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

This is the Accepted version of the following publication

Eynon, Nir, Banting, Lauren K, Ruiz, Jonatan, Cieszczyk, Pawel, Dyatlov, Dmitry A, Maciejewska-Karłowska, Agnieszka, Sawczuk, Marek, Pushkarev, Vladimir P, Kulikov, Leonid M, Pushkarev, Evgeny D, Femia, Pedro, Stepto, Nigel, Bishop, David and Lucia, Alejandro (2014) ACTN3 R577X polymorphism and team-sport performance: A study involving three European cohorts. *Journal of Science and Medicine in Sport*, 17 (1). pp. 102-106. ISSN 1440-2440 (print) 1878-1861 (online)

The publisher's official version can be found at
<http://dx.doi.org/10.1016/j.jsams.2013.02.005>
Note that access to this version may require subscription.

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1 ***ACTN3* R577X polymorphism and team-sport performance: a study involving three European**
2 **cohorts**

3
4 **Abstract**

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

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

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

14 **Results:** Genotype distributions of all control and athletic groups met Hardy-Weinberg Equilibrium (all
15 $p > 0.05$). Team-sport athletes were less likely to have the 577RR genotype compared to the 577XX
16 genotype than sprint/power athletes [odds ratio (OR): 0.58, 95% confidence interval (CI): 0.34-0.39, $p =$
17 0.045]. However, the *ACTN3* R577X polymorphism was not associated with team-sports athletic status,
18 compared to endurance athletes and non-athletic controls. Furthermore, no association was observed for
19 any of the genotypes with respect to the level of competition (elite vs. national level).

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

24
25 **Key words:** Genomics; alpha-actinin 3; exercise; athletes; genetics.
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29 **i. Introduction**

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

37 Team sports can be considered as mixed-energy system sports. Athletes engaged in these
38 disciplines are required to repeatedly produce maximal or near maximal efforts (i.e., sprints),
39 interspersed with brief recovery intervals (consisting of complete rest or low- to moderate-intensity
40 activity), over an extended period of time. In this situation, both the aerobic and anaerobic energy
41 systems are important to supply the muscle energy demands during the competition [2].

42 The *ACTN3* gene, which encodes for the α -actinin-3 protein, is a candidate to influence
43 individuals' performance in team-sports. The α -actinin-3 protein is almost exclusively expressed in fast,
44 glycolytic, type IIX fibres, which are responsible for producing powerful contractions [3]. North et al.
45 [4] have discovered a common null polymorphism (rs1815739) in the *ACTN3* gene, which results in
46 replacement of an arginine (R) residue with a premature stop codon (X) at amino acid 577.
47 Approximately 20% of the world population, and 18% of the European population, harbour the *ACTN3*
48 577XX genotype and consequently are completely deficient in α -actinin-3 [3].

49 The *ACTN3* R577X polymorphism has been investigated in the context of human athletic
50 performance, in both elite endurance and power athletes [5-13], and the general population [14-16], with
51 the overall conclusion that α -actinin-3 deficiency, as marked by the 577XX genotype, is detrimental to
52 power performance and possibly beneficial to endurance performance. Recently, we have shown, in a
53 large group of elite European athletes (n=633), that 'world-class' endurance athletes were 3.7 times
54 more likely to harbour the 577XX genotype than national-level counterparts, and that elite power
55 athletes were ~50% less likely to harbour the 577XX genotype compared to sedentary controls [17].

56 Few attempts have been made to investigate the association between the *ACTN3* R577X
57 polymorphism and team sport athletic status. Santiago et al. [18] showed higher proportions of the
58 577RR genotype in world-class professional soccer players (n=60) compared with non-athletic controls
59 and elite endurance athletes. In contrast, no association was found between the *ACTN3* R577X
60 polymorphism and athletic performance in a mixed group of elite Lithuanian athletes [19], in Welsh
61 rugby union players (n=102) [20], or in Italian team-sport athletes (i.e., football, basketball, and hockey
62 players; n=65) [21]. The inconsistent results in the aforementioned studies performed with elite team-
63 sports athletes may be due to an insufficient sample size, associated with the low number of elite
64 athletes available for analysis.

