Is the -174 C/G polymorphism of the IL6 gene associated with elite power performance? A replication study with two different Caucasian cohorts

Original research

Title: Is the -174 C/G polymorphism of the *IL6* gene associated with elite power performance? A replication study with two different Caucasian cohorts.

Running title: -174 C/G polymorphism and sports performance

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ABSTRACT

A functional -174 C/G polymorphism in the interleukin-6 gene (*IL6*) is a candidate to explain individual variations in exercise-related phenotypes. To replicate recent findings showing an association between the G allele and GG genotype of elite power sports performance in European (Spanish) Caucasian males (Ruiz *et al.* J Sci Med Sport, 2010;13:549-53), we compared allelic and genotypic frequencies of the *IL6* -174 C/G polymorphism among elite endurance athletes (N=74) and power athletes (N=81), and non-athletic controls (N=205) of both genders from Israel. All subjects were Israeli Caucasians [with an equivalent ratio of non-Ashkenazi and Ashkenazi descent in each group (2:1)]. We found no differences in the genotype or allele frequencies among groups (all P>0.3). We further compared the genotype and allele frequencies between national- (N=109) and international-level (N=46) Israeli athletes in the endurance and power group, and found no significant genotype or allele differences after adjusting for multiple comparisons. We repeated all the analyses after pulling together the Israeli and Spanish controls, endurance and power elite athletes, and found no genotypic and allelic differences among groups. The results did not change when the analyses were repeated including only the best Israeli athletes (i.e. the international level group) together with the group of elite Spanish athletes (P>0.2). In conclusion, the results of the present study did not show an association between the G allele of the *IL6* -174 G/C polymorphism and power sports performance in the Israeli (Caucasian) population. Our findings support the need to replicate association results between genetic polymorphisms and athletic status in populations of different ethnic backgrounds with the largest possible population samples.

INTRODUCTION

Interleukin-6 (IL-6) is not only involved in immune function (Amir *et al.*, 2007), but also in muscle repair and hypertrophy following exercise-induced damage (Cantini *et al.*, 1995; Helge *et al.*, 2003; Weigert *et al.*, 2007; McKay *et al.*, 2008; Serrano *et al.*, 2008). A functional C/G polymorphism at position -174 [rs1800795] was described in the 5' flanking region of the IL-6 gene *IL6* (Fishman *et al.*, 1998), with the mutant G allele, rather than the wild-type C allele being associated with increased transcriptional response *in vitro* (Fishman *et al.*, 1998; Terry *et al.*, 2000) and *in vivo* conditions (Bennermo *et al.*, 2004). The *IL6* -174 polymorphism is associated with numerous disease and disease-related phenotype traits such as cardiovascular disease (Sie *et al.*, 2006; Manginas *et al.*, 2008; Panoulas *et al.*, 2009), stroke (Tso *et al.*, 2007), obesity comorbidities (Goyenechea *et al.*, 2007), or fasting glucose levels (Huth *et al.*, 2009). This genetic variation is also associated with exercise-related phenotypes.

Ortlepp et al. found an association between the C allele and maximal work capacity in Caucasian smokers (Ortlepp *et al.*, 2003). The -174 C/G variant was found to be associated with high-density lipoprotein cholesterol levels (Halverstadt *et al.*, 2005), glucose tolerance (McKenzie *et al.*, 2004) and bone mass remodelling (Dhamrait *et al.*, 2003) in response to exercise. Yamin et al. reported a strong association between the C allele of the *IL6* -174 C/G polymorphism and skeletal muscle damage following eccentric contractions of the elbow flexor muscles in young adults (Yamin *et al.*, 2008). Ruiz et al. recently demonstrated that the GG genotype and G allele are overrepresented in elite power athletes, for whom muscle hypertrophy/strength is a key phenotype trait, compared with elite endurance athletes and non-athletic controls (Ruiz *et al.*, 2010b). Whether these results can be extrapolated to other populations remains to be elucidated. This is a question of interest because differences among the findings of studies in sports

genetics are partly attributable to between-studies differences in the size, gender and ethnic/geographic background of the cohorts. To replicate our recent findings (Ruiz *et al.*, 2010b) we compared allelic and genotypic frequencies of *IL6* -174 C/G polymorphism among elite endurance athletes and power athletes, and non-athletic controls from Israel. We also performed further replication analyses by pulling the two ethnic cohorts (i.e. Israeli and Spanish subjects) together.

