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The Distinguishing Factor in Soccer Players is Aerobic Performance, not the ACTN3 Gene

El Factor Distintivo en los Jugadores de Fútbol es el Rendimiento Aeróbico y no el gen ACTN3

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SUMMARY: The purpose of this study was to reveal the differences between ACTN3 genotype (RR, RX, XX) and aerobic performance [Yo-Yo IRT1 (m), $VO_{2\ max}$ (ml/kg/min)] in professional and regional amateur league soccer players and to reveal which of these parameters was a distinctive factor in these athletes.71 professional soccer players (age: 23.66 ±4.11 years; body height: 1.79 ± 6.99 m; body weight: 76.02 ± 6.76 kg; body fat: 11.59±3.11 %) and 62 regional amateur soccer players (age: 23.63 ±3.77 years; body height: 1.81 ± 5.77 m; body weight: 76.36 ± 7.53 kg; body fat: 15.60±4.65 %) volunteered for the study. After DNA extraction from buccal epithelial cells via a commercial kit was performed for the genetic background of the athletes, Real-Time PCR was carried out for genotyping. Furthermore, Yo-Yo IRT1 test was performed to determine the aerobic performance of the soccer players. SPSS 23 (SPSS Inc., Chicago, IL, USA) package program was used for the statistical analysis of the data obtained in the tests. Shapiro-Wilk test for normality and Levene's test for homogeneity of variance were performed. Chi-Square, Independent Sample T Test and One Way ANOVA test were used in the analysis of the parameters. Statistical significance was set as $p<0.05$. In the study, there found no meaningful statistical significance in terms of the rs1815739 polymorphism in the ACTN3 gene between the groups ($p>0.05$); however, there was a statistical significance in favor of professional soccer players in terms of aerobic parameters ($p<0.05$). Consequently, it can be said that aerobic performance is the distinguishing factor, not the ACTN3 gene, in soccer players.

KEY WORDS: Endurance; Sport Genetics; Polymorphism; Yo-Yo IRT1.

INTRODUCTION

Soccer is a sport that requires both low and high-intensity activities (Hulton *et al*., 2022). Though anaerobic energy is active during a soccer match, 90 % of energy spent is covered by the aerobic energy system (Hwang *et al*., 2022). Aerobic capacity is accepted to be essential for athletic performance. The higher an athlete's aerobic capacity is, the longer they can exercise at a particular intensity (Pickering *et al*., 2018). The ability to recover between two intense matches in athletes also depends on their aerobic capacity (Glaister, 2005; Stanula *et al*., 2014). It is determined by accurately measuring maximum oxygen uptake ($VO_{2\ max}$) (Astorino & White, 2010; Magnan *et al*., 2013). Athletic field tests are frequently used to determine $VO_{2\ max}$ to find out the athletes' performance (Jacob *et al*., 2018). However, direct VO_2 max testing is rarely performed in team sports due to the high costs and the necessity of

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special tools. Therefore, indirect $VO_{2\,max}$ measurements are more widely performed. These indirect measures are continuous timed runs or interval-based tests (Bangsbo *et al*., 2008; Buchheit, 2008). The soccer-specific Yo-Yo Intermittent Recovery 1 test has also been frequently used to determine $VO_{2\,max}$ in these days (Rossi *et al*., 2022; Rodrigues Lopes *et al*., 2022). Nevertheless, data obtained from field tests like VO2 max may not be sufficient to determine sportive performance since it is affected by many factors such as physical, technical, tactical, psychological, and genetic factors requiring endurance, speed, strength, coordination, and quickness.

Genetics have great influence on the components of athletic performance. Genetic factors explain 66 % of the variance of an athlete (De Moor *et al*., 2007). Therefore, athletes' genetics can play a key role in determining their success (Drozdovska *et al*., 2013). The ACTN3 gene is the most studied genetic polymorphism related to athletic performance (Dionisio *et al*., 2017; Ahmad Yusof *et al*., 2020). A common polymorphism in this gene causes a replacement of an arginine (R) with a premature stop codon (X) at amino acid 577 (MacArthur & North, 2007). Therefore, in the literature, the CC genotype is called RR, while the TT genotype is commonly referred as XX (referred to herein as R577X) (Jacob *et al*., 2018). Research show that the R allele is associated with sprint and power performance (MacArthur & North, 2004; Papadimitriou *et al*., 2016), while the X allele is associated with endurance (Pimenta *et al*. 2013).

