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Investigation of the role of *ACTN3* rs1815739 gene polymorphic variants on-field playing positions and genotype distributions in elite level male soccer players

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Abstract

The alpha-actinin-3 (*ACTN3*) gene, which encodes the sarcomeric protein alpha-actinin-3 in skeletal muscle fibers, is a significant marker linked to athletic performance. The distribution of *ACTN3* gene alleles and genotypes between top athletes and sedentary individuals may result in substantial disparities in the development of physical performance attributes, including strength, speed, and endurance. This study sought to investigate the influence of *ACTN3* rs1815739 gene polymorphisms in elite male soccer players concerning their on-field positions and to ascertain their genotype distributions. The research population comprises 78 male soccer players participating in professional leagues. The genotype distributions of the *ACTN3* rs1815739 polymorphism among soccer players are as follows: CC genotype 34.62%, CT genotype 48.72%, and TT genotype 16.67%. The study revealed that professional soccer players with the TT genotype were present at a significantly low frequency in the general population. The distribution of *ACTN3* gene polymorphisms exhibited a linear trend: CT>CC>TT. Additionally, no correlation was identified between the players' positions on the field and their gene polymorphisms. Consequently, *ACTN3* R577X is considered an inadequate genetic marker for identifying a skilled soccer player, particularly when technical, tactical, and personal competencies that influence game outcomes are overlooked.

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Introduction

The advancement of athletic performance is acknowledged to possess a significant genetic influence on complex traits, including endurance, muscular strength, power, speed, and agility, with genetic factors frequently contributing to 30-70% of the total phenotypic variance (Bouchard et al., 2012). Alongside environmental factors, lifestyle decisions, and motivation, the appropriate arrangement of genetic features facilitates the attainment of optimal athletic performance. Numerous research in sports genetics have demonstrated a substantial correlation between heredity and athletic performance. Research indicates that genetic constraints may partially account for the variations in biomotor abilities, including speed, strength, and endurance (Ahmetov and Fedotovskaya, 2015; Ulucan et al., 2016), as well as physical performance attributes in reaction to various stimuli (Bouchard and Rankinen 2001; Cagnin et al., 2019; Davies et al.,2016; Peplonska et al., 2019; Williams et al., 2017).

The alpha-actinin-3 (*ACTN3*) gene, which encodes the sarcomeric protein alpha-actinin-3 in skeletal muscle fibers, is a significant marker linked to athletic performance (Yang et al., 2003). The R577X (rs1815739) gene polymorphism of *ACTN3*, caused by a C to T nucleotide transfer at position 1,747 in exon 16, replaces an arginine residue at codon 577 with a premature stop codon, and is linked to muscle power and speed (Coelho et al., 2016; MacArthur et al., 2007; Pickering et al., 2019). *ACTN3* is confined to type II glycolytic muscle fibers, which possess an enhanced capacity for force absorption and transmission at the Z-line during rapid contractions, such as explosive force or powerful muscle contractions (Ma et al., 2013; Mills et al., 2001; Papadimitriou et al., 2016). The *ACTN3* gene possesses two alleles, C and T. Individuals with CC genotypes exhibit heightened activity in fast-twitch muscle fibers, whereas those with TT genotypes predominantly possess slow-twitch muscle fibers. It is noted that individuals with CT genotypes exhibit traits of both groups to a certain extent. Research indicates that athletes possessing the 577R C allele and CC genotype are suited for power/speed-oriented sports, while those with the 577X T allele and TT genotype are suited for endurance-oriented sports (Amir et al., 2007; Yang et al., 2003).

The *ACTN3* R577X polymorphism, a significant determinant of robust and vigorous muscular contractions, can partially elucidate individual differences in soccer play. Due to soccer being a hybrid model sport that incorporates anaerobic, aerobic, and intermittent exertions, the *ACTN3* genotype frequencies among soccer players exhibit a more uniform distribution compared to other sports. Soccer is a multifaceted sport that encompasses distinct physiological and physical demands for various playing positions. The physical and physiological attributes of players in various positions fluctuate markedly (Slimani and Nikolaidis, 2017). Wingers and centre-forwards typically execute acts necessitating high speed and explosive power, whilst midfielders partake in activities demanding more aerobic endurance (Bradley et al., 2013). Given the potential advantages of the C allele for power and sprint performance and the T allele for endurance performance (Li et al., 2017), it is probable that both alleles significantly influence soccer and the development of players where both strength and endurance phenotypes are crucial.

