Association of HLA-linked Hemochromatosis with Idiopathic Refractory Sideroblastic Anemia

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ABSTRACT Five of seven patients with idiopathic refractory sideroblastic anemia carried an HLA-A3 alloantigen (relative risk, 7.3; $P = 0.02$). The significance of this association was strengthened by study of two pedigrees. An abnormality in iron metabolism was found in two siblings who had an HLA-A3,B14 haplotype in common with the first proband. A second proband with idiopathic refractory sideroblastic anemia had clinically manifest hemochromatosis. His brother had clinically manifest hemochromatosis but not sideroblastic anemia. This proband and his brother shared only the HLA-A3, B12 haplotype. Our findings infer that patients with idiopathic refractory sideroblastic anemia carry a single allele for hemochromatosis, that this allele accounts for the increased iron loading in this form of anemia, and that clinically manifest hemochromatosis may develop in an occasional patient with only one allele for hemochromatosis in the presence of the sideroblastic factor.

INTRODUCTION

There is a strong positive association between hereditary hemochromatosis and the HLA-A3 alloantigen. This was shown in a study of 152 unrelated patients with the disease (1). The frequency of HLA-A3 was 72% in patients and 21% in controls, giving a relative risk value of 9.5. Close linkage of the HLA locus to the hemochromatosis locus was demonstrated in pedigree studies (2–6). We obtained a total lod score in eight pedigrees of +9.8 at a recombination fraction of 0.0 (7). This close genetic linkage indicates that recombination between the two loci will occur infrequently. Therefore, HLA genotyping of siblings of patients with hemochromatosis can be used to identify abnormal homozygotes (a pair of abnormal alleles), heterozygotes (one normal and one abnormal allele), and normal homozygotes (a pair of normal alleles). All male homozygotes over the age of 20 yr had transferrin saturations > 79% and hepatic iron concentrations > 400 μg/100 mg wet liver (major iron load). Transferrin saturation was abnormally increased (51–77%) in 31% of male heterozygotes and in 17% of female heterozygotes (minor iron load).

Idiopathic refractory sideroblastic anemia (IRSA)[1] is not known to be familial (8). Iron loading is always present and clinical manifestations of hemochromatosis have been observed in an occasional patient (9). Although it has been assumed that the iron loading is secondary to the high degree of ineffective erythropoiesis, one may ask whether such patients carry an allele for hemochromatosis. If such is the case, (*a*) an association between HLA-A3 and IRSA should be observed (*b*) abnormalities in iron metabolism should be detected in some siblings carrying one allele for hemochromatosis, and (*c*) in a sibship that is the product of a mating of two heterozygotes the occurrence of hemochromatosis in some siblings would be expected. The purpose of this paper is to report on such an association, on the detection of iron abnormalities in two siblings of one proband, and on the presence of clinically manifest hemochromatosis in a brother of a second proband.

METHODS

Seven unrelated male patients with IRSA were studied. All but one were descendants of Utah pioneers. The criteria used for the selection of patients were: (*a*) onset of anemia after 50 yr of age, (*b*) a dimorphic macrocytic anemia, (*c*) basophilic stippled hypochromic macrocytes in the blood smear, and (*d*) at least 40% ringed sideroblasts in the bone marrow (8). The leukocyte counts were 3,800–8,500/mm^3. The platelet counts were 250,000–427,000/mm^3. None of the patients were alcoholic. None received iron therapy or transfusions before evaluation of the iron status, except for SA-G-II-2, who re-

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[1] *Abbreviation used in this paper*: IRSA, idiopathic refractory sideroblastic anemia.

ceived 10 transfusions. The control population for the study of HLA alloantigens consisted of 318 descendants of Utah pioneers.

Relative risk (RR) was determined from the formula, RR = (P+)(C−)/(P−)(C+), where P is the number of patients and C the number of controls positive or negative for the HLA-A3 alloantigen (10). The frequency of the HLA-A3 antigen in the patient and in the control population were compared by chi-square analysis with Yates' correction. Published methods were used for the HLA typing (11), for the evaluation of iron status (12), and for the hematologic measurements (13).

RESULTS

The HLA haplotypes, hematologic characteristics, and iron status of the patients with IRSA are presented in Table I.