65 To overcome the problems of low sample size, we recruited over 200 elite team-sport athletes
66 from three different European countries (i.e., Spain, Poland and Russia). We then compared the
67 frequency distribution of the *ACTN3* R577X polymorphism between team-sport athletes, elite endurance
68 athletes, elite power athletes, and ethnically-matched, non-athletic controls, in a large cohort of
69 European athletes. Given that team-sport athletes perform multiple sprints and jumps during a match,
70 and the frequency distribution of the 577RR genotype is consistently higher in power athletes than it is
71 in controls [5], we hypothesised that the 577RR genotype frequency distribution would be higher in
72 team-sport athletes compared to the control group.

73

74 **ii. Methods**

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

79 A total of 888 athletes (305 endurance athletes, 378 sprint/power athletes, and 205 team sport athletes)
80 and 568 controls, from Poland, Russia and Spain, participated in this study. All participants were
81 unrelated European men and all Caucasians (self-reported) for ≥ 3 generations. According to their
82 individual best performances, we divided the athletes within each group into two subgroups: 'elite-level'

83 (competitors in European/World championships or in the Olympic Games) and 'national-level',
84 (competitors in national but not international level events) (Table 1). Of the athletes, 642 (72%) were
85 classified as elite athletes, and the remaining 246 (28%) athletes were classified as national-level
86 athletes. Control participants were required to be free of any diagnosed cardio-respiratory disease and
87 not participating regularly in any competitive or structured sport or physical activity (i.e. performing
88 less than 3 sessions per week of strenuous exercise such as running, swimming, bicycling or weight
89 lifting).

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

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

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

98 (iii) 154 endurance athletes aged 20-39 years. This sample included 50 elite endurance runners (the top
99 Spanish runners during the 1999-2009 period, i.e. mainly 5,000 m to marathon specialists), 50
100 professional road cyclists who were all Tour de France finishers (including stage winners), and 54
101 rowers. The rowers included 39 elite athletes who had in the lightweight category in the World
102 Championships held during 1997-2006. A total of 139 (90%) of these athletes were elite.

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

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

107 (i) 49 team-sport athletes. This group included ice hockey players (n=25), handball players (n=21), and
108 soccer players (n=3). Nine (18%) of these athletes were elite.

109 (ii) 178 sprint/power athletes. This group included weightlifters (n=43, including 2 Olympic champions,
110 3 World champions and 10 medalists in World or European championships), sprinters ($\leq 200\text{m}$, n=48,
111 including an Olympic champion and 9 medalists in Olympic games or World/European championships),
112 professional wrestlers (n=72), long jumpers (n=11), and volleyball players (n=4). The group included
113 118 elite athletes (66%).

114 (iii) 114 endurance athletes. This group included rowers (n=53, including 14 Olympic/World champions
115 and 22 medalists in Olympic Games or World/European championships), endurance road cyclists
116 (n=14, including 7 medalists in Olympic Games or World/European championships), 5,000m runners
117 (n=12, including 1 Olympic medalist), marathon runners (n=12), 800-1,500m swimmers (n=11,
118 including 2 medalists in Olympic Games or World/European championships)), 15-50 km cross-country
119 skiers (n=6, including 2 Olympic champions), and triathletes (n=6, all medalists in the European
120 championships). The group included 70 (61%) elite athletes.

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

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

124 (i) 106 team-sport athletes. This group included handball players (n=36), field hockey players (n=9),
125 ice hockey players from the *Kontinental Hockey League* (KHL), the highest ranked hockey league in
126 Europe (n=59), and water polo players (n=2). This group included 55 elite athletes (52%).

