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# Hemochromatosis in Ireland and HFE

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**ABSTRACT:** Sixty patients diagnosed with hereditary hemochromatosis with grade 3 or 4 hepatic iron overload and 18 patients diagnosed with hereditary hemochromatosis who had less than grade 3 hepatic iron overload were examined for the *HFE* gene mutations, 845A (C282Y) and 187G (H63D). Control samples were obtained from 109 randomly selected individuals. Fifty-six of 60 unrelated hereditary hemochromatosis patients (93%) with grade 3 or 4 hepatic iron deposition were homozygous for the C282Y mutation. Fourteen of the 18 hereditary hemochromatosis patients with <3+ iron deposition (76%) were homozygous for the C282Y mutation. Three of 8 patients who were heterozygous for the C282Y mutation were also heterozygous for the H63D mutation. Thirty-one of 109 control individuals were heterozygous for the C282Y mutation and 27 were heterozygous for the H63D mutation. Our finding that 93% of hereditary hemochromatosis patients who fulfil standard diagnostic criteria are homozygous for the C282Y mutation provides clear evidence that this mutation is strongly associated with hereditary hemochromatosis. The allele frequency of 14% for the C282Y mutation in our control population is the highest reported and supports the hypothesis of a Celtic origin for the hereditary hemochromatosis gene.



**Keywords:** hemochromatosis, HFE, C282Y, H63D, Ireland

## INTRODUCTION

Hereditary hemochromatosis, the most common autosomal recessive disease in individuals of North European descent has a carrier frequency estimated between 1:8 and 1:10, with a homozygote frequency between 1:200 and 1:400 (1). The disorder caused by an inborn error of iron metabolism, leads to increased intestinal absorption of iron and progressive iron overload. Excess iron is deposited in organs including the liver, heart and pancreas and may lead to liver cirrhosis, hepatocellular carcinoma, diabetes and heart disease. Early diagnosis is important, since treatment by regular phlebotomy prevents complications and confers normal life expectancy. Diagnosis may be difficult and delayed until irreversible complications have developed emphasizing the need for a specific test that allows early detection and thus prevent progressive disease.

Feder *et al* in the August 1996 issue of *Nature Genetics* described a candidate gene for hereditary hemochromatosis, originally designated *HLA-H* but later changed to *HFE* (2). Studies on patients of mixed European descent demonstrated that 83% of patients with hereditary

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hemochromatosis were homozygous for the C282Y missense mutation in the HFE gene (2,3). These findings were quickly substantiated by family based studies which showed an even higher frequency, nearly 91% in Brittany and 100% in Australia (4,5). Recently the UK hemochromatosis consortium has reported that this genetic test identifies 90% of UK patients with hemochromatosis (6). Homozygosity for the C282Y mutation accounts for about 70% of hereditary hemochromatosis in Italy and southern France suggesting that hereditary hemochromatosis is more heterogenous in southern Europe than in northern Europe (7,8).

A second missense mutation in the *HFE* gene, the H63D mutation has also been described. (2). Initial studies suggested that the role of the H63D mutation in hereditary hemochromatosis was minor and possibly just a polymorphism of the *HFE* gene (6,9). However, compelling evidence shows that this mutation is indeed associated with hereditary hemochromatosis (10,11,12,13). Compound heterozygotes (i.e. individuals who are heterozygous for both the C282Y and H63D mutations) and individuals who are homozygous for H63D mutation can develop significant iron overload (6,12,14).

Simon *et al* have hypothesized that the genetic mutation leading to iron overload originally occurred in peoples of Celtic origin and more recently this has been substantiated by a study demonstrating a greater prevalence of hereditary hemochromatosis in employees of the Polaroid Corporation in Massachusetts whose grandparents came from Ireland or Wales (15,16). This report outlines our analysis of the C282Y and H63D mutations in Irish hereditary hemochromatosis patients and in a control population. This is the first extensive report of investigations on hereditary hemochromatosis in Ireland, a putative source of the hereditary hemochromatosis mutation.

## MATERIALS AND METHODS

### *Patients*

Two groups of patients were studied. The first group consisted of 60 unrelated patients diagnosed with hereditary hemochromatosis. Diagnosis was made on the basis of clinical history, physical examination, persistently raised iron indices (% transferrin saturation and serum ferritin) and >3+ hepatic iron deposition. A second group of 18 patients diagnosed with hereditary hemochromatosis who had persistently raised iron indices but who had <3+ iron deposition on liver biopsy were also studied. Control samples were obtained from 109 randomly selected individuals from hospital staff. Informed consent was obtained from all patients and controls and the study protocol was approved by the Mater Misericordiae Hospital Ethics committee.

