**ACTN3 GENOTYPE IS ASSOCIATED WITH TESTOSTERONE LEVELS OF ATHLETES**

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**ABSTRACT:** α-Actinin-3 (ACTN3) has been proposed to regulate skeletal muscle differentiation and hypertrophy through its interaction with the signalling protein calcineurin. Since the inhibition of calcineurin potentiates the production of testosterone, we hypothesized that α-actinin-3 deficiency (predicted from the ACTN3 XX genotype) may influence serum levels of testosterone of athletes. Objective: To investigate the association of ACTN3 gene R577X polymorphism with resting testosterone levels in athletes. Methods: A total of 209 elite Russian athletes from different sports (119 males, 90 females) were genotyped for ACTN3 gene R577X polymorphism by real-time PCR. Resting testosterone was examined in serum of athletes using enzyme immunoassay. Results: The mean testosterone levels were significantly higher in both males and females with the ACTN3 R allele than in XX homozygotes (males: RR: 24.9 (5.7), RX: 21.8 (5.5), XX: 18.6 (4.9) ng·mL−1, P = 0.0071; females: RR: 1.43 (0.6), RX: 1.21 (0.71), XX: 0.79 (0.66) ng·mL−1, P = 0.0167). Conclusions: We found that the ACTN3 R allele was associated with high levels of testosterone in athletes, and this may explain, in part, the association between the ACTN3 RR genotype, skeletal muscle hypertrophy and power athlete status.

**KEY WORDS:** ACTN3, gene polymorphism, testosterone, calcineurin, muscle hypertrophy.

**INTRODUCTION**

α-Actins are important structural components of the Z-membrane, where they form crosslinks between the thin actin filaments and stabilize the muscle contractile apparatus [29]. Expression of α-actinin-3 (ACTN3) is limited to fast muscle fibres responsible for generating force at high velocity. A common genetic variation in the ACTN3 gene that results in the replacement of an arginine (R) with a stop codon at amino acid 577 (C-to-T transition in exon 16; rs1815739; R577X) had been identified. The X allele contains a sequence change that completely prevents the production of functional α-actinin-3 protein. α-Actinin-3 deficiency (the ACTN3 XX genotype) is common in humans (14-20%) and reduces strength, muscle mass, and fast-twitch fibre diameter, but increases the metabolic efficiency of skeletal muscle and the proportion of slow-twitch muscle fibres [2,16,17,22,31].

Several case-control studies have reported that ACTN3 RR genotype (or R allele) is over-represented or ACTN3 XX genotype is under-represented in strength/sprint athletes in comparison with controls [1,2,4,9,10,19,23,30]. The hypothesis that the ACTN3 R allele may confer some advantage in power performance events has been supported by several cross-sectional studies in non-athletes [3,7,8,12,18,27].

Sarcomeric α-actinins bind to the calsarcins [11], which interact with calcineurin, a signalling factor that plays a role in the specification of muscle fibre type and myocardium hypertrophy [5,6,24]. Recently, Henesy et al. [13] found that the inhibition of calcineurin potentiates atrial natriuretic peptide induced testosterone production. Interestingly, Pimenta et al. [20] found that immediately after eccentric training, soccer players with the ACTN3 RR and RX genotypes (n=28) presented higher levels of testosterone compared to XX homozygotes (n=9).

We therefore hypothesized that α-actinin-3 deficiency may influence resting levels of testosterone of athletes. The aim of the study was to investigate the association of ACTN3 gene polymorphism with testosterone levels in male and female athletes.

**MATERIALS AND METHODS**

The procedures followed in the study were conducted ethically according to the principles of the World Medical Association, the Declaration...
of Helsinki and ethical standards in sport and exercise science research. The Ethics Committee of the All-Russian Research Institute of Physical Culture and Sport approved the study and written informed consent was obtained from each participant.

In total, 209 elite Russian athletes (119 males, 90 females; age 22.2 ± 0.4 years) were recruited from the following sports: males: alpine skiing (n = 10), baseball (n = 21), cross-country skiing (n = 33), kayaking (n = 25), volleyball (n = 30); females: alpine skiing (n = 8), figure skating (n = 32), speed skating (n = 19), volleyball (n = 31). The athletes were all Caucasians.