127 (ii) 82 sprint/power athletes. This group included skaters competing in events $\leq 1000\text{m}$ (n=17, including
128 3 World champions and 3 European champions), boxers (n=34, including 8 World champions and 3
129 European champions), professional wrestlers (n=10, including 3 European champions), swimmers
130 competing in events $\leq 200\text{m}$ (n=8), weightlifters (n=6, including the World Powerlifting Congress man
131 record holder), figure skaters (n=6), weight lifters (n=6), one strongman (runner up at world
132 championship and three times Russia's Strongest Man). This group included 56 (68%) elite athletes.

133 (iii) 36 endurance athletes. This group included rowers (n=6), skaters competing in events $\geq 5000\text{m}$
134 (n=22), walkers (n=3, including one winner of the European Cup), mountain skiers (n=2), one swimmer

135 competing in events >400m (medalist in European championships, Olympian in 2008), one marathon
136 runner (European champion), and one duathlete. This group included 26 elite athletes.

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

139 We followed recent recommendations for genotype-phenotype association studies provided by Chanock
140 et al. [22] and Attie et al.[23] .

141 *Genotyping Spanish population.* Genomic DNA was isolated from buccal epithelium or peripheral
142 blood during the years 2004-2008 and genotyping was performed in the Genetics Laboratory of
143 Universidad Europea de Madrid, Spain. We used the polymerase chain reaction (PCR) method, which
144 has been applied in previous research [3]. We have replicated the genotype results (in 40% of samples)
145 in another laboratory (Progenika Biopharma, Parque Tecnológico de Zamudio, Vizcaya, Spain) using a
146 different method, i.e. a newly developed low-density DNA microarray based on allele-specific probes
147 [24]. The PCR products were fluorescently labelled and hybridized to the DNA microarray in an
148 automated platform (Tecan HS4800, Mannedorf, Switzerland), and the microarrays were scanned
149 (Innopsys S.A., Carbonne, France) using a developed software that converts the intensity of the spots
150 into the genotype of the polymorphism. For control genotyping, sample analysis was made together with
151 a DNA control processing with a known genotype of the *ACTN3* R577X polymorphism.

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

158 *Genotyping Russian population.* Genomic DNA was isolated from buccal epithelium or peripheral
159 blood, during the years 2009-2011, using the Diatom™ DNA Prep kit (Cat. # D 1025, IsoGene Lab ltd,
160 Russia). The kit is based on selective DNA on a surface of glass powder in the presence of high
161 concentration of guanidine isothiocyanate as chaotropic agent.

162 Genotyping was performed by using a TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster
163 city, CA, USA) by use StepOne™ Real-Time PCR System (Applied Biosystems, Foster city, CA,
164 USA). Assay ID was C____590093_1_. For replication purposes, 75% of the samples were analysed
165 with a different method, i.e. PCR-restriction length polymorphism (RFLP), according to a previously
166 described method [3]. The oligonucleotide primers for this method were synthesized by Evrogen Ru
167 JSC (Russia). K562 DNA High Molecular Weight from Promega Corp. (Cat # DD2011, Madison, WI,
168 USA) served as positive control sample at carrying out of both research methods. Genetic profile of
169 K562 DNA was 577XX in *ACTN3* R577X sequence variation.

170 Chi squared tests were used to test for the presence of Hardy-Weinberg equilibrium (HWE).
171 Multinomial logistic regression analyses were conducted to assess the association between genotype and
172 athletic status/competition level. In each case, nationality was controlled for; and analyses were made
173 comparing 577XX (reference group) vs. 577RX; 577XX vs. 577RR (co-dominant effect); 577XX vs.
174 577RR and 577RX combined (dominant effect); 577XX and 577RX combined (reference group) vs.
175 577RR (recessive effect). Significance was accepted when $p \leq 0.05$. Statistical analyses were conducted
176 using SPSS (v. 19).

177

178 **iii. Results**

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

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

185 Table 2 shows the association between genotype and athletic status for all participants. Team-
186 sport athletes were less likely to have the 577RR genotype compared to the 577XX genotype than
187 power athletes ($p = 0.045$), after controlling for the effects of nationality. Power athletes were

188 approximately 1.4 times more likely to have the 577RR genotype (as opposed to the 577XX genotype)
189 than team-sport athletes.

