ACE and ACTN3 Genes and Muscle Phenotypes in Nonagenarians

Abstract

We studied the association of ACE and ACTN3 polymorphisms with skeletal muscle phenotypes (i.e. upper and lower body muscular strength and functional tests) in Spanish nonagenarian subjects [n=41, 33 women, 8 men, age: 90–97 years]. Mean values of the study phenotypes were not significantly different (all P>0.05) between ACE and ACTN3 genotypes. The analyses of the combined effects between genotypes (ACE DD & ACTN3 RR/RX vs. ACE II/ID & ACTN3 XX) did not yield any significant difference. Our data suggest that, in the elderly, the influence of genetic factors on muscle phenotype traits is not reducible to a few single polymorphisms, including ACE and ACTN3 variants.

Introduction

In western societies people are living longer. This raises the importance of understanding how ageing, and the interaction of ageing with lifestyle and genetic factors affects functional capacity, especially skeletal muscle phenotypes. Indeed, in elderly people, functional capacity is directly dependent on muscular fitness as these persons experience age-associated declines in skeletal muscle mass and strength, i.e. sarcopenia [24]. Sarcopenia contributes to the decreased capacity for independent living and reduced ability to cope with activities of daily life and thus increases the burden for the caregiver and community [20]. Although the genetic factors that interact with the ageing process to influence muscle phenotypes are yet to be elucidated, two genetic variations that are candidates to explain individual variability in muscle phenotypes over most of the lifespan are the 287 bp Ins/Del (I/D) polymorphism of the angiotensin converting enzyme (ACE) gene, and (ii) the Arg577Ter (R577X) polymorphism of the actinin, alpha 3 (ACTN3) gene. The ACE gene is expressed in skeletal muscle, where it may influence its function and biomechanical properties [13,26]. The ACE D allele is associated with higher circulating levels of angiotensin II, which, besides regulating blood pressure, also acts as a skeletal-muscle growth factor [10,13]. As such, this allele would theoretically favour performance in more power or strength-oriented sports and exercise tasks. Indeed, the D allele has been associated with elite “sprint” athletic performance [17,28] and power-related phenotypes in non-athletic populations, e.g. preserved quadriceps muscle strength in chronic patients [12], lower risk of skeletal muscle damage induced by eccentric contractions [29], or greater gains in knee extensor strength after training in old (≥60 years) individuals [8].

The ACTN3 gene encodes for the synthesis of α-actinin-3 in skeletal-muscle fibres, a protein necessary for producing fast, powerful contractions [25]. A premature stop codon polymorphism (R577X) in ACTN3 was first described by North et al. [18] 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, jumping events), especially in women [30]. More discrepancy exists on the putative role of the ACTN3 R577X polymorphism on muscle power phenotypes in non-athletic population, with some authors reporting an unfavourable effect of the XX genotype [25], at least in women [27], and others reporting no effect [15,23]. 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 [5], while others found no genotype:phenotype association [22]. It was the purpose of this study to assess the association of ACE and ACTN3 polymorphisms with muscular strength and functional tests in Spanish nonagenarians. Based on previous research [8,29], we hypothesized that the D allele might be associated with greater strength in nonagenarians. Given the discrepancy of results concerning the ACTN3 R577X variation, especially in old people [5,22], we hypothesized that this polymorphism does not significantly influence muscle traits in nonagenarians.

Methods

Subjects

A total of 41 subjects [33 women, 8 men; mean (SD) age: 92 (2) years, range: 90–97] entered the study. All were recruited from a geriatric home (Los Nogales-Pacifico, Madrid, Spain) and received a comprehensive medical examination. Inclusion criteria were: age ≥ 90 years, able to ambulate, with or without assistance, able to communicate and being capable and willing to provide consent. Exclusion criteria were: acute or terminal illness, myocardial infarction in the past 3 months, unstable cardiovascular disease, upper or lower extremity fracture in the past 3 months, severe dementia and presence of neuromuscular disease or drugs affecting neuromuscular function.

The Medical Ethics Committee of the Hospital General Universitario Gregorio Marañón (Madrid, Spain) approved the study design, study protocols and informed consent procedure. All participants provided written informed consent. Our study was performed according to ethical standards in sport and exercise science research [11].