In the studies on the ACTN3 gene with male soccer players (Sanlısoy *et al*. 2011; Egorova *et al*. 2014; Ulucan *et al*. 2015; Mutlucan *et al*., 2017; Honarpour *et al*., 2017; Salgueirosa *et al*., 2017), only genotype distributions were reported, and in few studies (Santiago *et al*. 2008; Pimenta *et al*. 2013; Coelho *et al*. 2016) genotype and $VO_{2\,max}$ were compared. When the results of these studies are compared, there seen some discrepancies. When the literature is examined in this respect, it has been seen that there is a need for further studies comparing genotype and performance parameters.

The aim of the study, therefore, was to determine the differences between the aerobic performance [Yo-Yo IRT1 (m), $VO_{2\,max}$ (ml/kg/min)] and ACTN3 genotype (RR, RX, XX) in soccer players playing in professional and regional leagues and to reveal which parameter was the distinguishing factor in these athletes' performance.

MATERIAL AND METHOD

Participants. 71 professional male soccer players and 62 regional amateur male soccer players (as the control group) volunteered for the study. Üsküdar University, Ethical Committee approved the study protocol (2021/18-61351342), and the study was conducted in accordance with the principles of the Declaration of Helsinki II. A written consent form that explaining the study procedure and its aims was signed by all the participants. This study was supported by Gümüshane University Scientific Research Organization (GÜBAP2901-21.A0311.01.01).

Procedure. After collecting buccal epithelial cell samples for the genetic testing of the athletes in a room reserved for the soccer players, the Yo-Yo IRT1 test was performed outdoors on artificial turf. Before this test was conducted, a standard warm-up (5-min jogging, 3-min dynamic stretching and 7-min specific drills) was instructed and supervised by the coach (Diker *et al*., 2023).

Genotyping. DNA extractions from buccal cells of athletes participating in the study were performed with the commercially available PureLink DNA extraction kit (Invitrogen, Thermo Fisher Scientific, Inc.). Genotyping of the ACTN3 rs1815739 polymorphism was performed using StepOnePlus Real-Time PCR (Thermo Fisher Scientific, Inc.) and Taqman Genotyping Mater Mix (catalog no. 4371355, Thermo Fisher Scientific, Inc.) genotyping kits following the manufacturers' protocols. A 10µl total reaction volume consisted of 5µl Genotyping Master Mix (Applied Biosystems, Foster City, CA), 3.5µl nuclease-free H_2O (Thermofisher, the USA), 0.5µl ACTN3 genotyping assay (Applied Biosystems) and 1µl DNA. The TaqMan Probe sequences used for genotyping are shown in Figure 1.

Yo-Yo IRT1: Aerobic fitness of the participants was measured using the Yo-Yo Intermittent Recovery Test, level 1 (Yo-Yo IR1), which is a reliable and valid measure specifically designed for assessing soccer performance (Krustrup *et al*., 2003). The results from field tests are not the best criteria to determine physiological variables, but relevant, specific, and operative field tests are recommended to be conducted in team sports (Krustrup *et al*., 2005). VO2 max of the participants was measured by analysing the distance covered in the YYIRT1 using the equation recommended by Bangsbo *et al*. (2008):

Fig. 1. Sequences of the TaqMan probe used for genotyping the ACTN3 rs1815739 polymorphism.

