

New Diallelic Markers in the HLA Region of Chromosome 6

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ABSTRACT: Diallelic polymorphisms have been identified in the HLA-H gene and the ZNF192 gene located about 2 megabases centromeric to HLA-H. The three polymorphic sites in HLA-H together with the two hemochromatosis mutations in this gene give rise to 8 different haplotypes. The three polymorphic sites in ZNF192 give rise to 4 different haplotypes. The haplotypes in HLA-H are in complete linkage disequilibrium with the two common mutations in that gene, 845A (C282Y) and 187G (H63D). The 845A mutation is in weak linkage disequilibrium with the ZNF192 polymorphisms and the 187G mutation appears to be in equilibrium with this polymorphism. The 187G mutation therefore appears to be the older of the two HLA-H mutations.

Keywords: HLA-H, hemochromatosis, linkage, mutation

INTRODUCTION

The genetic diversity of the HLA system led to relatively early recognition that a considerable number of disease states were caused or influenced by genes in the 6p21.3 region. Among these was hereditary hemochromatosis, which was found to be linked to HLA-A and B in 1975 by Simon et al (1). It was only in 1996 that the putative gene for this disorder designated HLA-H was identified (2); over 80% of patients with hemochromatosis are homozygous for the 845A mutation in this gene (2,3). A major part of the difficulty in identifying the gene for hereditary hemochromatosis resulted from the fact that this region of the genome is one in which crossovers appear to be relatively rare. Moreover, although there are many polymorphic markers in this region (2,4-13), most of these are repeat sequences, unstable by their very nature, and therefore less suitable for studies of linkage

disequilibrium in populations than are diallelic polymorphisms.

In the course of sequencing the HLA-H gene and other candidate genes for hemochromatosis we have encountered a number of diallelic polymorphisms that may prove to be useful in genetic studies involving this important region.

MATERIALS AND METHODS

We studied 43 patients with primary hemochromatosis, presumably hereditary hemochromatosis, although in many of the cases there was no family history of iron storage disease. DNA was isolated from blood leukocytes using standard methods. Of the 39 Caucasian patients with hemochromatosis 24 were homozygous for the 845A(C282Y) mutation, the typical mutation in hereditary hemochromatosis. Another 4 of the Caucasian patients were compound heterozygotes for the 845A and 187G(H63D) mutations, a

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genotype that has been shown to cause hemochromatosis, but with low penetrance (2,3). Of the remaining 11 Caucasian patients one had the 845A/845G,187C/187C genotype, 4 the 845G/845G, 187C/187G genotype, 2 the 845G/845G, 187G/187G genotype, and 4 the "wild type" 845G/845G, 187C/187C genotype. All four hemochromatosis patients of Asian extraction also manifested the 845G/845G, 187C/187C genotype.

The control groups consisted of 35 Caucasian, 28 Asian and 13 Afro-American subjects.

All samples were typed for the 845A and 187G mutations in HLA-H as previously described (3). The microsatellite markers D6S265, D6S105, and D6S1260 were typed on DNA samples from the hemochromatosis patients and Caucasian control subjects (11). Most of the samples were also typed for three mutations of HLA-H located in introns 2, 4 and 5 (14) and for 3 polymorphic sites located at cDNA nt 480, 840, and 1220 in a gene that we have designated ZNF192 (15). These markers were evaluated by PCR and either restriction endonuclease digestion or allele specific oligonucleotide hybridization (ASOH) using the methods described in Table 1.

PCR amplifications were performed in a 100 μ l reaction containing the following: 33.5 mM Tris HCl pH8.3; 8.3 mM $(\text{NH}_4)_2\text{SO}_4$; 85 μ g/ml BSA; 3.35 mM MgCl_2 ; 5% DMSO; 0.2 mM dNTP's; 250 ng of each oligonucleotide primer (Table 1) and 0.5 to 1 μ g of genomic DNA. After denaturation at 95 C for 4 minutes, DNA amplification proceeded for 30 cycles of 94 C for 30 seconds, 55 to 58 C for 30 seconds and 72 C for 30 to 120 seconds.

Restriction endonuclease digests were performed on 10 to 15 μ l of unpurified PCR product according to the manufacturers recommendations (Table 1). The digested DNA was then electrophoresed on a 10% acrylamide vertical gel in $\frac{1}{2}$ x TBE and visualized by staining with ethidium bromide. Either pBR322/MspI or ϕ x/Hae III digested DNAs were

used as markers to estimate band sizes.

