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Original research

ACTN3 R577X polymorphism and team-sport performance: A study involving three European cohorts

Nir Eynon a,b,*, Lauren K. Banting b, Jonatan R. Ruiz c, Pawel Cieszczyk d, Dmitry A. Dyatlov e, Agnieszka Maciejewska-Karłowska f, Marek Sawczuk f, Vladimir P. Pushkarev g, Leonid M. Kulikov e, Evgeny D. Pushkarev e, Pedro Femia h, Nigel K. Stepto a,b, David J. Bishop a,h,1, Alejandro Lucia h,1

a School of Sport and Exercise Sciences, Victoria University, Australia
b Institute of Sport, Exercise and Active Living (ISEAL), Victoria University, Australia
c Department of Physical Education and Sport, School of Sport Sciences, University of Granada, Spain
d Department of Tourism and Recreation, Academy of Physical Education and Sport in Częstochowa, Poland
e Ural State University of Physical Culture, Russia
f Department of Physical Culture and Health Promotion, University of Silesia, Poland
g Department of Biostatistics, School of Medicine, University of Granada, Spain
h Universidad Europea de Madrid, Spain

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A B S T R A C T

Objectives: To determine the association between the α-actinin-3 (ACTN3) R577X polymorphism and elite team-sport athletic status in three cohorts of European team-sport athletes.

Design: We compared the genotype and allele frequencies of the ACTN3 R577X (rs1815739) polymorphisms between team-sport athletes (n = 205), endurance athletes (n = 305), sprint/power athletes (n = 378), and non-athletic controls (n = 568) from Poland, Russia and Spain; all participants were unrelated European men.

Methods: Genomic DNA was extracted from either buccal epithelium or peripheral blood using a standard protocol. Genotyping was performed using several methods, and the results were replicated following recent recommendations for genotype–phenotype association studies.

Results: Genotype distributions of all control and athletic groups met Hardy–Weinberg equilibrium (all p > 0.05). Team-sport athletes were less likely to have the 577RR genotype compared to the 577XX genotype than sprint/power athletes [odds ratio: 0.58, 95% confidence interval: 0.34–0.39, p = 0.045]. However, the ACTN3 R577X polymorphism was not associated with team-sports athletic status, compared to endurance athletes and non-athletic controls. Furthermore, no association was observed for any of the genotypes with respect to the level of competition (elite vs. national level).

Conclusions: The ACTN3 R577X polymorphism was not associated with team-sport athletic status, compared to endurance athletes and non-athletic controls, and the observation that the 577RR genotype is overrepresented in power/sprint athletes compared to team-sport athletes needs to be confirmed in future studies.

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1. Introduction

The field of genetics and elite athletic performance has made considerable progress in the last two decades, with studies suggesting a significant effect of genetics on athletic performance, even when adjusted for the manifest effect of the environment.1 The majority of studies, so far, have focused on genotyping predominantly power or endurance athletes, who represent the physiological end-points of the sporting continuum. However, the genetic contribution to success in sports that require a combination of anaerobic and aerobic qualities (e.g., team sports such as soccer and water-polo) has received limited attention.

Team sports can be considered as mixed-energy system sports. Athletes engaged in these disciplines are required to repeatedly produce maximal or near maximal efforts (i.e., sprints), interspersed with brief recovery intervals (consisting of complete rest or low- to moderate-intensity activity), over an extended period of time. In this situation, both the aerobic and anaerobic energy...
systems are important to supply the muscle energy demands during the competition.

The ACTN3 gene, which encodes for the α-actinin-3 protein, is a candidate to influence individuals’ performance in team-sports. The α-actinin-3 protein is almost exclusively expressed in fast, glycolytic, type IIx fibres, which are responsible for producing powerful contractions. North et al. have discovered a common null polymorphism (rs1815739) in the ACTN3 gene, which results in replacement of an arginine (R) residue with a premature stop codon (X) at amino acid 577. Approximately 20% of the world population, and 18% of the European population, harbour the ACTN3 577XX genotype and consequently are completely deficient in α-actinin-3.

