Original Research Article

Relationship between ACTN3 R577X polymorphism and maximal power output in elite Polish athletes

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Background and objective: The main purpose of this study was to examine the association between ACTN3 R577X polymorphism and the ability to produce peak power in young male athletes from various sports. Our hypothesis was that the ACTN3 R577X polymorphism is associated with jumping performance and athletes with RR genotype have better scores in tests than athletes with XX or RX genotype independently of the sport discipline.

Materials and methods: Two hundred young Polish male participants representing different disciplines were recruited for this study. Genotyping for ACTN3 gene was performed using polymerase chain reaction. The power output of lower extremities and the height of rise of the body mass center during vertical jumps were measured on a force plate.

Results: The genotype distribution of the ACTN3 gene did not differ significantly between groups of athletes. The significant difference in height of counter-movement jump was found between athletes with RR and XX genotype (0.446 ± 0.049 m vs. 0.421 ± 0.036 m, respectively, P = 0.026). The ACTN3 RR genotype was associated with greater muscle power and height of jump in young male athletes.
Conclusions: These results suggest that the ACTN3 gene may play a significant role in determining muscle phenotypes. However, this gene is only one of many factors which could contribute to athletes’ performance and muscle phenotypes.

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1. Introduction

It is well known that various factors determine athletic success and one of them is genetics. For many years researchers have examined genes associated with physical performance. Now more than 200 genes are thought to have a potential impact on physical performance [1]. One of the most studied genes is the R577X polymorphism of the α-actinin-3 gene (ACTN 3). The ACTN3 gene encodes the actin-binding protein α-actinin-3, which is one of the predominant components of the Z-disk in human skeletal muscles. ACTN3 protein anchors together actin-containing thin filaments and stabilizes the muscle contractile apparatus [2]. Expression of this protein is restricted to the fast glycolytic fibers, which are responsible for generating rapid, forceful contractions, for example in sprinting and weightlifting [2]. An R577X polymorphism in the ACTN3 gene, which results in conversion of the codon for arginine (R) at position 577 to a premature stop codon (X), was described by North et al. [3]. Because of this there are two different versions of the ACTN3 gene – a functional R allele (production of α-actinin-3 protein) and an X allele (lack of production of α-ACTN 3 protein). Some studies have demonstrated that the frequency of the RR genotype of the ACTN3 gene is higher in power/sprint athletes than in controls and/or endurance athletes in contrast to the XX genotype, which benefits endurance performance [4–7]. Studies on animal models [8,9] showed that α-actinin-3-deficient mice (knockout mice, KO) had a significant decrease in enzyme activity in the anaerobic glycolytic pathway and increased activity in the aerobic oxidative pathway. The ACTN3 KO mice had lower fat free mass than the wild type control (WT), which was due to a reduced diameter of the type IIb, fast glycolytic fibers. Furthermore, knockout mice were able to run a longer distance than wild type mice before reaching exhaustion and exhibited better recovery from fatigue, but grip strength was significantly lower in these mice compared with the WT mice.

Measurement of power output in jumps on a force plate is a routine method used for determination of muscle force and power in a laboratory testing [10–13]. Vertical jump tests were used, as a determinant of muscle power, to see if the ACTN3 R577X polymorphism influences explosive leg muscle power [14–16].

The main purpose of this study was to examine the association between ACTN3 R577X polymorphism and the ability to produce peak power in young Polish athletes from different disciplines. Based on the fact that previous findings on elite athletes suggested that allele R appears to confer an advantage in power performance [17] we hypothesized that the ACTN3 R577X polymorphism is associated with jumping performance in athletes, and athletes with RR genotype have better scores in tests than athletes with XX genotype, independently of the sport discipline. To verify this hypothesis, we decided to include disciplines which are trained in various jumping exercises and those who are not.

2. Materials and methods

2.1. Participants

Ethical approval for this study was provided by the local ethical committee (July 14, 2011). All participants were informed about the study aim and methodology, as well as the possibility of immediate resignation at any time of the experiment. Participants agreed on the above conditions in writing. The study was performed according to the Declaration of Helsinki.

Two hundred young male participants representing different disciplines (volleyball, swimming, ice hockey, canoe) were recruited for this study in 2009–2012. Most of them (82 canoeists, 43 swimmers and 25 volleyball players) were medalists of National/European/World championships in their age category in years from 2009 to 2014. Ice hockey players were students from school of Polish Ice Hockey Federation. All participants had Polish nationality and were Caucasians. Examined participants’ characteristics are presented in Table 1. Investigated sport disciplines differed significantly in relation to anthropometric measurements.