Allele specific oligonucleotides (Table 1), ^{32}P labeled with $\gamma^{32}\text{P}$ ATP and T4 polynucleotide kinase, were hybridized overnight at 42 C with 4 μ l of each amplified DNA spotted on nylon membranes (3). The blots were then washed in 6X SSC, 0.1% SDS to the temperature at which non-matched oligonucleotides melt from the immobilized DNA (Table 1) and visualized by exposure to XAR II Xray film.

A map of the location of HLA-H and ZNF192 in relation to other polymorphic markers, all variable length repeats, is shown in figure 1.

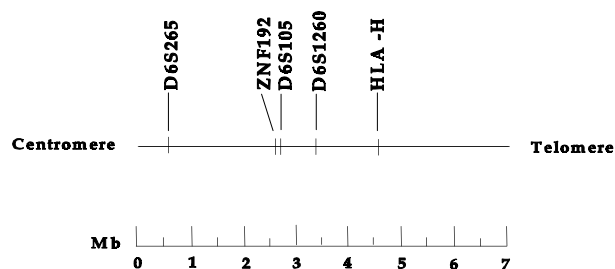


Figure 1. The relative locations of HLA-H, microsatellite markers, and ZNF 192 in the 6p21.3 region of chromosome 6 (2).

RESULTS

The results of our analyses are shown in Tables 2 and 3.

HLA-H Haplotypes

Table 4 presents the 8 possible combinations of the 3 diallelic intron polymorphisms in HLA-H. The most common (wild type) haplotype within the HLA-H gene of Caucasians was T,T,G (haplotype 1). All patients with the 845A/845A genotype had this haplotype, and it has been designated haplotype 1h when it includes the 845A mutation. In contrast, all of the patients who had the 845A/187G genotype were T/C at the intron 2 site and G/A at the intron 5 site, so

that their haplotype is deduced to be T,T,G/C,T,A (haplotype 1/haplotype 6). When the 187G was present this haplotype was designated haplotype 6h. The occurrence of other haplotypes was also apparent (Table 4). Of the eight possible combinations, only haplotypes 3 and 7 were not unequivocally represented. Among Asians haplotype 8 was the most

common. 14/24 Asian control subjects were homozygous for this haplotype and 11 were typed as C/T, C/T, A/G, suggesting heterozygosity for the common European haplotype 1 and the common Asian haplotype 8. The haplotypes of the four Asian patients with hemochromatosis did not differ from the distribution in the normal Asian population.

Table 1.

Gene	Mutation Position	Amplification Primers	Detection Method	Fragment Sizes, ASOH Oligonucleotides
HLA-H	IVS2(+4) t/c	5'-CATGAGAGTCGCCGTGTG-3'	Bsa A I digest	t = 232 bp c = 158, 74 bp
		5'-cagctgttctctcaaatgc-3'	Rsa I digest	t = 232 bp c = 156, 76 bp
	IVS4(-44) t/c	5'-gcctgaggaggaattatggc-3'	Hae III digest	t = 242 bp c = 58, 184 bp
5'-gagactcccccttgttct-3'		Sau 96 I digest	t = 242 bp c = 54, 188 bp	
IVS5(-47) g/a	5'-tgggtgaatgagaaaataagg-3'	5'-ctaggatcaccggcatg-3'	Ban I digest	a = 340 bp g = 42, 298 bp
Znf 192	488 C-T (P163L)	5'-cattgttcattacattcgcaaag-3'	ASOH	C 5'-ACCAGAGCCTCCAAATAC-3', 54 C T 5'-ACCAGAGCTTCCAAATAC-3', 52-53 C
		5'-gttcttcaggaaactagataggta-3'		
	840 G-A (Q280Q)	5'-tcctagcataaagaataggttg-3'	ASOH	G 5'-TGGAATCCAGCCACATG-3', 54 C A 5'-TGGAATCCAACCACATG-3', 51-52 C
5'-tgggcccaataactaaagag-3'				
1220 A-G (Q407R)	5'-tcctagcataaagaataggttg-3'	5'-tgggcccaataactaaagag-3'	ASOH	A 5'-TGATTGCACTGATATGGC-3', 54 C G 5'-TGATTGCACCGATATGGC-3', 52 C

Table 2.

