Mutations in the hereditary haemochromatosis gene HFE in professional endurance athletes

J L Chicharro, J Hoyos, F Gómez-Gallego, J G Villa, F Bandrés, P Celaya, F Jiménez, J M Alonso, A Córdova, A Lucia

Background: Hereditary haemochromatosis, a disease that affects iron metabolism, progresses with a greater or lesser tendency to induce iron overload, possibly leading to severe organ dysfunction. Most elite endurance athletes take iron supplements during their active sporting life, which could aggravate this condition.

Objective: To determine the prevalence and discuss potential clinical implications of mutations of HFE (the gene responsible for hereditary haemochromatosis) in endurance athletes.

Methods: Basal concentrations of iron, ferritin, and transferrin and transferrin saturation were determined in the period before competition in 65 highly trained athletes. Possible mutations in the HFE gene were evaluated in each subject by extracting genomic DNA from peripheral blood. The restriction enzymes SnaBl and BclI were used to detect the mutations 845G→A (C282Y) and 187C→G (H63D).

Results: Our findings indicate a high prevalence of HFE gene mutations in this population (49.2%) compared with sedentary controls (33.5%). No association was detected in the athletes between mutations and blood iron markers.

Conclusions: The findings support the need to assess regularly iron stores in elite endurance athletes.

Materials and methods

Subjects
Sixty five elite, male athletes (50 professional road cyclists and 15 Olympic class endurance runners) from Spain were enrolled in the study. Written consent was obtained from each subject according to the guidelines of the Universidad Complutense, Madrid.

The mean (SD) age, height, mass, and maximum oxygen consumption (V̇O2MAX) of the athletes were: 26 (3) years, 178 (5) cm, 66.7 (6.1) kg, and 71.8 (7) ml/kg/min respectively. The subjects were previously confirmed to be healthy by a medical examination including electrocardiography and cardiac ultrasonography. No subject had a familial or personal history of endocrine or metabolic disease. No exogenous substances had ever been detected in anti-doping checks performed in the subjects by the corresponding official organisations. Most (90%) of the athletes took iron supplements (not including the C282Y/H63D heterozygous subject (see the Results section)) at a mean dose of 105 mg Fe on alternate days for at least six months of the year. Doses and treatment regimens were similar in each subject.

A control group (n = 134) composed of random sedentary men from Spain also entered the study.

Measurement of blood iron markers in the athletes
Fasting blood samples were collected from all the athletes after at least three rest days during the period before competition. When available, serum ferritin concentrations were estimated in each subject by extraction of genomic DNA from peripheral blood. The restriction enzymes SnaBl and BclI were used to detect the mutations 845G→A (C282Y) and 187C→G (H63D).
(determined 12 and 6 months before the study) were recorded.

Serum iron concentrations were measured using a standard colorimetric method (Roche/Hitachi 714; Roche Diagnostics Corporation, Indianapolis, Indiana, USA). Transferrin concentration was determined by rate immunoturbidimetry (OSAX anti-serum for the Behring nephelometer; Dade Behring Marburg GmbH, Marburg, Germany). Serum transferrin saturation was calculated from these data as follows:

Transferrin saturation (%) = (serum iron concentration (mol/l)/(2 × transferrin concentration (mol/l))) × 100.

Serum ferritin concentrations were measured by chemiluminescence immunoassay (N-latex ferritin kit; Dade Behring Marburg GmbH). The coefficients of interassay and intra-assay variability averaged 1.2% and 1.8% for serum iron, 2.3% and 2.7% for transferrin concentration, and 1.2–3.1% and 1.0–4.6% for serum ferritin.

Serum ranges considered normal were 13–32 μmol/l for iron, 24–336 g/l for transferrin, 24–45% for transferrin saturation, and 20–300 μg/l for ferritin.19

Study of C282Y and H63D mutations in all subjects
Genomic DNA was extracted from peripheral blood using a standard phenol/chloroform procedure followed by alcohol precipitation. DNA amplification was performed using polymerase chain reaction (PCR) with specific primers for the two HFE gene mutations as described previously.20 The PCR conditions for both mutations were as follows: initial denaturation at 95°C for five minutes; 35 cycles at 95°C for one minute, 55°C for 45 seconds, 72°C for one minute, and a final extension at 72°C for five minutes. The PCR products were then subjected to enzymic digestion for two hours, with restriction endonucleases cleaving the DNA at specific points such that the presence or absence of the mutations could be detected. The restriction enzymes used were SnaBI for the 845G→A (C282Y) mutation and BclI for the 187C→G (H63D) mutation. The digested fragments were visualised by electrophoresis on 2% agarose gels stained with ethidium bromide (fig 1).

Figure 1  Agarose gel (2%) stained with ethidium bromide. Arrows (from top to bottom) indicate the fragments generated after digestion with BclI and SnaBI: 420, 300, 220, 160, and 120 pb. The samples applied were: lane L, molecular mass markers (ladder 100); lane 1, H63D (+/−); lane 2, H63D (+/−); lane 3, H63D (+/+); lane 4, C282Y (−/−); lane 5, C282Y (−/+).