2.2. ACTN3 genotyping

Genomic DNA was extracted from peripheral blood leucocytes using Blood Mini kit (A&A Biotechnology, Poland). The R577X polymorphism of ACTN3 was determined using polymerase chain reaction (PCR) [18]. The primers for exon 16 were as follows: forward 5’-CTGTTCGCTTGGTAAATTGCGG-3’ and reverse 5’-TGGTTCAGATATCGAGGG-3’. The PCR conditions were as follows: initial denaturation at 94 °C for 5 min; 35 cycles of denaturing at 94 °C for 30 s and annealing at 70 °C for 60 s and final extension at 72 °C for 10 min. After amplification PCR products were digested with Dde I enzyme (Thermo Scientific, USA) at 37 °C for 2 h. Digestion of the 577R allele results in fragments of 205 and 86 bp, whereas digestion of the 577X allele results in fragments of 108, 97, and 86 bp. The digested products for ACTN3 were then analyzed by electrophoresis at 120 V for 90 min through a 3% agarose gel containing SYBR® Safe DNA gel stain at 10,000× concentration in DMSO (Invitrogen, USA). To verify the genotyping results, selected samples were also analyzed by another investigator. The two analyses showed identical results.
Table 1 - Examined groups' characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Canoe, n = 87</th>
<th>Ice hockey, n = 39</th>
<th>Swimming, n = 43</th>
<th>Volleyball, n = 31</th>
<th>All, n = 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18.1 ± 1.6</td>
<td>17.5 ± 0.9</td>
<td>15.1 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.2 ± 0.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>17.2 ± 1.8</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>182.8 ± 6.0</td>
<td>179.3 ± 4.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>181.8 ± 7.2</td>
<td>196.6 ± 5.9&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>184.1 ± 8.2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>79.9 ± 7.7</td>
<td>75.2 ± 8.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.1 ± 7.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.3 ± 7.5&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>77.2 ± 9.1</td>
</tr>
<tr>
<td>BMI (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>23.9 ± 1.8</td>
<td>23.4 ± 2.2</td>
<td>20.9 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.5 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.8 ± 2.2</td>
</tr>
<tr>
<td>Training experience</td>
<td>7.5 ± 2.6</td>
<td>9.8 ± 2.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.5 ± 2.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.3 ± 2.1&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.4 ± 2.8</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation.  
* P < 0.05 as compared with the canoe group.  
<sup>b</sup> P < 0.05 as compared with the ice hockey group.  
<sup>c</sup> P < 0.05 as compared with the swimming group.

2.3. Power output and height of jump

All measurements of power, body weight, and jump height were performed on a Kistler tensometric force plate with a 400 Hz sampling rate. The force plate was connected via analog-to-digital converter to a PC with the MVJ v.3.4 software (“JBA” Z. Staniak, Poland). The vertical component of ground reaction force was used for calculating the peak power, body mass and jump height. The model applied for all calculations implies a closed system where the body mass of a human launching vertically from a force plate is reduced to a material point affected by vertical force components: gravity and the reaction force of the platform [11]. The velocity and spatial arrangement of a body launching from a tensometric platform was calculated using an integral equation. Mathematical formula used for estimation of force developed during jumping is presented by Eq. (1):

\[ F(t) = R(t) - Q \]  

(1)

where \( F(t) \) indicates force applied to force plate in a time domain; \( R(t) \), vertical component of ground reaction force in a time domain; \( Q \), gravity force of a body mass.

Using the measured force developed during jumping and a body mass, acceleration of the body’s center of mass can be estimated by means of formula (2):

\[ a(t) = \frac{1}{m[R(t) - Q]} \]  

(2)

where \( a(t) \) indicates acceleration in a time domain; \( m \), body weight; \( R(t) \), vertical component of ground reaction force in a time domain; \( Q \), gravity force of a body mass.

Having the acceleration, one can estimate velocity and spatial displacement of the body mass center by means of a calculus. The height of a jump is expressed by the maximum of the function presented in formula (3):

\[ y(t) = y_0 + \int_0^t V_0 dt + \frac{1}{m} \int_0^t \int_0^t F(t) dt dt \]  

(3)

where \( u(t) \) indicates velocity in a time domain; \( y(t) \), spatial placement in a time domain; \( V_0 \), \( L_0 \), and \( y_0 \), boundary conditions for velocity, time and spatial placement; \( m \), body weight.

