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Frequency and spectrum of hemochromatosis mutations in Tunisia

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The occurrence of the C282Y and H63D mutations of the HFE gene, responsible for toxic iron overload in the liver (hereditary hemochromatosis), was still unknown in Tunisia. We report the screening of 194 chromosomes from 97 randomly collected cord blood samples. The mutations were analyzed by PCR followed by DNA sequencing. The mild H63D and the severe C282Y mutations were found in $17.5 \pm 5.34\%$ and $0.5 \pm 0.97\%$ of the alleles, respectively. The allele frequency of the IVS $2+4$ T→C polymorphism is high ($46.4 \pm 7.01\%$) in this population. Risk for homozygosity for the severe C282Y mutation is present in the Tunisian population at a low theoretical incidence. However, due to the relatively high rate of consanguinity in the country, liver pathology due to HH is not to be disregarded.
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Introduction

Hereditary hemochromatosis (HH) is a recessive autosomal disorder of the iron metabolism, which usually manifests during the 4th or 5th decades of life and is induced by toxic iron overload in the liver. The disease is known to be HLA-linked[1] and the associated gene (HFE) was located on the short arm of chromosome 6.[2] The majority of the patients (83–100%) affected with severe pathology was found to be homozygous for the C282Y mutation, a G→A transition at nucleotide 845 of the HFE gene changing the cysteine to a tyrosine at position 282.[2,3] A second mutation (H63D), resulting from the transition C→G at position 187, changing histidine 63 to aspartic acid, was also detected in compound heterozygous C282Y/H63D patients, usually affected by less-severe symptoms.[4] The role of this mutation in the pathogenesis of the disease is still unclear. A third mutation (S65C), resulting from a transition (A→T 193), was also associated to a mild phenotype, in compound heterozygosity with the C282Y mutation.[5] HH is the most frequent monogenic recessive hereditary disease in north Europeans, and a Celtic origin of the mutated alleles was postulated.[6] The average allele frequencies measured in north Europeans are 4% for the C282Y and 18% for the H63D mutations, respectively.[7] These figures decrease from northern to southern Europe and allele frequencies measured in Italy are 1.65 and 13.3%, respectively.[8]

In northern Africa, the H63D mutation was detected with an allele frequency between 8.9%[9] and 13.2% in a mixed Moroccan/Algerian populations living in France (Aguilar-Martinez, Picot *et al*., 2001, 114/id). In the same mixed population, the C282Y mutation was found in 0.9% of the studied alleles {Aguilar-Martinez, Picot *et al*., 2001, 114/id} and it was not detected in Ethiopian, Senegalese and an isolated group of Algerians.[9] Before this screening, the incidence and spectrum of the HFE gene mutations in the Tunisian population were unknown.

Materials and methods

Population

The epidemiological study involved the random collection of 97 cord blood samples in Na_2 EDTA, in two large obstetric/gynecology departments in Tunis and Nabeul representing the average autochthonous Tunisian population.

Methods

DNA was extracted using the standard phenol/chloroform method. Screening of the HFE gene was performed by selective PCRs in order to amplify exons

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II and IV separately. Direct sequencing of both amplified fragments was used for the mutation's detection.

The first fragment covers the H63D mutation, the rare S65C mutation and the IVS-2 + 4 (T→C) polymorphism; it was amplified using 'H63D For' (5′CTCCCCTCCTACTACACATGGTT3′) as a forward primer and 'H63D Rev' (5′CAAGATGCATGAAAAGATGAAAGG3′) as a reverse one. Using a second pair of primers, 'C282Y For' (5′AGAAGGAAGTGAAAGTTCC-AGTCTT3′) and 'C282Y Rev' (5′CCCAATAGATTTTCTCAGCTCCT3′), exon IV was amplified to detect the C282Y mutation.

