ACE I/D and ACTN3 R/X polymorphisms as potential factors in modulating exercise-related phenotypes in older women in response to a muscle power training stimuli

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Abstract Genetic variation of the human ACE I/D and ACTN3 R577X polymorphisms subsequent to 12 weeks of high-speed power training on maximal strength (1RM) of the arm and leg muscles, muscle power performance (counter-movement jump), and functional capacity (sit-to-stand test) was examined in older Caucasian women [$n=139$; mean age 65.5

(8.2) years; 67.0 (10.0) kg and 1.57 (0.06) m]. Chelex 100 was used for DNA extraction, and genotype was determined by PCR-RFLP methods. Muscular strength, power, and functional testing were conducted at baseline (T1) and after 12 weeks (T2) of high-speed power training. At baseline, the ACE I/D and ACTN3 R/X polymorphisms were not associated with muscle function or muscularity phenotypes in older Caucasian women. After the 12-week high-speed training program, subjects significantly increased their muscular and functional capacity performance ($p<0.05$). For both polymorphisms, significant genotype-training interaction ($p<0.05$) was found in all muscular performance indices, except for 1RM leg extension in the ACE I/D ($p=0.187$). Analyses of the combined effects between genotypes showed significant differences in all parameters ($p<0.05$) in response to high-speed power training between the power (ACTN3 RR+RX & ACE DD) versus “non-power” muscularity-oriented genotypes (ACTN3 XX & ACE II+ID)]. Our data suggest that the ACE and ACTN3 genotypes (single or combined) exert a significant influence in the muscle phenotypes of older Caucasian women in response to high-speed power training. Thus, the ACE I/D and ACTN3 R/X polymorphisms are likely factors in modulating exercise-related phenotypes in older women, particularly in response to a resistance training stimuli.

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Introduction

Age-related decline in strength and especially in muscle power output leads to compromised mobility and an increased risk of falls (Izquierdo et al. 1999; Pereira et al. 2012). Lower extremity muscle power has been recently posited as a more discriminating variable for understanding the relationships between impairments, functional limitations, and resultant disability with aging (Reid and Fielding 2012). Several recent studies also suggest that high-speed training is clinically relevant to maintain functional abilities in older women (Sayers and Gibson 2010; Pereira et al. 2012; Reid and Fielding 2012).

Greater knowledge of the mechanisms and interaction of exercise-induced adaptive pathways in skeletal muscle is important for understanding the maintenance of functional capacity with aging (Coffey and Hawley 2007). Genetic variation has been used to answer questions as to how adaptations in skeletal muscle and interactions occur. It provides new frameworks to improve physical performance in older people (Ahmetov and Rogozkin 2009; Bustamante-Ara et al. 2010; McCauley et al. 2010; Garatachea et al. 2011) being clinically relevant to identifying training response association of different genotypes associated with decreases of strength and function with aging (Garatachea and Lucia 2011). Although previous association studies have suggested a relevant genetic contribution to individual variability in muscle-related phenotypes, published data on specific gene variants are controversial (Bouchard and Rankinen 2001; Garatachea and Lucia 2011). It is also likely that in an aged population, the genetic contribution to individual variability in training-induced effects also remains to be elucidated.

The angiotensin-converting enzyme (ACE) I/D and alpha-actinin 3 (ACTN3) R/X polymorphisms have been reported as influencing variations in skeletal muscle function (Gomez-Gallego et al. 2009; Gineviciene et al. 2011; Lima et al. 2010). ACE has a key role in the endocrine rennin–angiotensin system (RAS), being responsible for catalyzing the conversion of angiotensin I into angiotensin II (Costa et al. 2009). A few studies, however, have linked the potential importance of the RAS and ACE gene to skeletal muscle hypertrophy, a key determinant mechanism of strength and power enhancement in response to overload in healthy older men and women (Charbonneau et al. 2008; Gordon et al. 2001; Wagner et al. 2006; Puthuchery et al. 2011a). Historically,

research has centered on an insertion–deletion (I/D-allele) polymorphism in intron 16, which is characterized by the presence (I-allele) or absence (D-allele) of a 287-bp *Alu* repeat sequence (Rieder et al. 1999). It seems that the DD genotype may be associated with a greater proportion of fast-twitch fibers (Zhang et al. 2003) which could explain the possible influence of the ACE D-allele upon strength/power, particularly at high velocity tasks (Costa et al. 2009). However this evidence remains equivocal (Folland et al. 2000; Woods et al. 2001; Pescatello et al. 2006; McCauley et al. 2009; Garatachea et al. 2011). Moreover, to our knowledge, no studies have examined the influence of ACE DD genotype on high-speed power training-induced changes in older Caucasian women.

