Does the *ACE* I/D polymorphism, alone or in combination with the *ACTN3* R577X polymorphism, influence muscle power phenotypes in young, non-athletic adults?

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Abstract We investigated the association of the angiotensin converting enzyme gene (*ACE*) insertion/deletion (I/D) polymorphism, alone or in combination with the α-actinin-3 gene (*ACTN3*) R577X polymorphism, with jumping (vertical squat and counter-movement jump tests) and sprint ability (30 m dash) in non-athletic, healthy young adults [$N = 281$ (214 male), mean (SD) age 21 (2) years]. We did not observe any effect of the *ACE* I/D polymorphism on study phenotypes. We repeated the analyses separately in men and women and the results did not materially change. Likewise, the mean estimates of the study phenotypes were similar in subjects with the genotype combinations *ACE* II + ID and *ACTN3* XX or *ACE* DD and *ACTN3* RR + RX. We found no association between the *ACE* DD and *ACTN3* RR + RX genotype combination and performance (≥90th of the sex-specific percentile). In summary, though the *ACE* I/D polymorphism is a strong candidate to modulate some exercise-related phenotypes or athletic performance status, this polymorphism, alone or in combination with the *ACTN3* R577X polymorphism, does not seem to exert a major influence in the muscle 'explosive' power of young healthy adults, as assessed during multi-joint exercise tests.

Keywords Jump · Sprint · Genes · Genotype:phenotype association

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Introduction

One genetic variation that is a candidate to explain individual variability in exercise-related phenotypes, and particularly in muscle phenotypes is the 287 bp insertion/deletion (I/D) polymorphism of the angiotensin converting enzyme (*ACE*) gene. Besides regulating blood pressure, the ACE is expressed in skeletal muscle, where it may influence its function and biomechanical properties (Gordon et al. 2001; Jones et al. 2002; Wagner et al. 2006), e.g. its product, angiotensin II, acts as a muscle growth factor (Jones et al. 2002) that is necessary to induce overload-induced skeletal muscle hypertrophy (Gordon et al. 2001). The *ACE* D allele is associated with higher ACE activity and thus increased angiotensin II levels (Jones et al. 2002); thus, this allele would theoretically favour performance in power or strength-oriented versus more endurance exercise tasks. Indeed, the D allele has been associated with elite "sprint" athletic performance (Myerson et al. 1999; Woods et al. 2001) and power-related phenotypes in non-athletic populations, e.g. preserved quadriceps muscle strength in chronic patients (Hopkinson et al. 2004), lower risk of skeletal muscle damage induced by eccentric contractions

(Yamin et al. 2007), or greater gains in knee extensor strength after training in old (≥60 years) individuals (Giaccaglia et al. 2008).

Another strong candidate to influence muscle phenotypes is the *ACTN3* gene, which encodes for the synthesis of α-actinin-3 in skeletal-muscle fibres, a protein necessary for producing fast, powerful contractions (Yang et al. 2003). A premature stop codon polymorphism (R577X) in *ACTN3* was first described by North et al. (1999). Though this genetic variation is not associated with any known disease phenotype, the α-actinin-3-deficient XX genotype is believed to preclude top-level athletic performance in 'pure' power and sprint sports (e.g. sprinting and jumping events), especially in women (Yang et al. 2003). More discrepancy exists on the putative role of the *ACTN3* R577X polymorphism on muscle power phenotypes in non-athletic populations, with some authors reporting an unfavourable effect of the XX genotype (Vincent et al. 2007), at least in women (Walsh et al. 2008), and others reporting no effect (McCauley et al. 2009). Additional controversy stems from the fact that in older adults (mean age ~ 65 years), the XX genotype was associated with higher knee extensor concentric peak power compared with RR and RX genotypes (Delmonico et al. 2007), while others found no genotype:phenotype association (San Juan et al. 2006). We recently reported no association between leg explosive muscle power (jump and sprint tests) and the *ACTN3* R577X polymorphism in non-athletic young adults (Santiago et al. 2009). It was concluded that muscle phenotype traits (e.g. power) are complex and thus not likely reducible to a single polymorphism such as *ACTN3* R577X. For instance, it could be that *ACE* I/D and *ACTN3* R577X polymorphisms act in combination to exert their influence in exercise-related phenotypes.