Statistical analysis
All variables were tested for normality. We compared the distribution of HFE genotypes in both athlete and control groups with the Fisher exact test. Ferritin data for athletes were log transformed to normalise the distribution before analysis. Differences between genotypes in the athlete group were identified by one way analysis of variance. In this group, the paired t test was used to compare the variables according to the presence or lack of mutations. Correlation between paired quantitative data was assessed by the Spearman test. All statistical analyses were performed using SPSS 9.0 software for Windows. The level of significance was set at 0.05.

RESULTS
Table 1 shows the prevalence of the different HFE gene mutations in the two groups. The proportion of subjects without a HFE gene mutation was significantly higher in the control group than in the athlete group (66.5% vs 50.8%; p = 0.03). H63D heterozygosity occurred in 41.5% of the athletes and 24.6% of the controls (p = 0.01). No other significant difference was found between groups. No homozygote for the C282Y mutation was detected in athletes or controls.

Table 2 shows biometric variables, maximum aerobic capacity, and blood iron markers in subgroups of athletes established according to the type of HFE mutation carried. No significant differences in any of the variables (p>0.05) were detected among subgroups. The different variables were also compared in the athlete group in terms of the presence or absence of mutations (table 3). No differences were observed among the subgroups.

**DISCUSSION**
The most important finding of our study was the high proportion of endurance athletes with a mutation in the HFE gene (49.2%), 29 (44.6%) of whom carried an H63D mutation and three (4.6%) a C282Y mutation. The prevalence of H63D heterozygosity was significantly higher (p = 0.01) than in controls (41.5% vs 24.6%). Our results are in agreement with those of previous research showing that in the general population of Spain the prevalence of the H63D mutation ranges from 16% to 30.4%,21 and C282Y mutations occur in 2% to 4.4% depending in both cases on the geographical region.22

Thus, the prevalence observed here (especially for H63D) is much higher than that previously reported in non-athletic subjects, yet is similar to rates recently observed by Deugnier et al,15 who also warned of a higher prevalence of H63D mutations in French cyclists compared with healthy controls.15 It remains to be seen if these mutations afford any metabolic advantage to these athletes during exertion. We detected no significant differences in VO_{2\text{MAX}} between subjects with or without the mutation, thus we cannot confirm this hypothesis.

Although there appeared to be no relation in the athlete group between the presence and absence of mutation with respect to the blood iron markers, it is observed that 61.1% of

<p>| Table 1 | Percentage distribution of HFE genotypes in elite endurance athletes and sedentary controls |</p>
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Athletes (n = 65)</th>
<th>Controls (n = 134)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt/wt</td>
<td>50.8</td>
<td>66.5</td>
<td>0.03</td>
</tr>
<tr>
<td>C282Y/wt</td>
<td>3.1</td>
<td>4.5</td>
<td>NS</td>
</tr>
<tr>
<td>H63D/wt</td>
<td>41.5</td>
<td>24.6</td>
<td>0.01</td>
</tr>
<tr>
<td>C282Y/H63D</td>
<td>1.5</td>
<td>0.7</td>
<td>NS</td>
</tr>
<tr>
<td>H63D/H63D</td>
<td>3.1</td>
<td>3.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

wt/wt, Wild-type; C282Y/wt, heterozygotes for the C282Y mutation; H63D/wt, heterozygotes for the H63D mutation; C282Y/H63D, compound heterozygotes; H63D/H63D, homozygotes for the H63D mutation; NS, not significant.
athletes with high ferritin concentrations had a mutated HFE gene. It is known that long term exposure to small amounts of iron can lead to atherogenesis and ischaemia/reperfusion damage caused by free radical formation.23 Even minimally increased iron deposits in people heterozygous for the mutations could be detrimental and enhance the risk of increased iron deposits in people heterozygous for the mutation.

In conclusion, the prevalence of HFE gene mutations is high among elite endurance athletes (runners and professional cyclists). Regular determination of their iron stores is thus recommended.

ACKNOWLEDGEMENTS

We thank Ana Burton for translation of the manuscript.