ACTN3 is another candidate polymorphism that has been more extensively studied because of its potential associations with muscle phenotypes in older people. ACTN3 is an actin-binding protein with multiple roles in different cell types. This gene expression is limited to skeletal muscle (Mills et al. 2001). It is localized in the Z-disc and analogous dense bodies, where it helps anchor the myofibrillar actin filaments (Lek et al. 2010). Variations in ACTN3 gene can explain individual variability in muscle phenotypes by a substitution of arginine at an amino acid residue 577 (R-allele) with a premature stop codon (X-allele).

The association between R577X and loss in muscle function has previously been investigated (Lima et al. 2010; Seto et al. 2011). Most investigators report loss of ACTN3 genotype association with muscle traits in the elderly, although there is some indication that the XX genotype may be associated with faster muscle function decline.

The influence of genetic factors on muscle phenotypes is controversial (Garatachea and Lucia 2011; Puthuchery et al. 2011b) mainly because this is unlikely to be reducible to a few single genetic variants (Bustamante-Ara et al. 2010). However, the proportion of each gene variant in explaining the variance of a complex trait such as this is totally unknown. Concerning ACE I/D and ACTN3 R/X polymorphisms, some studies have examined their influence in conjunction (Bustamante-Ara et al. 2010; Garatachea et al. 2011) but the role of significant cumulative genotype–training effect remains to be elucidated.

The purpose of the present study was to analyze the influence of the ACE I/D and ACTN3 polymorphisms alone and in combination on muscle strength, power, and functional phenotype in older Caucasian women

following 12-week period of high-speed power training. One of the hypotheses argued is that the combination of ACE and ACTN3 genotypes has a higher skeletal muscle phenotype effect, which may partly explain the older individual variability in muscle performance adaptation to resistance training.

Methods

Subjects

One hundred and thirty-nine healthy older Caucasian women participated in this study (age: 65.5 ± 8.2 years, body mass: 67.0 ± 10.0 kg and height: 1.57 ± 0.06 m). Apart from normal daily routine tasks, older women underwent a resistance training program comprising three training sessions per week over 12 weeks (Pereira et al. 2012). None of the participants had a history of strength training. Before inclusion in the study, all candidates were thoroughly screened by a physician. Exclusion criteria included metallic prosthesis implants, artificial pACEmakers, smoking habit, hip replACement surgery, walking only with assistance, and metabolic or endocrine disorders known to affect the musculoskeletal system. Each volunteer answered a fACE-to-fACE questionnaire addressing medical history, hormone replACement therapy, lifestyle habits, and medication use. All participants were of the same Caucasian ancestry over at least three generations. A written informed consent was obtained from each participant. The experimental procedures were approved by the University of Trás-os-Montes and Alto Douro, Department of Sport Sciences, according to the Helsinki Declaration.

Genotype assessment

The present study was designed and performed according to the recommendations for human genotype–phenotype association studies recently published by the NCI-NHGRI Working Group on Replication in Association Studies (Chanock et al. 2007). During fall 2010, blood was collected in regular filter paper by finger blood spot (Albet, DP 400200), and the samples were dried at room temperature in the Polytechnic Institute of Bragança (IPB) and stored in separate plastic bags at 4 °C until DNA extraction. Chelex 100® protocol (Bio-Rad Laboratories, Hercules, CA) was used to extract DNA (Costa et al. 2009). To evaluate the extraction

technique, a negative control was always used. Genotyping of the ACE I/D and ACTN3 R577X polymorphism was performed during winter–spring 2011 in the laboratory of CGB/IBB/UTAD. 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; Lima et al. 2010).