It was the main purpose of our study to examine the influence of the *ACE* I/D polymorphism, alone or in combination with the *ACTN3* R577X polymorphisms, in the muscle power phenotypes of non-athletic adults. We hypothesized that, though the more 'endurance-oriented' *ACTN3* XX genotype is not associated with lower muscle explosive power (in jump and sprint tests) as we previously showed (Santiago et al. 2009), the combination of the *ACTN3* R577X and *ACE* ID polymorphisms may partly explain the individual variability in muscle strength phenotypes among young adults.

Methods

Subjects

Written consent was obtained from each subject. The study protocol was approved by the institutional ethics committee [Universidad Europea de Madrid (UEM), Spain] and was in accordance with the Declaration of Helsinki for Human Research. The study sample comprised 281 healthy young adults (University students) [mean (SD) age 21 (2) years (range 21, 32)] of both genders (214 men, 67 women) who took part in a previous study on *ACTN3* R577X polymorphism (Santiago et al. 2009). Inclusion criteria were to be free of any diagnosed cardiorespiratory disease, and not to be engaged in competitive sports such as (a) formal, supervised 'power' (e.g. weight lifting or alpine skiing) or jumping oriented type of training (e.g. plyometrics, volleyball or basketball) or (b) endurance training (e.g. running, swimming of bicycling), i.e., performing less than one (power) or three (endurance) structured weekly training sessions within the last year. All participants were of the same Spanish (Caucasian) ancestry for at least three generations.

Genotype assessment

Our study was designed and performed in accordance with the recommendations for the human genotype–phenotype association studies recently published by the NCI-NHGRI Working Group on Replication in Association Studies (Chanock et al. 2007). These recommendations include among others, the following items: indicating time period and location of subject recruitment, success rate for DNA acquisition, sample tracking methods or genotyping with a second technology in a second laboratory.

During winter–spring 2008, we extracted genomic DNA from saliva samples of the subjects in two different universities of the same city (Madrid, Spain): *Universidad Politécnica* and *UEM* ($N = 200$ and 84 subjects, respectively). Genotyping of the *ACTN3* R577X polymorphism was performed during fall 2008 in the genetics laboratory of the UEM and the results have been previously published (Santiago et al. 2009). The *ACE* I/D genotyping was performed during spring 2010 in the same laboratory.

We followed the *ACE* I/D (rs1799752) and *ACTN3* R577X (rs1815739) genotyping methods that have been applied in previous studies (Gomez-Gallego et al. 2009; Santiago et al. 2009). Sequences corresponding to each polymorphism were amplified by the polymerase chain reaction (PCR) and the resulting PCR products were genotyped using electrophoresis through agarose gel (*ACE*) or restriction fragment length polymorphisms (*ACTN3*). Primers used for the *ACE* I/D polymorphism were: 5′-TG GAGACCACTCCCATCCTTTCT and 5′-GATGTGGCC ATCACATTCGTCAGAT. The PCR conditions were as follows: initial denaturing at 95°C 5 min; 35 cycles at 95°C 30 s, 58°C 30 s, 72°C 1 min and a final extension at 72°C 5 min. The *ACE* I/D fragments without insertion (D allele) and with insertion (I allele) of 190 and 490 bp,

respectively, were detected on a 1.5% agarose gel containing ethidium bromide. In order to avoid a misclassification of ID heterozygotes as DD homozygotes, a second PCR reaction was performed in all of the samples initially classified as DD with the following insertion-specific primer pairs (Lindpaintner et al. 1995): 5′-TGGGACCACA GCGCCCGCCACTAC and 5′-TCGCCAGCCCTCCCAT GCCCATAA. The PCR conditions were similar as described above, except for the annealing temperature (64°C). Only the allele I produces a 335 bp amplicon, identified on an a 1.5% agarose gel stained with ethidium bromide. Figure 1 shows a representative example of visual detection of *ACE* genotypes.