### *Methods*

DNA was extracted from 10 µl blood collected in to 2.7ml EDTA tubes or from Guthrie cards using Chelex resin (17). Primers used to amplify the fragments encompassing the C282Y and H63D mutation sites included internal restriction enzyme control sites (2,18). Following amplification the PCR product generated using the primers for the C282Y mutation was digested with *Rsa*I. The 387bp PCR reaction product digested with *Rsa*I shows two fragments of 247bp and 140bp in normal DNA and mutant DNA digested with *Rsa*I generates two new fragments of 111bp and 29bp. The PCR product generated using primers for the His63Asp mutation was digested with *Mbo*I. The 294bp product digested with *Mbo*I generates fragments of 138bp, 99bp and 57bp in normal DNA and fragments of 237bp and 57bp in mutant DNA. PCR digests were analyzed on 3% agarose gels.

## RESULTS

Fifty-six of the 60 (93%) hereditary hemochromatosis patients who had grade 3 or 4 hepatic iron overload were homozygous for the C282Y mutation. H63D mutation analysis was not carried out on these individuals since the C282Y and H63D mutations are in complete linkage disequilibrium and can never be inherited on the same chromosome (19). Of the 4 who were not homozygous for C282Y (Table 1), patient 1 was heterozygous for both C282Y and H63D (i.e. a compound heterozygote), patient 2 was heterozygous for the C282Y mutation only and patients 3 and 4 were homozygous normal for both mutations (patient 4 has clinical characteristics consistent with juvenile hemochromatosis. Three of the 4 non-C282Y homozygotes do not have a family history of hereditary hemochromatosis. The fourth non-C282Y homozygote has a positive family history (patient 1 in Table 1), he has an affected brother who is also a compound heterozygote.

Fourteen of the 18 hereditary hemochromatosis patients (77%) with < grade 3 hepatic iron deposition were homozygous for the Cys282Tyr mutation. Ten of the 14 patients (71%) who were homozygous for C282Y had a positive family history of hereditary hemochromatosis, but the C282Y status of the index cases in these 10 families was not available. Two of 18 with <3+ hepatic iron were compound heterozygotes (patients 6 and 7), patient 5 was heterozygous for the C282Y mutation and patient 8 was heterozygous for the H63D mutation (Table 2). Diagnosis of hereditary hemochromatosis in all 4 of the patients who were not homozygous for the C282Y mutation was made incidentally following investigations for gastrointestinal complaints and none have a family history of hereditary hemochromatosis. Thirty-one of the 109 hospital based controls were heterozygous for the C282Y mutation. Twenty-seven were heterozygous and 4 were homozygous for the H63D mutation. Four of the control individuals were compound heterozygotes.

**Table 1.** Details of patients not homozygous for the C282Y mutation who had 3/4+ hepatic iron deposition

| Patient | Sex | Age at Diagnosis (years) | C282Y | H63D | Presentation | Liver histology | Family history | Trans. Sat. % | Serum Ferritin µg/l |
|---|---|---|---|---|---|---|---|---|---|
| 1 | M | 47 | +/- | +/- | diabetes | 3/4+ fib | Yes | NA | NA |
| 2 | M | 66 | +/- | -/- | abdominal pain | 3+ | No | 79 | 1186 |
| 3 | F | 47 | -/- | -/- | pigmentation, arthritis | 4+ cirr | No | 79 | NA |
| 4 | F | 16 | -/- | -/- | cardiomyopathy, diabetes hypogonadotrophic hypogonadism | 4+ cirr | No | 78 | 646 |

+ indicates that the mutation is present
- indicates that the mutation is absent

**Table 2.** Details of patients not homozygous for the C282Y mutation who had <3+ hepatic iron deposition

| Patient | Sex | Age at Diagnosis (years) | C282Y | H63D | Presentation | Liver Histology | Family History | Trans. Sat. % | Serum Ferritin µg/l |
|---|---|---|---|---|---|---|---|---|---|
| 5 | M | 47 | +/- | -/- | dyspepsia | 2+ | No | 36 | 417 |
| 6 | M | 23 | +/- | +/- | oesophagitis | 1+ | No | 63 | 143 |
| 7 | M | 60 | +/- | +/- | duodenitis | 2+ fib | No | 68 | 581 |
| 8 | M | 53 | -/- | +/- | peptic ulcer | 1/2+ | No | 68 | 361 |