Four ml of venous blood were collected in tubes containing EDTA. DNA extraction was performed using a Proba-GS kit according to the manufacturers’ instructions (DNA-Technology JSC, Russia). Genotyping for the ACTN3 gene R577X variant was performed by real-time PCR assays. Primers and probes were CACGATCAGTCAAGGCAACA (forward primer), CCCTGGATGCCCATGATG (reverse primer), CTGACCGAGAGCGA (probe for X allele), and AGGCTGAGTGDACTGAGAGC (probe for X allele). Allelic discrimination was performed by a DT-384 amplifier (DNA-Technology JSC, Russia, www.dna-technology.ru).

The thermal cycle protocol involved 90 s at 94°C, plus 10 s at 94°C and 20 s at 70°C for 10 cycles, plus 10 s at 94°C and 20 s at 64°C and 10 s at 72°C for 35 cycles.

Resting testosterone (i.e. testosterone which was measured on a day when the athlete did not train) was examined in serum of athletes. For these purposes, 10 ml of venous blood was collected the morning after an overnight fast and sleep into EDTA vacutainer tubes and placed at 4°C until processing. For each sporting discipline, blood was collected on the same day at least 15 h after the last training. Testosterone was analyzed on a Benchmark Plus Microplate Spectrophotometer (Bio-Rad, France) using enzyme immunoassay and commercial test systems (Alkor-Bio, Russia). The athletes were from the same team and trained under supervision of the same coach. None of them had overreaching symptoms.

Statistical analyses were conducted using GraphPad InStat software. Differences in testosterone levels between different ACTN3 genotype groups were analysed using ANOVA. The squared correlation coefficient R² was used as a measure of explained variance. For this purpose, Spearman’s correlation was used to assess the relationship (r) between the testosterone level and the ACTN3 genotypes (dummy coded as 1, 2 and 3 for XX, RX and RR, respectively). All values are means (SD). P values < 0.05 were considered statistically significant.

RESULTS

Genotype distributions of ACTN3 gene polymorphism in all athletes were in Hardy-Weinberg equilibrium (χ² = 0, P = 1.000). Genotype distribution amongst Russian athletes (RR – 38.7%, RX – 47.4%, XX – 14.4%, R allele frequency – 62.0%) was similar to that observed in other reported groups [14,23,28].

As expected, male athletes had significantly higher levels of testosterone than female athletes (P < 0.0001). The mean testosterone levels did not differ between athletes of different sporting disciplines of both genders (Table 1). Therefore, for the main analyses we used the combined data (i.e. combined groups of female and male athletes). The mean testosterone levels were significantly higher in both males and females with the ACTN3 R allele than in XX homoyzogotes (males: RR: 24.9 (5.7), RX: 21.8 (5.5), XX: 18.6 (4.9) ng · mL⁻¹, P = 0.0071; females: RR: 1.43 (0.6), RX: 1.21 (0.71), XX: 0.79 (0.66) ng · mL⁻¹, P = 0.0167). Accordingly, 12.5% and 14.8% of the variation in testosterone levels could be explained by the ACTN3 genotype in male and female athletes, respectively.

