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Absence of the hemochromatosis gene Cys282Tyr mutation in three ethnic groups from Algeria (Mzab), Ethiopia, and Senegal

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Abstract A Celtic origin for hemochromatosis, a common genetic iron metabolism disorder, has been postulated for a long time. To check whether the two mutations recently identified in the *HLA*-class I candidate gene for this disease were found only in Caucasians, we examined their frequencies in individuals originating from Algeria, Ethiopia, and Senegal. The presumably disease-causing mutation, responsible for the Cys282Tyr substitution, was not found in any member of these ethnic groups, although it was shown to be highly prevalent in populations of European ancestry. This geographic distribution supports the previously suggested Celtic origin for the disease. In contrast, the mutation responsible for the His63Asp substitution is not restricted to European populations. Although absent in the Senegalese, it was found on about 9% of the chromosomes of the Central Ethiopians and Algerians (Mzab) genotyped for this study. This second mutation, which probably represents a common variant unrelated to hemochromatosis, thus appears to have occurred earlier than that responsible for the Cys282Tyr substitution. More detailed population studies are needed to provide information on the age of these two mutations and eventually show how the hemochromatosis-causing mutation chronologically spread throughout Europe.

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Introduction

A candidate gene for hemochromatosis, a common genetic iron metabolism disorder, has recently been identified on the short arm of chromosome 6, about 4 megabases telomeric to the major histocompatibility complex [(*MHC*) (Feder et al. 1996)]. This gene encodes a 343 amino acid protein that exhibits significant similarity to the *HLA* class I molecules and is widely expressed. Although designated *HLA-H* by Feder and co-workers (1996), it should not be confused with the existing *HLA-H* gene located within the *MHC* cluster and will be referred to in this study as *HFE*, the name assigned to the hemochromatosis locus in the genome database.

A role of this gene in hemochromatosis is supported by the high frequency of a G→A transition at nucleotide 845 of the open reading frame in patients, predicted to substitute the cysteine residue 282 by a tyrosine in the α3 domain of the molecule. This mutation has been found in a high proportion of the patients tested so far in France, Australia, and in the United States (Beutler et al. 1996; Borot et al. 1997; Jazwinska et al. 1996; Jouanolle et al. 1996). A second transition at nucleotide 187 (G→C), responsible for the substitution of a histidine by an aspartic acid at residue 63, does not seem to be related to the disease (Borot et al. 1997; Jazwinska et al. 1996).

Hemochromatosis is the most common genetic disease in individuals of Northern European origin. A Celtic origin with spread of the mutated allele by migration has been postulated to account for the excess of *HLA-A3* in patients from Germany, France, Belgium, England, Scotland, and Ireland, as well as from Australia and the United States (Bothwell et al. 1989). If hemochromatosis is truly a disease of Celtic origin, the disease-causing mutation(s) in the *HFE* gene should be found only in Europeans. Examination of the mutation frequencies in individuals of non-European origin is therefore useful to confirm this hypothesis. As a first step, we evaluated the frequency of the two *HFE* mutations in individuals originating from three regions of Africa: Algeria (Mzab), Ethiopia, and

Senegal, and compared them with those observed in individuals of European origin.

Materials and methods

Population samples

DNA samples were obtained from 95 Mozabites, 181 Ethiopians, and 94 Senegalese.

The 95 *Mozabites* are members of a Berber-speaking (Afroasiatic) ethnic group in Algeria and were sampled in Ghardaïa, an oasis in the north of Sahara built by Ibadites, whose representatives are still found nowadays in Oman, Lybia, and Tunisia (Djerba). They have formed an isolated population based on the Muslim tradition since the 11th century and differ from the non-Mozabite population by their religious practices, social organization, and Berber language. *HLA* class II and *Ig GM* typings have demonstrated their close genetic relationship to populations from both sub-Saharan Africa and the Mediterranean area (Hariti et al. 1996).

The 181 *Ethiopians* are members of the Oromo (Afroasiatic Cushitic language) and Amhara (Afroasiatic Semitic language) ethnic groups. They have already been tested for several polymorphic erythrocyte and plasma protein systems, and differences between members of the two groups were not statistically significant (Scacchi et al. 1994; Tartaglia et al. 1996). With respect to most genetic systems tested so far, East Africans, among whom are Ethiopians, are closer to the populations from North Africa, the Near East, and Europe, than are other sub-Saharan African groups (Excoffier et al. 1987; Cavalli-Sforza et al. 1994). This is consistent with linguistic relationships, as most populations presently living in East Africa, North Africa, and the Near East speak an Afroasiatic language (Excoffier et al. 1991).