For the *ACTN3* R577X polymorphism, a fragment of 291 bp was amplified with the following primers: ACTN3-F 5′-CTGTTGCCTGTGGTAAGTGGG labelled a 5′ with VIC and ACTN3-R 5′-TGGTCACAGTATGCAGGAG GG. The PCR conditions were as follows: initial denaturing at 95°C 5 min; 35 cycles at 95°C 30 s, 60°C 30 s, 72°C 30 s and a final extension at 72°C 10 min. *ACTN3* genotypes were established by enzymatic digestion of amplicons with *Dde I*. The R577X change creates a restriction site resulting in fragments of 108, 97 and 86 bp. Digestion of the R577 allele results in fragments of 205 and 86 bp, and digestion of the 577X allele results in fragments of 108, 97 and 86 bp. The digestion products detected by capillary electrophoresis (ABI Prism 310 genetic analyzer; Applied Biosystems, Foster City, CA) were those labelled with VIC, i.e. 108 bp for 577X, and 205 bp for R577.

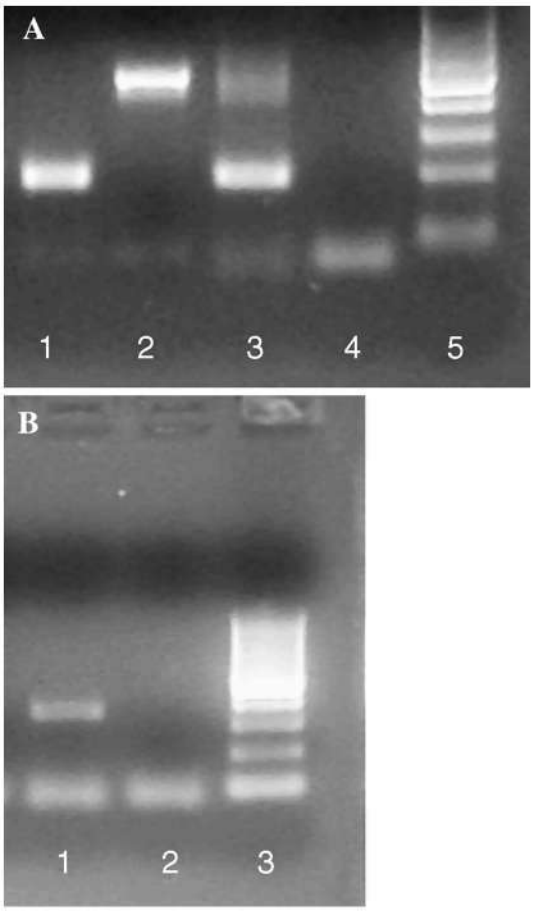

Fig. 1 Example of visual detection of *ACE* genotypes. **a** (*Upper figure*) Representative gel of angiotensin converting enzyme gene (*ACE*) genotyping. *Lane 1* Homozygous DD individual (190 bp), *lane 2* homozygous II individual (480 bp), *lane 3* heterozygous ID individual (190 and 480 bp), *lane 4* negative control, *lane 5* Ladder 100 bp (Biotools, Spain). **b** (*Lower figure*) Representative gel of internal *ACE* genotyping. *Lane 1* Positive internal *ACE* PCR (335 bp), *lane 2* negative internal *ACE* PCing enzyme R, *lane 3* Ladder 100 bp (Biotools, Spain)

Following recent recommendations (Chanock et al. 2007), genotype results of both *ACE* I/D and *ACTN3* R577X polymorphisms were replicated in 100 samples in a different laboratory (*Progenika Biopharma, Parque Tecnológico de Zamudio*, Derio-Vizcaya, Spain) using a different technology, i.e. 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 (Tejedor et al. 2005). In brief, the PCR products were fluorescently labelled and hybridized 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 we determined variants using a developed software that converts the intensity of the spots into the genotype of each variant.

