The $-174$ G/C polymorphism of the $IL6$ gene is associated with elite power performance

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Received 8 July 2009; received in revised form 11 September 2009; accepted 15 September 2009

Abstract

The $-174$ G/C polymorphism [rs1800795] of the $IL6$ gene is a candidate to explain individual variations in health and exercise related phenotypes. We compared $-174$ G/C genotypic and allelic frequencies in three groups of men of the same Caucasian (Spanish) descent: elite endurance athletes (cyclists, runners; $n=100$); elite power athletes (jumpers, throwers, sprinters; $n=53$) and non-athletic controls ($n=100$).

The frequency of the GG genotype ($P=0.030$) and G allele ($P=0.026$) was higher in the power athletes group compared with the control group. The frequency of the GG genotype ($P=0.033$) and G allele ($P=0.013$) was also higher in the power athletes group compared with the endurance athletes group. The odds ratio of being a power athlete if the subject had the GG genotype (dominant model) was 2.471 (95% confidence interval: 1.242–4.915) compared to the control group or the endurance athlete group. We did not find differences between the control and endurance athlete groups. In summary, our findings suggest that the G allele of the $IL6$ $-174$ G/C polymorphism might favour sprint/power sports performance.

Keywords: Interleukin 6; Genetics; Exercise; Endurance athletes

1. Introduction

Interleukin-6 (IL-6) is a multifunctional cytokine primarily involved in immune functions. Recent data also indicate a pivotal role of this protein in the processes of muscle repair and hypertrophy following exercise-induced damage. In addition, exercise can induce increases in muscle derived IL-6 mRNA and subsequent elevations in plasma IL-6.

The IL-6 gene, $IL6$, is located in the short arm of chromosome 7 (7p21). A functional G/C polymorphism at position $-174$ [rs1800795] was described in the 5’ flanking region of $IL6$, with the G allele being associated with increased transcripational response in vitro and in vivo conditions.

The $-174$ G/C variant is associated with numerous disease and disease-related phenotype traits including development of microvascular complications in diabetic patients, increased arterial stiffness, cardiovascular disease, obesity comorbidities, chronic obstructive pulmonary disease, fasting glucose levels, and circulating levels of C-reactive protein (CRP) and fibrinogen. It was also associated with longevity.

The $IL6$ $-174$ G/C polymorphism is also associated with fitness-related phenotypes. Ortlepp et al. reported that the C allele was associated with lower maximal work capacity in Caucasian smokers, yet no association was observed in their non-smoking referents. Furthermore, the $-174$ G/C variant influences high-density lipoprotein cholesterol levels (the CC genotype group increased high-density lipoprotein cholesterol more than the GG), glucose tolerance (decrease occurring only in the GG genotype group) and...
bone mass remodelling (GG homozygotes losing 6.8% in cortical area, GC gaining +5.5% and CC gaining +17.3%)\textsuperscript{16} in response to exercise. Yamin et al.\textsuperscript{17} reported a strong association between the C allele of the \textit{IL6} –174 G/C polymorphism and increased levels of total serum creatine kinase activity, an indicator of skeletal muscle damage, following eccentric contractions of the elbow flexor muscles in young adults.

Despite its association with some important exercise related phenotypes including muscle damage and repair following exercise, whether the \textit{IL6} –174 G/C polymorphism is associated with elite sports performance remains to be elucidated. It was the purpose of our study to compare allelic and genotypic frequencies of the \textit{IL6} –174 G/C polymorphism among Spanish male controls (non-athletes) and two groups of elite (world-class) male athletes who are at the two endpoints of the human sports performance continuum: endurance (professional road cyclists, endurance runners), and ‘power’ endpoint (throwers, jumpers, sprinters). Based on the finding that the C allele is strongly associated with increased risk of muscle damage following muscle eccentric contraction,\textsuperscript{17} we hypothesised that the C allele is underrepresented in power athletes and thus might impair, at least partly, performance in those athletic events in which muscle strength/power is one of the phenotype traits that determine competition success.

2. Methods

The population comprised 253 Spanish (Caucasian for ≥3 generations) healthy men:

(i) 100 endurance elite athletes aged 20–39 years. This sample included 50 elite endurance runners, of whom 27 were ‘Olympic-class’ (the top Spanish runners during the 1999–2009 period), having participated in at least one edition of the Olympic games (including Olympic finalists) and 23 were mostly ‘national’ class but with participation in international events as the cross-country world championships; the sample also included 50 professional road cyclists who were all Tour de France finishers, including top-3 finishers. Their mean $\pm$ SD maximal oxygen uptake (VO$_{2\text{max}}$) was $73.7 \pm 5.7$ ml kg$^{-1}$ min$^{-1}$.