The 94 *Senegalese* are issued from the Mandenka population in the Niokholo region of eastern Senegal, a small endogamous community represented by some 3200 individuals living in a dozen villages (Langaney and Gomila 1973). Their language belongs to the Niger-Congo Mande family, which includes several million people located between Nigeria and the Atlantic coast. Many serological and DNA polymorphisms have been analyzed in this population and have shown its close genetic relationship to other West African and, more generally, Niger-Congo populations (Blanc et al. 1990; Dard et al. 1992, 1996; Tiercy et al. 1992; Graven et al. 1995; Poloni et al. 1995).

HFE mutation analysis

DNA was prepared from peripheral blood by standard techniques. *HFE* mutation analysis was performed as described previously (Borot et al. 1997). Briefly, the two portions of the *HFE* gene containing the mutations described (Feder et al. 1996) were amplified separately with primers 5′-TGGCAAGGGTAAACAGATCC-3′ and 5′-CTCAGG-CACTCCTCTCAACC-3′. DNA samples (100 ng) were initially denatured at 94 °C for 1 min 30 s, before undergoing 35 cycles of 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 30 s. Ten microliters of the polymerase chain reaction (PCR) products were denatured in 200 µl 0.4 M NaOH and spotted in duplicate on nylon membranes Gene-Screen Plus (Biotechnology Systems, Boston, MA). The filters were then rinsed three times with 100 µl of 2X standard sodium citrate (SSC). Oligonucleotides matching the normal (5′-ATATACGTGC-CAGGTGG-3′) and mutant (5′-ATATACGTACCAGGTGG-3′) sequences were end-labeled with T4 polynucleotide kinase (Life Technologies, Gaithersburg, MD) at a specific activity of 2×10^9 cpm/µg and purified on Sephadex G-25 Quick Spin columns (Boehringer Mannheim, Indianapolis, IN). Filters were hybridized overnight at 37 °C in 5X SSC, 5X Denhardt's solution, 20 mM sodium phosphate pH 7, 0.1 mg/ml salmon sperm DNA, and 1% sodium dodecyl sulfate (SDS), in the presence of 2×10^6 cpm per ml of radiolabeled oligonucleotide probe. After hybridization, filters were washed in 2X SSC and 1% SDS for 30 min at room temperature and for 30 min at 51 °C (normal sequence) or 48 °C (mutant sequence).

Table 1 Frequency of the Cys282Tyr substitution

Nucleotide 845	Algerians (Mozabites)	Central Ethiopians	Senegalese (Mandenka)	French*	Americans of European ancestry+
Genotypes					
G/G	95	181	94	218	309
G/A	0	0	0	16	39
A/A	0	0	0	0	0
Total	95	181	94	234	348
Allele frequencies					
Allele *G*	190	362	188	452 (96.6%)	657 (94.4%)
Allele *A*	0	0	0	16 (3.4%)	39 (5.6%)

* Data from Borot and co-workers (1997) and Jouanolle and co-workers (1996) have been combined
+ Data from Feder and co-workers (1996) and Beutler and co-workers (1996) have been combined

Table 2 Frequency of the His63Asp substitution

Nucleotide 187	Algerians (Mozabites)	Central Ethiopians	Senegalese (Mandenka)	French*	Americans of European ancestry+
Genotypes					
C/C	78	149	94	167	242
C/G	17	30	0	58	98
G/G	0	2	0	9	7
Total	95	181	94	234	347
Allele frequencies					
Allele *C*	173 (91.1%)	328 (90.6%)	188 (100%)	392 (83.8%)	582 (83.9%)
Allele *G*	17 (8.9%)	34 (9.4%)	0	76 (16.2%)	112 (16.1%)

* Data from Borot and co-workers (1997) and Jouanolle and co-workers (1996) have been combined
+ Data from Feder and co-workers (1996) and Beutler and co-workers (1996) have been combined

Results

The genotypes at nucleotide 845 are summarized in Table 1, those at nucleotide 187 in Table 2. As shown in Table 1, the 845 G→A mutation was not found in any of the tested individuals from Algeria, Ethiopia, or Senegal. In contrast, it was observed on 3.4% (95% CI: 1.8–5.0) of the chromosomes of French individuals used as controls in the hemochromatosis case-control studies published so far (Borot et al. 1997; Jouanolle et al. 1996) and 5.6% (95% CI: 3.9–7.3) of those of American controls (Beutler et al. 1996; Feder et al. 1996). Interestingly, the 187 C→G mutation, although absent in the Senegalese population, was found on a significant proportion of Ethiopian (9.4 %; 95% CI: 6.4–12.4) and Mozabite (8.9%; 95% CI: 4.9–12.9) chromosomes (Table 2). For comparison, the frequency of this latter mutation was 16.2% (95% CI: 12.9–19.5) and 16.1% (95% CI: 13.4–18.8) on the chromosomes of French and American controls, respectively.