+ indicates that the mutation is present
- indicates that the mutation is absent

## DISCUSSION

That 93% of hereditary hemochromatosis patients fulfilling standard diagnostic criteria are homozygous for the C282Y mutation provides clear evidence that this mutation is strongly associated with hemochromatosis. This finding is in agreement with the other studies conducted in the UK, USA, Australia and France (2-6). Four of sixty (7%) patients (with 3/4+ hepatic iron) were not homozygous for the C282Y mutation, but 3 of these do not have a proven family history. Also, one of these 3 patients without a positive family history (patient 4 ) has clinical characteristics consistent with juvenile hemochromatosis (9). The UK hemochromatosis consortium has also described two patients with juvenile hemochromatosis who do not have the C282Y mutation (6). The fourth patient (patient 1 in Table 1) who is heterozygous for the C282Y mutation is also heterozygous for the H63D mutation and has an affected brother who is also a compound heterozygote. Individuals who are heterozygous for both C282Y and H63D and individuals homozygous for H63D can have significant iron overload but in general they do not have as severe an iron overload as the C282Y homozygotes (3,6,12,14). With regard to the group with <3+ hepatic iron deposition (n=18), the 4 individuals who were not homozygous for the C282Y mutation did not have a positive family history. Furthermore, their diagnosis was made incidentally to other investigations for gastrointestinal disorders and therefore they were asymptomatic from the hereditary hemochromatosis point of view. That 14/18 (77%) of patients with provisional diagnoses of hereditary hemochromatosis who are homozygous for the C282Y mutation and who do not fulfil standard diagnostic criteria (i.e. have <3-4+ hepatic iron) indicates that the inclusion criteria for hereditary hemochromatosis may change substantially in the future.

Thirty-one of 109 randomly selected hospital staff were heterozygous for the C282Y mutation. This indicates an allele frequency of 14% and a heterozygote or "carrier" frequency of ~1 in 4. This is surprisingly high since the "carrier" frequency is generally accepted to be about 1:8 - 1:10 but nevertheless in agreement with a recent study which demonstrates a heterozygote frequency of 1 in 5 in Irish people (9/45 were heterozygous for the C282Y mutation) (18,20). Three of the 31 C282Y heterozygotes in the randomly selected control population have a parent (the father in all 3 cases) who has been diagnosed with hereditary hemochromatosis and the mother of a fourth C282Y heterozygote whose family were subsequently screened for the C282Y mutation was found to be homozygous for the mutation but had normal iron indices on screening at age 55. The C282Y homozygote frequency determined from our 14% allele frequency indicates a homozygote frequency of 1 in 51 in Ireland. This is higher than that estimated from a recent study examining the global prevalence of the C282Y mutation which suggests a homozygote frequency of 1 in 100 in Ireland based on a heterozygote frequency of 1 in 5 (18). These findings support our previous observation of the high frequency of homozygous x heterozygous matings in Ireland and are in keeping with the hypothesis of a Celtic origin for the hemochromatosis gene (15,16,21)

Twenty-seven heterozygotes and 4 homozygotes for the H63D found in our control population indicates an allele frequency of 16% for this mutation which is also in agreement with the study which demonstrated that the highest allele frequencies for this mutation were observed in European populations (18).

The phenotypic expression of hereditary hemochromatosis in the Irish population has not been determined but the homozygote frequency of 1 in 51 for the C282Y mutation suggests that a large proportion of Irish hereditary hemochromatosis patients may remain undiagnosed. Our findings emphasize the necessity of firstly estimating the frequencies of the *HFE* genotypes in a larger control population and secondly of addressing the penetrance of the *HFE* genotypes in the Irish population. These studies will also permit us to address some of the factors including genetic, environmental or immune which may play a role in the expression of the *HFE* genotype. Furthermore, the results from such studies together with the fact that the high prevalence, morbidity and mortality as well as the benefits of early diagnosis and treatment make hereditary hemochromatosis an ideal target for screening will allow us to evaluate the use and implications of genetic population screening for hereditary hemochromatosis in Ireland (22,23).

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