	Sequencing 5'- 3'
FAM/VIC	CAAGGCAACACTGCCCGAGGCTGAC**[T/C]**GAGAGCGAGGTGCCATCATGGGCAT

$VO_{2\ max}$ prediction (ml/kg/min) = Yo-Yo IRT1 distance (m) × 0.0084 + 36.4

Data Analysis. SPSS 23 (SPSS Inc., Chicago, IL, USA) package program was used for the statistical analysis of the findings obtained in the tests. Shapiro-Wilk test for normality and Levene's test for homogeneity of variance were performed. Chi-Square was used in the genotype analysis of the parameters, the Independent Sample T Test was used in the analysis of the intergroup field tests, and the One-Way ANOVA test was used in the genotype-performance comparison. Statistical significance was set as $p<0.05$.

RESULTS

ACTN3 genotype (Table I) and performance [Yo-Yo IRT1 (m), $VO_{2\ max}$ (ml/kg/min)] (Table II) findings of professional soccer players and regional amateur soccer players are shown below.

There was no statistically significant difference in terms of genotype parameter between professional and regional amateur soccer players ($p<0.05$). While XX genotype was found to be more dominant than RR genotype in professional soccer players, RR and XX genotypes were equally distributed in regional amateur soccer players. In addition, professional soccer players had a higher rate of XX genotype compared to regional amateur soccer players (respectively 45.16 % and 49.29 %). A statistically significant difference was found between professional and regional amateur soccer players in all three parameters [Yo-Yo IRT1 (m), $VO_{2\ max}$ (ml/kg/min)] ($p<0.05$). There was no statistically significant difference between genotype and aerobic performance parameters of both professional and regional amateur soccer players ($p>0.05$).

Table I. ACTN3 genotype of soccer players.

	ACTN3				
Variance	RR	RX	XX	Total	p
Professional Soccer Players	21 29.58 %	15 21.13 %	35 49.29 %	71	0.08
Regional Amateur Soccer Players	28 45.16 %	6 9.68 %	28 45.16 %	62	

$p<0.05$

Table II. The average Yo-Yo IRT1 (m), $VO_{2\ max}$ (ml/kg/min), standard deviation (sd), t and p values of professional and regional amateur soccer players.

		n	$\bar{x}$	sd	t	p
Yo-Yo IRT1 (m)	Professional Soccer Players	71	2386.61	454.97	-7.16	0.01*
	Regional Amateur Soccer Players	62	1844.22	417.60		
$VO_{2\ max}$ (ml/kg/min)	Professional Soccer Players	71	58.22	6.36	-7.21	0.01*
	Regional Amateur Soccer Players	62	51.89	3.51		

*Significant at $p<0.05$ level. x: arithmetic mean. sd: standard deviation.

Table III. Aerobic performance and genotype comparisons of professional and regional amateur soccer players.

			n	$\bar{x}$	sd	f	p
Professional Soccer Players	Yo-Yo IRT1 (m)	RR	28	2482.14	396.13	2.27	0.11
		RX	6	2553.33	263.64		
		XX	28	2555.36	514.16		
	$VO_{2\ max}$ (ml/kg/min)	RR	28	59.55	5.54	2.27	0.11
		RX	6	60.55	3.68		
		XX	28	56.38	7.20		
Regional Amateur Soccer Players	Yo-Yo IRT1 (m)	RR	21	1887.62	454.82	0.16	0.85
		RX	15	1818.67	504.29		
		XX	35	1829.14	361.87		
	$VO_{2\ max}$ (ml/kg/min)	RR	21	52.25	3.82	0.16	0.85
		RX	15	51.68	4.23		
		XX	35	51.76	3.04		

*Significant at $p<0.05$ level. x: arithmetic mean. sd: standard deviation.

DISCUSSION

Taking the aerobic structure of soccer into consideration, it was aimed to attain several significant results with this study. The first one was professional soccer players having a higher rate of XX genotype compared to soccer players playing in the regional amateur league. The second was them showing higher rates of aerobic performance [Yo-Yo IRT1 (m), $VO_{2\,max}$ (ml/kg/min)], and the third result was those with the XX genotype having a higher rate of aerobic performance. In the light of these, it was aimed to reveal whether these factors showed distinctive results.