Transferrin saturation was increased in all seven patients. Serum ferritin was increased in five of the seven. Urinary iron excretion after deferoxamine was increased in four out of five.

Five of the seven patients carried an HLA-A3 alloantigen (frequency, 0.71). The frequency of this antigen in the control population was 0.255. The relative risk was 7.3. The difference between the two groups was statistically significant ($P = 0.02$).

Three siblings of the proband (II-2) in pedigree SA-B were studied along with his wife and four children (Fig. 1). The proband had no clinical manifestations of hemochromatosis. The three siblings (II-1, 4, and 5) of the proband (II-2) carried the A3,B14 haplotype in common with the proband. The transferrin saturation of two of the siblings (II-4, II-5) was increased (minor iron load). Their serum ferritin values were 174 (II-1), 124 (II-4), and 62 (II-5) ng/ml. Because we have observed a transferrin saturation > 51% in only one out of 96 genetically normal subjects (a pair of normal alleles) (7),

TABLE I

HLA Haplotypes, Hematologic Values, and Iron Status of Seven Unrelated Male Patients with Idiopathic Refractory Sideroblastic Anemia

Determination	Normal values*	Pedigree designation SA-A II-5	SA-B II-2	SA-C II-2	SA-D II-1	SA-E II-2	SA-F II-3	SA-G II-2
Age onset, *yr*		53	64	80	71	71	80	53
HLA haplotypes		A3,B12 A2,Bw22	A3,B14 A28,B5	A3,B7 A11,Bw35	A1,B8 A2,B15	A3,B7 Aw32,B5	A2,B18 Aw23,Bw21	A3,B5 Aw33,B14
Volume packed erythrocytes, *ml/100 ml*	49 44–54	32	36	36	32	35	22	21
Mean corpuscular, *volume, fl*	90 83–96	104	99	108	100	117	100	101
Reticulocytes, %	1.6 0.6–2.7	1.1	1.0	2.7	1.4	2.0	2.4	1.2
Erythrocyte porphyrins, *μg/100 ml*	29–58	66	63	33	60	30	48	68
Ringed sideroblasts, %	0.0	40	87	70	45	62	80	50
Serum iron, *μg/100 ml*	106 50–162	254	186	149	234	190	209	180
Transferrin saturation, %	32 14–50	86	74	60	82	76	82	100
Serum ferritin, *ng/ml*	93 16–542	1,331	395	625	1,340	915	215	830
Urinary iron‡ *mg/24 h*	1.3 0.4–2.2	6.9	3.3	2.8	—	2.1	—	11.7

* Mean and 98% confidence limits for male subjects.

‡ After the instramuscular injection of deferoxamine, 15 mg/kg body wt.

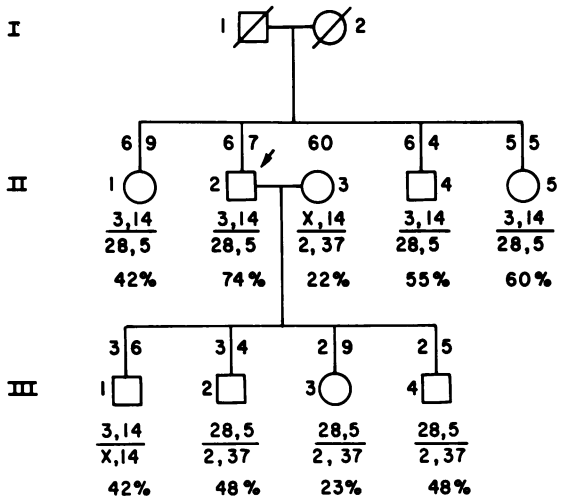

FIGURE 1 Pedigree SA-B. The squares represent males and the circles females. Pedigree numbers are given at the side, age above, and HLA haplotypes (HLA-A alloantigens followed by B alloantigens) and transferrin saturation below the squares and circles. A diagonal line through a square or circle indicates that the subject is not living. The arrow refers to the proband with IRSA. Solid squares designate clinically manifest hemochromatosis.

