Short communication

Eighty percent of French sport winners in Olympic, World and Europeans competitions have mutations in the hemochromatosis HFE gene

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Abstract

The HFE gene encodes a protein involved in iron homeostasis; individuals with mutations in both alleles develop hemochromatosis. 27% of the French population is heterozygous for mutations in this gene. We found that 80% of the French athletes who won international competitions in rowing, Nordic skiing and judo display mutations in one allele of HFE, thus demonstrating the existence of a favourable phenotype linked to this heterozygosity.

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1. Introduction

Elite athletes who compete at national or international levels benefit from a combination of both genetic potential and environmental factors. Recent genetic studies have identified individual variations that contribute to athletic performance [1,2]. In most sports, energy production and muscle oxygenation are required for efficient performance. Iron is an important dietary element which is essential for enzymes of the respiratory chain and for the oxygen carrier and storage proteins myoglobin and hemoglobin. It is critical...
for energy production by mitochondria [3] as well as for efficient erythropoiesis which is necessary for provision of oxygen to the working muscle and to the heart [4]. Iron absorption is tightly regulated by hepcidin, a peptide produced mainly by the liver [5].

Hemochromatosis is a hereditary disease that is characterized by iron overload due to a mutation of the HFE gene (HFE: High Fe, or high iron concentration), which is located on chromosome 6 (6p22.2). In Europe and North America, the disorder is associated mainly with the homozygous mutations C282Y and H63D or with the compound mutation C282Y/H63D. Literature shows some association between HFE variants and iron parameter [6] though heterozygous mutations are not associated with clinical hemochromatosis [7]. HFE participates to iron level sensing in the blood and participate to the modulation of hepcidin expression from the liver and, thereby, is an important regulator of iron bioavailability [8]. During the 2006 Olympic Games in Turin, two French athletes were suspected of doping. However, their higher hematocrit level was proven to be due to their mutation in the HFE gene.

Because high level sport may induce hepcidin synthesis, and thus decrease iron bioavailability, we tested the hypothesis that carriers of HFE mutations have a genetic advantage at the highest level of performance in sports which require either a large capacity for oxygen transfer (Nordic skiing or rowing, aerobic group) or high muscular performance (judo, fighting group).

2. Methods

2.1. Ethics statement

Each participant provided full written consent. The study was approved by the Necker Hospital Ethics Committee (n°ID RCB 2011_A00563-38).

2.2. Subjects

We performed a prevalence study of mutations in the HFE gene in 170 elite athletes (52 women, 118 men, 18–56 yo) from four French national teams. They competed in anaerobic sport [Group 1, Nordic skiing (n = 77; 28 women, 49 men), rowing (n = 18, 5 women, 13 men)], fighting [Group 2, judo (n = 34, 9 women, 25 men)], or low-energetic disciplines [Group 3, petanque (n = 41, 10 women, 31 men)]. They were compared to control subjects, matched by gender and geographic origin [n = 219; 116 women, 103 men, 18–87 yo] who were recruited from blood transfusion centres. The control subjects were all healthy with no medical disorders. Petanque players were chosen as an elite control group that did not have high energetic requirements. The recruitment started in 2009.

The performance level was assessed through the medals collected in international competitions (European or World championships; Olympic Games). The assessments of the international competitions were continued to 2012. In the energetic sports (groups 1 and 2), we compared athletes who reached the three best places in these competitions (defining a group of French athletes with international medals ie an “international podium group”: IPG, n = 46) to those who did not succeed at this level (members of the French teams who did not receive any medals in these competitions throughout their career ie. the “no international podium group”, NPG, n = 83).

2.3. HFE gene assays


2.4. Biological parameters

Red Blood Cell (RBC) parameters (RBC, hemoglobin, hematocrit, mean corpuscular volume -MCV-) and reticuloocytes were measured on a DXH800 (Beckman Coulter). Erythropoietin (EPO), soluble transferrin receptor (sTFR) and ferritin were analysed by chemo-luminescence on an Imunolite Xpi apparatus. Transferrin (immunoturbidimetry) and serum iron (ferrozin method) were assayed an a DXC analyser. From the measured serum iron and transferrin concentrations, total iron binding capacity and transferrin saturation were calculated (serum iron/transferin ratio).