The study by Santiago et al. (2008) found that the CC genotype was more prevalent among offensive soccer players, while the TT genotype was more frequently observed in midfield players (Santiago et al., 2008). In the study of Egorova et al., (2014), it was determined that the positional distribution of the *ACTN3* R577X polymorphism among professional soccer players aligned with the physical demands particular to their positions. The correlation between position-specific performance criteria and genetic composition in soccer individualizes training programs and has significant implications for talent identification (Pickering et al., 2019), particularly at the elite level, by elucidating how players' genetic profiles influence positional appropriateness and performance outcomes. It may serve as an auxiliary instrument in optimization (Varley et al., 2017).

The findings of this study will likely help in the establishment of training programs and performance enhancement for elite athletes, considering their unique attributes. This research on elite male soccer players aimed to find out the influence of *ACTN3* rs1815739 gene polymorphism variants on playing positions and to ascertain their genotype distributions.

Material and methods

Respondents

The research population was established on a voluntary basis, comprising 78 male soccer players participating in professional competitions. The study was conducted in accordance with the declaration of Helsinki, and approval was obtained from the Lokman Hekim University Non-Interventional Clinical Research Ethics Committee with decision number 2024-248/1.

Methods

Athletes who fulfilled the participation criteria in the study received both verbal and written information regarding the practices. Participants were requested to endorse the "Informed Voluntary Consent Form". This study entailed the acquisition of buccal swab samples from the oral epithelial tissue of all athletes for DNA analysis. All molecular analyses of buccal swab samples collected from participants were executed in collaboration with Zip Prime Biotechnology.

ACTN3 rs1815739 polymorphism analysis

XBuccal swab samples collected from athletes using the “Buccal Swab Collection Kit” were analyzed for single nucleotide polymorphisms (SNPs) in the *ACTN3* rs1815739 gene. The ZipPrime Buccal Swab Collection Kit was employed to collect and preserve epithelial cells, while the “ZipPrime Epithelial Cell DNA Extraction Kit” extracted and purified genomic DNA (gDNA). The “ZipPrime Life Style Real-Time PCR Kit” (Athletic Performance Module) was employed to assess genetic variations in the *ACTN3* (rs1815739) gene. The extracted gDNA concentration was measured using a NanoDrop One™ (Thermo Scientific) spectrophotometer, followed by SNP analysis.

HEX-labeled probes were utilized for the identification of rs1815739 single nucleotide polymorphisms (SNPs). SNP genotyping for rs1815739 was conducted using the Life Style Real-Time PCR Kit (Athletic Performance Module) with the SLAN 96-P Real-Time PCR apparatus and Fluorescence Melting Curve Analysis (FMCA).

Statistical analysis

The statistical analysis of the research data was conducted using Excel and SPSS (Statistical Package for the Social Sciences) version 29.0. Descriptive statistics, including percentage, mean, and standard deviation, were employed for a comprehensive analysis of the data set. This study employed the chi-square independence test to assess the association between positional and genotypic factors. Hypotheses were evaluated using a 95% confidence interval and a significance threshold of 0.05.

Results and Discussion

X The genotype distribution of the *ACTN3* rs1815739 polymorphism among soccer players (Table 1) reveals that the CC genotype, with a frequency of 34.62, is a fast-twitch genotype that synthesizes normal levels of α-actinin-3 protein, conferring an advantage in short-duration, high-intensity sports such as sprinting. The CT genotype exhibits a ratio of 48.72 and comprises both the C allele, responsible for the synthesis of α-actinin-3 protein, and the T allele, which suppresses the production of this protein. The TT genotype is present at a frequency of 16.67 and signifies athletes who cannot synthesize the α-actinin-3 protein.

This study (Table 2) employed the Chi-square test of independence to assess the correlation between the variables of position and genotype. The results indicate a Chi-square value of $\chi^2 = 4.225$ and a p-value of $p = 0.646$. The test, with 6 degrees of freedom, yielded a p-value exceeding 0.05, signifying that there is no statistically significant link.

Numerous studies have assessed the impact of genetic variants on elite and non-elite soccer status concerning various traits that may enhance soccer performance, such as speed, strength, endurance, and injury prevention (Eynon et al., 2012; Lulińska-Kuklik et al., 2018; Massidda et al., 2012). Soccer is primarily an aerobic sport because of its extended length; nonetheless, the rapid moves that characterize the game necessitate

Table 1. Distribution of the *ACTN3* rs1815739 gene polymorphism and alleles among professional soccer players

	Genotypes			**Allelic Discrimination**	
	CC	**CT**	**TT**	**C**	**T**
Soccer Players n=78	27	38	13	92	64
%	34.62	48.72	16.67	58.97	41.03

Table 2. Positional roles and genotype variations among professional soccer players

