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# ACTN-3 and ACE genotypes in elite male Italian athletes

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Abstract: The ACE I/D and the ACTN-3 R577X polymorphisms are the most studied genes associated with elite athlete status, even if this association has been often conflicting. The aim of the present study was to investigate the association between the ACE and the ACTN3 genotypes and elite performance in Italian male athletes. The ACTN-3 R577X and the ACE I/D genotype distributions of 59 elite male Italian athletes practicing gymnastics (G; n = 17), 100 m–400 m running (R; n = 12), and playing soccer (S; n= 30) were compared with controls from Italian (C; n = 31) populations. For ACE distribution, athletes did not differ from controls (G, $\chi^2 = 0.37$, df = 2, p = 0.82; R, $\chi^2 = 1.90$, df = 2, p = 0.45; S, $\chi^2 = 1.48$, df = 2, p = 0.47) and the DD genotype was at very high frequency in all groups (G = 53%, R= 50%, S = 60%, C = 45%). For ACTN-3 distribution, elite gymnasts showed a significant difference from controls ($\chi 2 = 6.57$, df = 2, p = 0.03), showing an absence of XX genotype. Soccer players and runners did not differ from controls in ACTN-3 genotype distribution (R, $\chi^2 = 0.43$, df = 2, p = 0.80; S, $\chi^2 = 1.25$, df = 2, p = 0.53). Even if the ACE DD genotype is often positively associated with elite sprint/power athlete status, its high frequency in Italian populations eliminates the possibility of its exclusive association in Italian athletes. The results of ACTN3 genotypes suggest that RR genotype of ACTN-3 gene is a determinant of elite gymnasts status but it is not the key factor for achieving a top-level performance in soccer or track events.

Key words: genetics, Italians, athletes, soccer, gymnastics, ACE I/D, ACTN-3 R/X, polymorphisms

Being a sports champion is a very complex attribute that results from the combined influences of hundreds of genetic polymorphisms (Ruiz et al. 2009; Santiago et al. 2009). Early family studies indicated that genetic factors may also contribute to the inter-individual differences in athletic performance (Bouchard et al. 1986), and the recent develop of technology for rapid DNA sequencing and genotyping has allowed the identification of 214 autosomal genes associated with elite athletic performance (Bray et al. 2009). Some authors have hypothesized that the "optimum" polygenic profile difference between power and endurance-oriented athletes is due to the different phenotype traits that determine performance in both types of athletes (Ruiz et al. 2009). Several genetic markers are candidates

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for the source of the high performances and muscle strength and, among them, the most studied are the ACE and the ACTN-3 polymorphisms. ACE is a key component in the renin-angiotensin system (RAS), generating simultaneously a potent vasoconstrictor (Ang II) and inactivating a potent vasodilator (BK) (Coates et al. 2003). Variants in the angiotensin-converting enzyme (ACE) gene have been associated with elite athletic performance and it can influence muscle mass and strength (Jones and Woods 2003; Jones et al. 2006). Generally, the D allele is associated with power phenotypes (Myerson et al. 1999; Nazarov et al. 2001; Woods et al. 2001), and the I allele with endurance performance (Gayagay et al. 1998; Myerson et al. 1999; Alvarez et al. 2001; Nazarov et al. 2001; Scanavini et al. 2001; Collins et al. 2004) in Caucasian populations, although findings have been equivocal (Taylor et al. 1999; Rankinen et al. 2000; Massidda et al. 2011). ACE activities are consistently higher in the DD subjects, intermediate in the ID and lower in the II subjects. An excess of II subjects was found in elite high altitude climbers (Montgomery et al. 1998), endurance swimming (Tsianos et al. 2004) and rowing (Gaygay et al. 1998), as well as among the British (Myerson et al. 1999), Spanish (Alvarez et al. 2000), Russian (Nazarov et al. 2001), Italian (Scanavini et al. 2002), elite long distance runners, and South African triathletes (Collins et al. 2004). Conversely, a high frequency of the D allele has been found in short distance runners (Myerson et al. 1999) and swimmers (Nazarov et al. 2001; Woods et al. 2001; Tsianos et al. 2004), although not all reports support these findings (Karjalainen et al. 1999; Taylor et al. 1999; Rankinen et al. 2000; Massidda et al. 2011). In exon 16 of the human a-actinin-3 (ACTN3) gene, some authors (North et al. 1999) identified a C-to-T transition at nucleotide 1747, which results in a stop codon (X) replacing the arginine (R) at aminoacid 577 (R577X). The single-nucleotide polymorphism R577X, associated with a complete absence of alpha-actin–3 (*a*-Atn3) protein (North et al. 1999), has been associated with physical performance in humans. The 577XX genotype occurs at significantly lower frequencies in elite sprint athletes than the general populations (Yang et al. 2003; Niemi et al. 2005; Massidda et al. 2009), and it has been associated with a decrease in muscular strength and sprinting performance among non-athletes (Clarkson et al. 2005; Vincent et al. 2007). Results from these studies suggest that absence of the *a*-Atn3 protein could have a negative effect on fast skeletal muscle fiber functions. In contrast, some authors have identified cases of high level sprint/power-oriented Olympic athlete carriers of XX genotype (Lucia et al. 2007; Druzhevskaya et al. 2008). Other authors reported an overrepresentation of the 577XX genotype in elite endurance athletes (Yang et al. 2003), suggesting that the absence of *a*-Atn3 protein could also provide an advantage for endurance activity.