No failures occurred in sample collection and DNA acquisition. Genotyping success rate was >98.9% (three missing data for *ACE* I/D and only one missing data for *ACTN3* R577X). Parallel genotyping results of the two polymorphisms showed 100% concordance between the two laboratories (UEM and Progenika).

Phenotype assessment

Assessment of leg muscle 'explosive' power was performed during spring 2008 in the same location (UEM) and all the tests were supervised by the same researchers, as detailed elsewhere (Santiago et al. 2009). Squat (SJ) and counter-movement jump (CMJ) tests were performed using an infrared contact timing platform (Globus Ergo Tester, Codognè, Italy) to evaluate leg muscles' ability to produce 'explosive' power (Young et al. 2001). Both tests were performed three times (each separated by a 2-min rest period) and the best score was retained.

Subjects also performed a 30 m sprint test in an indoor rubberized track under two conditions: (1) starting from the stationary (standing) position (Young et al. 2001) and (2) starting with a previous 15 m run thereby allowing achieving higher speeds in the first meters of the test

(Alcaraz et al. 2009). The difference in performance time between both tests (at 15 and 30 m, respectively) was used as an index of subject's ability to produce acceleration, i.e. lesser difference implies higher acceleration capacity. We used photoelectric gates at 0, 15 and 30 m to start and stop a digital timer. We previously showed the reliability of the aforementioned tests for explosive leg muscle power assessment with a subgroup of the present subjects (Santiago et al. 2009).

Statistical analysis

Hardy–Weinberg equilibrium was tested using a χ^2 test. We compared the genotype frequencies between males and females with the χ^2 test.

We analysed the differences in the study phenotypes among variants of the *ACE* I/D polymorphism by one-way analysis of covariance (ANCOVA), where the polymorphism was entered as a fixed factor, the phenotype was entered as a dependent variable, and age, weight and height were entered as covariates. We did not observe an interaction effect of sex × *ACE* I/D polymorphism on the phenotypes (all $P > 0.2$); therefore, all the analyses were performed with men and women together and sex was included as an additional covariate to the model.

We also analysed the combined effect of *ACE* I/D and *ACTN3* R577X polymorphisms on the study phenotypes by ANCOVA using two genotype combinations, i.e. *ACE* DD and *ACTN3* RR + RX (which, at least hypothetically, might be more suitable for power/hypertrophy-oriented exercise tasks) versus *ACE* II + ID and *ACTN3* XX group (Gomez-Gallego et al. 2009). We also compared the *ACE* DD and *ACTN3* RR + RX group against the rest of study participants by ANCOVA.

Finally, in order to examine whether the subjects with the best performance had also the 'best' genotype profile (i.e. *ACE* DD and *ACTN3* RR + RX), we classified the population into two groups based on an arbitrary cut-off point: ≥90th of the sex-specific percentile and <90th of the sex-specific percentile. The rationale for choosing 90th percentile was based on the fact that subjects in the top 10% might have a favourable genetic endowment to perform better. We performed logistic regression analysis to examine the association between the *ACE* DD and *ACTN3* RR + RX genotype combination and performance (≥90th of the sex-specific percentile) after adjusting for sex, age, weight and height. To further investigate the influence of the 90th percentile cut-off on the findings, we performed sensitivity analyses after varying these cut-offs (≥75th and ≥95th).

