LETTERS TO THE EDITOR

Frequency of the HFE C282Y and H63D mutations in distinct ethnic groups living in Spain

Hereditary haemochromatosis (HH), a disorder of iron metabolism, is one of the most common inherited diseases in white populations. A single amino acid change, C282Y, in the HFE gene product accounts for more than 90% of HH in this population. A second change, H63D, which has been observed in healthy controls, also appears to be indirectly related to the disease.1 In a population study, Merryweather-Clarke et al2 observed the presence of the C282Y allele in 3.8% of a sample of 1450 anonymous European subjects and the presence of the H63D allele in 13.6% of that population. As for western communities, their study, which included 78 Spanish subjects (28 Basques and 50 Catalans), showed a mean allele frequency of 3.2% for C282Y and 26.3% for H63D. According to these data, the frequency of H63D in Europe was highest in the Spanish and Dutch.

We investigated both substitutions in the HFE gene in two distinct Spanish ethnic groups: Basques and Catalans. A sample of voluntary blood donors living in Catalonia (a community whose present population is a mixture resulting from recent immigration from different parts of Spain) was also included in the study. Methods of collection and use of human samples were approved by the institutional review board at Sant Pau Hospital in Barcelona.

C282Y and H63D mutations were screened using enzymatic digestion of PCR products encompassing the mutation sites.3 The C282Y mutation creates a new RsaI restriction site. The 390 bp PCR reaction product (forward primer 5'-TGGCAAGGGTAAACAGATCC-3' and reverse primer 5'-CTCAGGCACTCCT-GATTTCC-3') digested with RsaI shows two fragments of 249 and 141 bp in normal DNA, while mutant DNA generates two new fragments (112 and 29 bp). The H63D mutation destroys an MboI site in the 294 bp PCR product (forward primer 5'-ACATGGTATACCC-3' and reverse primer 5'-CTTGCTTGTGTGTGATT-TTTCC-3'), while normal DNA generates three fragments of 138, 99, and 57 bp.

The genotype frequencies for mutations in the HFE gene found in the present study are shown in table 1. In the Basque population we have detected a frequency of 2% for the C282Y allele, a value that falls in the "south European range". The H63D allele in this population shows a frequency of 27.4%. This value, which is similar to that reported by Merryweather-Clarke et al2 is, together with the 29.5% found in the Dutch, the highest in the world population. Even today little is known of the origin of the Basques, a not very large population located in the west Pyrenees. It has been suggested that many of the mesolithic settlers of western Europe could have mixed with neolithic tribes to give rise to present day Europeans and a few groups of mesolithic people in the Pyrenean region could have remained shielded from subsequent invasions,4 thus giving rise to the present day Basques. It has also been stated that the Basques and a people who have successfully resisted absorption by a succession of conquering or neighbouring cultures, and in a more recent study, Aguirre et al5 reported that the Basque population has undergone limited genetic exchange with other Europeans and that the distribution of their genetic frequencies differentiates them from other populations. The high frequency of the H63D allele in the Basque population can be regarded as an additional genetic marker, which also lends support to the singularity of their genetic characteristics.

The Spanish Gypsy population represents the largest Gypsy community in western Europe with approximately half a million people distributed all over the country. In the group of Spanish Gypsies studied, we found a frequency of the C282Y allele within the range reported in Europe. As for the H63D allele, we found a low frequency (8.62%) compared with that of the European population. The arrival of Gypsies in Europe can be traced back to the 14th century and linguistic evidence suggests that Gypsies originally came from India where a trickle of small nomadic bands moved to the west.6 On the basis of the available data, the frequency of this allele in the Indian continent is 8.4%, which resembles the value found in Spanish Gypsies. Interestingly, similar frequencies are found in populations in the Middle East, on the route from India to the west.

The results obtained in both the group of blood donors show allele frequencies for both substitutions comparable with the frequencies of the European population as a whole.

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Mitochondrial DNA mutations and pathogenicity

We read with interest the case described by Dr Albin in the March issue of the Journal.7 We agree that some features of the clinical presentation of this 48 year old woman are highly suggestive of mitochondrial disease. A mitochondrial aetiology should be considered in any patient with bilateral sensorineural deafness and impaired glucose tolerance.8 Additional factors that affect multiple systems (such as the cerebellum, basal ganglia, and pyramidal tracts), coupled with the high signal in the deep white matter on MRI, add weight to the clinical diagnosis. It would be interesting to know whether there were oligoclonal bands in the CSF which were not matched in the serum, particularly because of the possible association of mitochondrial DNA (mtDNA) disease with multiple sclerosis.9 Being highly metabolically active, corneal endothelial cells may be particularly vulnerable to mitochondrial dysfunction,10 and although corneal dystrophy has been noted in patients harbouring established pathogenic mtDNA mutations,11 classical Fuch’s corneal dystrophy has not been described in this context. Fuch’s corneal dystrophy is a relatively common disorder, accounting for 15% of all corneal grafts12 and, as Dr Albin suggests, it is possible that the corneal disease was an incidental finding in the case that he described.

Despite the clinical evidence supporting a diagnosis of mtDNA disease, it is unlikely that the T4216C and G15257A transitions on their own are responsible for the symptoms of the patient described by Dr Albin. The T4216C and G15257A mutations are present in between 5 and 20% of the normal population,13 and phylogenetic analysis indicates that they are both ancient caucasian polymorphisms.14 There is a heavy phylogenetic clustering of patients who harbour the primary pathogenic G11778A and T14484C mutations (which cause Leber’s hereditary optic neuropathy (LHON)) in mtDNA haplotype J. This haplotype also carries the T4216C transition in all branches and the G15257A transition in one branch.15 However, there is no evidence that non-LHON neurological mtDNA disorders cluster in haplotype J. By contrast, there is an unexplained association of multiple sclerosis with haplotype T, which also carries the T4216C transition.16 Clearly the relationship between mtDNA haplotype and disease is highly complex, and it would be unwise to draw firm conclusions from any one individual case.

The investigation of possible mtDNA disease is difficult, particularly when the phenotype is not instantly recognisable. We advocate an integrated approach to the