

Haplotype Analysis of the HFE Gene: Implications for the Origins of Hemochromatosis Related Mutations

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A number of patients with genetic hemochromatosis do not present with the C845G→A (C282Y) nor C187C→G (H63D) mutations. Thus, efforts have been made to search for other mutations on the HFE gene (1). DNA sequencing of this gene did not result in the discovery of other relevant causative mutations, with the exception of the recently described C193A→T (S65C) variant (2). However, a small number of DNA polymorphisms have been documented (3,4,5). Beutler et al (3) described new diallelic markers on the HFE gene and used them to define intragenic haplotypes. They studied these haplotypes in 43 patients with genetic hemochromatosis, among which 39 were Caucasian C282Y homozygotes, and in a control group of 35 Caucasians, 28 Asians and 13 Afro-Americans.

During our own attempt to identify new mutations in hemochromatosis patients who were not homozygous for the C282Y mutation, we encountered 2 of these polymorphisms in a H63D heterozygote. We used both polymorphisms, namely IVS2 (+4) t/c and IVS5 (+907) g/a, to study intragenic haplotypes of the HFE gene in an additional group of individuals of various ethnic backgrounds. We randomly selected 25 unrelated patients among the C282Y homozygotes diagnosed in our laboratory. We also investigated fifty patients homozygous for the H63D mutation, as well as 51 individuals with a normal HFE genotype defined by the absence of both the C282Y and H63D mutations. Twenty five out of

51 normal subjects were healthy volunteers, whereas 26 were subjects referred for HFE genotyping. All previously described individuals were of Caucasian extraction. Additionally, we tested 24 African and 4 Asian subjects without hemochromatosis. Patients and controls had given written consent for DNA analysis according to the French law.

The C282Y or H63D mutations, and IVS2 (+4) t/c or IVS5 (+907) g/a, were investigated by the modification of natural restriction sites as described (3,6). Comparison of frequencies between the different groups were done by the chi-square test or the Fisher exact test for small samples.

All twenty five C282Y homozygotes had the IVS2 (+4) t/t and IVS5 (+907)a/a, haplotype (Table 1). This result is in agreement with the data reported by Beutler et al (3). This haplotype is schematically represented as *m1* in Figure. 1. We found the 50 H63D homozygotes to have a *c* at IVS2(+4), and a *a* at IVS5(+907) (Table 1). This corresponds to the *m2* chromosome illustrated in Figure 1. These findings were also suggested by Beutler (3) who studied 2 H63D homozygotes and 4 compound heterozygotes for the H63D and C282Y mutations. It is noteworthy that none of the 48 African or 8 Asian chromosomes tested in the present study bore the C282Y or H63D mutations.

Among the 51 Caucasians without the H63D or C282Y mutations, results obtained from healthy volunteers (n=25) or from subjects

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Table 1. Allelic Frequencies of HFE Gene Haplotypes in Normal Subjects of Caucasian and African Extraction

Haplotypes (this study)	Haplotype Number according to Beutler et al (3)	Normal HFE (Caucasians) n=102 chromosomes number (allelic frequency)	Normal HFE (Africans) n=48 chromosomes number (allelic frequency)
N1 (CTGG)	1 or 3	59 (0.578)	15 (0.313)
N2 (CCGA)	6 or 8	27 (0.265)	20 (0.417)
N3 (CTGA)	2 or 4	16 (0.157)	13 (0.271)
N4 (CCGG)	5 or 7	0	0
p=0.019			

Note: For definition of haplotypes N1, N2, N3 and N4 see Figure 1.

referred to our laboratory for HFE genotyping (n=26) were not statistically different. Thus, both groups were pooled. Allelic frequencies were calculated for each polymorphism, IVS2 (+4) and IVS5 (+907) in Caucasians and Africans. Significant differences were found for both polymorphisms between these two ethnic groups (Table 1). We then constructed *HFE* haplotypes using IVS2 and IVS5 polymorphisms and compared their frequencies in normal Caucasians and Africans. Four "normal" haplotypes may exist, N1, N2, N3 and N4 (Figure 1). The existence of the N1, N2 and N3 haplotypes in our sample, was clearly demonstrated by the presence of individuals homozygous for these haplotypes among both Caucasians and Africans. On the other hand, we never found homozygotes for the N4 haplotype among the 156 normal *HFE* chromosomes (Caucasian, African or Asian) we studied. A small number of chromosomes were identified by Beutler et al (3) with this haplotype which corresponds to haplotypes 5 or 7 in their paper. Although it could have been inferred in some chromosomes with an ambiguous haplotype, this haplotype was rare or absent in our sample. We thus hypothesized that the frequency of haplotype N4 is negligible in our sample and calculated haplotype frequencies only for the three other normal haplotypes. Haplotype N1 was the most prevalent among Caucasians followed by N2 and N3, whereas the N2 haplotype predominated within subjects of African extraction, followed by N1 and N3 (Table 1). There was a statistical difference between the

haplotype distribution among these two ethnic groups (p=0.019). The number of Asian individuals we tested was too small to allow any comparison with the other groups. However, Beutler et al examined 28 Asian subjects and found the N2 haplotypes (corresponding to haplotype 8 in their study) to be the most prevalent.