METHODS

Ethical approval

The study was approved by the Helsinki Committee of the 'Hillel-Yaffe' Medical Center (Hadera, Israel) following the Declaration of Helsinki. Written informed consent was obtained from each participant. Our study was performed according to ethical standards in sport and exercise science research (Harriss & Atkinson, 2009).

Subjects

Israeli cohort. A total of 155 elite athletes (119 men and 36 women, age=35.9±12.2 yrs) volunteered to participate in the study. Athletes were included in the study sample only if they had participated in national (n=109) or international (n=49) track and field championships. Athletes were divided into two groups: i) endurance athletes (n=74, 20 international level, which included long distance runners whose main event was the 10,000m run and the marathon), and (ii) power athletes (n=81, 26 international level, which included sprinters whose main event was the 100-200m dash). Furthermore, we divided the athletes within each group into two subgroups according to their individual best performance: international athletes (those who had represented Israel in a world track-and-field championship or in the Olympic Games) and national-level athletes. The main characteristics of the Israeli athletes are shown in Table 1. The control group consisted of 205 non-athletic Israeli healthy individuals (167 men and 38 women, age=34.9±12) who did not engage in physical activity on a regular basis. All subjects (athletes and controls) were Israeli Caucasians. The Israeli population includes Caucasians with mixed Jews coming from Arab countries (non-Ashkenazi), and Jews coming from Europe (Ashkenazi). In the present study the ratio of non-Ashkenazi and Ashkenazi descent in each group (2:1).

Spanish cohort. As detailed elsewhere (Ruiz *et al.*, 2010b), the Spanish population comprised:

(i) 100 male world-class endurance athletes aged 20-39 (50 endurance runners and 50 professional road cyclists). All the endurance runners (mostly specialists in 5,000m, 10,000m and marathon) had participated in at least one Olympiad, and some were Olympic finalists or Europe/World Champions; the cyclists were all Tour de France finishers, including top-3 finishers. Their mean±SD maximal oxygen uptake (VO_{2max}) was 73.7±5.7 $ml \cdot kg^{-1} \cdot min^{-1}$.

(ii) 53 elite male power athletes aged 20-33 years (jumpers, throwers and sprinters). This group included 40 top national level and 13 Olympic level athletes. Their VO_2max was 60.3±5.5 $ml \cdot kg^{-1} \cdot min^{-1}$.

(iii) 100 healthy male non-athletic controls aged 19-32 years (VO_2max: 50.1±2.6 $ml \cdot kg^{-1} \cdot min^{-1}$). All were 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 3 structured weekly sessions of strenuous exercise as running, swimming, bicycling, and weight lifting).

All the subjects in this cohort were of the same Caucasian (Spanish) descent for ≥3 generations.

Genotyping

Israeli cohort. We extracted genomic DNA from peripheral EDTA treated anti-coagulated blood using a standard protocol. Genotyping was performed for research purposes based on the hypothesis that the *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 bar-coding (Chanock *et al.*, 2007). We used the polymerase chain reaction (PCR). A 136-bp fragment of the *IL-6 -174* C and a 121 fragment of the *IL-6 -174* G polymorphism were amplified using 3 primers: *IL-6 GSP -F 5' ATAAATCTTTGTTGGAGGGTGAGG '3 and IL-6 C -R 5' ATGACGACCTAAGCTTTACTTTTCCCCCTAGTTGTGTCTTGAC '3 IL-6 G -R 5' GCACTTTTCCCCCTAGTTGTGTCTTACG '3.* The PCR was performed by denaturation at 95°C for 10 min, 39 cycles of denaturation at 94°C for 30 sec, annealing at 61°C for 45 sec, extension at 72°C for 1 min, and a final extension step of 7 min at 72°C. The PCR products were then electrophoresed in a 4% agarose gel. This method yields 136-bp fragments in the presence of the C allele, and 121-bp in the presence of the G allele.