Approximate Distance from HLA-H, Mb Gene / Microsatellite	0			1.25		1.8		2.0		4.0		
	IVS 2(+4)	IVS 4(-44)	HLA-H	D6S1260	D6S105	H 154	H 124	488	840	1220	ZNF 192	D6S265
Mutation / Allele size bp	845	187										
Hemochromatosis Patients												
Caucasian												
1	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	A/A	A/A	3/3	127/127
2	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/A	A/A	1/3	123/133
3	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/A	A/A	1/3	127/133
4	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/A	A/A	1/3	131/133
5	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/A	G/A	1/4	123/123
6	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/A	G/A	1/4	123/131
7	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	123/127
8	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	123/131
9	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	123/123
10	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	129/133
11	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	123/123
12	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	123/123
13	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	123/129
14	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	129/129
15	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	123/133
16	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	123/123
17	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	123/133
18	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	123/127
19	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	123/123
20	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	129/131
21	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/C	G/G	A/A	1/1	127/133
22	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/T	G/G	A/A	1/5	123/127
23	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/T	G/G	A/A	1/5	127/133
24	A/A	C/C	T/T	T/T	G/G	1b/1h	1b/1h	C/T	G/G	A/A	1/5	123/127

Approximate Distance from HLA-H, Mb Gene / Microsatellite	0			1.25		1.8		2.0		4.0			
	845	187	IVS 2(+4)	HLA-H IVS 4(-44)	IVS 5(-47)	Haplotype*	D6S1260 H 154	D6S105 H 124	488	840	1220	ZNF 192 Haplotype*	D6S265 H 123
Mutation / Allele size bp	G/A	C/C	T/T	T/T	G/G	1/1h	154/162	128/128	C/C	G/G	A/A	1/1	127/127
Hemochromatosis Patients	G/A	C/G	T/C	T/T	G/A	<i>1h/6h</i>	152/154	124/124	C/C	G/A	A/A	1/3	127/131
Caucasian	G/A	C/G	T/C	T/T	G/A	<i>1h/6h</i>	154/158	124/128	C/C	G/G	A/A	1/1	123/133
	G/A	C/G	T/C	T/T	G/A	<i>1h/6h</i>	154/154	124/124	C/C	G/A	A/A	1/3	123/131
	G/A	C/G	T/C	T/T	G/A	<i>1h/6h</i>	152/154	128/132	C/C	G/G	A/A	1/1	127/133
	G/G	C/C	T/T	T/T	G/A	1/2	152/162	126/128	C/T	G/A	A/A	1/7	127/127
	G/G	C/C	T/T	T/T	G/A	1/2	160/162	118/126	C/T	G/G	A/A	1/5	127/127
	G/G	C/C	T/T	T/T	G/G	1/1	156/164	136/136	C/C	G/G	A/A	1/1	127/129
	G/G	C/C	T/T	T/T	G/G	1/1	156/158	126/128	C/C	G/G	A/A	1/1	123/133
	G/G	C/G	C/C	T/T	A/A	6h/6		124/128	C/C	G/A	A/A	1/3	127/133
	G/G	C/G	T/C	T/T	A/A	<i>2/6h</i>	152/154	128/134	C/C	G/G	A/A	1/1	123/131
	G/G	C/G	T/C	T/T	A/A	<i>2/6h</i>	154/154	128/132	C/C	G/G	A/A	1/1	133/133
	G/G	C/G	T/C	T/T	G/A	<i>1/6h</i>	152/152	128/130	C/C	G/G	A/A	1/1	133/133
	G/G	G/G	C/C	T/T	A/A	6h/6h	152/160	124/128	C/T	G/G	A/A	1/5	127/133
	G/G	G/G	C/C	T/T	A/A	6h/6h	152/154	124/132	C/C	G/G	A/A	1/1	123/133
Asian													
	G/G	C/C	C/C	C/C	A/A	8/8	154/156	118/136	C/C	G/G	A/A	1/1	129/131
	G/G	C/C	C/C	C/C	A/A	8/8	154/156	122/124	C/C	G/A	A/A	1/3	129/131
	G/G	C/C	T/C	T/C	G/A	<i>1/8</i>			C/C	G/G	A/A	1/1	
	G/G	C/C	T/C	T/T	A/A	2/6	152/158	122/126	C/C	G/G	A/A	1/1	123/123

*deduced from the markers. Unambiguous haplotypes are in roman type, probable haplotypes in italics

Table 3.