Considering these expectations, here are the results of this study: professional soccer players (49.29 %) had, though not statistically significant, a higher rate of the XX genotype than amateur soccer players (45.16 %) (Table I). We also found out Yo-Yo IRT1 (m) and $VO_{2\,max}$ (ml/kg/min) values were found to be higher in professional soccer players (Table II). This difference was statistically significant. Since $VO_{2\,max}$ values were obtained via the Yo-Yo IRT1 test, it is natural that these data were similar to each other. Moreover, XX genotype did not make any difference in aerobic performance parameters (Table III). These findings show that we only achieved the first two goals. When the studies with male soccer players in the literature are examined, numerous inconsistent results have been encountered with.

Some of the contradictory results in the literature will be presented in this paragraph. Firstly, Mutlucan *et al*. (2017) in their study with soccer players found that 19 of the participants had RR (47.5 %), 15 of them had RX (37.5 %) and 6 of them had XX (15 %) genotypes. When the allelic distributions were analyzed, they noted that the R allele was 53 (66.25 %) and the X allele was 27 (33.75 %). In a similar study, Ulucan *et al*. (2015) examined the distribution of the same region polymorphism in 25 professional soccer players. They found the RR, RX, and XX genotype percentages to be 44, 36 and 20, respectively. Allelic distributions in the same study group were found to be 62 % for the R allele and 38 % for the X allele. They stated that ACTN3 genotypes were important biomarkers in terms of genetic counseling in successful soccer players (Ulucan *et al*., 2015). S¸anlısoy *et al*. (2011) stated the genotype values of the athletes as 32.4 % RR, 53.3 % RX, and 14.3 % XX in a study they conducted with 105 professional soccer players. They determined that the distributions of RR and RX genotypes of elite athletes were significantly different from sedentary individuals and emphasized that the ACTN3 gene could be a significant factor in the investigation of sport competence. Egorova *et al*. (2014) compared 246 Russian soccer players with a control group including 872 participants in their study and found the distribution of RR, RX, and XX genotypes as 46.25 %, 42.5 %, and 11.25 % respectively. As a result of the study, they attributed being a soccer player to having a positive gene variant. In another study, Lippi *et al*. (2010) found the frequency distributions of ACTN3 R577X genotypes in 60 elite soccer players as 48.3 %, 36.7 % and 15 % for RR, RX and XX, respectively. In all these studies, it can be seen that the RR genotype is higher than the XX genotype. These results contradict with the findings of our study. Since these studies are not supported by field tests, their results are not considered satisfactory. Therefore, we supported our genetic data with aerobic performance. Similar to the literature, we included the relationship between genotype comparison and the genotype-field test, and we also compared the field tests. We have discovered that field testing is a distinguishing factor. In the studies about this matter, inconsistent results have been obtained.

On the other hand, Coelho *et al*. (2016), investigated the relationship between ACTN3 R577X polymorphism and physical performance of 138 Brazilian professional U-20 and U-17vsoccer players and found out that there was not any significant difference between the ACTN3 gene and $VO_{2\,max}$ in the Yo-Yo IRT1 test, with which they determined endurance. Santiago *et al*. (2008), noted that the participants, 60 Brazilian soccer players, had 48.3 % RR, 36.7 % RX, and 15 % XX genotype, and sprinter characteristics, not aerobic processes, were dominant in elite soccer players. Koku *et al*. (2019), found that ACTN3 R577X genotype distribution did not have any effect on $VO_{2\,max}$ in amateur soccer players in their study with 100 soccer players. These studies support our findings.

Contrary to the results mentioned above, there are also studies that show results contradicting with the findings of our study. For example, Pimenta *et al*. (2013), reported ACTN3 RR, RX and XX genotypes as 45 %, 44 % and 22 %, respectively in their study with 200 professional soccer players. They noted that soccer players with ACTN3/XX genotype had the highest aerobic capacity in terms of the VO2 max values determined with the Yo-Yo IRT1 test. In Kasımay *et al*. (2009) study, which is accepted as a reference point for ACTN3 R577X studies, they determined the ACTN3 genotype distributions in 37 professional soccer players and compared the $VO_{2\,max}$ values. They stated that the $VO_{2\,max}$ values of the XX genotypes were statistically significantly higher when compared to other genotypes. In addition, they determined the genotype distributions and reported that 22 of them were RR (59 %), 11 of them were RX (30 %), and 4 of them were XX (11 %) genotype.

