CONCISE REPORT

# Genetic testing for haemochromatosis in patients with chondrocalcinosis

**A E Timms, R Sathananthan, L Bradbury, N A Athanasou, M A Brown**

*Ann Rheum Dis* 2002;**61**:745–747

Hereditary haemochromatosis (HH) is the most common lethal monogenic human disease, affecting roughly 1 in 300 white northern Europeans. Homozygosity for the C282Y polymorphism within the HFE gene causes more than 80% of cases, with compound heterozygosity of the C282Y and H63D polymorphism also increasing susceptibility to disease. The aim of this study was to determine the frequency of the C282Y and H63D polymorphisms in the disease, and to assess the risk of HH in heterozygotes for the C282Y polymorphism. 128 patients were recruited because of either radiographic chondrocalcinosis (at least bicompartmental knee disease or joints other than the knee involved) or CPPD pseudogout. Genotyping of the HFE C282Y and H63D mutations was performed using PCR/SSP and genotypes for the C282Y polymorphism confirmed by PCR/RFLP. Historical white European control data were used for comparison. Two previously undiagnosed C282Y homozygotes (1.6%), and 16 C282Y heterozygotes (12.5%), including four (3.1%) C282Y/H63D compound heterozygotes were identified. This represents a significant overrepresentation of C282Y homozygotes (relative risk 3.4, p=0.037), but the number of heterozygotes was not significantly increased. At a cost per test of £1 for each subject, screening all patients with chondrocalcinosis using the above ascertainment criteria costs only £64 for each case of haemochromatosis identified, clearly a highly cost effective test given the early mortality associated with untreated haemochromatosis. Routine screening for haemochromatosis in patients with appreciable chondrocalcinosis is recommended.

Hereditary haemochromatosis (HH) is one of the most common inherited diseases, affecting about 300 people of northern European descent.[1] It affects iron handling and if untreated can result in cirrhosis of the liver, hepatocellular carcinoma, diabetes mellitus, cardiomyopathy, and other conditions resulting in premature death. Three distinct genetic subsets of haemochromatosis have been characterised so far. Type 2 HH has been mapped to chromosome 1q (HFE2), is primarily involved with juvenile haemochromatosis,[2] and has not been related to the more common later onset disease. Type 3 is associated with mutations; Camaschella *et al*[3] identified a Tyr to stop signal substitution at codon 250 of the transferrin receptor-2 (TFR2) gene in two Sicilian families and linkage analysis subsequently established a locus at chromosome 7q22 (HFE3). Recently, two more genes involved in iron metabolism have been linked to HH. A mutation within the gene SLC11A3 (solute carrier family 11 A3) was found in all affected members of a large Dutch family.[4] A point mutation of the iron response element motif of H-ferritin mRNA[5] has also been reported to increase cellular iron uptake in a Japanese family. However, by far the most common form is type 1 HH, which is caused by mutations of the HFE gene, and is mapped to 6p21.3.[6] Over 80% of patients with HH are homozygous for a mutation at codon 282, which results in a Cys to Tyr substitution that inhibits HFE/β2-microglobulin binding.[7] A second mutation within the gene at codon 63 (His to Asp) is also associated with HH when inherited in combination with the C282Y mutation,[8] where compound heterozygosity for the two mutations results in increased susceptibility to disease. About 90% of C282Y homozygotes and 38% of H63D/C282Y heterozygotes develop haemochromatosis, and C282Y heterozygotes have also been shown to exhibit minor abnormalities of iron overload. Heterozygosity for the C282Y variant has been associated with porphyria cutanea tarda,[9] coronary heart disease,[10] and type 2 diabetes[11] in some but not all studies.

Chondrocalcinosis is a common rheumatic disease characterised by deposition of calcium salts in the joint cartilage, commonly calcium pyrophosphate dihydrate or calcium hydroxyapartite. Chondrocalcinosis may occur as a complication of various conditions including hyperparathyroidism, haemochromatosis, hypothyroidism, and Wilson's disease[12]; between 30% and 50% of patients with haemochromatosis also have chondrocalcinosis.