Gene / Microsatellite	Approximate Distance from HLA-H, Mb		0		1.25		1.8		2.0		4.0		
	187	845	IVS 2(+4)	IVS 4(-44)	IVS 5(-47)	Haplotype*	D6S1260	D6S105	H 124	D6S265	ZNF 192	Haplotype*	
Mutation / Allele size bp													
Control Subjects													
Caucasian													
1	G/A	C/C	C/T	T/C	G/A	8/1h	150/152	124/128	C/C	G/A	A/A	1/3	127/129
2	G/A	C/C	C/T	T/C	G/A	8/1h	154/154	124/130	C/C	G/G	A/A	1/1	131/131
3	G/A	C/C	C/T	T/T	G/A	6/1h	154/156	124/128	C/C	G/G	A/A	1/1	127/133
4	G/A	C/C	T/T	T/T	G/A	2/1h	154/154	130/130	C/C	G/G	A/A	1/1	127/133
5	G/A	C/C	T/T	T/T	G/G	1/1h	152/154	126/130	C/C	G/A	A/A	1/3	129/133
6	G/A	C/C	T/T	T/T	G/G	1/1h	154/154	124/126	C/C	G/G	A/A	1/1	127/133
7	G/G	C/C	C/C	T/C	A/A	6/8	156/158	124/126	C/T	G/G	A/A	1/5	123/127
8	G/G	C/C	C/C	T/C	G/A	6/7	154/158	130/134	C/C	G/A	A/A	1/3	127/127
9	G/G	C/C	C/T	T/C	A/A	8/2	154/154	130/136	C/C	G/G	A/A	1/1	127/133
10	G/G	C/C	C/T	T/C	A/A	8/2	152/158	124/134	C/T	G/G	A/A	1/5	127/133
11	G/G	C/C	C/T	T/C	G/A	1/8	154/158	126/130	C/C	G/A	A/A	1/3	135/139
12	G/G	C/C	C/T	T/T	A/A	6/1	152/154	126/130	C/C	G/A	A/A	1/3	129/133
13	G/G	C/C	C/T	T/T	A/A	6/1	154/162	126/128	C/T	G/G	A/A	1/5	127/133
14	G/G	C/C	C/T	T/T	A/A	6/1	154/158	124/128	C/C	G/G	A/A	1/1	129/133
15	G/G	C/C	C/T	T/T	A/A	6/1	150/156	128/134	C/C	A/A	A/A	3/3	127/131
16	G/G	C/C	C/T	T/T	G/A	5/1	152/156	124/128	C/C	G/A	A/A	1/3	127/131
17	G/G	C/C	C/T	T/T	G/A	5/1	152/154	128/130	C/C	G/G	A/A	1/1	127/127
18	G/G	C/C	C/T	T/T	G/A	5/1	154/162	126/128	C/T	G/A	A/A	1/5	127/127
19	G/G	C/C	T/T	T/C	A/A	2/4	154/160	124/130	C/T	G/G	A/A	1/5	127/131
20	G/G	C/C	T/T	T/T	G/A	1/2	154/154	128/128	C/C	G/G	A/A	1/1	131/133
21	G/G	C/C	T/T	T/T	G/A	1/2	154/162	124/134	C/C	G/G	A/A	1/1	123/131
22	G/G	C/C	T/T	T/T	G/G	1/1	154/154	128/132	C/C	G/G	A/A	1/1	129/133
23	G/G	C/C	T/T	T/T	G/G	1/1	154/162	126/130	C/T	G/G	A/A	1/5	127/131