There are also studies reporting a statistical difference in genotype frequency (RR, RX, XX) but no difference in

allele frequency (R, X) (Honarpour *et al*., 2017) or in both parameters (Salgueirosa *et al*., 2017) between being professional soccer player and being a member of the general population when the ACTN3 gene was into consideration.

LIMITATIONS. One of the most important limitations of this study was the number of soccer players included. Therefore, the results should be interpreted with caution. It is recommended that future studies include higher number of athletes to validate the results. Another significant limitation is the number of genes studied. If we had had a higher budget, we could have studied more of the genes associated with athletic performance. Therefore, it is recommended to conduct such studies with a higher budget.

CONCLUSION

It should not be forgotten that both physical and technical-tactical training affect performance in soccer (Coelho *et al*., 2016). Instead of discovering talents by using genetic information, it would be more accurate to direct people to the sports branch they are suitable for and to allow athletes to reach their maximum potential (Pickering *et al*., 2019). Conflicting results in the literature suggest that more multifaceted studies should be conducted in this area. In this sense, gene-field test results should be compared in studies targeted to reveal more clear distinction. Thus, according to our study results, although there is no relationship in terms of genotype between the groups, there is a difference in aerobic parameters. Therefore, we can say that aerobic parameters, not ACTN3 gene, are distinguishing factors in soccer players in our study.

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RESUMEN: El objetivo de este estudio fue revelar las diferencias entre el genotipo ACTN3 (RR, RX, XX) y el rendimiento aeróbico [Yo-Yo IRT1 (m), $VO_{2\,max}$ (ml/kg/min)] en jugadores de fútbol de ligas profesionales y amateurs regionales y determinar cuál de estos parámetros es un factor distintivo en estos deportistas. 71 futbolistas profesionales (edad: 23,66 ±4,11 años; altura corporal: 1,79 ± 6,99 m; peso corporal: 76,02 ± 6,76 kg; grasa corporal: 11,59±3,11 %) y 62 jugadores de fútbol amateur regionales (edad: 23,63 ± 3,77 años; altura corporal: 1,81 ± 5,77 m; peso corporal: 76,36 ± 7,53 kg; grasa corporal: 15,60 ± 4,65 %) se ofrecieron como voluntarios para el estudio. Después de realizar la extracción de ADN de las células epiteliales orales mediante un kit comercial para obtener los antecedentes genéticos de los atletas, se llevó a cabo una PCR en tiempo real para el genotipado. Además, se realizó la prueba Yo-Yo IRT1 para determinar el rendimiento aeróbico de los futbolistas. Para el análisis estadístico de los datos obtenidos en las pruebas se utilizó el programa SPSS 23 (SPSS Inc., Chicago, IL, EE. UU.). Se realizó la prueba de normalidad de Shapiro-Wilk y la prueba de homogeneidad de la varianza de Levene. En el análisis de los parámetros se utilizaron Chi-cuadrado, prueba T para muestra independiente y prueba ANOVA unidireccional. La significancia estadística se estableció en $p<0,05$. En el estudio no se encontró significancia estadística significativa en cuanto al polimorfismo rs1815739 en el gen ACTN3 entre los grupos ($p>0,05$); sin embargo, hubo significación estadística a favor de los futbolistas profesionales en cuanto a los parámetros aeróbicos ($p<0,05$). En consecuencia, se puede decir que el rendimiento aeróbico es el factor distintivo, no el gen ACTN3, en los jugadores de fútbol.

PALABRAS CLAVE: Resistencia; Genética Deportiva; Polimorfismo; Yo-Yo IRT1

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