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The Hemochromatosis 845 G→A and 187 C→G Mutations: Prevalence in Non-Caucasian Populations

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Summary

Hemochromatosis, the inherited disorder of iron metabolism, leads, if untreated, to progressive iron overload and premature death. The hemochromatosis gene, HFE, recently has been identified, and characterization of this gene has shown that it contains two mutations that result in amino acid substitutions—cDNA nucleotides 845 G→A (C282Y) and 187 C→G (H63D). Although hemochromatosis is common in Caucasians, affecting ⩾1/300 individuals of northern European origin, it has not been recognized in other populations. The present study used PCR and restriction-enzyme digestion to analyze the frequency of the 845 G→A and 187 C→G mutations in HLA-typed samples from non-Caucasian populations, comprising Australian Aboriginal, Chinese, and Pacific Islanders. Results showed that the 845 G→A mutation was present in these populations (allele frequency 0.32%), and, furthermore, it was always seen in conjunction with HLA haplotypes common in Caucasians, suggesting that 845 G→A may have been introduced into these populations by Caucasian admixture. 187 C→G was present at an allele frequency of 2.68% in the two populations analyzed (Australian Aboriginal and Chinese). In the Australian Aboriginal samples, 187 C→G was found to be associated with HLA haplotypes common in Caucasians, suggesting that it was introduced by recent admixture. In the Chinese samples analyzed, 187 C→G was present in association with a wide variety of HLA haplotypes, showing this mutation to be widespread and likely to predate the more genetically restricted 845 G→A mutation.

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Introduction

Hereditary hemochromatosis (MIM 235200), an autosomal recessive disorder of iron metabolism, leads to inappropriately high iron absorption. If hemochromatosis is left undiagnosed and untreated, the excess iron is stored in the parenchymal cells of major organs, primarily the liver, pancreas, heart, pituitary, and joints, eventually leading to severe tissue damage and premature death (Powell et al. 1994).

The hemochromatosis gene, HFE (GenBank accession number U60319), is located ~5 Mb distal of HLA-A and shows significant similarity to major histocompatibility class I genes (Feder et al. 1996). Two single-nucleotide changes have been described in HFE; a G→A transition at nucleotide 845 of the open reading frame, resulting in an amino acid substitution of cysteine by tyrosine (845 G→A; C282Y) and a C→G transition at nucleotide 187 resulting in a substitution of histidine by aspartic acid (187C→G; H63D).

Functional studies have shown that 845 G→A disrupts the interaction of HFE with β_2-microglobulin, resulting in intracellular sequestration of HFE, since the formation of a heterodimer between HFE and β_2-microglobulin is required for the correct cell-surface presentation of HFE (Feder et al. 1997). In contrast, 187 C→G does not disrupt binding of HFE with β_2-microglobulin, and the mature molecule is expressed on the cell surface; however, 187 C→G may disrupt binding of HFE to as yet unidentified ligands (Feder et al. 1997).

Some 80%–100% of hemochromatosis patients are homozygous for 845 G→A (Beutler et al. 1996; Callandro et al. 1996; Feder et al. 1996; Jazwinska et al. 1996; Jouanolle et al. 1996), providing strong evidence that 845 G→A in HFE is the primary causative mutation in hemochromatosis. 187 C→G has also been shown to be involved in hemochromatosis, with some homozygotes and compound heterozygotes—that is, subjects who carry both 845 G→A and 187 C→G—showing clinical expression (Beutler 1997*a*, 1997*b*; Martinez et al. 1997; Risch 1997; Sham et al. 1997).

Hemochromatosis is well recognized as a common disorder in populations of northern European origin,

Table 1

Results of 845 G→A Analyses

Population	No. of Chromosomes		
	Total	845G	845A
Australian Aboriginal:			
Cape York (n = 93)	186	184	2 (1.07%)
Groote Eylandt (n = 92)	184	184	0
Northern Chinese (n = 65)	130	130	0
Southern Chinese (n = 93)	186	186	0
Chinese Families (n = 84)	168	168	0
Javanese (n = 68)	136	136	0
Melanesian (n = 139)	278	277	1 (.36%)
Micronesian (n = 106)	212	212	0
Polynesian (n = 169)	338	335	3 (.89%)
Nepalese (n = 116)	232	232	0
Total (n = 941)[a]	1,882	1,876	6 (.32%)

[a] Excludes Chinese-family samples.

with ~1/300 Caucasians expressing the disorder (Edwards et al. 1988; Leggett et al. 1990). It has not been widely recognized in populations other than Caucasians; however, it is unclear whether this lack of expression in other populations is due to dietary differences affecting clinical expression or is due to differences in gene frequency. The recent identification of HFE has now made it possible to screen for the 845 G→A and 187 C→G mutations in HFE, in order to analyze their geographical distribution. In a recent study, 2,978 subjects from a variety of ethnic backgrounds were screened for 845 G→A and 187 C→G, and results showed worldwide allele frequencies of 1.9% for 845 G→A and 8.1% for 187 C→G (Merryweather-Clarke et al. 1997).