2.5. Statistical analysis

All analyses were made using R software (version 3.0.1. R Foundation for Statistical Computing, Vienna, Austria). The control sample size was determined using a sample size calculation with Type I error rate α = 0.05. Power 1−β = 0.8, Nordic skiing sample size (n = 77), a rowing HFE gene mutation proportion of 44% and a hypothesized control HFE gene mutation proportion of 30%.

To determine the impact of mutations in the HFE gene, we used a logistic regression with a generalized linear model. We first tested for an overall effect and then used a chi-squared test statistic of each group versus the control group. Interaction between group and gender was also tested; we exponentiated the coefficients and interpreted them as odds ratios (OR).

To analyse the effects of biological parameters, our data were analysed for normality with the Shapiro–Wilks test. The effects of mutation, group and sex were investigated on each measured blood parameter using a linear model approach, which corresponds to a three-way analysis of variance with crossed, fixed-effects factors. A test was considered to be significant if p < 0.05, after multiplicity corrections. The Holm approach was used considering 12 comparisons (one per parameter) to correct for multiplicity (uncorrected p-values must be lower than 0.004 to remain significant). We used a generalized linear fitting model to investigate the possible relation between mutation, biological parameters and international podiums.

3. Results and discussion

We assessed the frequency of mutations in the HFE gene (H63D, C282Y, S65C) in elite members of French sport teams (Nordic skiing = 77, rowing = 18 and judo = 34 individuals). Petanque players at the international level (n = 41) were chosen as an elite control group that did not require high energy expenditure (non-energetic group). These groups were compared to 219 control subjects matched for age, gender and geographical origin. This control group had a lower frequency of mutations in HFE compared with athletes (27% in controls vs 41% in athletes) (Supplemental table 1).

Among athletes who reached the three best places in international competitions (European or World championships; Olympic Games) (international podium group, IPG), the frequency of mutations in HFE was 80.4% (37 from 46) (p < 0.0001) (Fig. 1). In energetic sports, the frequency of any mutation was greater than that in the control group (Fig. 1). In contrast, no difference was found when the non energetic group was compared to the control group (Fig. 1, Supplemental table 2).

The group of athletes studied may appear too small compared to the usual sample size now investigated in genetic studies [9]. However, there is no higher level than Olympic or world champion in sport. Therefore there is no replication cohort in such cases. This
group is also highly representative of extreme effects of human genomic variation (ie under major constraints): variations of performance indicators might be large enough between this group and the general population [10] so that the demonstration of the underlying mechanisms might not require a large sample.

The control population exhibited a frequency of mutations in the HFE gene similar to that found in previous studies [11,12]. Our findings suggest that the HFE protein might then be a key regulating factor, involved in the oxygen transfer capacity and muscle functions. Furthermore, the frequency of mutations in the non-energetic group did not differ from that of the control population. This result suggests that, in sport with low energetic load, the access of athletes to the highest level of performance does not involve mutations in the HFE gene.

For the whole population, HFE mutations only increased hemoglobin (14.9 ± 1.4 vs 14.5 ± 1.1, p = 0.0007), hematocrit (45.5 ± 4.4 vs 44.6 ± 3.9, p = 0.002) and MCV (92.5 ± 5.3 vs 90.3 ± 5.4, p < 0.0001) (Supplemental table 3 and Fig. 2). In the IPG, there was an increase in the level of ferritin (p = 0.04), EPO (p = 0.05), serum iron (p = 0.07), RBC (p = 0.06), hemoglobin (p = 0.01), reticulocytes (p = 0.02), transferrin saturation (p = 0.05) and MCV (p = 0.09).

Mutations in the HFE gene may limit the exercise-induced increase of hepcidin, thus preventing the decrease in iron bioavailability. Here we further find an association between HFE mutation and serum iron in the aerobic group (19.9 ± 6.1 µmol L⁻¹ in athletes with mutation vs 17.0 ± 7.7 µmol L⁻¹ in the absence of mutation), that may contribute to higher iron bioavailability. Iron may enhance performance through a rise in erythropoiesis during endurance sports such as Nordic skiing and rowing [13] (Fig. 2). Our results also suggest that HFE mutations play a major role in sports considered as anaerobic such as judo. However, training loads have become so high that the physiological part of endurance could nowadays represent a much larger proportion than that what was previously estimated in so-called "power non aerobic" sports. Our findings in the population composed of judo athletes also indicate that the HFE mutations impact muscle function, which may include heart muscle, hence playing a different role than that observed in iron metabolism and erythropoiesis [4,13] (Fig. 2). Further, higher iron bioavailability may allow for a better recovery of the athletes between the high-intensity intermittent efforts in competition [14]. This may also improve muscle regeneration (repair and remodeling) and resistance to fatigue following micro-injuries or strains, a very frequent situation in a fighting sport [15].