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

194

195 **iv. Discussion**

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

206 In the present study, the *ACTN3* R577X polymorphism was chosen as a candidate to influence
207 team-sport athletic status as it has provided the most consistent results to date, being the only muscle
208 gene polymorphism to be associated with performance across multiple athlete cohorts [5]. The 577RR
209 genotype has been previously associated with elite, power-oriented athletic status (i.e., sprinters,
210 jumpers and throwers) in several cohorts of Caucasian athletes [7, 10-13, 19, 25], with one exception
211 [8]. With regards to this, a recent meta-analysis showed a strong association between the 577RR
212 genotype and power athletic performance especially among Europeans, regardless of the significant
213 heterogeneity among the groups of athletes [5].

214 Team sports are intermittent in nature and require the repetition of many powerful movements
215 such as short-distance sprinting and jumping [26], and these actions require the working muscles to

216 produce force at a high velocity [2]. We therefore hypothesis that the 577RR genotype frequency
217 distribution would be higher in team-sport athletes compared to the control group. We have shown that
218 team-sport athletes were less likely to harbour the *ACTN3* 577RR genotype, compared to the 577XX
219 genotype, than power athletes. Furthermore, when combining all groups of European athletes, compared
220 to the team-sport cohort the association between 577RR genotype and power athletic status remained
221 significant. An explanation for the overall clear association between the 577RR genotype and power
222 performance, and the unclear association with team-sport performance across multiple independent
223 cohorts, is that the original association study between the *ACTN3* 577RR genotype and elite power
224 performance [12] was performed with Australian (European decent) predominantly elite sprint/power
225 and endurance athletes. Most of the replication studies were also performed with predominantly
226 sprint/power athletes. Taken together with current literature, our data collected in the predominantly
227 power/sprint athletes demonstrate that across different ancestries the *ACTN3* 577RR polymorphism is
228 associated with the unique power/sprint muscle phenotype. This is not typical of association studies
229 involving the *ACTN3* R577X polymorphism and team-sport athletes [19-21] presumably due to the
230 mixed nature of team-sport events, which rely on both the aerobic and anaerobic energy systems [27].

231 Given that team-sport athletes perform multiple sprints and jumps during a match, we
232 hypothesised that the frequency distribution of the 577RR would be higher in team-sport athletes
233 compared to controls. However, once we explored this association in a relatively large cohort of team-
234 sport athletes (n=205), all European Caucasians, we found no association between the *ACTN3* R577X
235 polymorphism and team-sport athletic status. We assume that the inconsistent results provided by some
236 previous reports [19-21] can be attributed to the relatively small sample size of the studied cohorts, and
237 consequently low statistical power. This supports the need for larger cohorts with clearly-defined
238 phenotypes to reach more solid conclusion in human association studies.

239 The *ACTN3* R577X polymorphism association with sprint/power performance, in the present
240 study, is supported by the *Actn3* knock-out (KO) mouse model, which was developed to understand the
241 functional consequence of the *ACTN3* R577X polymorphism [28]. The KO mouse-model revealed,
242 among other findings, that compared with their wild-type (WT) counterparts, *Actn3* KO mice (i.e.

243 *ACTN3* 577XX genotype) have (1) lower muscle mass due to lower diameter of the fast twitch muscle
244 fibres (where α -actinin-3 is primarily expressed); and (2) A significant lower grip strength. Furthermore,
245 α -actinin-3 deficiency (the 577XX genotype) results in a shift in muscle properties towards those of
246 slow (type I) muscle fibre. Fast twitch muscles from KO mice have also significantly lower anaerobic
247 enzyme activity and higher oxidative/mitochondrial enzyme activity, without a shift in fibre-type
248 distribution [29]. These observations provide plausible explanation for the overall reduced sprint
249 capacity in humans with the 577XX genotype, and possibly increased in sprint capacity in humans with
250 the 577RR genotype [1, 30].