Assessment of muscular strength

We evaluated subjects’ upper body strength with the handgrip strength test using a digital dynamometer (T.K. K. 5101 Grip-D; Takey, Tokyo, Japan) [21]. We assessed dynamic muscular strength of the lower body following a standardized strength testing protocol, i.e. 6–7 repetition maximum (6–7RM) seated leg press (Technogym, Barcelona, Spain). The 1RM was estimated using the equation reported by Brzycki [4]: 1RM = 102.78–2.78×number of repetitions. Initial loads were 70–100% of body weight. Following a brief rest period, increments of 2–4 kg were added until maximal effort was achieved for each lift, usually after 5 trials or less. All participants were able to lift the initial load at least one time. They were instructed on proper breathing and lifting form for each movement.

Functional tests (ambulation ability)

Participants also performed the (i) 8-meter walk test and (ii) 4-step (20 cm height each) stairs test [20], both of which have proven (i) useful to determine leg muscle functional capacity in the elderly [1] and (ii) highly reliable [7]. We measured time (in sec) to walk 8 meters and to climb up and down 4 steps (using hand railing to diminish fall risk) respectively, at usual pace. Performance time was measured by the same experienced investigator with the same stopwatch to the nearest 0.1 s. All participants underwent a familiarization period with all the tests, consisting of three ~30 min sessions, and we assessed test-retest reliability of all phenotype evaluations [20].

Genotyping

During winter 2009, we extracted genomic DNA from saliva samples of the participants and performed genotype analyses in the genetics laboratory of the Universidad Europea de Madrid (Spain). We followed the ACE I/D (rs1799752) and ACTN3 R577X (rs1815739) genotyping methods that have been described in detail elsewhere [9]. 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’-TGGAGACCACTCCCCATCTTTTC and 5’-GATGTTGCCATCACACATCTCAGAT.

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 [14]: 5’TGGAGACCAACGCCGGCCCATAC and 5’TGGCAGCCTTCTCCTATCCATA.

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 a 1.5% agarose gel stained with ethidium bromide.

For the ACTN3 R577X polymorphism, a fragment of 291 bp was amplified with the following primers: ACTN3-F 5’-CTGTTGCTCTGTGTTAAGTGGG and labelled a 5’ with VIC and ACTN3-R 5’-TGCTCACAGTATGCAGGACC and labelled with FAM. 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 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.

Data analysis

We analysed the differences in the study phenotypes among variants of the ACE and the ACTN3 R577X polymorphisms by one-way analysis of covariance (ANCOVA), after adjusting for sex and age (there was no sex × genotype interaction, P > 0.2). Since the frequency of the ACE II genotypes was only of 3, we grouped II and ID genotypes (total n = 26). Following a recent approach [9], we also analysed the combined effect of ACE and ACTN3 R577X polymorphisms by ANCOVA, so that the ACTN3 RR + RX & ACE DD group represents the ‘power/hypertrophy-oriented’ genotype and the ACTN3 XX & ACE II + ID group represents the ‘non-power’ genotype. We also compared the ‘power group’ (ACTN3 RR + RX & ACE DD) against the rest of the study participants using ANCOVA.

All the analyses were performed with the SPSS, v. 16.0 (Inc, Chicago), and all the levels of significance was set at α ≤ 0.05.
Results

Genotype success was 100%, and genotypes were in Hardy-Weinberg equilibrium \( (P > 0.2) \). The ICC for test-retest assessment were \( \geq 0.91 \) \( (P < 0.01) \) in all phenotype evaluations. Mean values of the study phenotypes were not significantly different \( (all P > 0.05) \) between genotypes \( (\text{●} \rightarrow \text{Table 1}) \). Similar results were obtained in the \text{ACTN3} R577X polymorphism when analysing the dominant model \( (\text{●} \rightarrow \text{Table 1}) \). The analyses of the combined effects between genotypes did not yield any significant difference, i.e. no significant difference between the two extreme genotypes \{‘power’ (\text{ACTN3} RR + RX & \text{ACE} DD)\} vs. non-power \{\text{ACTN3} XX & \text{ACE} II + ID\} \( (\text{Fig. 1}) \), nor when comparing the ‘power group’ \((n = 9)\) vs. the rest of study participants \((n = 32)\): (Handgrip strength: 24.0 ± 1.5 vs. 21.4 ± 2.6 kg, respectively; Leg press: 65.9 ± 7.6 vs. 53.4 ± 4.4 kg, respectively; 8-meter walk test: 17.9 ± 4.4 vs. 26.3 ± 2.6 sec; Step test: 11.7 ± 2.8 vs. 13.2 ± 1.7 sec; all \( P > 0.1 \)). The statistical power for all cases ranged from 0.3–0.6.