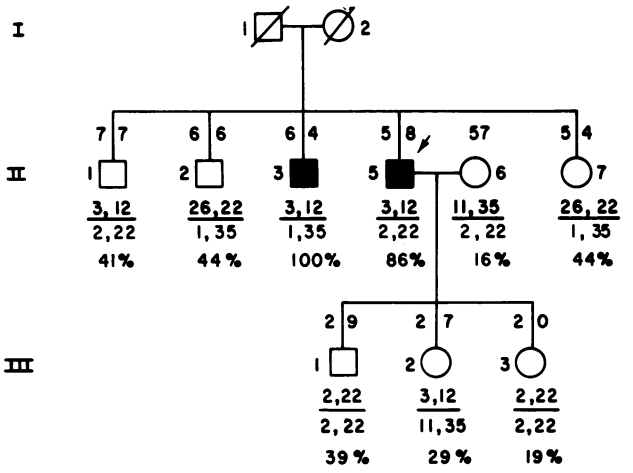

FIGURE 2 Pedigree SA-A. See legend for Fig. 1.

the presumption is that in this pedigree the A3,B14 haplotype carried an allele for hemochromatosis. All three siblings and the proband are heterozygous for the hemochromatosis allele. It is not surprising that the transferrin saturation of II-1 was not increased because we have observed an increased transferrin saturation in only 17% of female heterozygotes and the serum ferritin concentration of heterozygotes for hemochromatosis is usually normal (7).

Four siblings of the proband (II-5) in pedigree SA-A were studied along with his wife and three children (Fig. 2). The proband with sideroblastic anemia had clinically manifest hemochromatosis with skin pigmentation and histologic evidence of early cirrhosis of the liver. Hepatic parenchymal cell stainable iron was grade 4 (normal, 0–1) and the hepatic iron concentration was 602 μg/100 mg wet liver (normal, 0–29). His brother (II-3) had clinically manifest hemochromatosis with skin pigmentation and histologic evidence of cirrhosis of the liver but not sideroblastic anemia. His iron values were: serum iron, 336 μg/100 ml; transferrin saturation, 100%; serum ferritin, 3,300 ng/ml; urinary iron excretion after deferoxamine, 16.0 mg/24 h; hepatic parenchymal cell stainable iron, grade 4; and hepatic iron concentration 430 μg/100 mg wet liver. Because II-3 had clinically manifest hemochromatosis and carried the HLA-A3,B12 and A1,Bw35 haplotypes, the presumption is that these two haplotypes each carried an allele for hemochromatosis. The proband (II-5) carried only the HLA-A3,B12 haplotype in common with his brother. A second brother (II-1) carried the same two haplotypes as the proband. His transferrin saturation (41%) and serum ferritin (41 ng/ml) were within normal limits and he had none of the clinical manifestations of hemochromatosis.

Four of the other pedigrees (SA-C, D, E, and F) were too small to be informative and pedigree SA-G was not available to us for study.

DISCUSSION

Sideroblastic anemia is a heterogeneous group of disorders caused by drugs, alcohol, and various enzymatic defects (8, 9, 14). The studies reported in this paper relate to a specific and clearly defined subset of patients with sideroblastic anemia, those with IRSA (8). Only seven patients with this specific type of sideroblastic anemia were available to us for study. A larger population of patients needs to be studied to confirm the association with HLA-A3.

The frequency of the HLA-A3 alloantigen in this small group of unrelated patients with IRSA (71%) is similar to that observed in hereditary hemochromatosis (72%) (1). The significance of this association is strengthened by our finding clinically manifest hemochromatosis in a sibling of one proband and abnormalities in iron metabolism (minor iron load) in two siblings of a second proband. This suggests that patients with IRSA carry a single allele for hemochromatosis and that this allele plays a role in the increased iron loading observed in this form of anemia. However, because sideroblastic anemia is not a feature of hereditary hemochromatosis and since most heterozygotes for hemochromatosis do not develop sideroblastic anemia, it is apparent that a second factor, the sideroblastic factor, is required for the development of anemia. IRSA has not been observed in more than one member of a family in our studies or by others (8, 9). Whether the

sideroblastic factor is genetic or acquired cannot be stated from the limited information available. In any event, it seems that at least one allele for hemochromatosis and the sideroblastic factor are required for the expression of IRSA and that clinically manifest hemochromatosis may develop in an occasional patient with only one allele for hemochromatosis in the presence of the sideroblastic factor.

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