Spanish cohort. In the samples from Spanish subjects, genotyping was performed from saliva samples using a newly-developed low-density DNA microarray based on allele-specific probes as previously described (Gomez-Gallego *et al.*, 2010).

Data analysis

All statistical analyses were performed using the PASW (v. 18.0 for WINDOWS, Chicago). Using a χ^2 test, we performed the comparisons that are described below.

Israeli cohort. First, we compared genotype and allele frequencies of the *IL6* -174 G/C polymorphism by sex within each of the following groups: controls, national level athletes (endurance and power together), international level athletes (endurance and power together), endurance athletes (both national and international level), power athletes (both national and international level), and non-Ashkenazi and Ashkenazi subjects. Second, we compared genotype and allele frequencies among controls, endurance and power athletes. We also repeated the analyses using only international

level athletes. Third, we further compared genotype and allele frequencies between national and international level athletes within the endurance and power group respectively.

Israeli and Spanish cohorts. Finally, we repeated all the analyses after pulling together all the Israeli and Spanish controls, endurance and power elite athletes. We further conducted the analysis including only the best Israeli athletes (i.e. the international level group) together with the group of elite Spanish athletes. We used logistic regression analysis to determine the association between alleles and sports performance.

In all analyses, multiple comparisons were adjusted for mass significance (Holm, 1979).

RESULTS

There were no failures in sample collection, DNA acquisition or genotyping procedures. Genotype distributions met Hardy-Weinberg equilibrium in controls (P=0.931) and in endurance athletes (P=0.850), but not in the power athletes group (P=0.017).

Genotype and allele frequencies did not differ by sex in any of the study groups (Table 2). Likewise, genotype and allele frequencies were similar between non-Ashkenazi and Ashkenazi groups (χ^2 = 0.07, P=0.9).

Table 3 shows genotype and allele frequencies of the *IL6* -174 G/Cpolymorphism in controls, endurance and power athletes from Israel. There were no differences in genotype or allele frequencies among groups (all P>0.3). Likewise, there were no genotype and allele frequency differences when the analyses were repeated including only the international level group (Overall: χ^2=2.741, P=0.602; χ^2=2.069, P=0.355, respectively); endurance vs. power: (χ^2=1.274, P=0.529; χ^2=0.937, P=0.333, respectively) power vs. control: χ^2=2.167, P=0.338; χ^2=0.2.018, P=0.155, respectively; or endurance vs. control: χ^2=0.615, P=0.735; χ^2=0.004, P=0.951, respectively. When we further compared genotype and allele frequencies between national- and international-levels athletes in the endurance and power group, we found no genotype or allele differences (Table 4). We repeated the analyses by comparing C carriers vs. non-C carriers in national and international level, and found no significant differences between groups (χ^2=0.035, P=0.851; and χ^2=2.952, P=0.086; in endurance and power athletes, respectively).

Finally, to further confirm our findings, we repeated all the analyses after pulling together the Israeli and Spanish controls, endurance and power elite athletes. There were no genotype differences among groups (overall: χ^2=8.593, P=0.072; control vs. endurance: χ^2=8.593, P=0.072; controls vs. power: χ^2=3.825, P=0.148; endurance vs.

power: χ^2=5.850, P=0.054) (Figure 1). The odds ratio of being an elite power athlete if the subject had the G allele was 1.177 (95% confidence interval: 0.820-1.690) compared to the control group, and 1.329 (95% confidence interval: 0.900-1.961) compared to the endurance athlete group. The results did not change when the analyses were repeated including only the best Israeli athletes (i.e. only those of international level) together with the group of elite Spanish athletes (P>0.2). We repeated the analysis including the type of sport (cyclists or runners) as a covariate and the results remained the same (data not shown). It should be noted that there were between-country genotype and allele differences in the non-athlete and endurance athlete groups (all P<0.001), but not in the power athlete group (all P>0.1).