Pooled together our data and those of Beutler et al suggest that HFE haplotypes can be drawn out by using the nucleotide positions for both described mutations, respectively at positions 187 (H63D) and 845 (C282Y) of the HFE gene, and polymorphisms at IVS2 (+4) and IVS5 (+907). These polymorphic sites allow the identification of 4 normal or 2 abnormal chromosomes as represented in Figure 1. Beutler et al. (3) have studied a third polymorphism, IVS4 (-44) t/c, which allowed them to design eight haplotypes. The correspondence between these 8 haplotypes and the 4 haplotypes described in the present paper are indicated in Table 1.

In our study, the C282Y or H63D mutations were associated with a specific haplotype. The C282Y mutation was exclusively encountered associated with the *m1* haplotype which is derived from haplotype N1. Thus, the C282Y mutation is linked to an intragenic haplotype (N1) which is prevalent in Caucasians, but not in Africans (this study) nor in Asian subjects (3). This mutation is considered as a relatively recent event of less than 2000 years (7-8). The existence of an ancestral haplotype common to all patients with genetic hemochromatosis was demonstrated

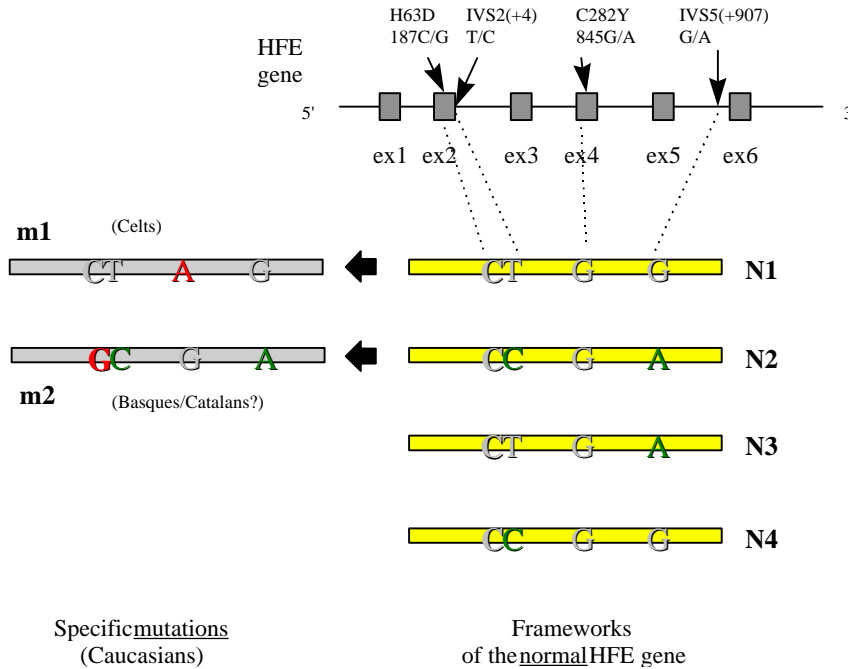


Figure 1: Haplotypes of the HFE gene in normal and mutated chromosomes. Possible origins of the different mutated HFE chromosomes.

and was the basis for the discovery of the gene (1).

Conversely we found the H63D mutation to be exclusively associated with the haplotype *m2*. This haplotype shares identities with the *N2* haplotype (Figure 1). The H63D mutation can be found in almost all groups of subjects of Caucasian origins (9). Its prevalence is very high among Basques and Spanish (20-21-22), whereas it has been found to be low among Africans (9-12-13), Asians (9) and Australasians (14). The presence of this mutation with a low frequency in some of these ethnic groups is suspected to be linked to Caucasian admixture (12-14). In our sample this mutation was absent in all subjects of Asian or African extraction. These subjects were

almost all recently settled in Europe and can thus be considered to have had little Caucasian admixture. Finally, we can hypothesize that the H63D mutation could have emerged in an Hispanic ancestor bearing the *N2* haplotype.

Very recently, Rochette et al (15) found the C282Y and H63D mutations to be present in different haplotypes in subjects from Sri Lanka. Their data bring evidence of a multiple origin of the two main *HFE* mutants. Such a result is not surprising, even for a severe mutation as the C282Y. Another well known example is given by the mutation responsible for sickle cell disease which arose in at least 4 different haplotypes on the β -globin gene. Thus, the four normal haplotypes (*N1* through *N4*) defined in this study

could be considered as genetic frameworks of the *HFE* gene as compared with the well known frameworks of the beta-globin gene. The β -globin gene frameworks have been the basis for the description of β -globin gene mutations and have also been linked to β -globin gene cluster haplotypes (16-17). In the same way, an intragenic *HFE* gene haplotype could be a useful tool to follow *HFE* gene mutations and evolution. These frameworks have served as a genetic background to *HFE* gene mutations, H63D on the *N2* haplotype and C282Y on the *N1* haplotype, both mutations having probably arisen in Caucasian ancestors.

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