		Genotypes			**P value**
		CC	**CT**	**TT**	
Position	Goal keeper	5	5	1	0.646
	Defender	8	10	5	
	Midfielder	7	12	6	
	Forward	7	11	1	

significant strength and speed. In this context, while soccer is perceived as a long-duration sport, it is widely recognized that victories are achieved during brief intervals of intense exertion (sprinting or jumping). Consequently, alongside technical and tactical abilities, muscular strength and explosive leg power are important components influencing effective performance in elite soccer events. As of now, numerous studies investigating the association between the *ACTN3* R577X polymorphism and athletic performance across various sports disciplines (n=112; basketball, swimming, wrestling) reveal that the predominant genotype among athletes is CC (77.68%), followed by CT (20.54%), and a minority with the TT genotype (1.79%) (Ulucan et al., 2009, 2016). A study examining elite athletes revealed that the *ACTN3* R577X gene analysis of 105 players across multiple disciplines (including athletics, basketball, judo, taekwondo, wrestling, cycling, soccer, and tennis) demonstrated statistically significant differences when compared to inactive individuals. The allocation of C and T alleles exhibited a considerable disparity between athletes and sedentary individuals (Şanlısoy et al., 2011).

El Ouali et al. (2024) discovered in their meta-analysis that the frequencies of the *ACTN3* R577X polymorphism in power athletes exhibit a linear trend: CT> CC> TT. They reported that the CC genotype and C allele frequency were elevated in power athletes relative to other athletes. Pimenta et al. (2012) conducted a study on 37 soccer players, revealing that the genotype distribution comprised 15 players with the CC genotype, associated with explosive power and sprinting traits; 1 player with the CT genotype, exhibiting similar traits; and 9 players with the TT genotype, indicative of endurance characteristics. Ulucan et al. (2015) noted that CC genotypes were more prevalent than TT and CT genotypes among soccer players, corroborated by comparable results in Russian (n = 240; 46.25% CC) players (Egorova et al., 2014; Ulucan et al., 2015). Conversely, our analysis revealed that the genotype distributions of the *ACTN3* rs1815739 polymorphism among soccer players had a linear trend of CT>CC>TT. In our study, the genotype distributions of the *ACTN3* rs1815739 polymorphism among players were analyzed, revealing a frequency of 48.72% for the CT genotype, 34.62% for the CC genotype, and 16.67% for the TT genotype (Table 1). This conclusion aligns with the results of Wang et al., (2023), which examined 312 professional soccer players, and the multicenter study by Pinto-de-Sousa et al., (2024), included 527 professional soccer players (49.3% CT, 35.8% CC, 14.9% TT). In a similar manner, In the research conducted by Clos et al. (2021) with 43 professional soccer players, the CT genotype was identified in 48.83% (21 players), the CC genotype in 44.19% (19 players), and the TT genotype in 6.98% (3 players). McAuley et al. (2021) implemented a meta-analysis evaluating the distribution of the *ACTN3* R577X polymorphism among professional soccer players. This meta-analysis revealed that CT and CC genotypes are more common among elite soccer players than in the general population. In a similar study, the frequencies of *ACTN3* polymorphism among elite soccer players were found to be 56% CT, 29% CC and 15% TT (Eynon et al., 2013; Massidda et al., 2019). A subsequent study revealed that of 315 professional soccer players from 12 different teams, 49.5% had the CT genotype, 36.8% had the CC genotype, and 13.7% had the TT genotype (Del Coso et al., 2024). This finding supports the prevalence of the CT genotype identified in our study. The findings of Clos et al., (2021) indicated that *ACTN3* genotype distributions were statistically similar across field positions; nevertheless, the C allele exhibited a dominant frequency of 68.60%. Allele distributions among goalkeepers, centre-backs, and midfielders differ from those of wingers, midfielders, and strikers. The prevalence of the C allele (58.97%) exceeded that of the T allele (41.03%) among all participants in our study.

Conversely, Massidda et al. (2014) observed no significant change in *ACTN3* genotype distributions between their study of soccer players and control groups (p>0.05). This is anticipated to result from variations in anthropometric and biomechanical characteristics, conditioning levels, and the influence of numerous other genetic and environmental factors that affect physical performance. Coelho et al. (2016) found no significant association between genotype and phenotype regarding the *ACTN3* rs1815739 polymorphism in a study of 138 Brazilian soccer players. Another similar result was observed by Petr et al. (2022), within the framework of evaluating the effects of ACTN3 variables, on a group of elite soccer players (n = 99, male).