Approximate Distance from HLA-H, Mb	0			1.25		1.8		2.0			4.0
	Gene / Microsatellite	HLA-H	D6S1260	D6S105	ZNF 192	D6S265					
Mutation / Allele size bp	845	187	IVS 2(+4)	IVS 4(-44)	IVS 5(-47)	Haplotype*					
Control Subjects											
Caucasian											
24	G/G	C/C	T/T	T/T	G/G	1/1					
25	G/G	C/G	C/T	T/C	A/A	6h/4					
26	G/G	C/G	C/T	T/T	A/A	6h/2					
27	G/G	C/G	C/T	T/T	A/A	6h/2					
28	G/G	C/G	C/T	T/T	G/A	6h/1					
29	G/G	C/G	C/T	T/T	G/A	6h/1					
30	G/G	C/G	C/T	T/T	G/A	6h/1					
31	G/G	C/G	C/T	T/T	G/A	6h/1					
32	G/G	C/C	C/T	T/T	G/G	5/1					
33	G/G	C/G	C/C	T/T	G/A	5/6h					
34	G/G	G/G	C/C	T/T	A/A	6h/6h					
35	G/G	G/G	C/C	T/T	A/A	6h/6h					
Asian											
36	G/G	C/C	C/C	C/C	A/A	8/8					
37	G/G	C/C	C/C	C/C	A/A	8/8					
38	G/G	C/C	C/C	C/C	A/A	8/8					
39	G/G	C/C	C/C	C/C	A/A	8/8					
40	G/G	C/C	C/C	C/C	A/A	8/8					
41	G/G	C/C	C/C	C/C	A/A	8/8					
42	G/G	C/C	C/C	C/C	A/A	8/8					
43	G/G	C/C	C/C	C/C	A/A	8/8					
44	G/G	C/C	C/C	C/C	A/A	8/8					
45	G/G	C/C	C/C	C/C	A/A	8/8					
46	G/G	C/C	C/C	C/C	A/A	8/8					
47	G/G	C/C	C/C	T/C	A/A	6/8					
48	G/G	C/C	C/C	T/C	G/A	5/8					
49	G/G	C/C	C/T		G/A						
50	G/G	C/C	C/T	T/C	A/A	8/2					
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Approximate Distance from HLA-H, Mb	0			1.25		1.8		2.0		4.0	
	HLA-H			D6S1260	D6S105	ZNF 192		D6S265		Haplotype*	
Gene / Microsatellite	845	187	IVS 2(+4)	IVS 4(-44)	IVS 5(-47)	Haplotype*		488	840	1220	Haplotype*
Mutation / Allele size bp											
Control Subjects											
Asian											
51	G/G	C/C	C/T	T/C	G/A	8/1		C/C	G/A	A/A	1/3
52	G/G	C/C	C/T	T/C	G/A	8/1		C/C	G/G	A/A	1/1
53	G/G	C/C	C/T	T/C	G/A	8/1		C/C	G/G	A/A	1/1
54	G/G	C/C	C/T	T/C	G/A	8/1		C/C	G/G	A/A	1/1
55	G/G	C/C	C/T	T/C	G/A	8/1		C/C	A/A	A/A	3/3
56	G/G	C/C	C/T	T/C	G/A	8/1		C/C	G/G	A/A	1/1
57	G/G	C/C	C/T	T/C	G/A	8/1		C/C	G/A	A/A	1/3
58	G/G	C/C	C/T	T/C	G/A	8/1		C/C	G/G	A/A	1/1
59	G/G	C/C	C/T	T/C	G/A	8/1		C/C	G/G	A/A	1/1
60	G/G	C/C	C/T	T/T	G/A	8/1		C/C	G/G	A/A	1/1
61	G/G	C/C	T/T	T/T	G/G	1/1		C/C	G/G	A/A	1/1
62	G/G	C/C	T/T	T/T	G/G	1/1		C/C	G/G	A/A	1/1
63	G/G	C/G	C/T	T/T	G/A	6/1		C/C	G/G	A/A	1/1
Blacks											
64	G/A	C/C	C/T	T/T	G/A	6/1h		C/C	G/A	A/A	1/3
65	G/G	C/C	C/T	C/T	A/A	2/8		C/C	G/A	A/A	1/3
66	G/G	C/C	C/T	C/T	A/A	2/8		C/C	G/A	A/A	1/3
67	G/G	C/C	C/T	C/T	G/A	8/1		C/C	G/G	A/A	1/1
68	G/G	C/C	C/T	C/T	G/A	8/1		C/C	G/A	A/A	1/3
69	G/G	C/C	C/T	T/T	G/A	6/1		C/C	G/G	A/A	1/1
70	G/G	C/C	C/T	T/T	G/A	6/1		C/C	G/G	A/A	1/1
71	G/G	C/C	C/T	T/T	G/A	6/1		C/C	G/G	A/A	1/1
72	G/G	C/C	T/T	T/T	G/A	1/2		C/C	G/G	A/A	1/1
73	G/G	C/C	T/T	T/T	G/A	1/2		C/C	G/G	A/A	1/3
74	G/G	C/C	T/T	T/T	G/G	1/1			G/A	A/A	
75	G/G	C/C	T/T	T/T	G/G	1/1		C/C	G/G	A/A	1/1
76	G/G	C/C	T/T	T/T	G/G	1/1		C/C	G/A	A/A	1/3