ACE I/D polymorphisms were amplified by polymerase chain reaction (PCR), and the resulting PCR products were genotyped using agarose gel electrophoresis. The primers used for the ACE I/D polymorphism were F-5'-CTGGAGACCACTCCCATCCTTTCT-3' and R-5'-GATGTGGCCATCACATTTCGTCAGAT-3'. The PCR conditions were as follows: initial denaturing at 95 °C for 3 min; 35 cycles at 95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 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.

For ACTN3 R577X polymorphism genotyping, a fragment of 291 bp was amplified with the following primers: ACTN3-F 5'-CTGTTGCCTGTGGTAAGTGGG-3' and ACTN3-R 5'-TGGTCACAGTATGCAGGAGGG-3' (Rodriguez-Romo et al. 2010). The PCR conditions were as follows: initial denaturing at 95 °C for 3 min; 35 cycles at 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, and a final extension at 72 °C for 10 min. ACTN3 genotypes were established by enzymatic digestion of amplicons with Dde I during 17 h at 37 °C. 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 fragments were detected on a 3 % agarose gel containing ethidium bromide.

Genotype results of both ACE I/D and ACTN3 R577X polymorphisms were replicated in a different laboratory (University of Beira Interior, Covilhã). Genotyping success rate was 99 % (two 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 (UTAD and UBI).

Phenotype assessment

The phenotype assessment (muscle strength, power output, and functional capacity) and subject characterization

were performed during fall 2010 in the same location (IPB), all of the measurements being supervised by the same qualified sports science researchers as detailed elsewhere (Pereira et al. 2012).

Specific protocols for evaluation of older musculoskeletal performance were selected in the present study. Tests were applied before (T1) and after the 12-week experimental period (T2). In the first session, all subjects were assessed on anthropometric measures and functional capacity [sit-to-stand test (STS)]. The second session (3 days later) involved measures of power (vertical jump) and maximum dynamic strength (one-repetition maximum bench press and leg extension) (Pereira et al. 2012). The test–retest reliability for all strength and power measurements taken was performed; the intra-class correlation coefficient (ICC) was always higher than 0.90. Before testing, subjects were familiarized with all strength testing procedures, preceded by a general warm-up routine. Verbal encouragement was given throughout the voluntary test and biofeedback provided in order to maximize motivation and to ensure that the participants made genuinely maximal contractions.

Measurements

A detailed description of the testing procedures has been given elsewhere (Pereira et al. 2012). In brief, body height (m) and body weight (kg) were assessed according to international standards for anthropometric assessment. To evaluate body height, a stadiometer (SECA, model 225, Germany) with a range scale of 0.10 cm was used, and body mass (kg) was measured to the nearest 0.1 kg using a digital scale (Philips, type HF 351/00). Lower- and upper-body maximal strength was assessed using one-repetition concentric maximum (1RM) actions in a leg extension (1RM_{LE}) and in bench press (1RM_{BP}) position, respectively. The power output of the leg muscles was measured concentrically in vertical jump using a trigonometric carpet (Ergojump Digitime 1000; Digitest, Jyväskylä, Finland) to assess maximum height in counter-movement jump (CMJ). Functional measure was evaluated using the 30-s sit-to-stand test (STS).

High-speed power training protocol

The resistance program (RT) consisted of three sessions per week (on non-consecutive days) over

12 weeks. The training consisted of progressive loads by three sets of ten reps with a load of 40 % of 1RM at the outset of their predetermined 1-repetition maximum up until three sets of four reps with a load of 75 % towards the end of the 12-week period in 1RM_{LE} and 1RM_{BP}. Two power exercises were then performed: the counter-movement jump and medicine ball throw (1.5 kg). Rest intervals of 2 min between sets and 3 min between exercises were deployed. The training programs utilized in the present study were similar to those reported previously (Pereira et al. 2012). Adherence to training averaged >95 % (mean of the total of 36 planned sessions).