The maximum error of the measurement channel is less than 0.5% (Kistler Instruments AG 1991). The maximal error of repeatability, expressed by the coefficient of variability, was 3.0% for maximal height of rise of the body mass center and 3.4% for maximal power output.

Each participant performed nine vertical jumps on the force plate: three akimbo counter-movement jumps (ACMJ), three counter-movement jumps (CMJ) and three spike jumps (SPJ). The characteristics of each jumping test are as follows [10]:

- ACMJ, a vertical jump from an upright standing position with hands on the hips and with lowering of the body mass center before the take-off;
- CMJ, a vertical jump from a standing erect position, preceded by a counter-movement of upper limbs and with lowering of the body mass center before the take-off;
- SPJ, a vertical jump which is performed with a three to four step run-up before the take-off.

The participants were told to jump as high as possible in every trial. There were 5 s breaks between the CMJs and ACMJs, and 1 min breaks between the SPJs. The jump with the highest elevation of the body’s COM was chosen for statistical analysis.

2.4. Anthropometric measurement

Body height was assessed using a Siber Hegner anthropometer (Switzerland) with an accuracy of 0.1 cm. Body weight was measured on an electronic scale (AXIS, Poland) with an accuracy of 0.1 kg. All measurements were taken by the same investigator, applying standard anthropometric methods according to the procedure of the International Biological Programme [19].

2.5. Statistical analysis

The significance of the observed differences was assessed using one-way or multivariate analysis of variance (MANOVA) with post hoc Fisher LSD test. Depended variables were: jumping height and maximal power output in CMJ, ACMJ and SPJ. Independent variables were: sport discipline and genotype variant. Distribution of all the investigated variables was assessed by Kolmogorov-Smirnov test. If the variable did not meet the assumptions of normal distribution, we used a nonparametric Mann–Whitney U test to assess the significance of differences between the mean values. Chi squared tests were used to test for the presence of Hardy–Weinberg equilibrium (HWE). We compared ACTN3 genotype frequencies between the
groups using the chi-square test. The level of statistical significance was set at \( P < 0.05 \). Statistics™ v. 10.0 software (StatSoft, USA) was used in data analysis.

3. Results

Table 2 shows the number of different variants of genotype within each of the disciplines. The ACTN3 genotype distribution in athletes was in Hardy–Weinberg equilibrium. The genotype distribution of the ACTN3 gene did not significantly differ between groups of athletes \((\chi^2 = 6.5, df = 6, P = 0.37)\) (Table 2).

The relationships between ACTN3 genotypes and athletes’ characteristics are illustrated in Table 3. Physical characteristics were independent of the ACTN3 genotype variant – we found no significant differences between particular genotype variants in relation to body height, body mass, age, training experience and body mass index (ANOVA, \( P = 0.53 \)).

Mean values of power output and height of jump are presented in Tables 4 and 5. ACTN3 genotype was associated with height of jump and power output in all three jump tests and athletes with XX genotype had lower values of power output and height of jump than RR homozygotes (ANOVA \( P = 0.04 \) with LSD post hoc test). No differences were observed between the RX variant and the other two variants of genotype (Table 5). Combined RR and RX groups demonstrated higher jump and greater power output than in XX genotypes in all tests (Mann–Whitney U test).

4. Discussion

The main finding of this study was that the ACTN3 R577X polymorphism is associated significantly with the ability of young male Polish athletes to produce peak power in the lower extremities.

In this study athletes with RR genotype had significantly greater explosive leg muscle power in all the jumping trials than those with XX genotype. However, we found no differences between RX and homozygous genotypes. In a study where young Chinese soldiers were examined, handgrip strength was significantly lower in participants with XX genotype than individuals with RR genotype [21], which is in accordance with our results. In addition, Pimenta et al. [22] suggested that elite football players with RR genotypes tend to exhibit greater explosive leg muscle strength than other genotype variants of ACTN3. In the latest study, it was found that ACTN3 R allele is related to larger muscle volume and greater power and isoinertial strength in comparison to individuals with XX genotype [23]. In contrast to the above-mentioned studies, Ginevičienė et al. [15] claimed that XX homozygous athletes had higher short-term explosive power than athletes with RR genotype. On the other hand, some papers state that there are no relationships between any ACTN3 genotype and maximal muscle power and height of jump in volleyball, basketball and rugby players [14,16,24], as well as in young nonathlete participants [25]. Ruiz et al. [14] described a similar tendency for jumping vs. genotype \((h_{RR} > h_{RX} > h_{XX})\) in volleyball players as in our work.