The aim of the present study was to investigate the association between the ACE and the ACTN3 genotypes and elite male Italian athletes from various sports.

## Materials and methods

The ACTN3 R577X and the ACE I/D genotype distributions of 59 elite male Italian athletes practicing gymnastics (G;n = 17), 100 m–400 m running

(R;n = 12), and athletes playing soccer (S;n = 30) were compared with controls from Italian (C;n = 31) populations. All the subjects were Italian descent for ≥ 3 generations and all examined athletes had reached National and International level of competition. All participants provided informed written consent and the study protocol was approved by a Medical Ethics Committee of Cagliari University. DNA was extracted from each participant using a buccal swab.

I/D polymorphism of ACE gene was amplified through PCR. The primers used to determine the ACE I/D polymorphism were the follows:

– Forward 5'-CTGGAGAC-CACTCCCATCCTTTCT–3',
– Reverse 5'-GATGTGGCCATCACAT-TCGTCAGAT–3'.

The amplified ACE gene fragments were detected on a 2% agarose gel stained with ethidium bromide.

The banding pattern of the 3 possible genotypes was as follows: DD, 210 bp fragment; II, 498 and 264 bp fragments; ID, 498, 264, 210 bp fragments.

Exon 16 of ACTN3 was amplified through polymerase chain reaction (PCR) using the following primers:

– Forward 5' – CTGTTGCCTGTGG-TAAGTGGG-3',
– Reverse 5' – TGGTCACAGTATG-CAGGAGGG-3'.

PCR products were digested with DdeI enzyme. The 577R and 577X allele (CGA and TGA codons, respectively) were distinguished by the presence (577X) or absence (577R) of a DdeI restriction site in exon 16. Allele 577X shows two fragments (205 and 85 bp), while allele 577R presents three fragments (108, 97, and 86 bp). The obtained fragments were separated by 10% polyacrilamide gel electrophoresis and stained with ethidium bromide (Mills et al. 2001).

Allele frequencies were calculated with gene counting from observed genotype frequencies with GENEPOP v.4.0 software. Contingency chi-squared ($\chi^2$) tests were used to determine any differences in genotype distribution and allele frequency between groups.

## Results

Figures 1, 2, 3 and 4 show the ACE and ACTN-3 allele and genotype frequencies in athletes and controls, respectively.

For ACE, the DD genotype and D allele were found to be at very high frequency in all athletes and control cohorts (DD; D: G = 53%; 70%, R= 50%; 75%, S = 60%; 73%, C = 45%; 66%). Athletes did not differ from controls in ACE allele and genotype distributions (G, $\chi^2 = 0.37$, df = 2, p = 0.82; R, $\chi^2 = 1.90$, df = 2, p = 0.45; S, $\chi^2 = 1.48$, df = 2, p = 0.47).