Statistical analysis

Standard statistical methods were used for the calculation of means and standard deviations. Differences in the distributions of the ACE and ACTN3 genotypes were examined using Pearson's χ^2 . For Hardy–Weinberg equilibrium calculations, χ^2 statistic (one degree of freedom) was computed from the observed distribution of genotypes and the distribution of genotypes expected from applying the Hardy–Weinberg equilibrium assumption to the observed allele frequencies in the population. Parameters measured during exercise strength/power testing and functional performance achieved at the beginning and end of high-speed power training were analyzed by one-way ANOVA after checking for normality by Kolmogorov–Smirnov and for homogeneity of variance by Levene's test. The gains in physical performance performed during training programs were also analyzed by two-way, repeated-measures ANOVA, using each performance measure as the within-subjects variable and ACE and ACTN3 genotype as the between-subjects variable. Post hoc testing for significant differences in the ANOVA was performed by the Tukey's honestly significant difference test. Combined effect of ACE I/D and ACTN3 R577X polymorphisms on the study phenotypes by ANOVA was analyzed 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). Test–retest reliabilities, as shown by ICC, ranged from 0.90 to 0.93 for all testing exercises. Statistical significance was accepted at $p \leq 0.05$ for all analysis. All data were analyzed using SPSS 17.0.

Results

At baseline, there were no significant differences ($P>0.05$) observed between ACE or ACTN3 genotypes for anthropometric, strength, muscle power, and functional performance. Likewise, no significant changes ($P>0.05$) in either ACE or ACTN3 genotypes were observed between first (T1) and second testing session (T2) in body height, body weight, or BMI (Table 1).

As expected, ACE I/D genotype distribution was in Hardy–Weinberg equilibrium ($P=0.125$). No significant differences were observed in genotype distributions for DD, II, and ID [52 (37.4 %), 35 (25.2 %), and 52 (37.4 %), respectively]. Allelic frequencies were 0.56 and 0.44 for the D- and I-alleles, respectively.

The genotype distribution of the ACTN3 R577X polymorphism also respected the Hardy–Weinberg equilibrium ($P=0.06$) and did not show differences between genotype distributions for RR, XX, and RX [52 (37.4 %), 33 (23.8 %), and 54 (38.8 %), respectively]. Allelic frequencies were 0.60 and 0.43 for the R- and X-alleles, respectively.

Training and ACE genotype effects are presented in Table 2. Significant group $\times$ time interactions were noted for all measures ($P<0.05$), with the DD genotype showing greater improvements in performance than those observed in the ID or II genotypes. Using post hoc analysis by Tukey's honestly significant difference test showed a significant difference between older women with both ID and DD genotypes having maximal strength in $1RM_{BP}$, power, and functional capacity than individuals with the II genotype (Table 2), but the difference between genotypes means was not significant in $1RM_{LE}$.

From the pre- to post-training period, subjects increased maximal dynamic strength of $1RM_{BP}$ (ID: 75.4 %; DD: 97.6 %; and II: 40.9 %) and $1RM_{LE}$ (ID: 59.6 %; DD: 80.9 %; and II: 43.3 %), as well all muscle power output measured by means of a CMJ (ID: 8.14 %; DD: 47.2 %; and II: 14.2 %) and the functional capacity in STS test (ID: 21.7 %; DD: 27.5 %; and II: 15.9 %). Significant training $\times$ genotype effects of the ACE I/D polymorphism were observed in $1RM_{BP}$ CMJ, and STS ($P=0.019$, $P=0.052$, and $P=0.013$, respectively) but not in $1RM_{LE}$ ($P=0.187$).

Training and ACTN3 genotype effect is presented in Table 3. Significant group $\times$ time interactions ($P<0.05$) were observed for ACTN3 R577X, with the RR genotype showing greater improvements in performance

than those observed in RX or XX genotypes. Post hoc analysis showed a significant difference between older women with both RR and RX genotypes having greater performance in all variables than individuals with the XX genotype (Table 3). From the pre- to post-training period, subjects significantly increased maximal dynamic strength in $1RM_{BP}$ (RX: 77.1 %; RR: 84.1 %; and XX: 56.2 %) and $1RM_{LE}$ (RX: 63.7 %; RR: 68 %; and XX: 53.7 %), as well as muscle power measured by means of CMJ (RX: 12.4 %; RR: 57.7 %; and XX: 8.3 %) and functional performance (RX: 13.8 %; RR: 25.2 %; and XX: 18.1 %). Significant training $\times$ genotype effects of the ACTN3 R577X polymorphism were found in all measures ($P<0.05$).