845 G→A was found to be most prevalent in northern European populations, with the highest frequency being seen in 90 Irish chromosomes (allele frequency 10%); and it was absent from the African, Asian, and Australasian populations studied. 187 C→G was found to be present in almost all populations, with the highest frequency in 56 Basque chromosomes (allele frequency 30.4%). Another study (Chang et al. 1997) found that 845 G→A was very rare in a Chinese population, showing a 0.33% carrier rate. Neither of these studies used any genetic markers to indicate the ancestry of the samples screened. The present study screened HLA-typed samples from various non-Caucasian populations, for both 845 G→A and 187 C→G, to determine the prevalence of these mutations in populations in which hemochromatosis is rare—and to use this in conjunction with the distribution of HLA alleles, in order to determine the likely ancestral origin of 845 G→A and 187 C→G.

Table 2

HLA Haplotypes of Subjects Carrying 845 G→A

Population	Haplotype of	
	HLA-A	HLA-B
Cape York Australian Aboriginal	0301[a]/3401	0702[a]/1301
	3201[a]/2402	4801[a]/3503
Melanesian (New Caledonia)	0201/2402	4002/5601
Polynesian (Cook Islands)	3002[a]/2402	0702[a]/5502
Polynesian (Western Samoa)	0301[a]/0206	0702[a]/4010
Polynesian (Western Samoa)	0301[a]/2402	...

[a] Caucasian specific or universal allele.

Subjects, Material, and Methods

Samples for 845 G→A Analysis

Samples were collected from Australian Aboriginal Cape York (n = 93), Australian Aboriginal Groote Eylandt (n = 92), northern Chinese (n = 65), southern Chinese (n = 93), Chinese family (n = 84), Javanese (n = 68), Melanesian (n = 139), Micronesian (n = 106), Polynesian (n = 169), and Nepalese (n = 116) subjects (total = 1,025). Subjects were unrelated except for 84 subjects from Chinese families. These samples were screened for 845 G→A by use of PCR and restriction-enzyme digestion, as described below.

Samples for 187 C→G Analysis

Australian Aboriginal Cape York population (n = 90), Australian Aboriginal Groote Eylandt population (n = 101), northern Chinese (n = 67), southern Chinese (n = 97), and Chinese family (n = 88) subjects were screened for 187 C→G (total = 443), by use of PCR and restriction-enzyme digestion, as described below. Subjects were unrelated except for 88 subjects from Chinese families.

DNA Extraction

Genomic DNA was extracted, by means of standard methods (Sambrook et al. 1989), from whole blood.

PCR Amplification and Restriction-Enzyme Digestion of HFE Mutations

PCR amplification of the HFE regions containing 845 G→A and 187 C→G was performed as described elsewhere (Cullen et al. 1997), by means of oligonucleotide primers described elsewhere (Feder et al. 1996). The annealing temperatures were 55°C, for amplification of the region containing 845 G→A, and 60°C, for amplification of the region containing 187 C→G. Detection of 845 G→A was by *Sna*BI digestion, as described elsewhere (Cullen et al. 1997).

The presence of 187 C→G results in the loss of a restriction site for the enzyme *Dpn*II. Samples were di-

gested with 2 U *Dpn*II (New England Biolabs) for 2 h at 37°C, in a total volume of 20 ml, according to the manufacturer's instructions. Restriction-enzyme digests were analyzed on a 2% agarose gel. Control samples of known HFE-mutation status were included, to test restriction-enzyme activity.

HLA Typing

All samples were typed for HLA-A and HLA-B by means of sequence-specific–oligonucleotide hybridization, as described elsewhere (Gao et al. 1994).

Results

845 G→A Analysis

Results of 845 G→A screening are shown in table 1. None of the samples analyzed were found to be homozygous for 845 G→A; however, two Australian Aboriginal (Cape York population) samples, one Melanesian sample, and three Polynesian samples were heterozygous, giving a combined allele frequency of 0.32% in the populations studied. Chinese-family samples were excluded from frequency calculations.

The HLA haplotypes of 845 G→A heterozygotes are shown in table 2. HLA typing indicated that five of the six subjects found to be heterozygous for 845 G→A carried HLA haplotypes common in Caucasians. In particular, HLA-A3, carried by three of these six subjects, is the HLA-A allele recognized as significantly associated with hemochromatosis in Caucasian populations (Simon et al. 1976).

The Melanesian subject heterozygous for 845 G→A carries the HLA-A alleles 0201 and 2402, both of which are common in Melanesian as well as in Caucasian populations. HLA-B 4002 is also common in Caucasian populations, whereas HLA-B 5601 is a typical Melanesian allele. The HLA class II haplotype of this Melanesian subject also contained Caucasian alleles (data not shown).