The ACTN3 R577X polymorphism has been investigated in the context of human athletic performance, in both elite endurance and power athletes, and the general population, with the overall conclusion that α-actinin-3 deficiency, as marked by the 577XX genotype, is detrimental to power performance and possibly beneficial to endurance performance. Recently, we have shown, in a large group of elite European athletes (n = 633), that ‘world-class’ endurance athletes were 3.7 times more likely to harbour the 577XX genotype than national-level counterparts, and that elite power athletes were ~50% less likely to harbour the 577XX genotype compared to sedentary controls.

Few attempts have been made to investigate the association between the ACTN3 R577X polymorphism and team sport athletic status. Santiago et al. showed higher proportions of the 577RR genotype in world-class professional soccer players (n = 60) compared with non-athletic controls and elite endurance athletes. In contrast, no association was found between the ACTN3 R577X polymorphism and athletic performance in a mixed group of elite Lithuanian athletes, in Welsh rugby union players (n = 102), or in Italian team-sport athletes (i.e., football, basketball, and hockey players; n = 65). The inconsistent results in the aforementioned studies performed with elite team-sports athletes may be due to an insufficient sample size, associated with the low number of elite athletes available for analysis.

To overcome the problems of low sample size, we recruited over 200 elite team-sport athletes from three European countries (i.e., Spain, Poland and Russia). We compared the frequency distribution of the ACTN3 R577X polymorphism between team-sport athletes, elite endurance athletes, elite powerathletes, and ethnically matched, non-athletic controls, in a large cohort of European athletes. Given that team-sport athletes perform multiple sprints and jumps during a match, and the frequency distribution of the 577RR genotype is consistently higher in power athletes than it is in controls, we hypothesised that the 577RR genotype frequency distribution would be higher in team-sport athletes compared to the control group.

2. Methods

The study was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants, and the study was approved by the Ethics Committees of Universidad Europea de Madrid, Spain, the Pomeranian Medical University, Poland, and the Ural State University of Physical Culture, Russia.

A total of 888 athletes (305 endurance athletes, 378 sprint/power athletes, and 205 team sport athletes) and 568 controls, from Poland, Russia and Spain, participated in this study. All participants were unrelated European men and all Caucasians (self-reported) for ≥3 generations. According to their individual best performances, we divided the athletes within each group into two subgroups: ‘elite-level’ (competitors in European/World championships or in the Olympic Games) and ‘national-level’, (competitors in national but not international level events) (Table 1). Of the athletes, 642 (72%) were classified as elite athletes, and the remaining 246 (28%) athletes were classified as national-level athletes. Control participants were required to be free of any diagnosed cardio-respiratory disease and not participating regularly in any competitive or structured sport or physical activity (i.e., performing less than 3 sessions per week of strenuous exercise such as running, swimming, bicycling or weight lifting).

The Spanish participants (n = 426) included 323 athletes and 103 controls. Of the athletes 308 were classified as elite and 15 were national level.

(i) 50 elite soccer players (team-sport athletes). Of these athletes, eleven played in teams that had won the Europe Champions League at least once and two had won the Soccer World Cup.