At baseline, analyses of the combined effects between genotypes ACTN3 RR + RX & ACE DD versus ACTN3 XX & ACE II + ID showed no significant difference ($P>0.05$) (Figs. 1, 2, 3 and 4). After 12 weeks of high-speed power training, analyses showed a significant influence ($P<0.05$) in all muscle phenotypes.

Discussion

This study was the first to examine the influence of the ACE and ACTN3 genotypes on training-induced changes in strength, power, and functional capacity before and after a high-speed power training period in older Caucasian women. A distinctive finding of the present study was the evidence that both genetic variants, individually or in combination, have a significant influence on muscle power gains and functional capacity in 60–70-year-old Caucasian women in response to a high-speed power training.

Potential associations between some genetic polymorphisms and individual performance in dynamic strength, muscle power, and functional capacity phenotypes in response to resistance training have previously been noted (Williams et al. 2005; Puthuchery et al. 2011b). Other studies, however, have failed to support such findings (Bustamante-Ara et al. 2010; Rodriguez-Romo et al. 2010). This is mainly because the effect of a single polymorphism on physical function is quite small and therefore difficult to identify (Williams and Folland 2008). In agreement with previous studies (Garatachea et al. 2011; Lima et al. 2010), a positive association was observed for the ACE I/D genotype with skeletal muscle strength and resistance training in older women.

Table 1 Mean $\pm$ standard deviation values regarding the subject's anthropometric characteristics

Variable	Moments				Moments			
	ACE genotype	Number of subjects	T1 $\bar{x} \pm \sigma$	T2 $\bar{x} \pm \sigma$	ACTN3 genotype	Number of subjects	T1 $\bar{x} \pm \sigma$	T2 $\bar{x} \pm \sigma$
Body weight (kg)	ID	52	66.3 $\pm$ 10.6	67.3 $\pm$ 9.6	RX	54	65.7 $\pm$ 11.2	65.8 $\pm$ 11.3
	DD	52	67.5 $\pm$ 9.2	67.8 $\pm$ 9.2	RR	52	68.5 $\pm$ 9.1	68.6 $\pm$ 9.2
	II	35	67.3 $\pm$ 10.2	67.4 $\pm$ 10.6	XX	33	66.6 $\pm$ 8.7	83.5 $\pm$ 9.2
p^*			0.820	0.547			0.355	0.213
BMI (kg m ⁻²)	ID	52	26.6 $\pm$ 3.9	26.0 $\pm$ 3.4	RX	54	26.6 $\pm$ 4.2	26.7 $\pm$ 4.3
	DD	52	27.4 $\pm$ 3.8	27.5 $\pm$ 3.8	RR	52	27.9 $\pm$ 3.4	27.9 $\pm$ 3.5
	II	35	27.5 $\pm$ 3.3	28.8 $\pm$ 2.5	XX	33	26.5 $\pm$ 3.7	28.5 $\pm$ 5.7
p^*			0.434	0.609			0.137	0.263
Height (m)	ID	52	1.57 $\pm$ 0.06	1.58 $\pm$ 0.06	RX	54	1.57 $\pm$ 0.06	1.57 $\pm$ 0.06
	DD	52	1.57 $\pm$ 0.05	1.57 $\pm$ 0.06	RR	52	1.56 $\pm$ 0.06	1.56 $\pm$ 0.06
	II	35	1.56 $\pm$ 0.07	1.57 $\pm$ 0.06	XX	33	1.58 $\pm$ 0.06	1.58 $\pm$ 0.06
p^*			0.502	0.503			0.422	0.422

Data presented are mean $\pm$ SD

BMI body mass index, a weight-to-height ratio, calculated by dividing one's weight in kilograms by the square of one's height in meters

* $P \leq 0.05$ - statistical differences between each genotype group (ACE or ACTN3) in pre-training and post-training evaluation (T1 and T2)

Table 2 Effect of ACE genotype in older Caucasian women in test performance gains during training