The following conditions were used: Hot start step at 94°C for 4′, denaturation at 92°C for 45″, annealing at 55°C for 60″ and extension at 74°C for 75″. After 30 cycles, a final elongation step of 5′ at 74°C, was applied. Two units of AMPLI *Taq* DNA Polymerase enzyme (Perkin-Elmer Foster City, CA, USA) were used for each reaction. The PCR was performed on a Robo Cycler 96 (STRATAGENE, La Jolla, CA, USA). DNA sequencing was performed on an ABI PRISM® 3700 DNA Analyzer (P-E Biosystems, Foster City, CA, USA) using ABI PRISM® Big Dye Terminator.

Results

The H63D mutation was found in a heterozygous form in 30 samples, and in a homozygous form in two samples (allele frequency 17.5±5.34%). Only one heterozygous case of the C282Y mutant was found, suggesting an allele frequency of 0.5±0.97%. The rare S65C mutation was not detected. The data are summarized in Table 1.

The IVS-2 + 4(T→C) polymorphism[10] was present in the heterozygous state (IVS-2 + 4T/C) in 59.8% and in the homozygous state (IVS-2 + 4C) in 16.5% of the samples (46.4±7.01% of the total alleles were IVS-2 + 4C). H63D homozygous were also homozygous IVS-2 + 4C, and all H63D heterozygous were either heterozygous (IVS-2 + 4T/C) or homozygous (IVS-2 + 4C) for this polymorphism. The only case found heterozygous for the C282Y mutation was also heterozygous IVS-2 + 4T/C.

Table 1 HFE genotypes and allele frequencies of the mutations found in 97 random individuals

Genotype	*Number of subjects (%)*	*Allele frequencies*
Exon II		
WT/WT	65 (66%)	H63D (17.5±5.34%)
H63D/WT	30 (30.5%)	
H63D/H63D	2 (2.5%)	
Exon IV		
WT/WT	96 (99%)	C282Y (0.5±0.97%)
WT/C282Y	1 (1%)	

Discussion

This screening in autochthonous newborns indicates that the H63D and the C282Y mutation are both present in the Tunisian population. The frequency of the H63D mutation (17.5±5.34%) is higher than the one previously reported in Moroccans and Algerians living in south France (13.2%).[7] The difference between the two figures is not statistically significant as such, but is significant when compared with the data reported for the isolated ethnic group (Mzab) living in Algeria and for the population from the Tunisian island of Djerba 8.9%.[9] In Italians, French North-Africans and Greeks the H63D mutation was observed at 13.3, 18.3 and 14.5% frequencies, respectively[7,8,11] showing no significant differences with our population, which was controlled for Tunisian ancestry. The only C282Y mutant allele detected in our screening indicates a very low allele frequency (0.5±0.97%) possibly due to ancestral gene flow. Although this frequency is obtained from a limited number of samples, it is similar to the one reported by other authors for Moroccans and Algerians (0.9%)[7] and to those reported in Italy (0.5±1%)[12] and in Greece (0.3%).[11] Our figures are also compatible with the frequency gradient, the highest in the Irish population (14%) (Ryan, O'Keane *et al*., 1998, 943/id) and decreasing in the average French (4.6%)[7] and Italian populations: 2.2 in the northern,[13] 1.6%,[8] in the central and 0.15% in the southern part,[14] respectively.

The IVS-2 + 4 T→C polymorphism observed in 46.4±7.01% of the chromosomes is associated with the IVS 4 and the ICS 5 polymorphisms corresponding to haplotype 6, which is linked to the European H63D mutation.[10]

We may conclude from our results that the C282Y mutation is extremely rare, but not absent in the Tunisian population. Therefore, homozygosity as well as combined heterozygosity for the C282Y and the H63D mutations could be expected, resulting in severe and intermediate HH phenotype. Moreover, both C282Y homozygosity and compound heterozygosity C282Y/H63D could be increased by a consanguinity factor due to the relatively high rate of first (36%) and second (13%) cousin marriages in the country.[15]

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