A. Groll, J.B. Simon, R.M. Barr and J.W.D. McDonald for helping to investigate some of the families, Dr. P. Flanagan for advice and Pat Chymowska for assistance in the preparation of the manuscript.

This study was supported by a grant from the Medical Research Council of Canada.

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