Performance measures	ACE genotype ($n=139$)	Moments		Repeated measures 2-way ANOVA	
		T1 $\bar{x} \pm \sigma$	T2 $\bar{x} \pm \sigma$	Training effect (within subjects) P	Genotype effect (between subjects) P
1RM _{BP} (kg)	ID ($n=52$)	17.9 $\pm$ 8.2	31.4 $\pm$ 11.7 ^a	0.000	0.019
	DD ($n=52$)	16.7 $\pm$ 7.2	33.0 $\pm$ 8.7 ^b		
	II ($n=35$)	17.1 $\pm$ 8.2	24.1 $\pm$ 6.4		
P^*		0.631	0.000		
1RM _{LE} (kg)	ID	19.3 $\pm$ 6.9	30.8 $\pm$ 7.5	0.000	0.187
	DD	17.8 $\pm$ 5.6	32.2 $\pm$ 6.3		
	II	18.7 $\pm$ 8.1	26.8 $\pm$ 7.5		
P^*		0.559	0.003		
CMJ (cm)	ID	13.5 $\pm$ 1.4	14.6 $\pm$ 3.0 ^a	0.002	0.052
	DD	10.8 $\pm$ 3.0	15.9 $\pm$ 3.0		
	II	10.6 $\pm$ 2.0	12.1 $\pm$ 2.0		
P^*		0.236	0.000		
STS (n.° reps)	ID	25.3 $\pm$ 5.6	30.8 $\pm$ 4.9 ^a	0.000	0.013
	DD	24.4 $\pm$ 5.6	31.1 $\pm$ 4.4 ^b		
	II	23.2 $\pm$ 5.9	26.9 $\pm$ 5.1		
P^*		0.284	0.000		

Values are mean $\pm$ SD. Pre-training and post-training, before (T1) and after (T2) 12 weeks of high-speed power training, respectively. All reported values were measured at maximum effort. P values ($P \leq 0.05$) were computed by one-way and two-way ANOVA

n number of subjects

^a $P < 0.05$, ID significantly different from II

^b $P < 0.05$, DD significantly different from II

* $P \leq 0.05$ - statistical differences between each genotype group (ACE or ACTN3) in pre-training and post-training evaluation (T1 and T2)

Table 3 Effect of ACTN3 genotype in older Caucasian women in test performance gains during training

Performance measures	ACTN3 genotype (<i>n</i> =139)	Moments		Repeated Measures 2-way ANOVA	
		T1 $\bar{x} \pm \sigma$	T2 $\bar{x} \pm \sigma$	Training effect (within subjects) <i>P</i>	Genotype effect (between subjects) <i>P</i>
1RM _{BP} (kg)	RX (<i>n</i> =54)	16.6±6.9	29.4±7.7 ^a	0.000	0.000
	RR (<i>n</i> =52)	18.9±8.5	34.8±11.6 ^b		
	XX (<i>n</i> =33)	15.3±7.5	23.9±6.9		
	<i>P</i> *	0.086	0.000		
1RM _{LE} (kg)	RX	18.2±7.5	29.8±7.6	0.000	0.011
	RR	19.7±5.8	33.1±6.4 ^b		
	XX	17.5±6.8	26.9±7.0		
	<i>P</i> *	0.322	0.001		
CMJ (cm)	RX	13.6±1.4	13.6±3.0 ^a	0.002	0.050
	RR	10.4±3.0	16.4±2.0		
	XX	10.8±2.0	11.7±1.0		
	<i>P</i> *	0.182	0.000		
STS (n.° reps)	RX	24.6±24.6	24.6±5.4	0.000	0.033
	RR	25.0±5.6	31.3±4.7 ^b		
	XX	23.2±5.2	27.4±4.1		
	<i>P</i> *	0.357	0.003		

Values are mean ± SD. Pre-training and post-training, before (T1) and after (T2) 12 weeks of high-speed power training, respectively. All reported values were measured at maximum effort. *P* values ($P \leq 0.05$) were computed by one-way and two-way ANOVA

n number of subjects

^a $P < 0.05$, RX significantly different from XX

^b $P < 0.05$, RR significantly different from XX

* $P \leq 0.05$ - statistical differences between each genotype group (ACE or ACTN3) in pre-training and post-training evaluation (T1 and T2)

Fig. 1 Bilateral 1RM bench press performance at the beginning of the protocol (T1) and after 12 weeks (T2). Data presented are mean ± SD. *Significantly different ($P \leq 0.05$) from T1–T2 weeks; †significant changes ($P \leq 0.05$) between the groups