REFERENCES


Table 2  Biometric variables, maximum aerobic capacity, and blood iron markers in athletes (n = 65) grouped by HFE genotypes

<table>
<thead>
<tr>
<th>HFE genotype</th>
<th>Number</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Mass (kg)</th>
<th>VO2MAX (ml/kg/min)</th>
<th>Fe (µmol/l)</th>
<th>Ferritin (µg/l)</th>
<th>Transferrin (g/l)</th>
<th>TSI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt/wt</td>
<td>26 (3)</td>
<td>178 (6)</td>
<td>67.4 (5.2)</td>
<td>70.8 (6.8)</td>
<td>193 (6.7)</td>
<td>18.8 (6.3)</td>
<td>224.7 (26.8)</td>
<td>35.8 (8.0)</td>
<td>24–45</td>
</tr>
<tr>
<td>C282Y/wt</td>
<td>26 (3)</td>
<td>178 (6)</td>
<td>67.4 (5.2)</td>
<td>70.8 (6.8)</td>
<td>193 (6.7)</td>
<td>18.8 (6.3)</td>
<td>224.7 (26.8)</td>
<td>35.8 (8.0)</td>
<td>24–45</td>
</tr>
<tr>
<td>H63D/wt</td>
<td>26 (3)</td>
<td>178 (6)</td>
<td>67.4 (5.2)</td>
<td>70.8 (6.8)</td>
<td>193 (6.7)</td>
<td>18.8 (6.3)</td>
<td>224.7 (26.8)</td>
<td>35.8 (8.0)</td>
<td>24–45</td>
</tr>
<tr>
<td>C282Y/H63D</td>
<td>26 (3)</td>
<td>178 (6)</td>
<td>67.4 (5.2)</td>
<td>70.8 (6.8)</td>
<td>193 (6.7)</td>
<td>18.8 (6.3)</td>
<td>224.7 (26.8)</td>
<td>35.8 (8.0)</td>
<td>24–45</td>
</tr>
<tr>
<td>H63D/H63D</td>
<td>26 (3)</td>
<td>178 (6)</td>
<td>67.4 (5.2)</td>
<td>70.8 (6.8)</td>
<td>193 (6.7)</td>
<td>18.8 (6.3)</td>
<td>224.7 (26.8)</td>
<td>35.8 (8.0)</td>
<td>24–45</td>
</tr>
</tbody>
</table>

Values are mean (SD). No significant differences in any of the variables (p>0.05) were detected among the athlete subgroups.

Table 3  Comparison of variables according to the presence or absence of HFE gene mutations

<table>
<thead>
<tr>
<th>Variable</th>
<th>HFE mutation</th>
<th>No HFE mutation</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>26 (3)</td>
<td>26 (3)</td>
<td>26 (3)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>26 (3)</td>
<td>26 (3)</td>
<td>26 (3)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178 (6)</td>
<td>178 (6)</td>
<td>178 (6)</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>67.4 (5.2)</td>
<td>67.4 (5.2)</td>
<td>67.4 (5.2)</td>
</tr>
<tr>
<td>VO2MAX (ml/kg/min)</td>
<td>70.8 (6.8)</td>
<td>70.8 (6.8)</td>
<td>70.8 (6.8)</td>
</tr>
<tr>
<td>Fe (µmol/l)</td>
<td>18.8 (6.3)</td>
<td>19.3 (5.7)</td>
<td>13-32</td>
</tr>
<tr>
<td>Ferritin (µg/l)</td>
<td>267.2 (151.3)</td>
<td>224.7 (153.2)</td>
<td>24-336</td>
</tr>
<tr>
<td>Transferrin (g/l)</td>
<td>224.4 (23.8)</td>
<td>224.4 (23.8)</td>
<td>24-336</td>
</tr>
<tr>
<td>TSI (%)</td>
<td>35.8 (8.0)</td>
<td>35.1 (12.1)</td>
<td>55.9</td>
</tr>
</tbody>
</table>

Values are mean (SD). No significant differences in any of the variables (p>0.05) were detected among the athlete subgroups.

Take home message

The prevalence of mutations in the HFE gene (responsible for hereditary haemochromatosis, a disease in which the body’s iron stores are increased) seems to be high among elite endurance athletes (about 49%). As most elite endurance athletes take iron supplements, regular assessment of their iron reserves is recommended to prevent iatrogenic iron overload.

F Gómez-Gallego, F Bandrés, Laboratorio de Biopatología, Departamento de Toxicología y Legislación Sanitaria, Universidad Complutense
J G Villa, INEF de Castilla y León, Universidad de León, León, Spain
P Celaya, Grupo Deportivo ONCE, Spain
F Jiménez, Universidad de Castilla-La Mancha, Spain
J M Alonso, Servicios Médicos RFEA, Madrid
A Córdova, Departamento de Fisiología, Universidad de Valladolid, Spain
A Lucia, Department of Physiology, Universidad Europea de Madrid, Madrid

www.bjsportmed.com
The cardiovascular stress imposed on an experienced elite basketball referee during national competition.

A S Leicht

This case report examined the cardiovascular stress imposed on an experienced elite basketball referee during national competition. The average heart rate was similar for all matches, approximated 73% of maximum heart rate, and was experienced for most (>63%) of the match.

An unusual presentation of immersion foot

D M Macgregor

We report a case of “green foot” in a child with a plaster cast applied for a fractured metatarsal who subsequently represented with circulatory compromise. The foot was green and smelly and profuse Pseudomonas aeruginosa was cultured. The infection cleared with simple exposure to air. Perhaps this diagnosis should be considered in patients presenting with circulatory compromise in a cast as severe infection can result in amputation.