**TABLE 1.** THE MEAN SERUM TESTOSTERONE LEVELS (ng·mL⁻¹) IN FEMALE AND MALE ATHLETES WITH DIFFERENT ACTN3 GENOTYPES

<table>
<thead>
<tr>
<th>Sport</th>
<th>n</th>
<th>All</th>
<th>ACTN3 genotypes</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>RR</td>
<td>RX</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volleyball</td>
<td>31</td>
<td>1.17 (0.56)</td>
<td>1.21 (0.35)</td>
<td>1.09 (0.67)</td>
</tr>
<tr>
<td>Alpine skiing</td>
<td>8</td>
<td>1.16 (0.8)</td>
<td>1.66 (0.79)</td>
<td>0.73 (0.53)</td>
</tr>
<tr>
<td>Speed skating</td>
<td>19</td>
<td>1.33 (0.51)</td>
<td>1.73 (0.42)</td>
<td>1.29 (0.42)</td>
</tr>
<tr>
<td>Figure skating</td>
<td>32</td>
<td>1.27 (0.88)</td>
<td>1.46 (0.8)</td>
<td>1.34 (0.9)</td>
</tr>
<tr>
<td>All females</td>
<td>90</td>
<td>1.24 (0.7)</td>
<td>1.43 (0.6)</td>
<td>1.21 (0.71)</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseball</td>
<td>21</td>
<td>22.1 (4.2)</td>
<td>23.5 (4.8)</td>
<td>21.8 (3.9)</td>
</tr>
<tr>
<td>Volleyball</td>
<td>30</td>
<td>22.5 (5.6)</td>
<td>22.9 (5.4)</td>
<td>23.3 (5.7)</td>
</tr>
<tr>
<td>Alpine skiing</td>
<td>10</td>
<td>22.4 (4.1)</td>
<td>24.3 (3.2)</td>
<td>21.1 (4.4)</td>
</tr>
<tr>
<td>Kayaking</td>
<td>25</td>
<td>22.2 (5.5)</td>
<td>24.9 (5.5)</td>
<td>22.5 (6.2)</td>
</tr>
<tr>
<td>Cross-country skiing</td>
<td>33</td>
<td>22.7 (7.5)</td>
<td>27.6 (6.9)</td>
<td>20.1 (6.5)</td>
</tr>
<tr>
<td>All males</td>
<td>119</td>
<td>22.4 (5.8)</td>
<td>24.9 (5.7)</td>
<td>21.8 (5.5)</td>
</tr>
</tbody>
</table>

Note: Values are mean (SD). Comparisons between athletes with different genotypes were performed by ANOVA. *P < 0.05, statistically significant differences
This is the first study to demonstrate that the ACTN3 genotype is associated with resting testosterone levels of both male and female athletes. We therefore confirmed in some manner the results of the previous study by Pimenta et al. [20], where ACTN3 XX genotype was shown to be associated with low testosterone levels of soccer players immediately after eccentric training.

Testosterone has an anabolic effect and stimulates the growth of muscle by increase in muscle protein synthesis and inhibition of protein breakdown via a ubiquitin-mediated pathway [25]. Several studies have confirmed that testosterone increases muscle mass, strength and endurance [25]. The possible mechanism underlying the association of the ACTN3 R577X polymorphism with testosterone levels might be explained by the findings that sarcomeric α-actinins interact with calcineurin, and its inhibition potentiates testosterone production [13]. It can be speculated that the ACTN3 RR/RX genotypes, due to their anabolic effect, might be favourable for increasing skeletal muscle mass. Indeed, Walsh et al. [27] found that carriers of the ACTN3 R allele had higher levels of both total body and lower limb fat-free mass. In addition, Zempo et al. [31] observed larger thigh muscle cross-sectional area in women with RR/RX genotypes compared with XX homozygotes.

Since the ACTN3 R allele is associated with higher testosterone levels, increased muscle mass [27, 31] and increased proportion of fast-twitch muscle fibres [2,26], it may be favourable for power and strength sports. Accordingly, numerous studies have reported that the ACTN3 R allele is associated with power and strength athlete status. More specifically, Yang et al. [30] for the first time showed that the frequency of the ACTN3 XX genotype was reduced in Australian power athletes compared to controls, whereas none of the Olympians or female power athletes had an XX genotype. These findings have been supported by the independent replications in case-control studies of elite Greek track and field athletes [19], elite-level strength athletes from across the United States [23], Russian power-oriented athletes [9], Israeli sprinters [10], and Russian short-distance speed skaters [2].

There are already other genetic variants that have been reported to show associations with muscle mass [15,21], and we strongly suspect that many additional common polymorphisms, and probably rare mutations as well, will be shown to be associated with skeletal muscle hypertrophy and testosterone levels in due course.

In conclusion, we found that the ACTN3 R allele was associated with high levels of testosterone in athletes. These data provide some mechanistic insights into the association between α-actinin-3 deficiency, skeletal muscle hypertrophy and power athlete status.

Conflict of interest
The authors report no conflicts of interest.

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REFERENCES
13. Henesy M.B., Britain A.L., Zhu B., Amabile L., Honkanen R.E., Corbin J.D.,