An association has been found between the performance at the international level and the presence of several mutations [16].

Fig. 1. HFE mutation odds ratios (OR) among athletes and controls. (A) Aerobic sports include Nordic skiing (n = 77) and rowing (n = 18). Fighting sport includes judo (n = 34), Low-energetic includes petanque (n = 41). (B) Athletes include Nordic skiing, rowing and judo. (C) International podium group (IPG) of energetic sports (Nordic skiing, rowing and judo) vs those who did not succeed at this level (NPG). Controls (n = 219) were matched by geographic origin, age and gender. Significant differences are indicated with ** for p < 0.01 and *** for p < 0.001.
specifically those involved in muscle metabolism and function [2], energy production or erythropoiesis [17]. Chicharro et al. [12] found an increase in the frequency of HFE mutations in elite Spanish male athletes (professional road cyclists and Olympic class endurance runners) when compared with controls. A similar result was detected in elite French male cyclists among whom iron supplementation was a regular practice [18]. However, none of these studies showed an association with the winners. We establish here that HFE mutations present the best association ever demonstrated with podium winners in sport.

Our findings raise ethical issues. One might be tempted to search for individuals with favourable genotypes when recruiting members of athletic teams [19]. However, the complexity of the gene–environment interactions makes it extremely unlikely that a sport career can be planned based on a single genetic criterion tested at a young age. Many other genotypes may have been included in our study population.

The high frequency of mutations in elite athletes shows that the genetic diagnosis in the general population is definitely not sufficient to predict a restrained phenotype or a potential disease [20]. In the French teams evaluated, 10 athletes were homozygous for H63D or compound heterozygous, but no athletes had clinical symptoms or signs of hemochromatosis. Three of them reached the international podium group, suggesting that HFE homozygosity might also be favourable for performance in some cases and that high level training may play a role in iron storage and availability.

The detection of mutations that may lead to hemochromatosis should raise physician awareness about the potential risks of iron supplementation (a common practice in sports) among high level athletes. In contrast to the whole population with simple heterozygous mutations [7], athletes who possess an HFE mutant genotype (hetero- or homozygous) might risk clinically significant iron overload. In addition, these mutations might explain an increase in the hematocrit level per se [8], that does not result from doping.

4. Conclusions

In conclusion, our study reveals that a phenotype linked to international levels of performance (Olympic, World and Europeans podiums) in energetic sports is associated with a higher frequency of mutations in the HFE gene. More generally, the studying of both the physiology and the analysis of gene mutations in athletes may reveal unexpected genetic influences on fundamental biological functions with potential impacts in public health.

Conflict of interest disclosure

The authors agree with manuscript results and conclusions, and report no potential conflicts of interest.

Ethics approval

The study was approved by the Necker hospital Ethic committee (n°/ID RCB 2011_A00563-38).

Fig. 2. Impact of HFE mutation on plasma hemoglobin concentration in athletes and controls. Aerobic sports include Nordic skiing (n = 77) and rowing (n = 18). Fighting sport includes judo (n = 34). Controls (n = 219) were matched by geographic origin, age and gender. In each group, plasma hemoglobin concentration is represented by mean ± SD for subjects who present at least one assayed mutation (HFE) and for those who do not (No); (all) represents all subjects from the 3 groups. Each circle represents one subject. Significant differences are indicated with an horizontal bar and * for p < 0.05.
Author contributions

OH and GD were the principal investigators and take primary responsibility for the paper. OH, GD, JFT conceived and designed the experiments. VG, GF, FVL, MPRB, JCL, JPC, AF performed the experiments and enrolled the patients. PN, VG, LAM, GF, FVL, FG, MT, XJ analysed the data. OH, GD, PN, VG, LAM, JFT contributed to the writing of the manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biochi.2015.09.028.

References