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## Haemochromatosis mutation analysis in a normal Irish population

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els that lie mostly within the toxicity-enhancement range for both cations. This should alert all potential users of this agent to take necessary precautions against its possible cytolytic side-effects.

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Haemochromatosis is an inborn error of metabolism that develops into progressive iron overload, which, if untreated, causes high morbidity and mortality.<sup>1</sup> Hereditary haemochromatosis (HH) is the most common genetic disease in the Caucasian population. It is inherited as an autosomal-recessive condition, and a high incidence is found in northern European populations.<sup>2</sup> The affected gene, *HFE*, is located on chromosome 6, telomeric to the HLA-A region.<sup>3</sup> Two mutations, C282Y and H63D, are the main missense mutations identified within the gene.

A single base substitution, 845G→A (C282Y), is the main mutation responsible for HH, and 81-90% of patients of northern European origin are homozygous for it. Compound heterozygotes (C282Y/H63D) account for approximately 7% of haemochromatosis patients. Currently, it remains unclear whether or not H63D homozygotes are at increased risk of developing HH; however, the H63D substitution may be considered a genetic variant that increases the risk of developing a mild form of the disease.<sup>4,5</sup>

The HFE protein is a 343-residue transmembrane glycoprotein, the proposed role of which is the regulation of the interaction of the transferrin receptor with transferrin. Functional data suggest that HFE protein with the C282Y mutation is unable to associate efficiently with  $\beta$ 2-microglobulin, and, hence, fails to reach the cell surface.<sup>3</sup> In the presence of the C282Y mutation, transferrin receptor regulation is absent, and unregulated iron absorption occurs. It is speculated that the C282Y mutation results in only a partial loss of HFE function.<sup>6</sup>

The HFE protein with the H63D mutation can associate with  $\beta$ 2-microglobulin. The protein reaches the cell surface but the defect lies in its failure to modify the affinity of the transferrin receptor for transferrin, which is decreased by a factor of five- to ten-fold.<sup>7</sup> H63D is thought to disrupt a salt bridge and lead to a more subtle change in HFE structure.

A high incidence of HFE mutations has been reported in Celtic populations,<sup>2</sup> with a 10-14% allele frequency for C282Y reported in the chromosomes of a normal Irish population. Previous studies have shown that 90%<sup>8</sup> and 93%<sup>9</sup> of HH patients in Ireland are homozygous for the C282Y mutations.

From two previous studies carried out on smaller normal Irish populations, the C282Y homozygote range was estimated at zero to 1.24%, the heterozygote range at 14.9-28%, and C282Y/H63D compound het-

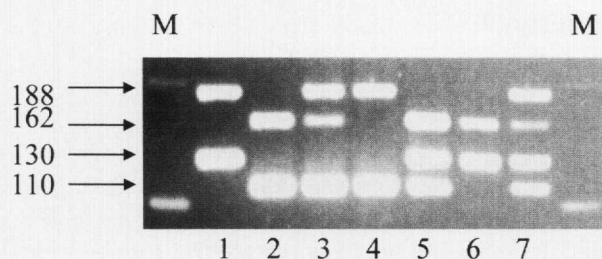


Fig. 1 Representative results of multiplex PCR analysis of C282Y and H63D mutations. Restriction fragments were separated on a 2.5% agarose gel containing 0.5 µg/mL ethidium bromide. M: 100 bp ladder. Lane 1: uncut PCR products. Lanes 2–7: Bbr PI-digested products from individuals, as follows — lane 2: wild-type at both loci; lane 3: heterozygous C282Y, wild-type H63D; lane 4: homozygous C282Y, wild-type H63D; lane 5: wild-type C282Y, heterozygous H63D; lane 6: wild-type C282Y, homozygous H63D; and lane 7: heterozygous C282Y, heterozygous H63D.

**Table 1.** Results of C282Y and H63D mutation analysis of 187 Irish blood donors. Numbers of individuals in each category (with percentage of total population) are presented. Figures presented for heterozygous C282Y and H63D do not include the single compound C282Y / H63D heterozygote.

Mutation	Heterozygous	Homozygous	Allele frequency
C282Y	37 (19.7%)	4 (2.1%)	12.3%
H63D	36 (19.3%)	5 (2.7%)	12.6%
Compound	1 (0.5%)		

erzygotes at 2.5–3.6%. The results obtained for the H63D mutation were 1.5–3.6% (homozygotes) and 23–25% (heterozygotes).<sup>8,9</sup> The study reported here utilises a larger randomised population obtained from blood donors.

Blood was drawn from 187 blood donors, who had given informed consent. DNA was isolated from peripheral blood leucocytes, using a standard salting-out procedure.<sup>10</sup> The DNA was tested for the presence of the C282Y and H63D mutations using a multiplex polymerase chain reaction (PCR), followed by a restriction digest protocol.<sup>11</sup> Briefly, wild-type 282 and 63 loci produced PCR products of 188 bp and 130 bp, respectively, which were cut by Bbr P1 into 162/26 bp and 110/20 bp amplicon fragments, respectively. The C282Y and H63D mutations resulted in the loss of the Bbr P1 restriction sites in the respective PCR products. The procedure has been verified using standard individual PCR and restriction digest protocols for C282Y and H63D mutations, both by Stott *et al.*<sup>11</sup> and in our laboratory.

The PCR reaction for the C282Y locus was not influenced by the primer binding site polymorphism recently described<sup>12</sup> because the primer set used in the

present study binds to a different site. A representative result for C282Y and H63D multiplex mutation analysis is presented in Figure 1. The C282Y and H63D allele frequencies for the 187 blood donors were determined and are presented in Table 1.

The allele frequency for C282Y of 12.3% found in this study was higher than that reported in other studies<sup>13</sup> and, when related to a worldwide allele frequency of 1.9%, supports a Celtic origin for this mutation. The homozygous value of 2.1% for C282Y in normal Irish individuals is higher than previously reported. The finding of four homozygotes and one compound heterozygote, without clinical symptoms, supports the concept of incomplete penetrance for these mutations, and may augur well for regular blood donations. Other environmental factors may be necessary to trigger clinical symptoms. It has been suggested that the maintenance of such high *HFE* mutation levels in the Celtic populations may be because *HFE* mutations confer a selective advantage in the prevention of iron deficiency. This may have protected against anaemia or lack of iron associated with multiple pregnancies.

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