For ACTN3,only elite gymnasts showed a significant difference from controls ($\chi^2 = 6.57$, df = 2, p = 0.03), displaying an absence of XX genotype and an excess of RR genotype (G=59%; C=32%) than those of controls. Soccer players and runners did not differ from controls in ACTN3 allele and genotype distribution (R, $\chi^2 = 0.43$, df = 2, p = 0.80; S, $\chi^2 = 1.25$, df = 2, p = 0.53).

## Discussion

Our results indicate that the ACE I/D polymorphism is not significantly associated with top-level performance in Italian male soccer players, gymnasts and short distance runners. As far as the ACTN-3 R577X polymorphism is concerned, we observed that genotype and allele distributions were similar between athletes

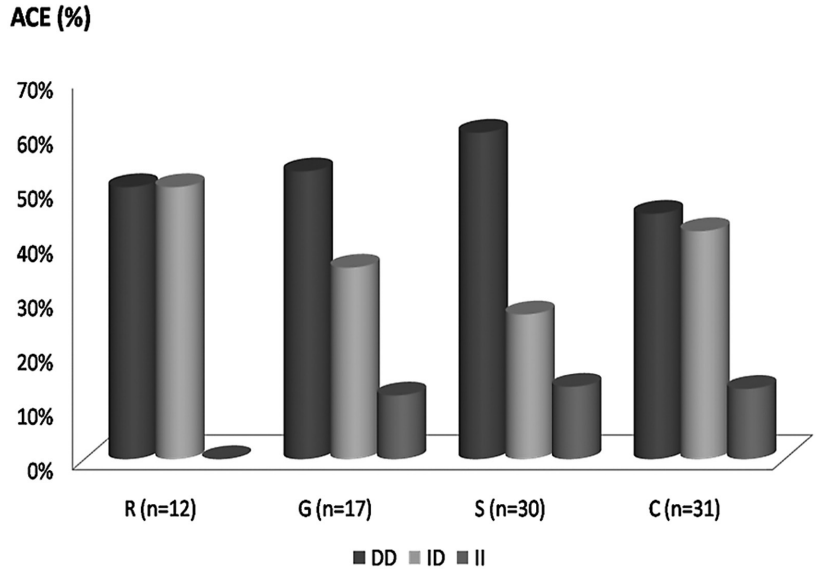


Fig. 1. ACE genotype frequencies (%) in athletes (R=Runners; G=Gymnasts; S=Soccer players) and controls (C)

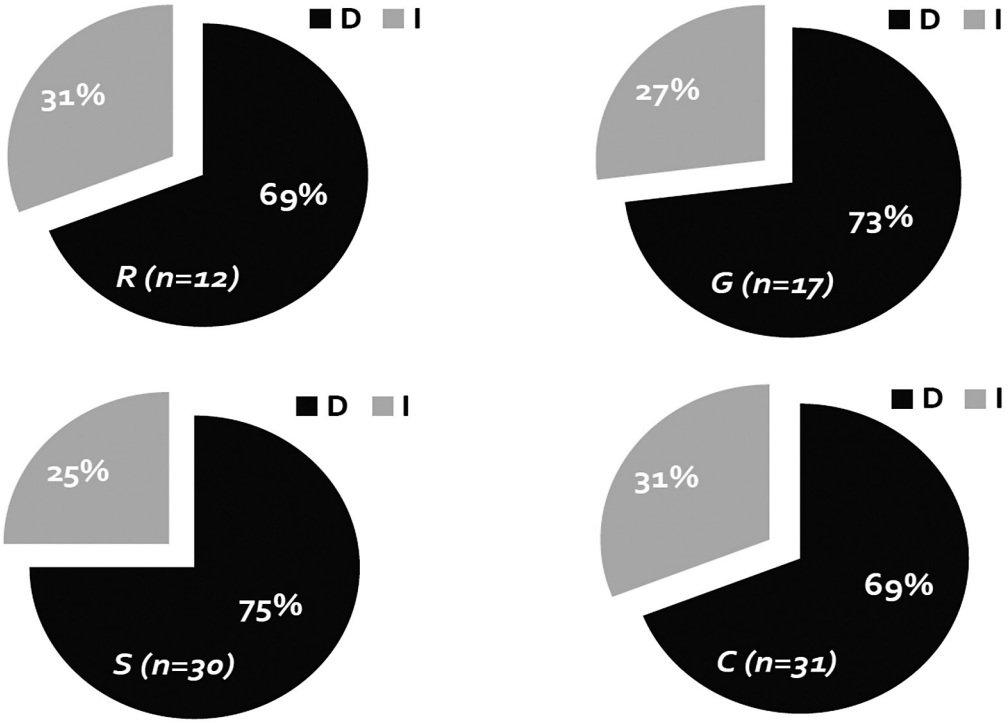


Fig. 2. ACE allele frequencies (%) in athletes (R=Runners; G=Gymnasts; S=Soccer players) and controls (C)