Discussion

The hypothesis that hemochromatosis was predominantly due to a single mutation with subsequent modifications of the ancestral haplotype by recombinations (Bothwell et al. 1989) is now supported by the presence in two copies of the *HFE* 845A mutation in 72% to 95% of the patients tested so far (Beutler et al. 1996; Borot et al. 1997; Feder et al. 1996; Jazwinska et al. 1996; Jouanolle et al. 1996). This mutation is quite frequent in the general population: 5.8 to 15% of the individuals used as controls in these studies are indeed heterozygotes. This result contrasts with the virtual absence of the *845A* allele in three populations from Algeria, central Ethiopia, and eastern Senegal genotyped for this study.

The fact that the disease mutation is found in Europeans as well as in Americans and Australians of European ancestry, but not in the three African groups tested here, supports the idea that hemochromatosis is a Celtic disease. Celts indeed spread through much of Europe between the 2nd millennium and the 1st century BC: from a heartland in central Europe, they settled the area of France, penetrated northern Spain, and crossed to the British Isles in the 8th and 7th centuries BC; moving south and southwest, they sacked Rome ca. 390 BC, attacked Delphi in 279 BC, crossed into Anatolia, and established the state of Galatia. Genotypes obtained from various individual samples across Europe would allow one to establish a picture of the 845G→A mutation frequency and eventually to match the mutation frequency map to the historic Celtic distribution. However, to ascertain with more confidence the Celtic origin hypothesis for hemochromatosis, testing of additional samples from other world regions, such as the Asiatic continent, is required.

In contrast to the 845G→A mutation, the 187C→G transition is observed in a significant proportion of the individuals belonging to the Mozabite and Ethiopian ethnic groups studied here, but lower than that observed in the French and American controls of European ancestry tested so far (Beutler et al. 1996; Borot et al. 1997; Feder et al. 1996; Jouanolle et al. 1996). According to this result, the Algerians and Ethiopians are genetically closer to the Europeans than are the Senegalese. This is in agreement with data obtained from the study of other polymorphisms. For example, the *GM* haplotype *GM*1,17;.. ;5,10,11,13,14, 26,27*, which is very rare in Europe, reaches a frequency of about 70% in the Senegalese Mandenka and in many Niger-Congo-speaking populations (Blanc et al. 1990), while the frequency of this haplotype is close to 40% in East Africans, and between 5% and 30% in North Africans. The presence of the *187G* allele in North and East Africans and its virtual absence in the Senegalese may be explained by gene flow between Caucasoid and East African populations.

It is noteworthy that an iron overload syndrome clinically distinct from hemochromatosis is quite common among sub-Saharan Africans. This syndrome has been attributed to high concentrations of iron found in the traditional home-brewed beer consumed in rural areas of Africa (Bothwell et al. 1964). Gordeuk and co-workers (1992), after carefully surveying the families of African beer drinkers with iron overload, concluded that this syndrome was associated with a familial, probably genetic, factor which interacts with the beer drinking. *HLA* typing studies of these families did not show any linkage to *HLA* phenotypes, and they postulated the existence of a gene distinct from the gene responsible for hemochromatosis, that was a contributing factor to this syndrome. This is in agreement with the lack of the mutations potentially responsible for hemochromatosis in the Senegalese genotyped for this study.

The two mutations identified in the *HLA* class I-like gene candidate for hemochromatosis very probably have different origins. The observation of the first one, responsible for a Cys282Tyr substitution, only in populations of European ancestry so far is consistent with hemochromatosis being a Celtic disease. In contrast, the second mutation identified, responsible for a His63Asp substitution, is not restricted to populations from European origin and is very likely much older than that responsible for the Cys282Tyr substitution. More detailed population studies should provide information on the age of the hemochromatosis-causing mutation and show how it chronologically spread throughout Europe.

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