DISCUSSION

The main finding of the present study was that the wild-type G allele of the *IL6* -174 G/C polymorphism is not associated with elite power athletic status in the Israeli population. Recent findings on a different Caucasian (Spanish) cohort, showing that the GG genotype and the G allele of the *IL6* -174 G/C variant are overrepresented in elite power athletes compared with endurance athletes and sedentary controls (Ruiz *et al.*, 2010b) were not corroborated in the present study with a different population.

The majority of published data in sports genetics comes from studies performed in European and North-American Caucasian populations. Our results support the need to replicate genotype:phenotype associations in this field of research with the largest possible cohorts, including populations of different ethnic backgrounds. Differences in the ethnic origin of the subjects might indeed help explain between-studies differences. This is well illustrated by the existing disparities on the possible influence of the insertion (I)/deletion (D) polymorphism in the angiotensin-converting enzyme (*ACE*) gene, arguably the most extensively studied polymorphism in sports genetics. In European Caucasians, the D allele has been associated with elite performance in power-oriented events, e.g., short-distance swimming (Woods *et al.*, 2001; Costa *et al.*, 2009), whereas the I allele seems to favour endurance events, e.g., running (Myerson *et al.*, 1999; Woods *et al.*, 2001). In contrast, the D allele might be unfavourable for power athletic events in Koreans (Kim *et al.*, 2010). Further, Amir et al. reported a positive association between *ACE* DD genotype and endurance (marathon) performance in the present athletic cohort (Amir *et al.*, 2007).

Besides the putative multifunctional role of *IL6* gene, including modulation of body immune responses and muscle repair following damage, the rationale for studying the influence of the -174 G/C variation in exercise or disease phenotypes lies in the fact

that mutations/polymorphisms that are located in gene promoter regions can affect mRNA expression and protein levels (Yamin *et al.*, 2008). The previous finding that the C allele is underrepresented in Spanish power athletes (Ruiz *et al.*, 2010b) concurs with previous findings reported by Yamin et al. 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 (Yamin *et al.*, 2008). Both findings could be explained, at least partly, by the pivotal role that IL-6 plays on muscle repair and hypertrophy (Cantini *et al.*, 1995; Helge *et al.*, 2003; Weigert *et al.*, 2007; Serrano *et al.*, 2008; McKay *et al.*, 2009) in response to acute exercise (Bamman *et al.*, 2001; Yang & Goldspink, 2002; Tomiya *et al.*, 2004; Mourkioti & Rosenthal, 2005; McKay *et al.*, 2008; O'Reilly *et al.*, 2008). However, in the present study allelic and genotypic frequencies were very similar in the three groups.

The current finding that the *IL6* -174 G/C polymorphism is not associated with endurance performance concurs with those by Ruiz *et al.* in Spanish athletes (showing no differences in the allelic or genotypic frequencies between elite endurance athletes and sedentary controls) (Ruiz *et al.*, 2010b). Ortlepp et al. likewise observed similar levels of cardiorespiratory fitness levels in young male healthy non-smokers across *IL6* -174 G/C genotypes (Ortlepp *et al.*, 2003).

Finally, to determine those genetic polymorphisms that allow for excelling in power sports (vs. endurance events) across different ethnic groups as we did here is of potential interest for two reasons. First, elite athletes with a pure power oriented phenotype are not usually gathered in genotype:phenotype association studies. The majority of studies in the field have typically focused on endurance-related phenotype traits in North-American or European Caucasians. Yet, the ‘optimum’ genotype profile does probably differ between athletes excelling in endurance vs. more power-oriented

sports because the phenotype traits that determine performance in both types of events are likely different. For instance, in the Decathlon, performance by the world's best competitors in 100m sprint or the long jump is negatively correlated with performance in the 1,500m race (Van Damme *et al.*, 2002). We recently showed that it is very unlikely to find an individual with a genetic profile suitable to excel in both pure power and endurance oriented sport events (e.g., 100m race and marathon respectively) (Ruiz *et al.*, 2010a). Secondly, humans are approaching locomotory limits in sprint races, which considerably reduces the ability of natural or artificial selection to produce faster athletes (Denny, 2008). Thus, given the difficulty to find more powerful athletes in coming generations, sports professionals worldwide might be interested in identifying those athletes who are endowed with the most suitable genetic profile to excel in competitive power sports.