Fig. 2 Bilateral 1RM leg extension performance at the beginning of the protocol (T1) and after 12 weeks (T2). Data presented are mean ± SD. *Significantly different ($P \leq 0.05$) from T1–T2 weeks; †significant changes ($P \leq 0.05$) between the groups

Fig. 3 Vertical jump (CMJ) performance at the beginning of the protocol (T1) and after 12 weeks (T2). Data presented are mean $\pm$ SD. *Significantly different ($P \leq 0.05$) from T1–T2 weeks; †significant changes ($P \leq 0.05$) between the groups

Dynamic strength and power, rather than isometric strength, are critical aspects of an optimal muscle function achievement in older age. This physical component has been defined as clinically relevant for risk of falls prevention and loss of independence in the community-active older population (Reid and Fielding 2012; Pereira et al. 2012). As such, identifying genetic variations in

Fig. 4 Sit-to-stand test performance at the beginning of the protocol (T1) and after 12 weeks (T2). Data presented are mean $\pm$ SD. *Significantly different ($P \leq 0.05$) from T1–T2 weeks; †significant changes ($P \leq 0.05$) between the groups

response to high-speed power training that influence elderly dynamic strength and power, and functional capacity declines, in comparison with previous reports that used single-joint movements tests for muscle power assessment (e.g., leg extension) (Marsh et al. 2009).

The results of this investigation concerning the ACE I/D genotype indicate a positive association with skeletal muscle strength and resistance training in older women after 12 weeks of high-speed power training, with the DD genotype showing greater improvements in performance than those observed in the ID or II genotypes.

As previous studies have hypothesized, the ACE DD genotype should be associated with a greater proportion of fast-twitch fibers (Zang et al. 2003) and consequently with optimal muscle function, particularly in fast strength at high velocity (McCauley et al. 2010). According to this, our data showed that individuals with genotype II had the lowest mean performances on dynamic strength and explosive tests both before and after training. Controversially, the DD and ID genotype showed an advantage in both power and functional tests (STS and CMJ, respectively), corresponding to the time course of the twitch response thought to reflect fiber-type composition only in young and non-athletic adults (Rodriguez-Romo et al. 2010). It may be that the association of the ACE genotype in lower limbs is only apparent in the extreme phenotypes of elite athletes and might indicate an interaction with long-term training in older people.

The likely mechanism through which the ACE genotype affects skeletal muscle is in the production of angiotensin II. ACE catalyzes the production of angiotensin II which mediates the hypertrophic response of overloaded cardiac muscle, likely via the AT-1 receptor (Costa et al. 2009). This has been found to promote skeletal muscle hypertrophy, particularly important for the hypertrophy of slow-twitch fibers (Gordon et al. 2001). Zhang et al. (2003) support this notion, reporting that the ACE D-allele may influence skeletal muscle function, particularly strength/power at high velocities. Conversely, Ahmetov and Rogozkin (2009) found that ACE D-allele carriers have a higher proportion of slow-twitch fibers, and it is well recognized that about 45 % of the fiber type proportions are determined by genetic factors (Vincent et al. 2007). These results may suggest that genetic factors that interact with aging and thus modulate functional capacity and skeletal muscle phenotypes in older people may depend on the level of physical activity (Costa et al. 2009). It is also likely that

along with effects resulting from complex interactions between genes and type of exercise stimuli, the ACE DD genotype did not influence maximal leg extension strength. Post hoc analysis by Tukey's honestly significant difference test doesn't showed significant differences between ACE I/D genotypes. To perform everyday activities, leg muscles are frequently used, and possibly in older women, this would not be a determinant in the expression of significant genotype differences. Also, since this is directly associated with lower extremity performance and a common weakness in older adults, it will likely lead to further inactivity and deterioration of functional status (Marsh et al. 2009) with increasing age. Within the context of previous studies, the present investigation suggests that the ACE genotype plays a minor role in maximal strength in lower limbs and its adaptation to resistance training, but potentially only in certain subgroups within the population.