(ii) 119 elite sprint/power athletes. This group included track and field jumpers (n = 13), track and field sprinters (n = 40), and 66 volleyball players. All volleyball players belonged to the Spanish national team and competed at the international level (including 4 medalists in Olympic Games or World/European championships), and thirteen track and field sprinters were Olympians during the period 2000–2008.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Spanish (n = 426)</th>
<th>Polish (n = 695)</th>
<th>Russian (n = 335)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Team-sport</td>
<td>Power</td>
<td>Endurance</td>
</tr>
<tr>
<td></td>
<td>Power</td>
<td>Endurance</td>
<td>Control</td>
</tr>
<tr>
<td>All (n)</td>
<td>50</td>
<td>119</td>
<td>154</td>
</tr>
<tr>
<td>XX</td>
<td>18%</td>
<td>13%</td>
<td>26%</td>
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<td>RX</td>
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<td>47%</td>
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<tr>
<td>RR</td>
<td>43%</td>
<td>31%</td>
<td>27%</td>
</tr>
<tr>
<td>MAF</td>
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<td>0.412</td>
<td>0.497</td>
</tr>
<tr>
<td>MAF</td>
<td>0.360</td>
<td>0.412</td>
<td>0.500</td>
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<td>HWE-p value</td>
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<td>Elite (n)</td>
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<td>119</td>
<td>139</td>
</tr>
<tr>
<td>XX</td>
<td>18%</td>
<td>13%</td>
<td>27%</td>
</tr>
<tr>
<td>RX</td>
<td>36%</td>
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<td>45%</td>
</tr>
<tr>
<td>RR</td>
<td>43%</td>
<td>31%</td>
<td>27%</td>
</tr>
<tr>
<td>MAF</td>
<td>0.360</td>
<td>0.412</td>
<td>0.500</td>
</tr>
<tr>
<td>MAF</td>
<td>0.360</td>
<td>0.412</td>
<td>0.500</td>
</tr>
<tr>
<td>National Level (n)</td>
<td>–</td>
<td>15</td>
<td>–</td>
</tr>
<tr>
<td>XX</td>
<td>–</td>
<td>13%</td>
<td>–</td>
</tr>
<tr>
<td>RX</td>
<td>–</td>
<td>67%</td>
<td>–</td>
</tr>
<tr>
<td>RR</td>
<td>–</td>
<td>20%</td>
<td>–</td>
</tr>
<tr>
<td>MAF</td>
<td>–</td>
<td>0.467</td>
<td>–</td>
</tr>
<tr>
<td>MAF</td>
<td>–</td>
<td>0.467</td>
<td>–</td>
</tr>
</tbody>
</table>
(iii) 154 endurance athletes aged 20–39 years. This sample included 50 elite endurance runners (the top Spanish runners during the 1999–2009 period, i.e., mainly 5000 m to marathon specialists), 50 professional road cyclists who were all Tour de France finishers (including stage winners), and 54 rowers. The rowers included 39 elite athletes who had in the lightweight category in the World Championships held during 1997–2006. A total of 139 (90%) of these athletes were elite.

(iv) 103 healthy, non-athletic controls aged 19–32 years. All were undergraduate students from the same university (Universidad Europea de Madrid, Spain).

The Polish participants (n = 695) included 341 athletes and 354 controls. Of the athletes, 197 were classified as elite and 144 were national-level athletes:

(i) 49 team-sport athletes. This group included ice hockey players (n = 25, male, age 28 ± 4), all are members of the best three ice hockey clubs in Poland, and nine are members of the 2010–2011 National Team. Handball players (n = 21 male, age 26 ± 2), all are members of the best three handball clubs in Poland, and 11 are member of the 2009 National Team. Soccer players (n = 3 male, age 26 ± 6), all are members of one of the best soccer teams in Poland. Nine (18%) of these athletes were elite.

(ii) 178 sprint/power athletes. This group included weightlifters (n = 43, including 2 Olympic champions, 3 World champions and 10 medalists in World or European championships), sprinters (≤200 m, n = 48, including an Olympic champion and 9 medalists in Olympic Games or World/European championships), professional wrestlers (n = 72), long jumpers (n = 11), and volleyball players (n = 4). The group included 118 elite athletes (66%).

(iii) 114 endurance athletes. This group included rowers (n = 53, including 14 Olympic/World champions and 22 medalists in Olympic Games or World/European championships), endurance road cyclists (n = 14, including 7 medalists in Olympic Games or World/European championships), 5000 m runners (n = 12, including 1 Olympic medalist), marathon runners (n = 12), 800–1500 m swimmers (n = 11, including 2 medalists in Olympic Games or World/European championships), 15–50 km cross-country skiers (n = 6, including 2 Olympic champions), and triathletes (n = 6, all medalists in World/European championships). The group included 70 (61%) elite athletes.

