

## COMMENTARY

Feder et al. have recently identified a candidate gene for hereditary hemochromatosis (HH) (1). The gene is located on the short arm of chromosome 6 telomeric to the major histocompatibility complex and exhibits sequence homology to HLA class I markers, leading to its designation by Feder et al. as HLA-H. Feder et al. noted two mutations in their patient population: a G→A transition at nucleotide 845 and a C→G transversion at nucleotide 187. In a patient population of 178 individuals, 148 were homozygous and 9 were heterozygous for the G845A variant; 8 of the 9 heterozygotes carried the C187G variant. In contrast, the allele frequencies of the two variants in a control population were 3.2% and 17%.

The accompanying paper by Beutler et al. confirms the strong association between these HLA-H variants and HH. We take this opportunity to add our own findings with regard to

the occurrence of the 845A variant in a HH patient population; we have not tested for the 187G variant. Our patient population consists of 56 individuals initially identified by transferrin saturation and serum ferritin testing; most diagnoses have been verified by liver biopsy. We have assayed for the 845A variant by restriction enzyme digestion of the PCR amplification product obtained using the Feder et al. Cys282Tyr primer set (1); the 845G→A nucleotide substitution creates a *RsaI* restriction site. (Experimental details can be provided on request.) Of the 56 individuals in our patient population, 45 are A/A homozygotes, 6 are A/G heterozygotes, and 5 are G/G homozygotes. These numbers translate to an allele frequency for the 845A variant of 0.857; ironically, the exact same frequency value has been found both by Feder et al. (305 of 356 chromosomes) and by Beutler et al. (252 of 294 chromosomes).

Table 1. Haplotype Analysis in Hemochromatosis

Locus	Allele	Allele Frequency, Control Subjects	Allele Frequency, HH Subjects	p(excess)	Allele Frequency, 845A/845A Subjects	p(excess)
s265	123	0.207	0.386	0.226	0.433	0.285
HLA-F3'	206	0.247	0.491	0.324	0.522	0.365
MOGc	130	0.28	0.464	0.256	0.511	0.321
D6S258	200	0.51	0.64	0.265	0.722	0.433
D6S306	239	0.556	0.711	0.349	0.767	0.475
D6S105	124	0.155	0.561	0.480	0.622	0.553
D6S464	202	0.571	0.76	0.441	0.798	0.529
D6S1260	151	0.15	0.754	0.711	0.822	0.791
D6S2238	105	0.36	0.798	0.684	0.878	0.809
D6S1281	199	0.3	0.368	0.097	0.411	0.159
"	203	0.28	0.404	0.172	0.367	0.121
D6S276	222	0.331	0.43	0.148	0.456	0.187

Genetic typing at the indicated microsatellite loci was performed as previously described (1,2).

Alleles are designated by amplified product size in bp.

It is of interest to review retrospectively the status of the genetic markers on chromosome 6 used to map the HH gene in the light of these HLA-H typings. Table 1 shows the frequencies of HH associated alleles at 11 loci for the total patient population and for the population subset defined by the A/A type; p(excess) values relative to a control population are shown for both. It is clear that the D6S2238 locus shows the strongest association in both the HH patient population and the A/A population subset. This locus was one of the several identified by Feder et al. as tightly associated with HH (1). Comparison of allele frequencies in the two populations shows the disease associated allele to be at higher frequency in the A/A population at all but the D6S1281 locus. This supports the case that the indicated alleles represent the ancestral haplotype. With regard to the D6S1281 locus, two alleles are associated with HH and this dual association is not dissolved by HLA-H typing; possibly a mutation or recombination event occurred at this locus shortly after the mutation that gave rise to HH.

Although the findings reported by Beutler et al. and in this commentary confirm the very strong association between HH and the 845A variant of HLA-H, they do not prove that a defect in HLA-H

is the determinative cause of HH. This proof must await studies demonstrating a functional relationship between HLA-H and iron metabolism.

#### REFERENCES

1. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary hemochromatosis. *Nature Genetics* 13:399-408, 1996.
2. Calandro LM, Baer D, Sensabaugh GF. Characterization of a recombinant that locates the hereditary hemochromatosis gene telomeric to HLA-F. *Hum Genet* 96:339-342, 1995.

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