**HFE mutations in an inflammatory arthritis population**

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Abstract

**Objectives.** To determine the value of screening patients with inflammatory arthritis for haemochromatosis-associated mutations in the *HFE* gene.

**Methods.** We screened 1000 patients with inflammatory arthritis and 1000 controls for the *HFE* gene mutations that are associated with haemochromatosis. The arthritis patients were diagnosed between 1989 and 1995 and their blood DNA was archived as part of the Norfolk Arthritis Register project.

**Results.** Five out of 1000 (0.005) patients in the arthritis group were homozygous for the *HFE* C282Y mutation. This frequency is the same as the frequency of 5/1000 (0.005) for C282Y homozygosity observed in the normal population. It is slightly above the predicted frequency of homozygosity of 0.0044 derived from the gene frequency in the normal population.

**Conclusions.** These data suggest that most of the C282Y homozygotes occurred in this arthritis group by chance and that their arthritis was incidental to their *HFE* genotype. This implies that screening for *HFE* mutations among patients with inflammatory arthritis would infrequently identify patients whose arthritis might benefit from additional treatment.

**KEY WORDS:** HFE, HLA-H, Arthritis, Haemochromatosis, Iron, C282Y, H63D, 845A, 187G.

Hereditary haemochromatosis is an autosomal recessive condition in which excess iron is absorbed by the intestine. If untreated, affected individuals accumulate excess available iron over many years of adult life, and this can result in tissue damage that causes the disease manifestations of haemochromatosis. The discovery of mutations in *HFE* gene that are present in most hereditary haemochromatosis patients [1] has provided a useful diagnostic test that is independent of the elevated ferritin levels caused by inflammatory changes [2] and is a means to study the epidemiology of haemochromatosis from DNA archives [3]. Two *HFE* genotypes are commonly associated with haemochromatosis. The most important is homozygosity for the C282Y (845A) mutation; an apparently less penetrant genotype is compound heterozygosity with the C282Y and H63D (187G) mutations [1].

Arthritis was not widely recognized as a manifestation of haemochromatosis until 1964 [4], almost 100 yr after the disease was first described. In many series of haemochromatosis patients, joint involvement [5–8] is the commonest feature, found in about half the patients. Arthritis has also been found to be the greatest cause of morbidity among haemochromatosis patients [9]. The typical arthritis seen in haemochromatosis most frequently involves the index and middle metacarpophalangeal (MCP) and proximal interphalangeal joints [5], although there are many reports of more widespread non-specific joint disease [5, 8, 10].

The high frequency of *HFE* mutations [1–3] and biochemical iron overload [11] among Caucasian populations has led to the suggestion that undiagnosed haemochromatosis may be a significant health problem. This would imply that some cases of arthritis may be caused by undiagnosed haemochromatosis; such cases are particularly likely to be underdiagnosed if they do not present with the characteristic arthropathy of haemochromatosis.

Screening of arthritis patients by measuring serum transferrin saturation and ferritin identified patients with haemochromatosis who had the classical arthritis of haemochromatosis and patients with other forms of arthritis [10]. Reviewing the disease features of arthritis in 5000 arthritis out-patients identified 11 patients...
with typical haemochromatotic arthropathy; all had previously undiagnosed haemochromatosis [12].

The phlebotomy treatment of haemochromatosis is apparently ineffective in reversing arthritic manifestations [13, 14], and some progression of arthritic symptoms has been described in iron-depleted patients [13]. However, it is logical to infer that phlebotomy may reduce disease progression and prevent the development of arthritis in patients treated before damage from iron overload has occurred. This would imply that the early diagnosis of haemochromatosis which is possible with a genetic test could prevent some progression of arthritis. Moreover, screening of the arthritic population for haemochromatosis might allow the prevention of life-threatening manifestations, such as liver disease and cardiomyopathy, a supposition that is supported by the observation that arthritis as a referring symptom of haemochromatosis is a good prognostic indicator for survival [15].

We tested the large, unselected inflammatory arthritis population collected by the Norfolk Arthritis Register (NOAR) for HFE mutations and compared the prevalence of the haemochromatosis-associated HFE genotypes with a large sample from the normal population.