(ii) 53 power elite athletes aged 20–33 years, including jumpers, throwers and sprinters; 40 top national level and 13 Olympic level. Their VO$_{2\text{max}}$ was $60.3 \pm 5.5$ ml kg$^{-1}$ min$^{-1}$.

(iii) 100 healthy, non-athletic controls aged 19–32 years (VO$_{2\text{max}}$: 50.1 ± 2.6 ml kg$^{-1}$ min$^{-1}$). All were undergraduate Physical Education students from the same university (Universidad Europea de Madrid, Spain). Inclusion and exclusion criteria for this group were to be free of any diagnosed cardiorespiratory disease and not to be engaged in competitive sports or in formal, supervised exercise training (i.e. performing less than three structured weekly sessions of strenuous exercise as running, swimming, bicycling, and weight lifting).

The VO$_{2\text{max}}$ values of endurance and power athletes were obtained using a breath-breath system (Oxycon Pro System, Jaeger, Wuerzburg, Germany) in laboratory ramp tests performed until volitional exhaustion. Cyclists performed the tests (25 W + 25 W min$^{-1}$) on an electrically braked cycle-ergometer (Ergometrics 900; Ergo-line; Bitz, Germany) while runners and power athletes performed the tests (11 + 0.5 km h$^{-1}$ every 30 s, at a constant 1% inclination) on a treadmill (Technogym Run Race 1400 HC, Gambettola, Italy). All the tests were performed under similar laboratory conditions (temperature, $\sim$20°C; relative humidity, 45–55%; barometric pressure, $\sim$720 mmHg). The VO$_{2\text{max}}$ of controls was estimated from the time to complete 2000 m tests.\textsuperscript{18} The tests were performed inside a 400-m outdoor track under similar environmental conditions (temperature, $\sim$23–24°C; relative humidity, 45–55%; barometric pressure, $\sim$720 mmHg).

Written consent was obtained from each participant. The study was approved by the ethics committee of Universidad Europea de Madrid, Spain.

We obtained DNA from athletes’ blood or saliva samples over years 2004–2008. All genotyping was performed in the same laboratory (Progenika Biopharma, Parque Tecnológico de Zamudio, Derio-Vizcaya, Spain) using a newly developed low-density DNA microarray based on allele-specific probes. The design, fabrication, validation and analysis of the arrays were performed following the procedure described elsewhere\textsuperscript{19} with minor modifications. Briefly, the polymerase chain reaction (PCR) products were fluorescently labelled and hybridised to the DNA microarray in an automated platform (Ventana Medical Systems, Inc., Tucson, AZ, USA). The microarrays were scanned (Innopsys S.A., Carbonne, France) and absolute values of hybridisation signal of the replicates for each oligonucleotide probe were processed automatically by the software (Progenika Biopharma, Zamudio, Spain). Each variant was genotyped based on the mean of hybridisation signal.\textsuperscript{19}

Genotyping was performed for research purposes based on the hypothesis that the \textit{IL6} –174 G/C polymorphism influences sports performance. The researchers in charge of genotyping were totally blinded to the subjects’ identities as blood samples were tracked solely with barcoding.

Hardy–Weinberg (H–W) equilibrium within the study groups was tested using a $\chi^2$ test. Genotypic and allelic frequencies were compared among the three groups using a $\chi^2$ test with $\alpha$ set at 0.05. We used logistic regression analysis to analyse the association between genotypes and sports performance, using the dominant model (GG vs. GC + CC). All the analyses were performed with the SPSS, v. 16.0 (SPSS Inc, Chicago).
Table 1
Genotype and allele frequencies of \( \text{IL6} -174 \) G/C polymorphism (rs1800795) in Spanish (Caucasian) controls (\( n = 100 \)), elite endurance athletes (\( n = 100 \)) and elite power athletes (\( n = 53 \)).