To note is that the genotype distributions of power athletes did not meet H-W equilibrium, as it was the case in a previous study of Spanish power athletes (Ruiz *et al.*, 2010b). There are several reasons that are likely to explain deviation from H-W expectations including genetic drift, migration, 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 however difficult to determine, without speculating, which of the aforementioned conditions occurred in our study.

In summary, the results of the present study did not show an association between the G allele of the *IL6* -174 G/C polymorphism and power sports performance in the Israeli (Caucasian) population. Previous data on a different cohort (Ruiz *et al.*, 2010b), could not be corroborated with the present ethnic group.

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Table 1. Main characteristics of the two athlete groups from Israel.

	Endurance athletes (n=74): Personal best marathon time		**Sprinters (n=81): Personal best 100m time**	
	Men (n=60)	Women (n=14)	Men (n=59)	Women (n=22)
Elite-level (n=46)	2h 19 min 57 s ± 2 min (n=14)	2 h 44 min 20 s ± 3 min (n=6)	10.43 ±0.15 s (n=15)	11.80±0.1 s (n=11)
National-level (n=109)	2 h 44 min 6 s ± 25 min (n=46)	3 h 5min 20 s ± 35 min (n=8)	10.85± 0.26 s (n=44)	12.22±0.34 s (n=11)

Data are means ± standard deviation.

Non-athletic (controls) Israeli healthy individuals included 167 men and 38 women.

Table 2. Genotype and allele frequencies by sex in the Israeli cohort.

	Men		Women		P (χ^2)
Controls	N	%	N	%	
GG	122	73.1	25	65.8	0.662 (0.826)
GC	(41)	24.6	12	31.6	
CC	(4)	2.4	1	2.6	
p (G)		0.85		0.82	0.413 (0.670)
q (C		0.15		0.18	
Endurance International					
GG	10	76.9	4	57.1	0.357 (0.848)
GC	3	23.1	3	42.9	
CC	-		-		
p (G)		0.88		0.79	0.403 (0.698)
q (C		0.12		0.21	
Power International					
GG	7	46.7	8	72.7	0.126 (4.149)
GC	8	53.3	2	18.2	
CC	-		1	9.1	
p (G)		0.73		0.82	0.473 (0.515)
q (C		0.27		0.18	
Endurance National					
GG	34	72.3	5	71.4	0.833 (0.366)
GC	11	23.4	2	28.6	
CC	2	4.3	-		
p (G)		0.84		0.86	0.873 (0.026)
q (C		0.16		0.14	
Power National					
GG	34	77.3	8	72.7	0.246 (2.805)
GC	5	11.4	3	27.3	
CC	5	11.4	-		
p (G)		0.83		0.86	0.699 (0.149)
q (C		0.17		0.14	

Table 3. Genotype and allele frequencies of *IL6* -174 G/C polymorphism (rs1800795) in Israel (Caucasian) controls (n=205), endurance athletes (n=74) and elite power athletes (n=81).

	Controls (C)	Endurance (E)	Power (P)	P (χ^2) Overall	P (χ^2) C *vs.* E	P (χ^2) C *vs.* P	P (χ^2) E vs. P
Genotype							
GG	71.7% (n=147)	71.6% (n=53)	70.4% (n=57)	0.340 (4.520)	0.992 (0.016)	0.132 (4.049)	0.395 (1.860)
GC	25.9% (n=53)	25.7% (n=19)	22.2% (n=18)				
CC	5% (n=5)	2.7% (n=2)	7.4% (n=6)				
Allele							
p (G)	0.85	0.85	0.82	0.638 (0.899)	0.960 (0.003)	0.357 (0.848)	0.487 (0.484)
q (C)	0.15	0.15	0.18				

Table 4. Genotype and allele frequencies of the *IL6* -174 G/C polymorphism (rs1800795) in Israel (Caucasian) National (n=109), and International athletes (n=46).

