A simple genetic test identifies 90% of UK patients with haemochromatosis

The UK Haemochromatosis Consortium

Abstract

Background—The diagnosis of genetic haemochromatosis (GH) before iron overload has developed is difficult. However a convincing candidate gene for GH, HFE (previously HLA-H), has been described recently.

Aims—To determine the prevalence of the haemochromatosis associated HFE mutations C282Y and H63D in United Kingdom affected and control populations.

Methods—The prevalence of the HFE C282Y and H63D mutations was determined by polymerase chain reaction amplification and restriction enzyme digestion in a cohort of 115 well characterised patients with GH and 101 controls from the United Kingdom.

Results—One hundred and five of 115 (91%) patients with GH were homozygous for the C282Y mutation. Only one of 101 (1%) controls was homozygous for the C282Y mutation and this individual currently shows evidence of iron overload. Two of five patients who did not have either of the two described mutations of HFE had early onset iron overload (ages 16 and 24). One had a family history of cardiac failure and the second was subsequently hospitalised due to cardiac failure. These are the first phenotypic observations for patients without either C282Y or H63D mutation of HFE.

Conclusion—This simple genetic test promises to be a highly effective tool in the diagnosis of GH.

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Keywords: haemochromatosis; genetic testing; HFE; HLA-H; C282Y; H63D; iron overload

Genetic haemochromatosis is an autosomal recessive disorder resulting in iron overload. In northern European populations, where the disease is believed to have originated, as many as 1 in 300 individuals are affected.^{1,2} Unrecognised, the iron loading results in tissue damage affecting the liver, pancreas, joints, anterior pituitary, and heart.³ Early diagnosis and treatment by repeated venesection is effective and restores normal life expectancy.⁴ However, detection of sporadic genetic haemochromatosis in the early, asymptomatic phase may be difficult in the absence of a reliable test.

Simon *et al* showed that the gene responsible for genetic haemochromatosis mapped close to the major histocompatibility complex (MHC) locus HLA-A (6p21.3).⁵ About 50% of genetic haemochromatosis chromosomes carry an ex-

tended "ancestral" haplotype of chromosome 6 microsatellite marker alleles (D6S265-1, D6S105-8, D6S1260-4 which includes HLA-A3), reflecting the haplotype of the founder mutation.⁶ In the absence of information about the biochemical defect in genetic haemochromatosis, positional cloning has been used in the search for the gene. Recently, we showed that the gene mapped telomeric to HLA-A, close to the microsatellite marker D6S1260.⁶ Extension of this approach led to the identification of a strong candidate gene for genetic haemochromatosis, termed HFE⁷ (previously HLA-H). The direct evidence on which this claim was based centred on the high frequency (83%) of US patients with genetic haemochromatosis homozygous for a single mutation (C282Y), the association of this mutation with the ancestral haplotype, and the proposed functional consequences of this mutation. We have studied the prevalence of mutations in this gene in a cohort of UK patients with genetic haemochromatosis identified by strict diagnostic criteria.

Patients and Methods

DNA was prepared from blood samples obtained from patients with haemochromatosis diagnosed and managed at four UK centres (King's College Hospital, Royal Free Hospital, John Radcliffe Hospital, and the University Hospital of Wales). The diagnosis of genetic haemochromatosis was confirmed on the basis of a hepatic iron index of greater than 1.9, or greater than 5 g mobilisable iron by quantitative phlebotomy, in the absence of any other cause of iron loading. Thirty five per cent of the 115 patients with genetic haemochromatosis studied had at least one other known affected family member. Control samples were obtained from a series of 101 healthy blood donors from South Wales.⁸

HFE gene analysis was performed by polymerase chain reaction (PCR) amplification of total genomic DNA of the two regions of the HFE gene carrying the mutations C282Y and H63D.⁷ The G to A transition (aa282) creates a new RsaI site; there is a second RsaI site in this fragment which acts as an internal control in the restriction fragment length polymorphism (RFLP) analysis. The C to G transversion (aa63) results in the loss of BclI and MboI sites in the amplified product allowing typing by RFLP. Results were confirmed by relocating the 3' primer (5' CTT GCT GTG GTT GTG ATT TTC C 3') to include a second MboI site, thus providing an internal control.