*deduced from the markers. Unambiguous haplotypes are in roman type, probable haplotypes in italics

Table 4. HLA-H Haplotypes Deduced from the Data in Tables 2 and 3

Haplotype Designation	Nucleotides (IVS 2,4,5)	Haplotype Frequency		
		Normal European	Hemochromatosis European	Asian
1	T,T,G	0.329	0.103	0.278
1h	T,T,G & 845A	0.086	0.680	0
2	T,T,A	0.114	0.050	0.019
3	T,C,G	0	0	0
4	T,C,A	0.029	0	0
5	C,T,G	0.071	0	0.019
6	C,T,A	0.100	0.013	0.037
6h	C,T,A & 187G	0.171	0.154	0
7	C,C,G	0.014	0	0
8	C,C,A	0.086	0	0.648

Table 5. ZNF 192 Haplotypes Deduced from the Data in Tables 2 and 3

Haplotype Designation	cDNA nt 488,840,1220	Haplotype Frequency		
		Normal European	Hemochromatosis European	Asian
1	C,G,A	0.743	0.795	0.852
2	C,G,G	0	0	0
3	C,A,A	0.157	0.102	0.148
4	C,A,G	0	0.026	0
5	T,G,A	0.100	0.064	0
6	T,G,G	0	0	0
7	T,A,A	0	0.012	0
8	T,A,G	0	0	0

ZNF 192 Haplotypes

The most common (wild type) haplotype within the ZNF 192 gene was C,G,A, and this is designated haplotype 1. This was apparently the ancestral haplotype in which the HLA-H 845A arose. 15/24 (62.5%) of hemochromatosis patients with the 845A/845A genotype were homozygous for this haplotype compared with 19/35 (54.3%) of the normal Caucasian controls. Of the 9 845A/845A hemochromatosis patients who were not homozygous for the C,G,A haplotype, 8 *could* have been heterozygotes for this haplotype, while among the Caucasian controls 14/35 were heterozygotes. Thus, assuming the these genotype assignments are correct the P_{excess} is only 0.19 in the hemochromatosis patients with the most common haplotype. The ZNF 192 A-G mutation at nt1220 was relatively rare, being found in only two subjects, both of them with the typical 845A/845A genotype. Assuming that the other allele in these patients was C,G,A, both would be classified as having the C,A,G haplotype (haplotype 4).

DISCUSSION

There is a paucity of known diallelic markers between HLA-A and HLA-H. We have found three such markers in the HLA-H gene itself and three located in the exons of the ZNF 192 gene, located about midway between HLA-H and HLA-A. Not surprisingly, the 6 haplotypes of the HLA-H gene were in complete linkage disequilibrium with the 845A mutation in this gene. The linkage disequilibrium between HLA-H and the haplotypes ZNF 192 was weak, weaker, as expected than that between HLA-H and the microsatellite D6S105, which is closer to the HLA-H gene.

Feder et al(2) found no linkage disequilibrium between the 187G mutation of HLA-H and the previously known markers. However, there is complete disequilibrium between this mutation and the haplotypes of the HLA-H gene: all cases

were in the context of haplotype 6. On the other hand, as indicated by the findings of Feder et al(2), this mutation was in complete equilibrium with the diallelic markers of ZNF 192 and of all of the microsatellites at which the subjects were typed. This, as well as the panethnic distribution of this mutation in contrast with that of the exclusively European 845A mutation indicates that the 845A is of relatively ancient origin. Recently developed epidemiologic evidence(16) suggests that the 187G mutation itself may slightly augment iron absorption. If this turns out to be the case, it may prove to be an old polymorphism that decreases the incidence of iron deficiency.

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