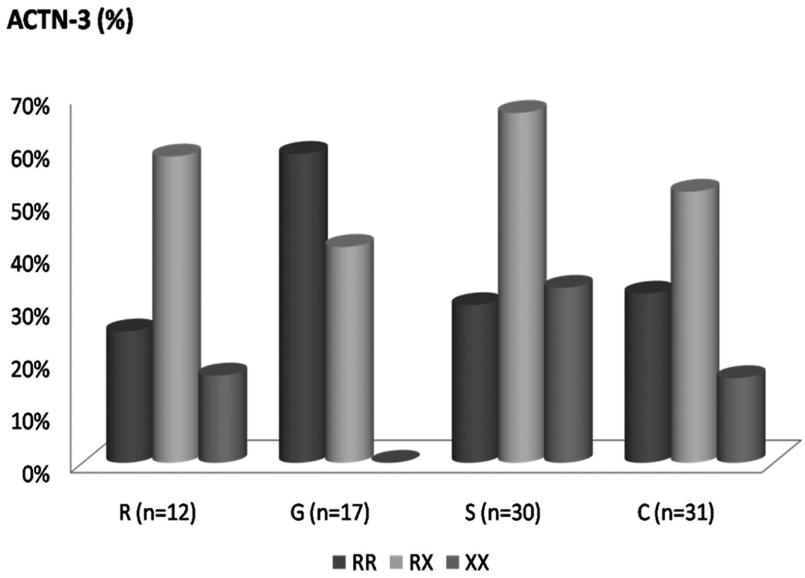


Fig. 3. ACTN-3 genotype frequencies (%) in athletes (R=Runners; G=Gymnasts; S=Soccer players) and controls (C)

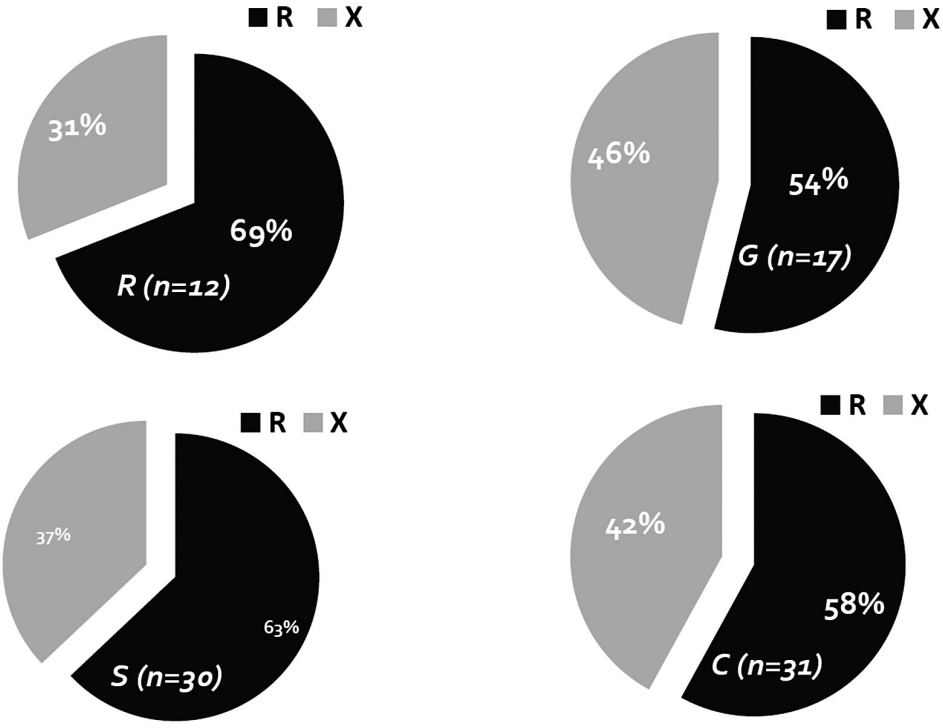


Fig. 4. ACTN-3 allele frequencies (%) in athletes (R=Runners; G=Gymnasts; S=Soccer players) and controls (C)