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls (C)</th>
<th>Endurance (E)</th>
<th>Power (P)</th>
<th>( P (\chi^2) ), overall</th>
<th>( P (\chi^2) ), C vs. E</th>
<th>( P (\chi^2) ), C vs. P</th>
<th>( P (\chi^2) ), E vs. P</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>42% (( n = 42 ))</td>
<td>42% (( n = 42 ))</td>
<td>65% (( n = 34 ))</td>
<td>0.067 (8.788)</td>
<td>0.805 (0.434)</td>
<td>0.030 (7.015)</td>
<td>0.033 (6.800)</td>
</tr>
<tr>
<td>GC</td>
<td>46% (( n = 46 ))</td>
<td>43% (( n = 43 ))</td>
<td>26% (( n = 14 ))</td>
<td>( \chi^2 = 5.877, P = 0.015 )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>12% (( n = 12 ))</td>
<td>15% (( n = 15 ))</td>
<td>9% (( n = 5 ))</td>
<td>( \chi^2 = 5.877, P = 0.015 )</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Allele

| \( p \) (G) | 0.65 | 0.63 | 0.77 | 0.037 (6.614) | 0.754 (0.098) | 0.026 (4.972) | 0.013 (6.146) |
| \( q \) (C) | 0.35 | 0.37 | 0.23 | \( \chi^2 = 5.877, P = 0.015 \) |

3. Results

We did not experience any failure in sample collection, DNA acquisition or genotyping procedures.

Genotype distributions met H–W equilibrium in controls (\( \chi^2 = 0.012, P = 0.912 \)) and in endurance athletes (\( \chi^2 = 0.523, P = 0.469 \)), whereas it did not in the power athletes group (\( \chi^2 = 5.877, P = 0.015 \)).

Table 1 shows the genotype and allele frequency of the \( \text{IL6} -174 \) G/C polymorphism in Spanish controls, elite endurance athletes and elite power athletes. The frequency of the GG genotype (\( P = 0.030 \)) and G allele (\( P = 0.026 \)) was higher in the power athletes group compared with the control group. Likewise, frequency of the GG genotype (\( P = 0.033 \)) and G allele (\( P = 0.013 \)) was higher in the power athletes group compared with the endurance athletes group. We did not find differences between the control and endurance athlete groups (\( P > 0.1 \)). Genotype frequencies were similar between cyclists (GG: 44%; GC: 42%; CC: 14%) and runners (GG: 40%; GC: 44%; CC: 16%).

The odds ratio of being an elite power athlete if the subject had the GG genotype (dominant model) was 2.471 (95% confidence interval: 1.242–4.915) compared to the control and endurance athlete groups. The results did not change after adjusting for VO\(_2\)max (data not shown).

4. Discussion

The main finding of the present study was that the G allele of the \( \text{IL6} -174 \) G/C polymorphism is more frequent in elite power athletes than in elite endurance athletes or non-athletic referents. We also observed that this allele does not seem to influence endurance sports performance, since the genotype and allele frequencies were similar between endurance and control groups.

Besides the putative multifunctional role of \( \text{IL6} \) gene, including modulation of body immune responses and muscle repair following damage, the rationale for studying the \( -174 \) G/C variation lies on the fact that mutations/polymorphisms that are located in gene promoter regions can affect mRNA expression and protein levels. Further, the G to C change in position \(-174\) of the \( \text{IL6} \) gene creates a potential site for the transcriptional factor NF-1, which can repress gene expression.\(^{20}\) The G allele is in fact associated with increased gene transcription\(^{3,20}\) and increased plasma IL-6 in response to standard inflammatory stimuli.\(^{4}\)

Our finding that the C allele is underrepresented in power athletes concurs with recent findings reported by Yamin et al.,\(^{17}\) who indicated a strong, dose-dependent association between the C allele/CC genotype, and increased muscle damage in response to unaccustomed eccentric exercise in non-athletes. Our findings together with those reported by Yamin et al.\(^{17} \) might be explained, at least partly, by the pivotal role that IL-6 plays on muscle repair in response to acute exercise. First, it is well established that IL-6 can be derived from skeletal muscle and released into circulation following acute exercise.\(^{2}\) The IL-6 acutely released from contracting skeletal muscle fibres (and thus becoming a myokine during exercise) has a beneficial anti-inflammatory (rather than pro-inflammatory) effect, e.g. by inhibiting tumour-necrosis factor-\( \alpha \) (TNF-\( \alpha \)) or interleukin-1 (IL-1) production.\(^{21}\)

Together with other cytokines and growth factors, IL-6 released in response to muscle repair is an essential component of the muscle repair process.\(^{22}\) In humans, IL-6 is fundamentally important to the contribution of satellite cells to muscle repair following muscle contractions and may, at least in part, contribute to muscle hypertrophy.\(^{1}\)