(iv) 354 healthy sedentary controls aged 19–32 years (all students of the University of Szczecin).

The Russian participants (n = 335) included 111 controls and 224 athletes. Of the athletes 137 were classified as elite and 87 were classified as national-level athletes:

(i) 106 team-sport athletes. This group included handball players (n = 36), all are members of handball clubs participating in the first division handball league (Super League of Russia). Field hockey players (n = 9), all are members of one of the best field hockey team in Ural region of Russia. Ice hockey players, members of the Kontinental Hockey League (KHL), the highest ranked hockey league in Europe (n = 59), and water polo players (n = 2). This group included 55 elite athletes (52%).

(ii) 82 sprint/power athletes. This group included skaters competing in events ≤1000 m (n = 17, including 3 World champions and 3 European champions), boxers (n = 34, including 8 World champions and 3 European champions), professional wrestlers (n = 10, including 3 European champions), swimmers competing in events ≤200 m (n = 8), weightlifters (n = 6, including the World Powerlifting Congress man record holder), figure skaters (n = 6), weight lifters (n = 6), one strongman (runner up at world championship and three times Russia’s Strongest Man). This group included 56 (68%) elite athletes.

(iii) 36 endurance athletes. This group included rowers (n = 6), skaters competing in events >5000 m (n = 22), walkers (n = 3, including one winner of the European Cup), mountain skiers (n = 2), one swimmer competing in events >400 m (medalist in European championships, Olympian in 2008), one marathon runner (European champion), and one duathlete. This group included 26 elite athletes.

(iv) 111 healthy sedentary controls aged 19–32 years. All were students or employees of the Ural State University of Physical Culture.

We followed recent recommendations for genotype–phenotype association studies.16,17 In the Spanish population genomic DNA was isolated from buccal epithelium or peripheral blood during the years 2004–2008 and genotyping was performed in the Genetics Laboratory of Universidad Europea de Madrid, Spain. We used the polymerase chain reaction (PCR) method, which has been applied in previous research.3 We have replicated the genotype results (in 40% of samples) in another laboratory (ProgenikaBiopharma, ParqueTecnológico de Zamudio, Vizcaya, Spain) using a different method, i.e., a newly developed low-density DNA microarray based on allele-specific probes.3 The PCR products were fluorescently labelled and hybridised to the DNA microarray in an automated platform (Tecan HS4800, Mannedorf, Switzerland), and the microarrays were scanned (Innopsys S.A., Carbone, France) using a developed software that converts the intensity of the spots into the genotype of the polymorphism.

In the Polish population genomic DNA was isolated from buccal epithelium using GenElute Mammalian Genomic DNA Miniprep Kit (Sigma, Germany), during the years 2008–2010, according to the manufacturer’s instructions. We again used the polymerase chain reaction (PCR) method, which has been applied in previous research.3 To ensure proper internal control, for each genotype analysis we used positive and negative controls from different DNA aliquots that were previously genotyped with the same method.

In the Russian population genomic DNA was isolated from buccal epithelium or peripheral blood, during the years 2009–2011, using the Diatom™ DNA Prep kit (Cat. # D 1025, IsoGene Lab Ltd., Russia). The kit is based on selective DNA on a surface of glass powder in the presence of high concentration of guanidine isothiocyanate as chaotropic agent.

Genotyping was performed by using a TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) by use StepOne™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Assay ID was C__590093_1. For replication purposes, 75% of the samples were analysed with a different method, i.e., PCR restriction length polymorphism (RFLP), according to a previously described method.1

Chi squared tests were used to test for the presence of Hardy–Weinberg equilibrium (HWE). Multinomial logistic regression analyses were conducted to test the association between genotype and athletic status/competition level. In each case, nationality was controlled for; and analyses were made comparing 577XX (reference group) vs. 577RX; 577XX vs. 577RR (co-dominant effect); 577XX vs. 577RR and 577RX combined (dominant effect); 577XX and 577RX combined (reference group) vs. 577RR (recessive effect). Significance was accepted when p ≤ 0.05. Statistical analyses were conducted using SPSS (v. 19).
Table 2
Odds ratios of genotypes for athletes and control participants according to sport type.