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TABLE 1 Genotype frequencies for mutations in HLA-H

	Genotypes						Total
	HH/YY	HH/CC	HD/CC	HD/CY	HH/CY	DD/CC	
GH	105	5	0	3	1	1	115
Controls	1	65	22	4	6	3	101

Genotypes are given for amino acid 63/amino acid 282 of the polypeptide (C, cysteine; D, aspartic acid; H, histidine; Y, tyrosine). HH/CC = normal (wild type) and HH/YY = homozygosity for the C282Y mutation. GH, genetic haemochromatosis.

Results

One hundred and fifteen unrelated patients with genetic haemochromatosis and 101 controls were genotyped (table 1). The frequency of the C282Y mutation was 93% and 6% in patient and control chromosomes respectively. Homozygosity for this mutation was seen in 91% of patients and only 1% of controls. Homozygosity for the C282Y mutation was seen in 38/40 patients with genetic haemochromatosis with a positive family history, which was not significantly different from 67/75 patients with genetic haemochromatosis without a family history ($\chi^2=0.046$, $p=0.50$). Of the 99 patients for which we had full haplotype information, 23 were homozygous for the extended ancestral haplotype (D6S265-1, D6S105-8, D6S1260-4 which includes HLA-A3) and homozygous for the C282Y mutation.

The H63D variant was present in 2% of haemochromatosis and 16% of control chromosomes. One patient and three controls were homozygous for this variant. There was no evidence of iron loading in these three controls.

None of the patients homozygous for the C282Y mutation carried the H63D variant. However, three patients and four controls were heterozygous for both the C282Y and H63D mutations (compound heterozygotes). These three compound heterozygote patients had mild disease as evidenced by initial quantitative phlebotomy removing circa 5 g iron. The four controls who were compound heterozygotes showed no signs of iron loading.

Four of the six haemochromatotic patients without the C282Y mutation had clinical and biochemical features typical of genetic haemochromatosis (table 2). The remaining two however had early onset of iron overload. Patient 1 was diagnosed with heart failure at 29 years of age. On reviewing his history, he had displayed features of hypogonadotropic hypogonadism five years earlier (age 24). The diagnosis of genetic haemochromatosis was made on the basis of serum iron studies (serum iron 31 $\mu\text{mol/l}$, total iron binding capacity

(TIBC) 33 $\mu\text{mol/l}$, ferritin 3350 $\mu\text{g/l}$), myocardial biopsy, and the quantitation of iron overload. Initial desferrioxamine treatment for 52 days removed 3.5 g iron. Venesection therapy, commenced in parallel with the iron chelation, was continued over 13.5 months removing an additional 11 g iron. In total, 14.5 g iron was removed in 13.5 months. Patient 1 has had no transfusions, and has no known family history, as he is adopted. His genotype (D6S265 1,3; D6S105 6,8; D6S1260 3,4; D6S1621 5,6) does not provide sufficient information to exclude the possible presence of one copy of the ancestral haplotype, but reveals that he is not homozygous for the ancestral haplotype.

Patient 2 was diagnosed at the age of 16 during family screening. His elder brother had presented at age 21 with gross heart failure and on investigation was found to have micronodular cirrhosis and massive iron overload on liver biopsy. The brother had died of an asystolic cardiac arrest while in hospital before treatment could be started. On biochemical screening patient 2 was found to have a serum iron concentration of 42 $\mu\text{mol/l}$, TIBC of 44 $\mu\text{mol/l}$, ferritin of 2082 $\mu\text{g/l}$, and on liver biopsy grade 4 siderosis but minimal fibrosis. He has no abnormality of copper metabolism as determined by biochemical analysis. He became iron deficient after the removal of 10 g iron by venesection and during the follow up period of 20 years required regular venesection to maintain normal iron indexes. Microsatellite analysis for patient 2 revealed a haplotype (D6S265 3,6; D6S105 6,8; D6S1260 4,4; D6S1621 7,10) in which neither chromosome demonstrates the founder haplotype.