	National	International	P (χ^2)
Endurance			
Genotype			
GG	70.0% (n=14)	72.2% (n=39)	0.622 (0.950)
GC	30.0% (n=6)	24.1% (n=13)	
CC	0% (n=0)	3.7% (n=2)	
Allele			
p (G)	0.84	0.85	0.912 (0.012)
q (C)	0.16	0.15	
Power			
Genotype			
GG	76.4% (n=42)	57.7% (n=15)	0.048 (6.074)
GC	14.5% (n=8)	38.5% (n=10)	
CC	9.1% (n=5)	3.8% (n=1)	
Allele			
p (G)	0.84	0.77	0.304 (1.055)
q (C)	0.16	0.23	

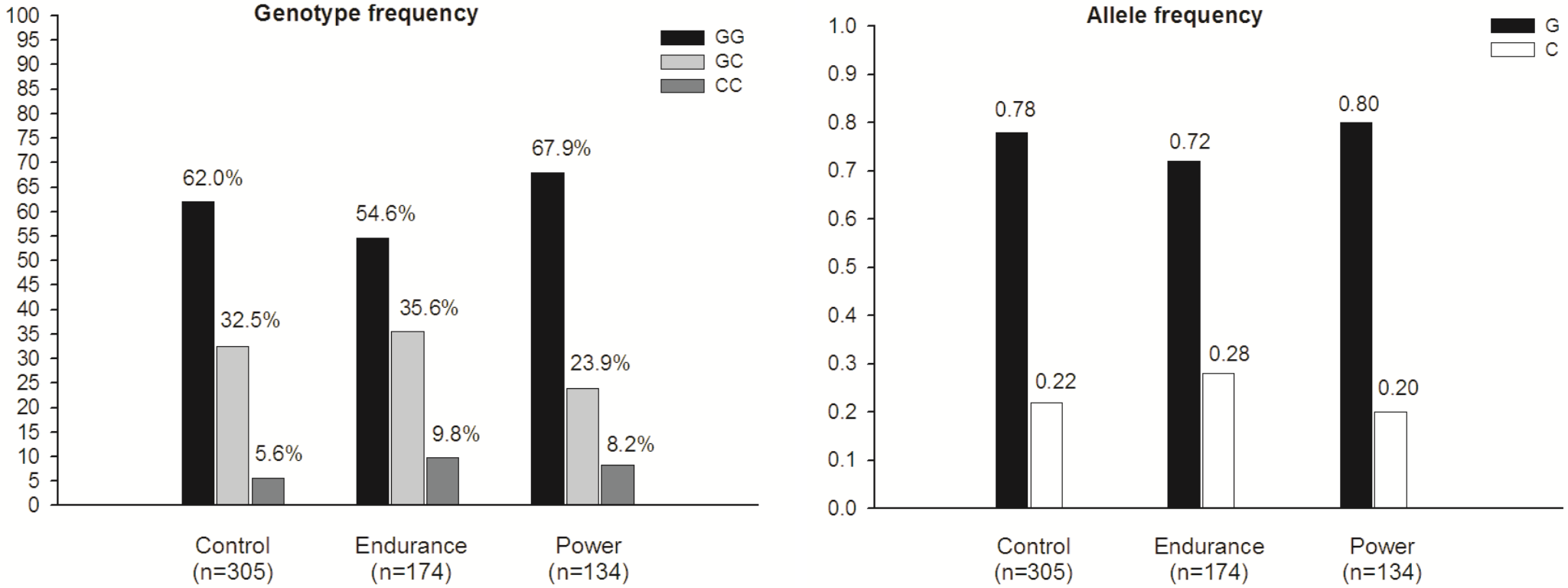

Figure 1. Genotype and allele frequencies of the *IL6* -174 G/C polymorphism (rs1800795) in Israel and Spanish (Caucasians) non-athletes (controls), elite endurance and power athletes. Note: data from Israel and Spanish are combined.