Previous studies examining the frequency of haemochromatosis in chondrocalcinosis have not used genetic screening. The aim of this study was to determine the frequency of genetic haemochromatosis in chondrocalcinosis and to assess the risk of disease in heterozygotes for the C282Y polymorphism.

## PATIENTS AND METHODS

### Patients

A total of 128 patients (70 male and 58 female) were recruited from the Nuffield Orthopaedic Centre. Patients were recruited because of either radiographic chondrocalcinosis (at least bicompartmental knee disease or involvement of joints other than the knee (82 (64%) patients)) or calcium pyrophosphate dihydrate pseudogout (46 (36%) patients). Ages ranged from 28 to 89 years, mean 64.7 years. Patients were recruited irrespective of whether they had previously been screened for metabolic diseases associated with haemochromatosis. None of the patients had previously been diagnosed as having iron overload.

### Laboratory and statistical methods

A polymerase chain reaction using sequence specific primers (PCR/SSP)[13] was used to identify alleles at both polymorphisms within codons 63 and 282 (fig 1). Results at codon 282 were confirmed by PCR using restriction fragment length polymorphism (PCR/RFLP).[14] Historical white controls of European descent were used for comparison.[15] The controls

**Abbreviations:** HH, hereditary haemochromatosis; PCR/RFLP, polymerase chain reaction using restriction fragment length polymorphism; PCR/SSP, polymerase chain reaction using sequence specific primers;

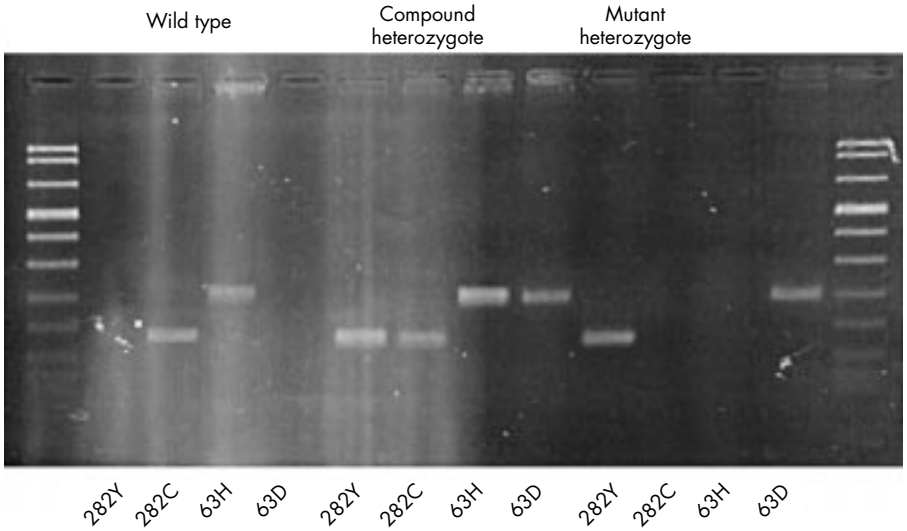


**Figure 1** A polymerase chain reaction with sequence specific primers (PCR/SSP)[13] was used to identify alleles at both polymorphisms within codons 63 and 282.

**Table 1** HFE polymorphism genotypes in patient and historical control populations

| Genotype | Patients n (%) | Historical controls n (%) |
|---|---|---|
| C282Y/C282Y | 2(1.6)* | 16(0.5) |
| C282Y/wild-type | 16(13) | 359(12) |
| C282Y/H63D | 4(3.1) | 65(2.2) |
| Other | 110(86) | 2636(88) |
| Total | 128 | 3011 |

*Relative risk 3.4, p=0.037.

were genotyped as part of a large community survey and are therefore not significantly affected by ascertainment bias. This problem may lead to underestimation of haemochromatosis gene frequencies when exclusion of diagnosed cases may inadvertently have occurred.

