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 80% 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 large population study, Merryweather-Clarke *et al*² observed the presence of the C288Y 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 C288Y 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 Gypsies. 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.¹ The C282Y mutation creates a new *RsaI* restriction site. The 390 bp PCR reaction product (forward primer 5'-TGGCAAGGGTAAACAGATCC-3' and reverse primer 5'-CTCAGGCACTCCTCTCAACC-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'-ACATGGTTAAGGCCTGTTGC-3' and reverse primer 5'-CTTGCTGTGGTTGTGATTTTCC-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 al*,² 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 sheltered from subsequent invasions,³ thus giving rise to the present day Basques. It has also been stated that the Basques are a people who have successfully resisted absorption by a succession of conquering or neighbouring cultures, and in a more recent study, Aguirre *et al*⁴ reported that the Basque population has undergone little 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 C288Y 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 from where a trickle of small nomadic bands moved to the west.^{5,6} On the basis of the available data, the frequency of this allele in the Indian subcontinent 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 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.¹ We agree that some features of the clinical presentation of the 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.² Additional neurological features 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.³ 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.⁴ Being highly metabolically active, corneal endothelial cells may be particularly vulnerable to mitochondrial dysfunction,⁵ and although corneal dystrophy has been noted in patients harbouring established pathogenic mtDNA mutations,⁶ 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 grafts⁷ 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 transitions are present in between 5 and 20% of the normal population,^{8,9} and phylogenetic analysis indicates that they are both ancient caucasian polymorphisms.¹⁰ 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.^{8,9} 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.⁴ 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

Table 1 Genotype frequencies for mutations in the HFE gene

Genotypes	Basques (n=51)	Gypsies (n=58)	Blood donors (n=108)
HH/CC	25	45	70
HH/CY	2	3	6
HD/CC	20	10	28
DD/CC	4	—	2
HD/CY	—	—	2
C282Y*	1.96±2.7	2.58±2.9	3.70±2.5
H63D*	27.4±8.7	8.62±5.1	15.7±4.9

*Allele frequencies (% ± 95%CI).

Genotypes are given for amino acid 63 (H63D)/amino acid 282 (C282Y) of the protein. CC/HH corresponds to the wild type.

investigation of these patients, incorporating both clinical and laboratory evidence.¹ It would be of great value to carry out a skeletal muscle biopsy on the patient described by Dr Albin, looking for histochemical and biochemical evidence of mitochondrial respiratory chain dysfunction. These investigations are often abnormal in patients with neurological abnormalities resulting from mtDNA disease, and the characteristic mosaic pattern of cytochrome c oxidase activity in muscle or a biochemical complex deficiency may provide clues as to the nature of the underlying genetic defect. It is often difficult to ascribe pathogenicity to a mtDNA mutation, particularly if the disease has an unusual phenotype.¹¹ Therefore, although the clinical evidence presented by Dr Albin is compatible with a mitochondrial disorder, the inference that the T4216C and G15257A nucleotide transitions are the primary aetiological factor responsible for Fuch's corneal dystrophy is unfounded.

PFC is a Wellcome Trust Research Fellow. RA is a MRC (UK) Research Fellow. NH is supported by the Wellcome Trust, The National Eye Institute (RO1 EY10758), and the John Sealy Memorial Endowment Fund.

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"Cataplexy" in Coffin-Lowry syndrome

Crow *et al*¹ reported an unusual, non-epileptic, cataplexy-like phenomenon in three males with Coffin-Lowry syndrome (CLS). The authors also provided evidence of

neuromuscular dysfunction as part of the phenotype by showing abnormalities on muscle ultrasound in four gene carriers, and they commented on our observation of marked distal muscle wasting in two affected brothers, aged 15 and 14 years at the time of our report.² In that report, we described the frequent occurrence of generalised epileptic seizures without pathognomonic epileptic elements on EEG after the age of 6 years. We had occasion to follow these CLS brothers up to their sudden death at the age of 32 and 34 years, respectively. During this follow up it became evident that the "epileptic episodes" were episodes of sudden, non-epileptic collapses with atonia; as in case 1 reported by Crow *et al*,¹ these episodes were precipitated by a loud noise or excitement. The frequency and severity of these drop attacks worsened with age and was correlated with a further progression of the peripheral muscle wasting and of a severe thoracolumbar torsion scoliosis, which finally resulted in acute cardiorespiratory failure.

Over the last 25 years we have had the opportunity to examine 20 other CLS males. In one of these patients the same type of sudden, non-epileptic attacks were noted from the age of 4 years. When walking this CLS boy suddenly dropped, always in a forward position, hurting himself. No epileptic discharges have ever been noted on repeated 24 hour EEG monitoring. Also, a progressive thoracolumbar scoliosis was noted in this boy, and after the experience in the two brothers we decided to operate on the scoliosis at the age of 14 years with satisfactory correction and stabilisation of the curvature. On that occasion a muscle biopsy was performed with normal results. Much to our surprise, the frequent episodes of sudden and reversible loss of muscle tone have completely disappeared after the scoliosis fusion.

In conclusion, our experience in CLS males confirms that "cataplexy" is not rare in this XLMR syndrome, as we observed it in three of 22 male patients. The aetiology and pathogenesis of this sudden collapse phenomenon remains unclear. In this perspective, it is of interest to note that these cataplexy-like symptoms increased in frequency and severity in the two brothers, together with progression of the torsion scoliosis and muscle wasting. In contrast, the collapse symptoms disappeared completely in the third male after surgical correction of the scoliosis.

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Autoclaving Guthrie cards does not prevent their use in PCR reactions!

Doctors Rahman, Emery, and Poulton (*J Med Genet* 1998;35:263) point out their problems in obtaining neonatal screening dried blood spots or Guthrie cards from patients with the MELAS 3243 mutation. They comment that of the four cards they

were able to obtain, one had been autoclaved. This, they claim, destroys the DNA.

Dried blood spot cards are commonly autoclaved or steamed before performing the bacterial inhibition assay for phenylalanine in screening for phenylketonuria (PKU).¹ We have used such blood spots for analysis of the common mutations for medium chain acyl CoA dehydrogenase deficiency (MCADD), hereditary fructose intolerance, and the NARP 8993 mutation. One has also been successfully used for identification purposes by DNA fingerprinting. In a study of autoclaved blood spots from 5014 neonates in the West Midlands Region² for the common MCADD mutation, a failure rate of PCR of only 0.3% was obtained.¹

Hence there is no reason to believe autoclaving per se substantially contributes to a poor PCR. In fact it may be that denaturing contaminating protein by autoclaving may help to reduce the amount of protein carried over during extraction and hence reduce the risk of PCR failure. However, we fully support the authors' suggestions for central funding for the storage of this important medical resource.

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Genetic Disorders and Pregnancy Outcome. Editors L D Platt, R Koch, F de la Cruz. (£55.00, \$98.00.) London: Parthenon Publishing. 1997. ISBN 1-85070-721-9.

Many clinicians who deal with high risk obstetrics or have specialised in maternal-fetal medicine, obstetric physicians, and geneticists will welcome a book on the approach to management of people with genetic disorders who intend to have