251 We believe that the results of this carefully controlled study are valid, as we strictly followed the
252 latest genotype:phenotype study recommendations [22] and all of the following criteria have been met:
253 all studied participants presented the main study phenotype (i.e., being a professional team sports
254 athletes). Although we studied three cohorts, participants within and between each cohort were both age
255 and ethnically-matched (all European Caucasians), genetic assessment was accurate and unbiased, with
256 genotype distribution being in Hardy-Weinberg equilibrium (HWE) in both cases and controls.

257

258 **v. Conclusion**

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

262

263 **vi. Practical implications**

- 264 • The results of the present study can assist to understand which genetic profiles contribute to team-
265 sport performance.
- 266 • Discovering the complex relationship between gene variants and team-sport performance may assist
267 coaches to optimize training.

268

269

270 **vii. Acknowledgments**

271 The study was supported by grants from:

272 1) Consejo Superior de Deportes, Spain (CSD, grant # 001/UPR10/12).

273 2) Ministry of Sport of the Russian Federation.

274

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346

347

ix. Tables

Table 1. Genotype distribution (Frequency and percentages) of genotypes according to nationality, sport type and level of competition.

	Spanish (n=426)				Polish (n=695)			Russian (n=335)				
	Team-sport	Power	Endurance	Control	Team-sport	Power	Endurance	Control	Team-sport	Power	Endurance	Control
All (n)	50	119	154	103	49	178	114	354	106	82	36	111
XX	18%	13%	26%	14%	18%	8%	9%	11%	13%	12%	8%	23%
RX	36%	55%	47%	57%	45%	52%	50%	50%	54%	48%	42%	41%
RR	46%	31%	27%	29%	37%	40%	41%	39%	33%	40%	50%	35%
MAF	0.360	0.412	0.497	0.422	0.408	0.339	0.338	0.356	0.401	0.360	0.292	0.441
HWE- <i>P</i> value	0.302	0.287	0.813	0.210	0.885	0.089	0.454	0.284	0.469	0.958	0.999	0.243
Elite (n)	50	119	139		9	118	70		55	56	26	
XX	18%	13%	27%		11%	8%	13%		11%	9%	12%	
RX	36%	55%	45%		67%	55%	40%		53%	46%	42%	
RR	46%	31%	27%		22%	36%	47%		36%	45%	46%	
MAF	0.360	0.412	0.500		0.444	0.360	0.328		0.373	0.321	0.327	
National Level (n)	-	-	15		40	60	44		51	26	10	
XX		-	13%		20%	7%	2%		16%	19%	0%	
RX		-	67%		40%	47%	66%		55%	50%	40%	
RR		-	20%		40%	47%	32%		29%	31%	60%	
MAF		-	0.467		0.400	0.300	0.352		0.431	0.442	0.200	

Table 2. Odds ratios of genotypes for athletes and control participants according to sport type.

Sport Type	XX (ref)	RX			RR			RX&RR (XX ref)			RR (RX & XX ref)		
	OR	OR	CI	<i>p</i>	OR	CI	<i>p</i>	OR	CI	<i>p</i>	OR	CI	<i>p</i>
Team-sport vs. Power	1	0.64	0.37-1.12	0.115	0.58	0.34-0.99	0.045	0.60	0.36-1.01	0.053	0.99	0.69-1.42	0.955
Team-sport vs. Endurance	1	0.80	0.46-1.39	0.436	0.85	0.48-1.52	0.589	0.83	0.48-1.39	0.467	1.00	0.67-1.50	0.998
Team-sport vs. Control	1	0.88	0.55-1.43	0.606	0.93	0.56-1.52	0.765	0.90	0.57-1.42	0.652	1.02	0.73-1.43	0.905

Abbreviations: CI: Confidence intervals; ref, reference; OR, odds ratio. Significant *p*-value is in bold.

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

Genotype	Team-sport		
	OR	CI	<i>p</i>
XX (ref)	1	-	-
RX	1.64	0.58-4.64	0.355
RR	1.58	0.53-4.73	0.412
RX-RR (XX ref)	1.61	0.59-4.39	0.348
RR (XX-RX ref)	1.07	0.53-2.19	0.846

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