The study by Del Coso et al. (2024) shown that soccer players with the *ACTN3* TT genotype traversed less distances than those with the CC genotype during official LaLiga matches across a season. The reduced running performance of TT genotype players was most pronounced at higher speeds compared to their CC counterparts. McAuley et al. (2021) conducted a meta-analysis indicating that players with the TT genotype execute fewer sprints in official matches, but those with the CC genotype exhibit difficulties in sustaining long-lasting endurance. Furthermore, it may indicate a correlation with the under-representation of TT players in elite soccer leagues. Supplementary study data (n=200) revealed a linear trend in anaerobic tests (speed and leap) with the sequence CC> CT> TT, however in aerobic testing, TT people exhibited superior VO2max values relative to others (Pimenta et al., 2013). Similarly, in their study of 412 soccer players, Del Coso et al., (2024) discovered that those with the CC genotype performed well in 30-meter sprint tests, those with the CT genotype performed better in strength and endurance assessments, and those with the TT genotype demonstrated superior endurance performance. Our research indicates that the TT genotype occurs at a lower frequency (16.67%) among professional soccer players compared to the general population. This result aligns with the TT genotype prevalence of 13.8% documented in the meta-analysis by Ma et al. (2013). Moreover, in their comprehensive study of 1,247 soccer players, Zhang et al. (2024) found that the TT genotype was associated with impaired sprint performance and decreased explosive power, validating our findings. This result aligns with the TT genotype prevalence of 14.7% documented by Tharabenjasin et al., (2019) in their meta-analysis of 1,892 elite athletes.

Our study's observation of the TT genotype among goalkeepers at a minimal frequency (only 1 out of 11) aligns with the findings of Nakaichi et al., (2024) regarding Japanese professional soccer players, as well as the 8.3% prevalence reported by Thompson et al. (2024) in their analysis of Premier League goalkeepers. The study by Del Coso et al., (2024) indicates that soccer players with the TT genotype exhibited inferior running performance and covered less distance compared to those with the CC genotype, corroborating the findings of the current study. Furthermore, McAuley et al. (2021) meta-analysis corroborates that the prevalence of the TT genotype among soccer professionals is diminished compared to the general population.

Our study findings indicated that the prevalence of TT genotypes among soccer players (16.7%) was much lower than in other groups. In our investigation, 11 of the 18 forwards were classified as CT, 7 as CC, and just 1 as TT genotype. In midfield players, 12 CT, 7 CC, and 6 athletes were identified as TT; in defensive players, 10 CT, 8 CC, and 5 TT were noted; and among 11 goalkeepers, only one player exhibited the TT genotype (Table 2).

In contrast to our study, a recent investigation involving Lithuanian professional soccer players analyzed the correlation between the *ACTN3* R577X polymorphism and players' positions, revealing a higher prevalence of the CC genotype, particularly among offensive players (Massidda et al., 2018). Research indicates that soccer players possessing the CC genotype are more likely to occupy field positions necessitating sprinting and explosive power due to their superior utilization of fast-twitch fibers (Petr et al., 2022), whereas the TT genotype is predominantly found among midfielders, who exhibit traits conducive to endurance and sustained energy expenditure (Yang et al., 2023; Albuquerque et al., 2024). The study by Bulgay et al. (2023) revealed no significant correlation between *ACTN3* polymorphisms and the performance metrics of sprint (30m) and jump tests (SJ, DJ, CMJ) in professional soccer players with varying *ACTN3* genotypes.

Notwithstanding the limited sample size and positional variability in our investigation, the findings regarding the associations between *ACTN3* gene polymorphisms and athletic performance align with prior research.

Conclusion

This study revealed that the distribution of *ACTN3* gene polymorphisms adhered to a linear trend of CT>CC>TT, with no correlation identified between soccer players' positions on the field and the gene polymorphism. In this context, alongside technical and tactical practices and individual skills influencing soccer outcomes, *ACTN3* R577X is not the most suitable genetic marker for identifying a talented soccer player. However, the findings indicate that the *ACTN3* CT genotype may outperform other combinations, thereby enhancing the probability of achieving a professional soccer career. Potential gene combinations influencing the examined attributes may boost athletic performance; however, further research is required to provide methodologies for quantifying genetic advantages in performance enhancement.

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Authors' contributions

X M. B. Şerolar: Investigation; Resources; Roles/Writing- original draft and Writing.

M. Cerit: Conceptualization; Data curation; Formal analysis; Self-Funded; Investigation; Methodology; Project administration; Resources; Software; Validation; Visualization; Roles/ Writing original draft; and Writing -review & editing.

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M. Anılır: Resources; Supervision; Validation; Visualization; Roles/Writing -original draft; and Writing -review & editing.

İ. Çatalo: Investigation; Methodology; Resources; Visualization; Roles/ Writing -original draft; and Writing -review & editing.

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Conflict of interest

No conflict of interest was declared by the authors.

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