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

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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.

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25 **Key words**: Genomics; alpha-actinin 3; exercise; athletes; genetics.

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i. Introduction

The field of genetics and elite athletic performance has made considerable progress in the last two decades, with various 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 [2].

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 [3]. North et al. [4] 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 [3].

The *ACTN3* R577X polymorphism has been investigated in the context of human athletic performance, in both elite endurance and power athletes [5-13], and the general population [14-16], 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 [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 $\geq$ 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 (≤200m, 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 ≤1000m (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 ≤200m (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 ≥5000m

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 metod, 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.

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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-P 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	*p*	OR	CI	*p*	OR	CI	*p*	OR	CI	*p*
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	*p*
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.