The above-mentioned studies provide ambiguous statements about the relationship between ACTN3 genotype and muscular strength and power output. Differences in these results may be due to many reasons, e.g. training experience, type of physical effort (intensity, frequency), muscle fiber type composition, gender, anthropometric variables or different testing batteries used for examinations [25,26].

It was suggested that α-actinin could play a role in the determination of muscle fiber type [4]. It is well known that muscle fiber type composition influences physical performance. In general, endurance athletes have a remarkably higher number of type I (slow-twitch, fatigue-resistant; ST) fibers in their muscles, whereas power-oriented individuals have more Ila/Ilx (fast-twitch) fibers [27]. Vincent et al. [28] reported that muscle cross-section and number of IIX fibers was greater in individuals with RR genotype than XX genotype carriers. The relationship between ACTN3 R577X polymorphism and muscle fiber composition was also observed in Russian participants (speed skaters and active men), where individuals with XX genotype have a higher proportion of ST fibers than carriers of other genotypes [29]. Expression of α-actinin-3 is limited to fast-twitching muscle fibers [3], which are responsible for generating force at high velocity. Probably

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### Table 2 – Distribution of study and control participants by different variants of the ACTN3 genotype and different sports [20].

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>RR</th>
<th>RX</th>
<th>XX</th>
<th>R</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canoe</td>
<td>87</td>
<td>30 (34.5)</td>
<td>42 (48.3)</td>
<td>15 (17.2)</td>
<td>102 (58.6)</td>
<td>72 (41.4)</td>
</tr>
<tr>
<td>Ice hockey</td>
<td>39</td>
<td>15 (38.5)</td>
<td>19 (48.7)</td>
<td>5 (12.8)</td>
<td>49 (62.8)</td>
<td>29 (37.2)</td>
</tr>
<tr>
<td>Swimming</td>
<td>43</td>
<td>21 (48.8)</td>
<td>20 (46.5)</td>
<td>2 (4.7)</td>
<td>62 (72.1)</td>
<td>24 (27.9)</td>
</tr>
<tr>
<td>Volleyball</td>
<td>31</td>
<td>14 (45.2)</td>
<td>15 (48.4)</td>
<td>2 (6.4)</td>
<td>43 (69.4)</td>
<td>19 (30.6)</td>
</tr>
<tr>
<td>All</td>
<td>200</td>
<td>80 (40.0)</td>
<td>96 (48.0)</td>
<td>24 (12.0)</td>
<td>256 (64.0)</td>
<td>144 (36.0)</td>
</tr>
<tr>
<td>Control</td>
<td>354</td>
<td>140 (39.0)</td>
<td>176 (50.0)</td>
<td>38 (11.0)</td>
<td>456 (64.0)</td>
<td>252 (35.6)</td>
</tr>
</tbody>
</table>

Values are number (percentage).

### Table 3 – Characteristics of all participants by different ACTN3 R577X polymorphisms.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>RR, n = 80</th>
<th>RX, n = 96</th>
<th>XX, n = 24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>17.1 ± 1.8</td>
<td>17.1 ± 1.7</td>
<td>17.8 ± 1.8</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>184.4 ± 8.2</td>
<td>184.0 ± 8.3</td>
<td>183.2 ± 8.4</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>77.6 ± 9.1</td>
<td>76.7 ± 8.6</td>
<td>78.1 ± 10.7</td>
</tr>
<tr>
<td>Training experience (years)</td>
<td>7.2 ± 2.2</td>
<td>7.7 ± 1.8</td>
<td>7.4 ± 3.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.8 ± 2.5</td>
<td>22.6 ± 2.1</td>
<td>23.2 ± 1.9</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation.
Table 4 – Height of rise of body mass center (h) and relative maximal power output (P) during akimbo countermovement jumps (ACMJ), countermovement jumps (CMJ), and spike jumps (SP) by different sports.