Relative risk of genotypes was determined by standard methods.[16]

## RESULTS

Results for both polymorphisms in comparison with the historical white controls are shown in table 1. We identified two previously undiagnosed C282Y homozygotes (1.6%) in patients compared with 16 of 3011 controls (0.5%). This represents a significant overrepresentation of C282Y homozygotes (relative risk 3.4, p=0.037). We also identified four C282Y/H63D compound heterozygotes (3.1%), a similar proportion to the normal control population (2.2%). There was also no significant difference in the number of C282Y heterozygotes (12.5% in the patient group *v* 11.9% in the control group). Genotyping results of codon 282 were consistent with PCR/SSP and PCR/RFLP in all 128 patients.

The genotyping cost for each patient was £1, representing the consumable costs for DNA extraction, PCR/RFLP, and PCR/SSP; thus in this subset of patients with chondrocalcinosis the cost for each C282Y homozygote discovered was £64.

## DISCUSSION

This study confirms the association of haemochromatosis caused by homozgosity for the C282Y HFE variant, with significant chondrocalcinosis, as defined by ascertainment criteria for this study. Although haemochromatosis remains an uncommon cause of chondrocalcinosis, it is sufficiently frequent that screening for haemochromatosis is clearly worthwhile in patients with appreciable chondrocalcinosis. However, whether screening patients with less severe chondrocalcinosis is worthwhile is uncertain. Previous studies have shown that patients with haemochromatosis and chondrocalcinosis are younger and more likely to be male than patients with chondrocalcinosis not associated with haemochromatosis.[17 18] Screening elderly patients with mild chondrocalcinosis may not therefore be as worthwhile.

We found no association of chondrocalcinosis with genotypes causing less severe concentrations of iron overload, such as C282Y/wild-type and C282Y/H63D heterozygote genotypes. This indicates that unlike the situation in porphyria cutanea tarda, mild degrees of iron overload are not sufficient to cause chondrocalcinosis. The study had 80% power to detect an association (for $p<0.05$, one tailed) with C282Y heterozygotes with an odds ratio of less than 1.9, and an association with C282Y/H63D compound heterozygotes with an odds ratio of less than 3.2. Thus associations of smaller magnitude may not have been found in this study.

Previous modelling of cost effectiveness of screening for haemochromatosis has suggested that screening is cost effective if the prevalence of disease in the screened population was equal to 3/1000, the positive predictive value of the diagnostic test was less than 0.4, and the test cost was less than $12.[19] The positive predictive value of C282Y screening is more than 80%, and the cost of the genetic test in our laboratory is about £1, well within the values required for screening to be cost effective. Although further studies are required to confirm our findings, this study suggests that there is a strong argument for widespread genetic screening for haemochromatosis in patients with appreciable chondrocalcinosis and onset of chondrocalcinosis at an early age.

**Authors' affiliations**
**A E Timms, R Sathananthan, L Bradbury, M A Brown,** Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Headington, Oxford, OX3 7BN, UK
**N A Athanasou,** Nuffield Orthopaedic Centre, Windmill Road, Headington, Oxford, OX3 7LD, UK

Correspondence to: Dr M Brown, Spondyloarthritis and Bone Disease Research Group, Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Headington, OX3 7BN, UK; mbrown@well.ox.ac.uk

Accepted 25 February 2002

## ACKNOWLEDGEMENT

This work was funded by the Arthritis Research Campaign, UK.