Discussion

We confirm the remarkably strong association between the C282Y mutation in HFE and genetic haemochromatosis as recently reported.⁷ The observed homozygosity for this mutation was higher in this sample of UK haemochromatosis patients than that reported in the original sample from the USA (91% versus 83%, $\chi^2=3.95$, $p=0.047$). Recent reports have confirmed the high frequency of homozygosity for the C282Y mutation in patients with genetic haemochromatosis, and reveal consistent results in similar populations. Thus, two American studies have now reported 83% and 82% homozygosity for C282Y⁹; 92% homozygosity was observed in a French Breton series,¹⁰ similar to this UK study (91% homozygosity) and 100% occurred in an Aus-

TABLE 2 Clinical details of patients not carrying the C282Y mutation

Patient	Genotype	Known family history	Age at onset of symptoms (y)	Iron removed by initial venesection/desferrioxamine (g)	Liver histology/grade of siderosis	Serum Fe/TIBC ($\mu\text{mol/l}$)	Transferrin saturation (%)	Serum ferritin ($\mu\text{g/l}$)
1	HH/CC	No (adopted)	24	14	—	31/33	94	3350
2	HH/CC	Yes	16*	10	Fibrosis/4	42/44	95	2082
3	HH/CC	No	60	7	Fibrosis/2 (HII-5,6)	37.6/44.9	84	1350
4	HH/CC	No	55	NA†	Cirrhosis	NA	80	595
5	HH/CC	No	54	5	Fibrosis/3	40/54	74	1400
6	DD/CC	No	36	> 10	Cirrhosis/heavy siderosis	60/65	92	7000

* Diagnosed at age 16 during family screening.

† Died shortly after diagnosis.

tralian study,¹¹ possibly reflecting recent migration and a secondary founder effect.

The frequency of the C282Y mutation in control chromosomes observed by us is compatible with the predicted carrier rate for the haemochromatosis allele of approximately 10%.¹² In the present study we found one control who was homozygous for the C282Y mutation. This subject was found to have a transferrin saturation of 70% and is currently being investigated. These data together support the proposal that the C282Y mutation in HFE represents the ancestral genetic haemochromatosis mutation. Final confirmation requires functional analyses.

The significance of compound heterozygosity (heterozygosity for both the C282Y and H63D mutations) is not clear as we found similar numbers in patients and controls. Three haemochromatosis patients and four controls were compound heterozygotes. Two additional patients with suspected genetic haemochromatosis failed to meet diagnostic criteria for entry into the study but were subsequently investigated and found to be compound heterozygotes.

Five patients lacked either mutation. Three of these presented classically in middle age. However, the remaining two patients presented early (ages 16 and 24 years). One patient developed haemochromatotic cardiomyopathy and the second had a family history of cardiac disease. Neither of these two patients were homozygous for the ancestral haplotype. Possible causes for heterogeneity of genetic haemochromatosis include: other mutations of HFE; mutations in other causative genes; functional polymorphism of genes which modify the phenotype; environmental effects; and combinations of the above.

The screening method we and others¹⁰ have used involves RFLP analysis of PCR products with *RsaI*. This method is rapid and straightforward, and can be subjected to quality control as the PCR product has a built in control *RsaI* site. This method can therefore be easily transferred to any regional genetics or routine hospital laboratory as it does not require sophisticated automated technology. The high sensitivity and specificity of this genetic test make it ideally suited for the preliminary investigation of family members of affected patients and for screening individuals with liver disease or biochemical evidence of iron overload.

Although the majority of patients with genetic haemochromatosis are homozygous for C282Y, it remains to be established whether all homozygous individuals would develop iron overload. Data from a group of patients with porphyria cutanea tarda (PCT) provide the first evidence on this question of development of iron overload. PCT is another iron dependent condition in which iron removal by phlebotomy produces prolonged biochemical and clinical remission. However, patients with PCT rarely accumulate as much iron as do patients with genetic haemochromatosis. Roberts *et al* have recently shown that over 40% of patients with sporadic PCT have at least one

copy of the C282Y mutation.¹³ Seven of the 41 patients were homozygous for this mutation. These patients presented at a mean age of 67 years, but none had developed clinically significant iron overload. This shows that an undetermined proportion of C282Y homozygotes may live for 60 years or more without developing iron overload leading to tissue damage. To determine this proportion will require large scale screening for this mutation along with measures of iron status. This group of patients may prove helpful in elucidating the biology of genetic haemochromatosis.

