Global prevalence of putative haemochromatosis mutations

Alison T Merryweather-Clarke, Jennifer J Pointon, Jeremy D Shearman, Kathryn J H Robson

Abstract

Haemochromatosis is a genetic disease associated with progressive iron overload, and is common among populations of northern European origin. HLA-H is a recently reported candidate gene for this condition. Two mutations have been identified, a substitution of cysteine for tyrosine at amino acid 282 (C282Y, nucleotide 845) and of histidine for aspartate at amino acid 63 (H63D, nucleotide 187). Over 90% of UK haemochromatosis patients are homozygous for the C282Y mutation. We have examined 5956 chromosomes (2978 people) for the presence of HLA-H C282Y and H63D by PCR followed by restriction enzyme analysis. We have found world wide allele frequencies of 1.9% for C282Y and 8.1% for H63D. The highest frequencies were 10% for C282Y in 90 Irish chromosomes and 30.4% for H63D in 56 Basque chromosomes. C282Y was most frequent in northern European populations and absent from 1042 African chromosomes, 484 Asian chromosomes, and 644 Australasian chromosomes. The distribution of the C282Y mutation coincides with that of populations in which haemochromatosis has been reported and is consistent with the theory of a northern European origin for the mutation. The H63D polymorphism is more widely distributed and its connection with haemochromatosis remains unclear.

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Keywords: haemochromatosis; HLA-H; northern European/Celtic populations.

Haemochromatosis is an autosomal recessive disorder in which increased iron absorption causes iron overload, eventually resulting in tissue damage which can be fatal if undetected. However, life expectancy is normal if iron is removed by venesection in the precirrhotic stage of the disease. Patients often do not present until middle age and genetic testing of populations of northern European origin, in which the disease frequency has been estimated at 1/300,11 would identify those at risk before the development of tissue damage because of iron overload. Feder et al.9 have recently identified two mutations in HLA-H, a candidate gene for haemochromatosis on chromosome 6 (6p22.1): a G to an A at nucleotide 845 in the codon of amino acid 282, changing cysteine to tyrosine, and a C to a G at nucleotide 187 in the codon of amino acid 63, changing histidine to asparte. Feder et al. found that 83% of 178 haemochromatosis patients were homozygous for the C282Y mutation. The UK Haemochromatosis Consortium (submitted for publication) has found that over 90% of 115 UK patients were homozygous for the mutation, with H63D allele frequencies of 2% in patients and 16% in controls. Jawinska et al. found that 100% of 112 Caucasian Australian patients with a positive family history of haemochromatosis were homozygous for the C282Y mutation, and Beutler et al. and Jouanolle et al. found that 82.3% and 92.4% of Caucasian haemochromatosis patients in the USA and Brittany, respectively, were homozygous for C282Y, and found H63D allele frequencies of 5.4% and 3.4% in patients and 15-16.5% in controls. Calandro et al. reported that 80% of 56 patients in the USA were homozygous for the C282Y mutation. Therefore, C282Y is an excellent marker for the disease, whether or not HLA-H is confirmed to be the haemochromatosis gene. What role, if any, H63D plays in haemochromatosis remains to be established.

We have analysed 5956 chromosomes for these mutations, in order to ascertain the relationship between their prevalence and that of the disease in populations with different frequencies of haemochromatosis.

Methods

The origin of DNA samples is shown in the footnotes to table 1. All populations screened were anonymous to the authors. The two regions of the HLA-H gene containing the proposed mutations were amplified by PCR before restriction fragment length polymorphism (RFLP) analysis. For both PCR products, primers were designed to include an internal restriction enzyme site in the product, to act as a control for complete digestion. Both
PCR reactions were performed using an annealing temperature of 58°C.

For the H63D mutation, the following primers gave a product of 294 bp: H63DF: 5' ACA TGG TTA AGG CCT GTT GC; H63DR: 5' CCT GCT GTG GTT GTG ATT TTC C. Following digestion with Mbol, those products carrying the mutation gave restriction fragments of 237 and 57 bp, while fragments lacking the mutation contained an extra Mbol site and gave restriction fragments of 138, 99, and 57 bp.

For the C282Y mutation, the following primers gave a product of 343 bp: C282YF: 5' CAA GTG CCT CCT TTG GTG AAG GTG ACA CAT; C282YR: 5' CTC AGG CAC TCC TCT CAA CC. Following digestion with RsaI, those fragments containing the mutation carried an additional RsaI site, resulting in products of 203, 111, and 29 bp, whereas those

<table>
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<th>Table 1 Genotype frequencies for mutations in HLA-H</th>
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<tr>
<td>Europe</td>
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<td>Total</td>
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<tr>
<td><strong>Australia</strong></td>
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<tr>
<td>Total Papua New Guinea</td>
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<td><em>Australian Aboriginals</em></td>
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<td>Total Vanuatuans</td>
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<td>Pacific Islands</td>
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<td>Americas</td>
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<td><em>Mexicans</em></td>
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<td><em>Jamaicans</em></td>
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<td><em>Cubanians</em></td>
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<td><em>Venezuelan Indians</em></td>
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H63D allele frequencies significantly greater in Europe than in Africa/Middle East (p<0.0001), Indian subcontinent (p=0.0025), Asia (p<0.0001), Australasia (p<0.0001), and Americas (p<0.0001) (χ² test).

C282Y frequencies significantly greater in Europe than in Africa/Middle East (p<0.0001), Indian subcontinent (p<0.0001), Asia (p<0.0001), Australasia (p<0.0001), and Americas (p<0.0001) (χ² test).