and controls with the exception of the elite gymnasts who showed a complete absence of XX genotype. These findings indicate that ACE I/D and ACTN3 R577X genotypes do not seem to influence the performance in sprint/power oriented sports such as short distance running and in mixed aerobic-anaerobic performance such as soccer. Nevertheless, the absence of the XX genotype in elite male Italian gymnasts does not exclude the possibility that the presence of *a*-Atn3 represents an advantage in a sport such as artistic gymnastics.

Our data are in agreement with those of other previous studies that found a lack of association between ACE I/D polymorphism and elite sprint/power oriented events (Scott et al. 2010; Massidda et al. 2011). Our data also accord with the equivocal recent finding on the association of ACTN3 R577X and elite sprint/power performance. In fact, despite some studies that showed no effect of the ACTN3 R577X polymorphism on muscular strength and power phenotypes and sprint/power performance (Hanson et al. 2010; Santiago et al. 2010; Ruiz et al. 2011) others reported an advantageous effect of RR genotype (Clarkson et al. 2005; Moran et al. 2007; Vincent et al. 2007; Druzhevskaya et al. 2008; Santiago et al. 2008).

For the ACE gene, the frequencies of our control group are in line with those of other Italian samples (Aucella et al. 2000; Scanavini et al. 2002) but different from other control groups of the Caucasian population (Myerson et al.1999; Nazarov et al. 2001; Woods et al. 2001; Tsianos et al. 2004), due to higher frequencies of the D allele and DD genotype in the Italian population. This fact is very important because it eliminates the possibility of replicating the association of ACE I/D polymorphism and elite sprint/power performance in Italian athletes population.

As for the ACTN-3 gene, it is interesting to underline that the *a*-Atn3 is a sarcomeric protein that is almost exclusively expressed in fast muscle fibers, where it constitutes one of the major components of the Z-disc, and seems crucial for producing fast and powerful muscle contractions. This protein also stabilizes the muscle contractile apparatus, which may confer a higher capacity for force absorption/transmission compared with the slow (type I) subtype (Mills et al. 2001). Muscle strength is a factor that strongly limits the performance in all of the sport disciplines considered in this study. An optimal development of muscle strength is particularly relevant in the acceleration phase of the 100 m sprint, as well as for elevating the body under the gravity force in artistic gymnastics, and in a lot of skills critical to soccer (for example kicking the ball, jumping, sprinting and changing pace) (De Proft et al. 1988; Reilly and Doran 1999). However, while muscle performance shows a multi factorial character, it is impossible to explain its variability with only a single polymorphism (Bustamante-Ara et al. 2010). Moreover, the same protein could be implicated in different muscle mechanisms, as reported in a recent study in which the new role of *a*-Atn3 in muscle metabolism emerges (Berman and North 2010). The authors conclude that *a*-Atn3 deficiency reduces the activity of glycogen phosphorylase and results in a fundamental shift toward more oxidative pathways of energy utilization.

Finally, our findings could indicate as the *a*-Atn3 may pose like a limiting factor for some sprint/power oriented performances such, for example, artistic gym-

nastics, but not for all sprint/power or mixed aerobic/anaerobic events.

## Conclusion

Given that the ACE DD genotype is positively associated with elite athlete status, its underlying high frequency in Italian populations eliminates the possibility of replicating this association in Italian male athletes. The results of ACTN3 genotypes suggest that RR genotype of ACTN3 gene is a determinant of elite gymnast status but it is not the key for achieving a top-level in soccer or in the 100–400 meters sprint run.

The identification of genetic predisposition of different athletes brings important information to trainers and coaches for individual training loads adjustment.

### Authors' contribution

MM ran statistical analysis, interpretation of results and writing of the manuscript; CL carried out laboratory analysis; SE sampling; VG revised critically the manuscript; CCM developed research design and study protocol.

### Conflicting interests

The authors declare that they have no conflicts of interest in the research.

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