All statistical analyses were performed using the PASW. We used the Bonferroni and Holm method to correct for multiple testing (Holm 1979; Shaffer 1995). This method proceeds as follows: Sort the P values of the k tests in increasing order, i.e., $P_1, P_2, \ldots, P_i, \ldots, P_k$. If $P_1 > \alpha/k$, none of the k tests are significant, and the test procedure is finished. If $P_1 \leq \alpha/k$, test 1 is significant, and now P_2 is examined. If $P_2 > \alpha/(k-1)$, none of the $(k-1)$ remaining tests are significant, but if $P_2 \leq \alpha/(k-1)$, test 2 is significant and P_3 is examined. This procedure goes on until $P_i > \alpha/(k-i+1)$.

Results

ACE I/D genotype distributions met Hardy–Weinberg equilibrium ($\chi^2 = 0.050$, $P = 0.821$). We did not observe differences ($\chi^2 = 2.225$, $P = 0.329$) in the genotype distributions in men [30 (13.9%), 103 (47.7%) and 81 (37.5%) for II, ID and DD, respectively] and women [9 (13.4%), 26 (38.8%) and 32 (47.8%) for II, ID and DD, respectively]. The genotype distribution of the *ACTN3* R577X polymorphism also respected the Hardy–Weinberg equilibrium and did not differ by sex (Santiago et al. 2009).

The association between the *ACE* I/D polymorphism and study phenotypes is presented in Table 1. We did not observe any significant effect of the polymorphism on study phenotypes, although a 'minor trend' ($P = 0.063$ and $P = 0.062$) was found for the SJ tests. We repeated the analyses separately in men and women and the results did not materially change (data not shown). Likewise, the mean estimates of the study phenotypes were similar in the *ACE* II + ID and *ACTN3* XX and *ACE* DD and *ACTN3* RR + RX groups (Table 2). We performed separate analyses comparing the *ACE* DD and *ACTN3* RR + RX group versus the rest of subjects and the results remained the same (data not shown). The observed power ranged between 0.78 and 0.85.

We found no association between the *ACE* DD and *ACTN3* RR + RX genotype combination and performance (≥90th of the sex-specific percentile) (Fig. 2). The results remained the same after changing the cut-off point to ≥75th percentile or to ≥95th percentile, or after repeating the analysis by sex (data not shown).

Discussion

Our data suggest that the *ACE* I/D polymorphism, individually or in combination with the *ACTN3* R577X polymorphism, does not have a major influence on explosive muscle power in young, non-athletic adults, at least with the multi-joint exercise tests we used (see below). We only found a 'minor trend' for an influence of the *ACE* I/D polymorphism on squat jump tests (P values of 0.063 and 0.062 for the dominant model in flight time and vertical

Table 1 Mean estimates of study phenotypes by genotypes of *ACE* I/D (rs1799752) polymorphism

	II (n = 39)		ID (n = 129)		DD (n = 113)		P add	P recess	P dom
	Mean	SEM	Mean	SEM	Mean	SEM			
Vertical jump tests									
SJ									
Flight time (s)	556.1	6.3	541.1	3.5	545.9	3.8	0.432	0.819	0.063
Vertical displacement of CG (cm)	38.2	0.9	36.2	0.5	36.9	0.5	0.451	0.773	0.062
CMJ									
Flight time (s)	564.6	6.6	552.8	3.7	556.5	4.0	0.546	0.863	0.155
Vertical displacement of CG (cm)	39.4	0.9	37.7	0.5	38.3	0.5	0.609	0.771	0.160
Sprint tests									
30 m running start									
Time at 15 m (s) (A)	1.99	0.02	2.00	0.01	1.99	0.01	0.801	0.566	0.765
Time at 30 m (s) (B)	3.89	0.04	3.91	0.02	3.91	0.02	0.727	0.972	0.525
30 m standing start									
Time at 15 m (s) (C)	2.58	0.02	2.61	0.01	2.61	0.01	0.439	0.821	0.229
Time at 30 m (s) (D)	4.53	0.04	4.56	0.02	4.56	0.02	0.686	0.924	0.510
Acceleration index									
C − A (s)	0.59	0.02	0.61	0.01	0.62	0.01	0.332	0.455	0.385
D − B (s)	0.65	0.03	0.65	0.02	0.65	0.02	0.944	0.941	0.972