Origins of samples: *Family studies of collagen disorders and polycystic kidney disease; †autophenological community based surveys; ‡blood donors; §referrals for diagnosis of haemoglobinopathies; ||=community based surveys of haemoglobinopathies; #=community based malarial survey; **=neonatal survey of haemoglobinopathies.

Genotypes are given for amino acid 63 (H63D) and amino acid 282 (C282Y) of the polypeptide (C = cysteine, D = aspartic acid, H = histidine, Y = tyrosine). CC/HH is normal (wild type). The combinations DD/CY, DD/YY, and HD/YY were not found.
lacking the mutation yielded products of 203 and 140 bp.

Ninety-five percent confidence intervals were calculated assuming a binomial distribution, and $\chi^2$ analysis was performed using Statview 4.1 (Abacus Concepts Inc, Berkeley, CA, USA).

Results
Results are shown in table 1. A total of 2900 European chromosomes was analysed and the C282Y and H63D mutations were observed at frequencies of 3.8% and 13.6%, respectively.

The H63D mutation was present in all European populations included in the study, at allele frequencies greater than 6%, and in Saudi Arabian, Indian Asian, and Mexican populations at 8.5%, 8.4%, and 6.5% respectively. It was present at lower frequencies in Africans, Asians, and native Americans, and was not found in Colombians, most Australasians (except a single Vanuatuuan), Taiwanese Aboriginals, or Senegalese.

The C282Y mutation was found in all European populations studied except Udmurts, Ashkenazi Jews, Finns, Greek Cypriots, and Turkish Cypriots, and, with the exception of one Indian heterozygote and two Jamaican heterozygotes, was absent from all other populations. The highest allele frequencies were found in the UK (6.4% overall, including 10% in Irish chromosomes), Danes (9.6%), Icelanders (6.7%), Norwegians (6.4%), and Bavarians (5.6%).

Discussion
Feder et al. suggested that the H63D mutation increases the risk of haemochromatosis for C282Y heterozygotes, and that compound heterozygotes have a low penetrance iron storage disease. The UK Haemochromatosis Consortium (submitted for publication) found that three of 115 patients were compound heterozygotes suffering from mild disease, and four of 101 control subjects were compound heterozygotes who showed no signs of iron loading. In the present study, we have found a frequency of compound heterozygotes in the UK, Denmark, Iceland, and the Netherlands of approximately 1/30, which is much higher than previous estimates of haemochromatosis frequency. Therefore, if enhanced risk of disease exists for this genotype, our data support the hypothesis that it is low penetrance. An alternative explanation of the variation in the phenotype of compound heterozygotes is that the H63D polymorphism may be associated with more than one haplotype, only one of which carries a second mutation causing haemochromatosis. We have found high H63D frequencies in populations not previously reported to suffer from haemochromatosis. This finding sheds doubt on the significance of the mutation in this disease, and indicates that it is unlikely to be implicated independently of another mutation.

The C282Y mutation was most prevalent in north European populations, and absent from 3056 non-European chromosomes studied except for three chromosomes (one Indian and two Jamaican). These results strongly suggest that the mutation originated in northern Europe, which is where haemochromatosis is generally accepted to have arisen. Simon et al. have postulated that the geographical distribution of haemochromatosis is similar to the migration pattern of Celtic peoples, and Smith et al. concluded that there was a significantly higher prevalence of haemochromatosis in Americans of British/Irish descent compared with that of Americans of other Caucasian descent. The distribution of the C282Y mutation is therefore similar to that of haemochromatosis. The presence of the allele in Indian and Jamaican populations at trace levels may be because of admixture with Europeans in the history of these peoples.

The high percentage of C282Y heterozygotes found in north European populations indicates homozygote frequencies of 1/100 in Ireland, 1/278 in the rest of the UK (we found 2/368 = 1/184), 1/111 in Denmark, 1/223 in Iceland, and 1/244 in Norway. A previous estimate of the haemochromatosis frequency in Denmark is 1/217-1/270, considerably lower than our extrapolated frequency of 1/111. This discrepancy may have arisen because of the small sample number of 37 Danes included in the present study, or may indicate that a large number of Danish haemochromatotics are undiagnosed. A haemochromatosis frequency of 1/270 indicates an allele frequency of 6.1%, which is within our 95% confidence limits.

These observed allele frequencies are comparable with previous estimates of haemochromatosis frequencies in populations of north European origin of 1/200-1/300 in predominantly Caucasian Australians, French Bretons, Swedes (Jutlanders), and North Americans. Low frequencies have previously been observed in Finns, who are known to differ genetically from other northern European populations, and our findings are consistent with this.

Haemochromatosis has not been confirmed in those populations lacking the C282Y mutation. There have been isolated reports of non-chromosomal iron overload in a Chinese woman, in a Melanesian kindred, and many accounts of African siderosis. This condition was first recognised in the Bantu tribe of southern Africa. Bantu siderosis may be distinguished from haemochromatosis in that the levels of iron deposited in the liver and bone marrow are comparable in siderosis, but in haemochromatosis, the liver is the major site of iron storage. An additional example of non-chromosome 6 linked hyperferritinaemia with normal serum iron has recently been described in overweight French subjects. There have also been reports of a hereditary hyperferritinaemia in France and Italy which is associated with a congenital cataract condition, in which raised serum ferritin is not related to iron overload. It is important not to confuse haemochromatosis with other iron overload disorders. In 1965, a world study of haemochromatosis distribution concluded that the highest incidence of haemochromatosis occurred in Africans of the Bantu tribe, while the
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Global prevalence of putative haemochromatosis mutations.

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