Materials and methods

Arthritis patients
All work was approved by Norwich District Ethics Committee (references 96/140 and 97/090). Samples from arthritis patients were collected from the DNA archive of the NOAR [16]. The NOAR is a population-based register of cases of inflammatory arthritis. The criterion for inclusion is more than one swollen joint lasting for more than 6 weeks. All new cases of arthritis in Norfolk since 1990 have been included in the register. These include many patients in the community with arthritis who have not been referred to hospital. DNA has been archived from patients who gave consent. This study used 1000 sequential samples from patients (average age 54 yr) for whom adequate DNA samples remained and who were first diagnosed with arthritis between 1989 and 1995.

Normal population
DNA was extracted by standard methods from blood collected from 1000 individuals (average age 54 yr), excluding haemochromatosis patients and people with foreign names selected from the catchment area of the Norfolk and Norwich Hospital, which is a large subset of the area covered by the NOAR. This included 373 normal screening trial volunteers aged 55–64 yr and 541 patients undergoing full blood counts. In 1991, 94% of the population resident in the parliamentary constituencies that approximately constitute this area were both white and born in England [17].

Archive DNA amplification

The amino acid 282 region of the HFE gene was amplified in 30-μl polymerase chain reaction (PCR) reactions containing 15 μl of 2 × PCR master (Boehringer Mannheim, Lewes, UK) reagents with approximately 200 ng DNA and 0.2 μl of each of the appropriate primers described by Feder et al. [1] and Worwood et al. [2]. Thermal cycling was carried out in a programmable heating block as follows: 94°C for 4 min then 40 cycles of 94°C for 40 s, 55°C for 40 s and 70°C for 40 s, followed by incubation at 70°C for 10 min. DNA from patients with one C282Y mutation was further amplified with primers specific for the amino acid 63 region. All PCR work was carried out in rooms dedicated to either preparation or product analysis.

Restriction digestion analysis

Ten microlitres of PCR product was digested in 20-μl reactions containing 10 U RsaI (282 region) or MboI (63 region) at 37°C for 3 h. The products were run on a TBE/3% MetaPhor agarose (FMC Bioproducts, Rockland ME, USA) gel containing 0.5% ethidium bromide. The bands in apparent heterozygotes were analysed using a GDS8000 image analysis system (Ultra Violet Products, Cambridge, UK) to confirm that the ratios of intensity of the bands were equal to those in controls.

Serum iron, transferrin and ferritin analysis

Serum samples taken at the time of first diagnosis of arthritis were stored at −40°C. Samples from the 19 patients who were homozygous for the C282Y mutation or compound heterozygous for the C282Y and H63D mutations were analysed for levels of transferrin using a BNII nephelometer (Dade Behring, Newark, DE, USA), for ferritin using an AutoDELFIA automated immunoassay system (Wallac Oy, Turku, Finland) and for iron using a dimension clinical chemistry system (Dade Behring).

Statistics

Confidence intervals (CI) were calculated as exact binomial CIs. P values were calculated as one-tailed exact binomial probabilities using the homozygote frequency predicted from the group gene frequency as the population proportion.

Results

Five of the 1000 (0.005, 95% CI = 0.0016–0.012) patients from the arthritis group were homozygous for the HFE C282Y mutation, which is the same as the frequency of five out of 1000 observed in the normal group (Table 1).

The overall frequency of the C282Y mutation in the arthritis group was 118 among the 2000 chromosomes tested, or 0.059. The square of this figure gives a prediction for the C282Y homozygote frequency, 0.0035, that has a lower standard error than the figure.
Based on the observed number of homozygotes in the normal group. The observed frequency of C282Y homozygosity in the arthritis group of 5/1000 homozygotes is not significantly \((P = 0.27)\) higher than this predicted frequency; i.e. the arthritis group is in Hardy–Weinberg equilibrium. Similarly, the normal population is in Hardy–Weinberg equilibrium (Table 1).

Fourteen of the 108 patients from the arthritis group who were heterozygous for the C282Y mutation also carried an H63D mutation. The overall frequency of compound heterozygosity of 14 out of 1000 (0.014, 95% CI = 0.008–0.023) is similar to the frequency of 13 out of 1000 (0.013) observed in the normal population (Table 1). The frequency of the C282Y mutation was similar in the volunteer and patient subsets of the normal group (not shown).