Analyses adjusted for sex, age, weight and height. P values before applying correction for multiple testing

recess recessive (DD + ID vs. II), *dom* dominant (II + ID vs. DD), *SJ* squat jump, *CMJ* counter-movement jump, *CG* centre of gravity, *SEM* standard error of the mean

Table 2 Estimates of study phenotypes in the *ACE* II + ID and *ACTN3* XX and *ACE* DD and *ACTN3* RR + RX groups

	ACE II + ID and *ACTN3* XX (n = 36)		*ACE* DD and *ACTN3* RR + RX (n = 97)		P*
	Mean	SEM	Mean	SEM	
Vertical jump tests					
SJ					
Flight time (s)	538.3	7.1	548.3	4.4	0.383
Vertical displacement of CG (cm)	35.8	1.0	37.2	0.6	0.373
CMJ					
Flight time (s)	549.6	7.3	558.2	4.5	0.641
Vertical displacement of CG (cm)	37.3	1.0	38.5	0.6	0.636
Sprint tests					
30 m running start					
Time at 15 m (s) (A)	2.0	0.0	2.0	0.0	0.612
Time at 30 m (s) (B)	4.0	0.0	3.9	0.0	0.420
30 m standing start					
Time at 15 m (s) (C)	2.6	0.0	2.6	0.0	0.152
Time at 30 m (s) (D)	4.6	0.0	4.6	0.0	0.109
Acceleration index					
C − A (s)	0.6	0.0	0.6	0.0	0.340
D − B (s)	0.7	0.0	0.7	0.0	0.626

SJ squat jump, *CMJ* counter-movement jump, *CG* centre of gravity, SEM standard error of the mean

* Analyses adjusted for sex, age, weight and height; P values before applying correction for multiple testing

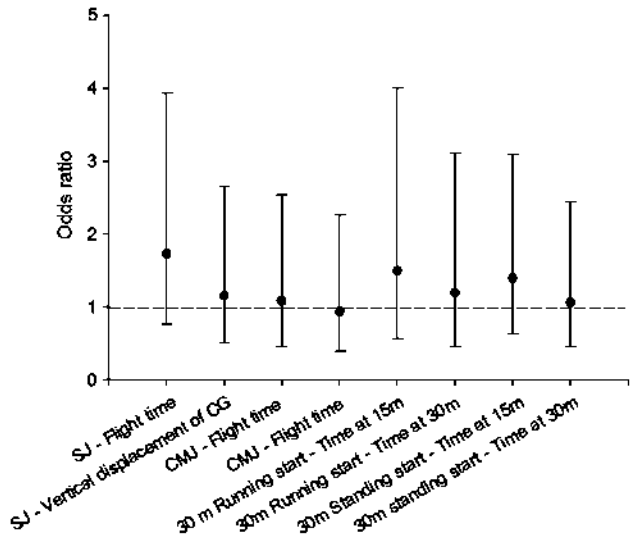

Fig. 2 Association between the *ACE* DD and *ACTN3* RR + RX genotype combination and performance (≥90th of the sex-specific percentile). Values are odds ratio and 95% confidence intervals

displacement of centre of gravity, respectively). Thus, with the present design for phenotype assessment, the hypothesis that *ACE* I/D and *ACTN3* R557X genotypes might exert a combined influence on leg muscle phenotypes was not corroborated.

Though controversy exists (McCauley et al. 2009, 2010; Moran et al. 2006), the *ACE* D allele has been associated with power-related phenotypes in non-athletic populations (Giaccaglia et al. 2008; Hopkinson et al. 2004; Yamin et al. 2007); this allele has also been associated with elite "sprint" athletic performance (Myerson et al. 1999; Woods et al. 2001), though more recent data do not corroborate such association (Amir et al. 2007; Scott et al. 2005, 2010). However, the association between leg 'explosive' muscle power and the *ACE* I/D polymorphism had not been studied to date.