<table>
<thead>
<tr>
<th>Sport Type</th>
<th>XX (ref)</th>
<th>RX</th>
<th>RR</th>
<th>RX and RR (XX ref)</th>
<th>RR (RX and XX ref)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>OR</td>
<td>Cl</td>
<td>p</td>
<td>OR</td>
</tr>
<tr>
<td>Mixed vs. control</td>
<td>1</td>
<td>0.88</td>
<td>0.55–1.43</td>
<td>0.606</td>
<td>0.93</td>
</tr>
<tr>
<td>Mixed vs. endurance</td>
<td>1</td>
<td>0.80</td>
<td>0.46–1.39</td>
<td>0.436</td>
<td>0.85</td>
</tr>
<tr>
<td>Mixed vs. power</td>
<td>1</td>
<td>0.64</td>
<td>0.37–1.12</td>
<td>0.115</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Note: OR: odds ratio; CI: Confidence intervals; p: two-tailed p value. Significance is assumed when p < 0.05.

Table 3
Odds ratios of genotypes for elite athletes compared to national level athletes in team sports.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Team-sport</th>
<th>OR</th>
<th>Cl</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>XX (ref)</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RX</td>
<td>1.64</td>
<td>0.58–4.64</td>
<td>0.355</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td>1.58</td>
<td>0.53–4.73</td>
<td>0.412</td>
<td></td>
</tr>
<tr>
<td>RX-RX (XX ref)</td>
<td>1.61</td>
<td>0.59–4.39</td>
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<td></td>
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<tr>
<td>RX-RX (XX ref)</td>
<td>1.07</td>
<td>0.53–2.19</td>
<td>0.846</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI: Confidence intervals; ref: reference; OR odds ratio.

3. Results

Replication of genotyping within Spanish, Polish and Russian cohorts with the abovementioned methods gave comparable results (data not shown).

Table 1 shows the genotype and allele frequency distributions amongst all participants according to their nationality. Genotype distributions of all control and athletic groups in each of the three populations met HWE (all p > 0.05). No significant differences in genotype distribution were observed across nationalities in control, team sport, power or elite athletes groups respectively.

Table 2 shows the association between genotype and athletic status for all participants. Team-sport athletes were less likely to have the 577RR genotype compared to the 577XX genotype than power athletes (p = 0.045), after controlling for the effects of nationality. Power athletes were approximately 1.4 times more likely to have the 577RR genotype (as opposed to the 577XX genotype) than team-sport athletes.

Table 3 shows the association between genotype and competition level (elite vs. national level) for the team-sport athletes from all countries. No association was observed for any of the genotypes with respect to the level of competition (elite vs. national level). As above, nationality was controlled for in the regression analyses.

4. Discussion

We studied the association between the ACTN3 R577X polymorphism and team-sport athletic status, in a relatively large group of elite and national-level athletes, comprising three cohorts of European Caucasian athletes. Our main findings were as follows: (i) team-sport athletes were less likely to harbour the 577RR genotype than the 577XX genotype, compared to power athletes (p = 0.045), (ii) the ACTN3 R577X polymorphism genotype distribution was similar in the team-sport athletes, endurance athletes and the control group, and (iii) the ACTN3 R577X genotype distribution was similar in the elite-level team-sport athletes and in their national-level counterparts. These findings suggest that team-sport performance is not significantly influenced by the ACTN3 R577X polymorphism, and that the 577RR genotype is probably a more important achievement factor for predominantly power performance events, than it is for team-sport events.

In the present study, the ACTN3 R577X polymorphism was chosen as a candidate to influence team-sport athletic status as it has provided the most consistent results to date, being the only muscle gene polymorphism to be associated with performance across multiple athlete cohorts. The 577RR genotype has been previously associated with elite, power-oriented athletic status (i.e., sprinters, jumpers and throwers) in several cohorts of Caucasian athletes, with one exception. With regards to this, a recent meta-analysis showed a strong association between the 577RR genotype and power athletic performance especially among Europeans, regardless of the significant heterogeneity among the groups of athletes.