Significant interactions of genotype-training across all measures of muscle performance were observed in relation to the ACTN3 R577X polymorphism. These results may provide weak support for a potential influence on the decline of physical function in older people. In the present study, at baseline, the homozygous women for the ACTN3 mutant allele 577X (XX) had lower dynamic strength and functional capacity compared to the other genotypes. In the latter case, women homozygous for the wild type (RR) demonstrated greater 1RM_{LE} and 1RM_{BP} gains, jump ability, and functional capacity in STS test compared with the homozygous XX after 12 weeks of high-speed power training. Indeed, because gains were greatest for RR and least for RX or XX polymorphisms, a trend for a dose response in the R577X genotype was observed in the present study. These findings are consistent with previous reports indicating that alpha-actinin-3 deficiency appears to impair muscle performance (in different population studies) (Clarkson et al. 2005; Scott et al. 2010; Judson et al. 2011). Recent research (Seto et al. 2011) concluded that genotype differences in fast muscle force production result in fast-twitch fibers developing slower properties, suggesting that the lack of alpha-actinin-3 may cause faster decline in muscle function with increasing age. The loss of type II muscle fibers may be particularly important concerning the influence of the ACTN3 R577X in older persons, as ACTN3 is mainly expressed in this fiber type. However, it also seems that the selective atrophy of type II

muscle fibers with age could attenuate any influence of the ACTN3 genotype on whole body muscle function (Clarkson et al. 2005). Moreover, subjects without ACTN3 seem better able to adapt to a stressful condition (Clarkson et al. 2005), but they are unable to develop power to the same extent as persons with the 577R allele. Yu et al. (2003) proposed that strain-dependent processes acting on the Z disk during forceful contractions may lead to a release of α -actinin and other cytoskeletal proteins and allow the α -actinin to initiate formation of additional sarcomeres elsewhere along the myofibril. These results may suggest that the present intervention program based on high-speed execution was essential to focus on the training that induced particular adaptations between the subjects of different genotypes. A large-scale study found no relationship between the ACTN3 genotype and muscle size, strength, power, mobility, or walking performance in 70–79-year-old men and women (Delmonico et al. 2008). However, after 5 years, the decline in 400-m walking performance was significantly greater in XX genotype subjects.

A unique finding of the present study was that the ACE I/D and ACTN3 R577X polymorphisms might exert a combined influence on dynamic muscle strength, power, and functional phenotypes in older Caucasian women. The actual differences between homozygote genotype polymorphisms may indicate a significant influence on human muscle function. Indeed, ACE II + ID & ACTN3 XX versus ACE DD & ACTN3 RR + RX showed significant differences ($p < 0.05$) in all muscular phenotypes evaluated after 12 weeks of high-speed power training. However, we did not observe a difference among genotypes in baseline ($p > 0.05$) that might indicate an interaction only with long-term training. Based on previous studies (Folland et al. 2000; Pescatello et al. 2006), D-allele of the ACE gene would not be associated with baseline measures of skeletal muscle strength, and this hypothesis was partially supported. In contrast, Williams et al. (2005) reported a significant association between the ACE genotype and pre-training differences, although Thomis et al. (1998) suggested that this expression is improved by training-induced adaptations. These authors noted that the genetic factors that served to explain part of the variance in increased 1RM strength in response to resistance training differed from those that explained variation in pre-training phenotypes. Besides,

the lower average levels of testosterone in older women may cause a variation in ACE I/D and ACTN3 R557X genotypes and other parameters fundamental in determining muscular performance (MacArthur and North 2004).

Nevertheless, our study has a potential limitation. We were unfortunately not able to measure the activity of the ACE gene product (i.e., ACE) or circulating levels of the muscle growth factor angiotensin II. Moreover, in future studies, it would be interesting to assess muscle damage during resistant strength training because the lack of alpha actin (as occurs in the null homozygote) may impair the stability of sarcomeres, making them more susceptible to damage.

In summary, our results suggest that both the ACE I/D and ACTN3 R577X polymorphisms (alone or in combination) are strong potential factors in modulating some exercise-related phenotypes induced by training-induced responses that affects muscle adaptation to resistance training. Indeed, the ACE I/D and ACTN3 R/X polymorphisms influenced muscle performance phenotypes including maximal strength, power, and functional capacity in response to high-speed power training in older Caucasian women after a high-speed power training program. These genotypes may be important for targeting individuals who may be more susceptible to the decrease of strength and function with aging and may need specific interventions.

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