We assessed 'explosive' muscle power using jumping and sprinting testing tests in young, non-athletic adults. Jumping and sprinting are naturally occurring multi-joint movements in humans that involve the coordinated participation of the majority of lower limb muscles (Ashley and Weiss 1994; Brown and Weir 2001). On one hand, we believe that the tests we used for muscle phenotype assessment represent both a novelty and a strength of our study versus previous reports in the field that used other tests for muscle power assessment, e.g. isokinetic tests involving single-joint movements (e.g. knee-extension) at relatively low angular velocities (≤240° s^{-1}) (McCauley et al. 2009, 2010). Indeed, during actual, *natural* high muscle power actions (e.g. sprint and jumps), angular velocities at the hip or knee joints can approach 800°–1,000° s^{-1} (Bosco et al. 1983). The fact that we only used multi-joint tests can also be viewed as a limitation of our study because other factors aside from the skeletal muscle tissue per se (see below) could have influenced the results of our tests and masked any potential genotype effect. This is particularly true for sprinting. In this type of exercise task, owing to the short duration of the foot contact on the ground, the rate of force development (RFD) is the critical performance determinant. The RFD is, in turn, influenced by several factors such as muscle fibre type, synchronization of motor units, tendon stiffness, or lean mass of lower extremities (Perez-Gomez et al. 2008). In contrast, the ability of leg muscles (quadriceps) to produce power during the concentric phase of muscle contraction is the critical factor affecting stationary vertical jumps as the ones we used here (Ham et al. 2007). Future research in the field is necessary combining both single and multi-joint exercise tasks.

Scarce data are available on the *combined* influence of *ACE* I/D and *ACTN3* R577X polymorphisms on muscle power phenotypes. In elite athletes, data are rather controversial. We showed no significant combined influence of *ACTN3* R577X and *ACE* I/D variations on the peak muscle power output generated by elite endurance athletes during cycle-ergometer testing until exhaustion (Gomez-Gallego et al. 2009). No differences were found in peak power output between those cyclists with the *ACE* DD and *ACTN3* RR + RX and *ACE* II + ID and *ACTN3* XX genotype combinations. More recently, Eynon et al. (2009) reported an association between the combination of both *ACE* and *ACTN3* polymorphisms and elite sprint athletic status (Eynon et al. 2009), yet they found that the most 'favourable' allele combination for a sprinter was the *ACTN3* R allele (i.e., theoretically power oriented) + the *ACE* I allele [i.e. theoretically more endurance oriented according to other studies (Jones et al. 2002; Williams et al. 2000)]. With regard to this, in our study, we found no significant association between the *ACTN3* R allele + *ACE* I allele combination (data not shown). As for non-athletic populations, our findings concur with recent data on elderly people showing no association (individually or combined) between power muscle phenotypes and *ACE* I/D + *ACTN3* R577X genotype combinations (Bustamante-Ara et al. 2010).

Besides the aforementioned lack of data on single-joint tests for muscle power assessment, our study has other potential limitations. We did not measure the activity of the *ACE* gene product (i.e. ACE) or circulating levels of the muscle growth factor angiotensin II. With regard to this, although serum ACE activity was suggested to be significantly dependent on *ACE* genotypes in young Caucasian adults (with the highest enzymatic levels been found in the DD genotype), it does not seem to influence their muscle functional or contractile properties (McCauley et al. 2009). Further research is also necessary to assess the combined

influence of other candidate genes with a potential effect on muscle phenotypes, e.g. the myostatin gene.

In summary, though the *ACE* I/D polymorphism is a strong candidate to modulate some exercise-related phenotypes or athletic performance status, this polymorphism, alone or in combination with the *ACTN3* R577X variation, does not seem to exert a major influence in the muscle 'explosive' power of non-athletic young healthy adults.

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