Team sports are intermittent in nature and require the repetition of powerful movements including short-distance sprinting and jumping, and these actions require the working muscles to produce force at a high velocity. We therefore hypothesised that the 577RR genotype frequency distribution would be higher in team-sport athletes compared to the control group. We have shown that team-sport athletes were less likely to harbour the ACTN3 577RR genotype, compared to the 577XX genotype, than power athletes. Furthermore, when combining all groups of European athletes, compared to the team-sport cohort the association between 577RR genotype and power athletic status remained significant. An explanation for the overall clear association between the 577RR genotype and power performance, and the unclear association with team-sport performance across multiple independent cohorts, is that the original association study between the ACTN3 577RR genotype and elite power performance was performed with Australian (European decent) predominantly elite sprint/power and endurance athletes. Most of the subsequent studies were also performed with predominantly sprint/power athletes. Taken together with current literature, our data collected in the predominantly power/sprint athletes demonstrate that across different ancestries the ACTN3 577RR polymorphism is associated with the unique power/sprint muscle phenotype. This is not typical of association studies involving the ACTN3 R577X polymorphism and team-sport athletes presumably due to the mixed nature of team-sport events, which rely on both the aerobic and anaerobic energy systems.

Given that team-sport athletes perform multiple sprints and jumps during a match, we hypothesised that the frequency distribution of the 577RR would be higher in team-sport athletes compared to controls. However, once we explored this association in a relatively large cohort of team-sport athletes (n = 205), all European Caucasians, we found no association between the ACTN3 R577X polymorphism and team-sport athletic status. We assume that the inconsistent results provided by some previous reports could be attributed to the relatively small sample size of the studied cohorts, and consequently low statistical power. This supports the need for larger cohorts with clearly defined phenotypes to reach more solid conclusion in human association studies. The ACTN3 R577X polymorphism association with sprint/power performance, in the present study, is supported by the Actn3 knock-out (KO) mouse model, which was developed to understand the functional consequence of the ACTN3 R577X polymorphism.
The KO mouse-model revealed, among other findings, that compared with their wild-type (WT) counterparts, Actn3 KO mice (i.e., ACTN3 577XX genotype) have (1) lower muscle mass due to lower diameter of the fast twitch muscle fibres (where α-actinin-3 is primarily expressed); and (2) A significant lower grip strength. Furthermore, α-actinin-3 deficiency (the 577XX genotype) results in a shift in muscle properties towards those of slow (type I) muscle fibre. Fast twitch muscles from KO mice have also significantly lower anaerobic enzyme activity and higher oxidative/mitochondrial enzyme activity, without a shift in fibre-type distribution. 26 These observations provide plausible explanation for the overall reduced sprint capacity in humans with the 577XX genotype, and possibly increased in sprint capacity in humans with the 577RR genotype. 15,27

We believe that the results of this carefully controlled study are valid, as we strictly followed the latest genotype: phenotype study recommendations 16 and all of the following criteria have been met: all studied participants presented the main study phenotype (i.e., being a professional team sports athletes). Although we studied three cohorts, participants within and between each cohort were both age and ethnically matched (all European Caucasians), genetic assessment was accurate and unbiased, with genotype distribution being in Hardy–Weinberg equilibrium (HWE) in both cases and controls.

5. Conclusion

In conclusion, the ACTN3 R577X polymorphism was not significantly associated with team sport athletic status, compared to endurance athletes and non-athletic controls. However, the 577RR genotype was overrepresented in power/sprint athletes compared with team sports athletes.

Practical implications

• The results of the present study can assist to understand which genetic profiles contribute to team-sport performance.
• Discovering the complex relationship between gene variants and team-sport performance may assist coaches to optimise training.
• The ACTN3 R577X should not be considered to influence team-sport performance.

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