<table>
<thead>
<tr>
<th></th>
<th>Canoe, n = 87</th>
<th>Ice hockey, n = 39</th>
<th>Swimming, n = 43</th>
<th>Volleyball, n = 31</th>
<th>All, n = 200</th>
</tr>
</thead>
<tbody>
<tr>
<td>h_{ACMJ} (m)</td>
<td>0.369 ± 0.040</td>
<td>0.380 ± 0.041</td>
<td>0.365 ± 0.039</td>
<td>0.419 ± 0.046</td>
<td>0.378 ± 0.045</td>
</tr>
<tr>
<td>P_{ACMJ} (W/kg)</td>
<td>22.2 ± 3.8</td>
<td>23.7 ± 3.7</td>
<td>24.1 ± 4.5</td>
<td>25.5 ± 4.4</td>
<td>23.4 ± 4.2</td>
</tr>
<tr>
<td>h_{CMJ} (m)</td>
<td>0.429 ± 0.045</td>
<td>0.435 ± 0.043</td>
<td>0.426 ± 0.045</td>
<td>0.497 ± 0.056</td>
<td>0.440 ± 0.052</td>
</tr>
<tr>
<td>P_{CMJ} (W/kg)</td>
<td>28.5 ± 6.1</td>
<td>31.3 ± 5.9</td>
<td>29.7 ± 5.2</td>
<td>37.7 ± 6.7</td>
<td>30.7 ± 5.7</td>
</tr>
<tr>
<td>h_{SPJ} (m)</td>
<td>0.509 ± 0.062</td>
<td>0.521 ± 0.063</td>
<td>0.499 ± 0.050</td>
<td>0.616 ± 0.066</td>
<td>0.525 ± 0.072</td>
</tr>
<tr>
<td>P_{SPJ} (W/kg)</td>
<td>40.7 ± 10.3</td>
<td>40.2 ± 9.2</td>
<td>40.9 ± 7.9</td>
<td>54.8 ± 10.4</td>
<td>42.8 ± 10.9</td>
</tr>
</tbody>
</table>

* P < 0.05 as compared with the canoe group.
* P < 0.05 as compared with the ice hockey group.
* P < 0.05 as compared with the swimming group.

more dynamic muscle power (power output) obtained by athletes with RR genotype in our study could be associated with muscle fiber composition and a predominance of fast-twitch fibers in muscles of these individuals. It may confirm the results of Vincent et al. [28], in which healthy young men with RR genotype showed significantly higher relative dynamic quadriceps torques at 300/s velocity, and a greater percentage of fast twitching muscle fibers than XX homozygotes. We can only speculate on this association between ACTN3 and muscle fiber type because not all studies support these findings [26].

In the present study we found no association between ACTN3 genotype and anthropometric characteristics, which is in accordance with the findings of other researchers [21,24, 28].

In this study we decided to employ three kinds of vertical jump test. The reason was the assumption that the maximal power output developed in a vertical jump is related to jumping technique (kind of jump; P_{max_{SST}} > P_{max_{CMJ}} > P_{max_{ACMJ}}) [11]. This relationship is a direct result of the impact of two physiological muscle phenomena: stretch reflex and accumulation of elastic energy in the muscle-tendon complex (eccentric phase of jumping). The explosive force of lower extremities is strongly dependent on these two mechanisms. The differences in average values of maximal power and height of jump were significant for all the jumping trials between XX and RR genotypes. In our opinion, this observation is an argument which confirms the hypothesis that individuals with RR genotype are predisposed to develop greater explosive muscle force.

The second interesting observation in our study is the fact that sport disciplines seem to be unrelated to ACTN3 genotype variant ($\chi^2 = 7.03, \text{df} = 8, P = 0.32$). Investigating the differences in genotype frequency distribution, Ginevičienė et al. [15] found no such relation between endurance and power-oriented athletes. Similar results were presented by Lucia et al. [30]. Moreover, in our study, the frequency distribution of ACTN3 genotypes and alleles in athletes from various sports was similar to that observed in the Polish male control (nonathletes) [20] ($\chi^2 = 7.03, \text{df} = 8, P = 0.32$ and $\chi^2 = 9.65; \text{df} = 4, P = 0.14$, respectively, for genotype and allele variants). Those results are in accordance to those presented by Eynn et al. [20], but Cięciszczyk et al. [5] found significant differences in the genotype distribution between Polish power oriented athletes and control group. Thus, it is not clear whether the ACTN3 genotype distinguishes athletes and non-athletes individuals. It seems that ACTN3 genotype is not a major factor responsible for predisposing individuals to a particular sport discipline.

5. Conclusions

These results suggest that the ACTN3 gene may play a significant role in determining muscle phenotypes of Polish elite athletes. However, this gene is only one of many factors (epigenetic factors, environment, and complex gene-gene and gene-environment interactions) that could contribute to athletes’ performance and muscle phenotypes.

Conflict of interest

The authors state no conflict of interest.

Acknowledgments

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