Genetic haemochromatosis should no longer be considered a rare condition^{1 2} and this has important health implications. In this study, one C282Y homozygote was identified out of 101 controls, and this individual showed evidence of iron loading. In another study, two out of 368 random UK samples were found to be homozygous for C282Y.¹⁴

Prior to the identification of the candidate gene, universal biochemical screening was proposed based on cost benefit analysis.¹⁵ The Center for Disease Control has recommended that the entire US population be screened for haemochromatosis by transferrin saturation.¹⁶ Genetic analysis will provide a rapid and specific way of identifying those at risk from iron overload in the general population.

Genotyping individuals for the C282Y mutation makes it possible to identify those at risk in the early asymptomatic phase of the disease. Individuals who are homozygous for this mutation should be considered at risk for developing iron overload. Whether or not all such subjects develop major iron overload remains to be determined. Before this genetic test is adopted, ethical issues need to be addressed, such as how insurance companies will view homozygous C282Y individuals without iron overload or tissue damage. Powell¹⁷ has stated that if normal iron concentrations are maintained by regular phlebotomy, these patients should not be denied life insurance or have their premiums raised. However, the advantage of this genetic test will lie in early detection. It is widely accepted that venesection carried out before the onset of tissue damage will restore normal life expectancy.⁴

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1 Worwood M. Genetics of haemochromatosis. *Baillieres Clin Haematol* 1994; 7: 903-18.

2 Leggett BA, Halliday JW, Brown NN, Bryant S, Powell LW. Prevalence of haemochromatosis amongst asymptomatic Australians. *Br J Haematol* 1990; 74: 525-30.

- 3 Bothwell TH, Chariton RW, Motulsky AG. Hemochromatosis. In: *The metabolic and molecular bases of inherited disease*. New York: McGraw Hill, 1995: 2237–69.
- 4 Niederau C, Fischer R, Purschel A, Stremmel W, Haussinger D, Strohmeyer G. Long-term survival in patients with hereditary hemochromatosis. *Gastroenterology* 1996; **110**: 1107–19.
- 5 Simon M, Pawlotsky Y, Bourel M, Fauchet R, Genetet B. Hemochromatose idiopathique: maladie associee a l'antigene tissulaire HLA-A3? *Nouvelle Presse Medicine* 1975; **4**: 1432.
- 6 Raha-Chowdhury R, Bowen DJ, Stone C, Pointon JJ, Terwilliger JD, Shearman JD, *et al.* New polymorphic microsatellite markers place the haemochromatosis gene telomeric to D6S105. *Hum Mol Genet* 1995; **4**: 1869–74.
- 7 Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, *et al.* A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996; **13**: 399–408.
- 8 Worwood M, Darke C. Serum ferritin, blood donation, iron stores and haemochromatosis. *Transfus Med* 1993; **3**: 21–8.
- 9 Beutler E, Gelbart T, West C, Lee P, Adams M, Blackstone R, *et al.* Mutation analysis in hereditary haemochromatosis. *Blood Cells Mol Dis* 1996; **22**: 187–94.
- 10 Jouanolle AM, Gandon G, Blayau M, Campion ML, Yaouanq J, Mosser J, *et al.* Haemochromatosis and HLA-H. *Nat Genet* 1996; **14**: 251–2.
- 11 Jazwinska EC, Cullen LM, Busfield F, Pyper WR, Webb SI, Powell LP, *et al.* Haemochromatosis and HLA-H. *Nat Genet* 1996; **14**: 249–51.
- 12 Powell LW, Jazwinska E, Halliday JW. Primary iron overload. In: *Iron metabolism in health and disease*. 1994: 227–70.
- 13 Roberts AG, Whatley SD, Morgan R, Lawless S, Worwood M, Elder GH. Increased frequency of the haemochromatosis Cys282Tyr mutation in sporadic porphyria cutanea tarda. *Lancet* 1997; **349**: 321–3.
- 14 Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJH. Global prevalence of putative haemochromatosis mutations. *J Med Genet* 1997; **34**: 275–8.
- 15 Adams PC, Gregor JC, Kertesz AE, Valberg LS. Screening blood donors for hereditary hemochromatosis: decision analysis model based on a 30-year database. *Gastroenterology* 1995; **109**: 177–88.
- 16 Barton JC, Bertoli LF. Hemochromatosis: the genetic disorder of the twentyfirst century. *Nat Med* 1996; **2**: 394–5.
- 17 Powell LW. Hemochromatosis: the impact of early diagnosis and therapy. *Gastroenterology* 1996; **110**: 1304–7.