Discussion

Our data suggest that, overall, \text{ACE} and \text{ACTN3} do not have a major influence on muscle phenotypes in nonagenarians. Though \text{ACTN3} is the first structural skeletal-muscle gene for which a genotype-elite sports performance phenotype association has been clearly demonstrated, especially in women [30], controversy exists on the putative role of the \text{ACTN3} R577X polymorphism on muscle phenotypes in the non-athletic population, especially in older people. We previously observed no association between \text{ACTN3} genotypes and functional capacity and muscle phenotypes \{included 1RM leg press\} in women aged 61–80 years [22], whereas others found (i) that in adults with a mean age ~ 65 years, the XX genotype was associated with higher knee extensor concentric peak power compared with RR/RX genotypes, especially in women [5], and (ii) in women (aged ~60 years on average) the XX genotype is associated with lower peak torque in knee extensor muscles [27]. Differences in age, baseline physical capacity and ethnic origin make comparison between studies difficult. In any case, it must be emphasised that the potential effect of the \text{ACTN3} R577X polymorphism in muscle phenotypes across most of the human population is minor.
lifespan, which appears to be more marked in women, seems to disappear in those who become nonagenarians. One possible explanation may be the fact that age-induced sarcopenia predominantly affects those muscle fibres in which α-actinin-3 is expressed, i.e. fast fibres [6].

The ACE gene is by far the most extensively studied gene with regard to human exercise capacity [2], yet controversy exists and its role in the muscle fitness of the elderly has not been clearly established. Though controversy exists [16, 19], the ACE D allele has been associated with power-related phenotypes in non-athletic populations, e.g. preserved quadriceps muscle strength in patients with chronic obstructive pulmonary disease [12], lower risk of skeletal muscle damage induced by eccentric contractions [29], or greater gains in knee extensor strength [12], lower risk of skeletal muscle damage induced by eccentric exercise capacity [2], yet controversy exists [16, 19], the ACE D allele has been associated with power-related phenotypes in non-athletic populations, e.g. preserved quadriceps muscle strength in patients with chronic obstructive pulmonary disease [12], lower risk of skeletal muscle damage induced by eccentric contractions [29], or greater gains in knee extensor strength [12], lower risk of skeletal muscle damage induced by eccentric exercise capacity [2], yet controversy exists [16, 19], the ACE D allele has been associated with power-related phenotypes in non-athletic populations, e.g. preserved quadriceps muscle strength in patients with chronic obstructive pulmonary disease [12], lower risk of skeletal muscle damage induced by eccentric contractions [29], or greater gains in knee extensor strength [12], lower risk of skeletal muscle damage induced by eccentric contractions [29], or greater gains in knee extensor strength [12], lower risk of skeletal muscle damage induced by eccentric contractions [29], or greater gains in knee extensor strength [12], lower risk of skeletal muscle damage induced by eccentric contractions [29], or greater gains in knee extensor strength [12], lower risk of skeletal muscle damage induced by eccentric contractions [29], or greater gains in knee extensor strength [12], lower risk of skeletal muscle damage induced by eccentric contractions [29], or greater gains in knee extensor strength [12], lower risk of skeletal muscle damage induced by eccentric contractions [29], or greater gains in knee extensor strength [12], lower risk of skeletal muscle damage induced by eccentric contractions [29], or greater gains in knee extensor strength.

The Western societies are ageing and thus, sarcopenia, i.e. the loss of muscle mass and function that is commonly associated with ageing [3], is becoming a growing health problem. To identify those lifestyle and genetic factors that influence muscle fitness at the end of the human lifespan, and that could be associated with more severe sarcopenia, is of clinical and public health interest. Though they are strong candidates to modulate exercise-related phenotypes in adults, ACE I/D and ACTN3 R577X polymorphisms do not seem to exert a major influence in the muscle phenotypes of the oldest humans. However, it should be noted that our results should be taken as preliminary due to the relatively small sample size and limited statistical power.

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