Four out of five of the patients who were homozygous for the C282Y mutation had a ferritin or transferrin saturation level that exceeded the normal threshold values of 300 \(\mu g/l\) for ferritin or 55% transferrin saturation for men and 50% for women (Table 2). Only two out of 14 patients who were compound heterozygous for the C282Y and H63D mutations had an elevated ferritin or transferrin saturation level.

Involvement of the index and middle MCP joints (defined by swelling or tenderness at these joints at first clinical examination) was present in all the C282Y homozygous and C282Y/H63D compound heterozygous patients for whom data were available. However, only two of the 11 such patients for whom data were available had any changes on X-ray (Table 2).

### Discussion

We have observed the same frequency of individuals with the haemochromatosis-associated C282Y homozygous genotype in a large group of patients with inflammatory arthritis as that seen in a large group of controls. This suggests that most of the C282Y-homozygous patients in the arthritis group were not recruited into that group as a result of their HFE muta-

### Table 1. HFE genotypes in 1000 arthritis patients and 1000 normal controls

<table>
<thead>
<tr>
<th>C282Y genotype</th>
<th>Arthritis patients</th>
<th>Normal population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age (yr)</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>282C/282C normal subjects</td>
<td>887</td>
<td>875</td>
</tr>
<tr>
<td>282C/282Y heterozygotes</td>
<td>108</td>
<td>120</td>
</tr>
<tr>
<td>C282Y homozygotes</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>C282Y allele frequency</td>
<td>0.059</td>
<td>0.065</td>
</tr>
<tr>
<td>Predicted C282Y homozygote frequency</td>
<td>1 in 287</td>
<td>1 in 236</td>
</tr>
<tr>
<td>282C compound heterozygotes</td>
<td>14</td>
<td>13</td>
</tr>
</tbody>
</table>

### Table 2. Features of patients with two HFE mutations from the arthritis group

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Genotype</th>
<th>MCP involvement</th>
<th>MCP changes on X-ray</th>
<th>Ferritin ((\mu g/l))</th>
<th>Transferrin saturation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M 48</td>
<td>hom</td>
<td>2,3L 3R</td>
<td>n.a.</td>
<td>378</td>
<td>29</td>
</tr>
<tr>
<td>M 61</td>
<td>hom</td>
<td>3R</td>
<td>n.a.</td>
<td>1349</td>
<td>57</td>
</tr>
<tr>
<td>F 51</td>
<td>hom</td>
<td>3L</td>
<td>No</td>
<td>233</td>
<td>63</td>
</tr>
<tr>
<td>F 67</td>
<td>hom</td>
<td>2,3R</td>
<td>n.a.</td>
<td>24</td>
<td>46</td>
</tr>
<tr>
<td>F 71</td>
<td>hom</td>
<td>2L 2R</td>
<td>No</td>
<td>1075</td>
<td>84</td>
</tr>
<tr>
<td>M 52</td>
<td>c het</td>
<td>2,3L</td>
<td>No</td>
<td>81</td>
<td>33</td>
</tr>
<tr>
<td>M 71</td>
<td>c het</td>
<td>2,3L 2,3R</td>
<td>Erosions 2R</td>
<td>98</td>
<td>14</td>
</tr>
<tr>
<td>M 58</td>
<td>c het</td>
<td>2L 2,3R</td>
<td>No</td>
<td>288</td>
<td>26</td>
</tr>
<tr>
<td>M 35</td>
<td>c het</td>
<td>2L</td>
<td>No</td>
<td>97</td>
<td>18</td>
</tr>
<tr>
<td>M 71</td>
<td>c het</td>
<td>2,3L 2,3R</td>
<td>No</td>
<td>80</td>
<td>26</td>
</tr>
<tr>
<td>M 51</td>
<td>c het</td>
<td>2L 2,3R</td>
<td>n.a.</td>
<td>158</td>
<td>54</td>
</tr>
<tr>
<td>M 32</td>
<td>c het</td>
<td>2L</td>
<td>n.a.</td>
<td>13</td>
<td>67</td>
</tr>
<tr>
<td>F 72</td>
<td>c het</td>
<td>2,3L 2,3R</td>
<td>n.a.</td>
<td>47</td>
<td>34</td>
</tr>
<tr>
<td>F 47</td>
<td>c het</td>
<td>2,3L 2,3R</td>
<td>No</td>
<td>113</td>
<td>14</td>
</tr>
<tr>
<td>F 64</td>
<td>c het</td>
<td>2,3L 2,3R</td>
<td>No</td>
<td>42</td>
<td>13</td>
</tr>
<tr>
<td>F 73</td>
<td>c het</td>
<td>2L 2,3R</td>
<td>No</td>
<td>372</td>
<td>30</td>
</tr>
<tr>
<td>F 69</td>
<td>c het</td>
<td>n.a.</td>
<td>n.a.</td>
<td>38</td>
<td>25</td>
</tr>
<tr>
<td>F 34</td>
<td>c het</td>
<td>2,3L</td>
<td>n.a.</td>
<td>15</td>
<td>38</td>
</tr>
<tr>
<td>F 65</td>
<td>c het</td>
<td>2R</td>
<td>Changes 2R</td>
<td>83</td>
<td>29</td>
</tr>
</tbody>
</table>