## REFERENCES

1 **Edwards CQ**, Griffen LM, Goldgar D, Drummond C, Skolnick MH, Kushner JP. Prevalence of hemochromatosis among 11 065 presumably healthy blood donors. *N Engl J Med* 1988;318:1355–62.
2 **Roetto A**, Totaro A, Cazzola M, Cicilano M, Bosio S, D'Ascola G, *et al*. Juvenile hemochromatosis locus maps to chromosome 1q. *Am J Hum Genet* 1999;64:1388–93.
3 **Camaschella C**, Roetto A, Cali A, De Gobbi M, Garozzo G, Carella M, *et al*. The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. *Nat Genet* 2000;25:14–5.
4 **Njajou OT**, Vaessen N, Joosse M, Berghuis B, van Dongen JW, Breuning MH, *et al*. A mutation in SLC11A3 is associated with autosomal dominant hemochromatosis. *Nat Genet* 2001;28:213–4.
5 **Kato J**, Fujikawa K, Kanda M, Fukuda N, Sasaki K, Takayama T, *et al*. A mutation, in the iron-responsive element of H ferritin mRNA, causing autosomal dominant iron overload. *Am J Hum Genet* 2001;69:191–7.
6 **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.
7 **Feder JN**, Tsuchihashi Z, Irrinki A, Lee VK, Mapa FA, Morikang E, *et al*. The hemochromatosis founder mutation in HLA-H disrupts beta2-microglobulin interaction and cell surface expression. *J Biol Chem* 1997;272:14025–8.
8 **Risch N**. Haemochromatosis, HFE and genetic complexity. *Nat Genet* 1997;17:375–6.
9 **Santos M**, Clevers HC, Marx JJ. Mutations of the hereditary hemochromatosis candidate gene HLA-H in porphyria cutanea tarda. *N Engl J Med* 1997;336:1327–8.
10 **Rasmussen ML**, Folsom AR, Catellier DJ, Tsai MY, Garg U, Eckfeldt JH. A prospective study of coronary heart disease and the hemochromatosis gene (HFE) C282Y mutation: the atherosclerosis risk in communities (ARIC) study. *Atherosclerosis* 2001;154:739–46.
11 **Sampson MJ**, Williams T, Heyburn PJ, Greenwood RH, Temple RC, Wimperis JZ, *et al*. Prevalence of HFE (hemochromatosis gene) mutations in unselected male patients with type 2 diabetes. *J Lab Clin Med* 2000;135:170–3.
12 **Jones AC**, Chuck AJ, Arie EA, Green DJ, Doherty M. Diseases associated with calcium pyrophosphate deposition disease. *Semin Arthritis Rheum* 1992;22:188–202.
13 **Mullighan CG**, Bunce M, Fanning GC, Marshall SE, Welsh KI. A rapid method of haplotyping HFE mutations and linkage disequilibrium in a Caucasoid population. *Gut* 1998;42:566–9.
14 **Jeffrey GP**, Chakrabarti S, Hegele RA, Adams PC. Polymorphism in intron 4 of HFE may cause overestimation of C282Y homozygote prevalence in haemochromatosis. *Nat Genet* 1999;22:325–6.
15 **Olynyk JK**, Cullen DJ, Aquilia S, Rossi E, Summerville L, Powell LW. A population-based study of the clinical expression of the hemochromatosis gene. *N Engl J Med* 1999;341:718–24.
16 **Lathrop GM**. Estimating genotype relative risks. *Tissue Antigens* 1983;22:160–6.
17 **Atkins CJ**, McIvor J, Smith PM, Hamilton E, Williams R. Chondrocalcinosis and arthropathy: studies in haemochromatosis and in idiopathic chondrocalcinosis. *Q J Med* 1970;39:71–82.
18 **Huaux JP**, Geubel A, Koch MC, Malghem J, Maldague B, Devogelaer JP, *et al*. The arthritis of hemochromatosis. A review of 25 cases with special reference to chondrocalcinosis, and a comparison with patients with primary hyperparathyroidism and controls. *Clin Rheumatol* 1986;5:317–24.
19 **Phatak PD**, Guzman G, Woll JE, Robeson A, Phelps CE. Cost-effectiveness of screening for hereditary hemochromatosis. *Arch Intern Med* 1994;154:769–76.