Table 3

Results of 187 C→G Analyses

Population	No. of Chrommosomes		
	Total	187C	187G
Australian Aboriginal:			
Cape York (n = 90)	180	171	9 (5.0%)
Groote Eylandt (n = 101)	202	202	0
Northern Chinese (n = 67)	134	130	4 (2.99%)
Southern Chinese (n = 97)	194	188	6 (3.09%)
Chinese Families (n = 88)	176	171	5
Total[a] (n = 355)	710	691	19 (2.68%)

[a] Excludes Chinese-family samples.

Table 4

HLA Haplotypes of Subjects Carrying 187 C→G

Population	Haplotype of	
	HLA-A	HLA-B
Cape York Australian Aboriginal[a]	0201/3101	2705[b]/5801[b]
	1101/2402	3501[b]/5601
	3303[b]/3401	5801[b]/4001
	0101[b]/0301[b]	0801[b]/3901[b]
	0301[b]/1101	0702[b]/4001
	0101[b]/2402	5701[b]/4001
	0301[b]/3401	0702[b]/3901
	0101[b]/0301[b]	0702[b]/0801[b]
Northern Chinese	2301/3001	1302/4403
	1101/3001	4001/4006
	2601/2402	4002/4402
	1101/0207	.../...
Southern Chinese	0201/0203	4001/4001
	0206/1101	1527/5502
	0201/2901	4001/0705
	0207/1101	4006/3901
	0203/2402	4001/3802
	1101/2402	4001/1301
Family A	0301[b]/1101	4402[b]/3901
	0301[b]/3303	4402[b]/5801
	0301[b]/3303	4402[b]/5801
Family B	3303[b]/1101	5801[b]/1301
	3303[b]/0206	5801[b]/4601

[a] Homozygous for 187 C→G.
[b] Caucasian-specific or universal allele.

187 C→G Analysis

Results of 187 C→G screening are shown in table 3. In the Cape York Australian Aboriginal population, one sample was homozygous for 187 C→G, and seven were heterozygous (allele frequency 5.0%). 187 C→G was not present in the Groote Eylandt Australian Aboriginal population. Fifteen Chinese samples were heterozygous for 187 C→G, as were four northern Chinese (2.99%) samples, six southern Chinese (3.09%) samples, and five samples from Chinese families. This gives an overall allele frequency of 2.68% (when the Chinese subjects belonging to families are excluded).

HLA typing of subjects carrying 187 C→G (shown in table 4) indicated that all Australian Aboriginal subjects with this mutation also carry HLA haplotypes that are Caucasian. It is therefore likely that 187 C→G was introduced into the Cape York Australian Aboriginal population by Caucasian admixture.

HLA typing of the Chinese subjects heterozygous for 187 C→G showed that this mutation was present on chromosomes carrying a wide variety of HLA haplotypes, several of which are rare in Caucasians, making it unlikely that this mutation was introduced into the northern and southern Chinese populations studied. 187 C→G in the Chinese families, however, is associated with HLA haplotypes common in Caucasians.

Discussion

The 845 G→A mutation causative in hemochromatosis was found to be present at a very low frequency in Australian Aboriginal, Melanesian, and Polynesian populations and to be absent from Chinese, Javanese, Micronesian, and Nepalese populations. HLA typing indicated that 845 G→A was found in association with common Caucasian haplotypes, suggesting that this mutation has been introduced into these populations by Caucasian admixture. These results are consistent with the observations of Merryweather-Clarke et al. (1997) and Chang et al. (1997) and support the widely held view that this mutation arose on a single chromosome in a Celtic population that spread by migration throughout northern Europe, the United States, and Australia.

The 187 C→G mutation was found to be present in two dissimilar non-Caucasian populations—the Cape York Australian Aboriginal population and the Chinese; however, HLA typing indicated that the origin of 187 C→G differed in the two populations. 187 C→G in the Cape York Aboriginal population was always seen in conjunction with HLA haplotypes common in Caucasians, suggesting that it was introduced by recent admixture. 187 C→G was not present in the Groote Eylandt, the other Australian Aboriginal population studied.

In the Chinese samples studied, 187 C→G was associated with a wide variety of HLA haplotypes, some of which are rare in Caucasians. The frequency of 187 C→G in Chinese was low compared with that seen in Caucasians; however, the variation in HLA haplotypes makes it unlikely that this mutation was introduced into the Chinese population by Caucasians and suggests that 187 C→G probably predates 845 G→A. 187 C→G is clearly much more widespread than 845 G→A; however, the affect of this mutation on the function of HFE remains to be elucidated.

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Electronic-Database Information

URLs and accession numbers for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for hemochromatosis gene [HFE])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/htbin-post/Omim (for hereditary hemochromatosis)

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