Age, age at first diagnosis of arthritis; hom, homozygous for the C282Y mutation; c het, compound heterozygous with C282Y and H63D; MCP involvement refers to swelling or tenderness of the index or middle MCP joints at first clinical examination; L, left; R, right; n.a., data not available.

We have observed the same frequency of individuals with the haemochromatosis-associated C282Y homozygous genotype in a large group of patients with inflammatory arthritis as that seen in a large group of controls. This suggests that most of the C282Y-homozygous patients in the arthritis group were not recruited into that group as a result of their HFE mutation. This implies that, in most cases, the arthritis was incidental to the patient’s HFE genotype and would further imply that treatment of iron overload would not affect the course of the arthritis in these cases. However, the upper confidence limit for the frequency of C282Y homozygosity in the arthritis group (0.012) does not exclude the possibility that some of these C282Y homozygotes developed arthritis as a direct consequence of their HFE genotype.

During the period when the NOAR samples used in this study were collected, a small number of patients, who are not represented in the NOAR sample, were diagnosed with haemochromatosis and arthritis. This observation, taken with the data presented here, suggests that only a minority of the large number of C282Y homozygotes in our hospital’s catchment area develop arthritis.

This is consistent with our estimates that the penetrance of the HFE C282Y-homozygous genotype is very low in this population with respect to liver cancer [18], cirrhosis [3] and diabetes [19] and the observation that C282Y-homozygous men can survive to old age without treatment for haemochromatosis [20]. Thus, while C282Y homozygosity is common, relatively few homozygous individuals develop disease manifestations, including arthritis, despite not being treated [3].

This finding is apparently at odds with the observation that there is a high frequency of arthritis among patients with manifest haemochromatosis [5–8]. The most likely explanation is that haemochromatosis patients are a highly selected subset of C282Y homozygotes.

In two studies that examined the prevalence of the classic arthropathy of haemochromatosis and biochemical iron overload in arthritis populations, Gottschalk et al. [12] found 0.22% and Olynyk et al. [10] found 1.5% of arthritis patients to have haemochromatosis, respectively. These figures are significantly below and above...
the expected frequency of HFE C282Y homozygosity in the German [21] and Australian [22] populations. The frequency of C282Y homozygosity that we observed in an inflammatory arthritis population falls between the figures observed by Gottschalk et al. [12] and Olynuk et al. [10] and is consistent with the fact that we used a sensitive genetic test but studied a population that was not significantly enriched in individuals with haemochromatosis.

Four of the five homozygotes in the arthritis group could have been detected by screening for both ferritin and transferrin saturation; the remaining patient had a transferrin saturation just below the threshold value. There was no apparent association between the extent of iron overload measured by these tests and the extent of arthritis involving the MCP joints of the index and middle fingers.

Our observations would be consistent with the observation that arthritis as a referring symptom was a good prognostic indicator for survival among haemochromatosis patients [15] if high diagnostic vigilance identified patients who were homozygous and mildly iron-overloaded but in whom arthritis had occurred incidentally to their HFE genotype.

Our data suggest that the value of screening patients with inflammatory arthritis for HFE mutations may be similar to that of screening the general population. It is, however, very important that similar studies are carried out in other populations, particularly those that might have diets rich in available iron. It also remains important that physicians should remain alert to the possibility of haemochromatosis being present incidentally in the arthritis clinic, and to rare cases of haemochromatosis-associated arthritis of the characteristic pattern. Treatment of these cases may reverse life-threatening disease manifestations and prevent progression of arthritis.

Acknowledgements

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References