Interestingly, the beneficial effects of the anti-inflammatory myokine IL-6 released in response to acute exercise are in contrast with the overall deleterious effects of chronically elevated pro-inflammatory IL-6 derived from other sources, i.e. adipocytes. Chronically increased levels of inflammatory proteins such as IL-6 are involved in the functional decline of older persons,\(^{23}\) partly through their catabolic effects on skeletal muscle.\(^{24}\) The chronic systemic IL-6 increases that accompany numerous diseases can promote a catabolic state leading to muscle atrophy.\(^{25}\) A cross-sectional study showed a negative association between levels of CRP, IL-6 and TNF-\( \alpha \), and muscle strength.\(^{26}\) Additionally, one prospective study reported that higher levels of IL-6 and CRP are associated with loss of muscle strength in older persons.\(^{27}\)

We did not observe an association between the \( \text{IL6} -174 \) G/C polymorphism and endurance performance, which concurs with the findings reported by Ortlepp et al.\(^{13}\) in non-smokers. They observed similar levels of cardiorespiratory fitness levels in young male healthy non-smokers across
IL6 −174 G/C genotypes, yet cardiorespiratory fitness was reduced in a subgroup of male smokers carrying the C allele.

Though the main limitation of the present study lays on the small sample size, we believe this is justifiable given the uniqueness of this type of sportsmen. We gathered almost all Spanish elite (world-class) athletes with a ‘pure’ power phenotype (weightlifters, sprinters or throwers) and a high proportion of the best athletes in the country with an endurance phenotype (runners or cyclists). Further studies using large sample sizes, different ethnicities, and in women should confirm these results. In addition, further research in the field might determine IL-6 plasma levels in response to standard stimuli (e.g. endotoxin injection) across the different IL6−174 G/C genotypes. Although this more mechanistic approach is desirable, it would be very difficult to conduct with elite athletes as the ones studied here.

Though we believe it does not necessarily represent a major methodological limitation of our study, the fact that in the power group genotype distributions did not meet H–W expectations in a given population are the following: genetic drift, migration (i.e. gene flow), mutation (e.g. change in the rate of mutation from the C to the G allele of the IL6−174 G/C polymorphism), selection, and non-random mating. It is difficult to determine, without speculating, which of the aforementioned conditions occurred in our group of power athletes, especially given its limited sample. Nevertheless, we cannot discard a change in the rate of the C to G mutation in this group as the G allele of the IL6−174 G/C polymorphism could favour performance in power-oriented sports.

A methodological strength from our study stems from the fact that we gathered the top-level athletes who are at both endpoints of the human sports performance continuum. Elite athletes with a pure power/strength oriented phenotype are seldom gathered in genotype:phenotype association studies and the majority of reports in the field have focused on endurance phenotype traits (e.g. VO2max). The ‘optimum’ genotype does probably differ between endurance and more power-oriented sports because the phenotype traits that determine performance in both types of events are likely different. For instance, in world-class decathletes performance in explosive power specialities such as the 100-m sprint or the long jump is negatively correlated with performance in the more endurance-oriented 1500-m race.28

Previous reports also showed differences between endurance and power-oriented athletes in the genotype frequencies of other polymorphisms, i.e. ACTN3 R577X29 and ACE I/D.30

5. Conclusions

While it obviously does imply a true cause–effect relationship, the results of the present study suggest that the G allele of the IL6 −174 G/C polymorphism might favour, at least partly, power sports performance. We observed no association between this polymorphism and endurance performance. Further studies, using larger sample sizes or mechanistic approaches, might confirm our results.

Practical implications

- The −174 G/C polymorphism [rs1800795] of the IL6 gene is a candidate to explain individual variations in health and exercise related phenotypes.
- The G allele of the IL6 −174 G/C polymorphism might favour, at least partly, power sports performance.
- This polymorphism does not seem to influence endurance sports performance.

Disclaimer

This manuscript has not been published elsewhere, and is not being considered, or will be submitted for publication elsewhere until a final decision has been made as to its acceptability by this journal.

Acknowledgements

This study was funded by funded by Progenika Biopharma and Sabiobbi S.L. (Spain) and by the Consejo Superior de Deportes (CSD, ref # UPR10/08), Fondo de Investigaciones Sanitarias (FIS, ref. # PI061183), Spanish Ministry of Education (EX-2007-1124), and Swedish Council for Working Life and Social Research (FAS).

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