Sprint and Anaerobic Power with the Soccer-Specific ACTN3 Gene: A Distintive Example

Sprint y Potencia Anaeróbica con el Gen ACTN3 Específico del Fútbol: Un Ejemplo Distintivo

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SUMMARY: The aim of this study is twofold: (1) to identify differences in certain anaerobic parameters (10m sprint, 30m sprint, anaerobic power, and Illinois agility tests) between professional and amateur soccer players, and (2) to determine whether there is a difference in the *ACTN3* gene polymorphism between professional and amateur soccer players. Ultimately, the goal is to reveal which parameters contribute to the differentiation in these two aspects. A total of 133 volunteer soccer players, including 71 professionals and 62 amateurs, participated in the research. DNA extraction from buccal epithelial cells was performed using a commercial kit to determine the genetic background of the athletes, and Real-Time PCR was conducted for genotyping. Statistical analysis of the findings obtained from the test results was performed using the SPSS 23 (SPSS Inc., Chicago, IL, USA) package program. The homogeneity of variance of the data was assessed using the Levene Test, and normal distribution analyses were conducted using the Shapiro-Wilk Test. Chi-square and Mann-Whitney U tests were employed for parameter analysis. The significance level was set at p<0.05. Evaluation of the data in our study revealed no statistically significant difference in *ACTN3* rs1815739 gene polymorphism between the groups (p>0.05). However, there is a statistically significant difference in anaerobic parameters (10m sprint, 30m sprint, and anaerobic power) except for the Illinois test (p<0.05). In conclusion, our study found that gene polymorphism is not a differentiating factor between professional and anaerobic power) except players, but speed (10m and 30m) and anaerobic power parameters are differentiating factors.

KEY WORDS: Gene; Power; Polymorphism; Sprint.

INTRODUCTION

In recent times, the concept of sporting performance or athletic performance has been widely discussed in soccer. When examined, it can be defined as the entirety of efforts put forth for the success of an athletic task that a soccer player must perform or accomplish. Moreover, it is comprised of factors that can influence the outcome in a short period, viewed as a whole (Clos *et al.*, 2019). In soccer, it would be appropriate to consider the concept of sporting performance as the ability of a soccer player to generate athletic work, occurring despite all positive and negative factors. From this perspective, the evaluation of sporting performance requires taking into account all factors and components influencing performance (Massidda *et al.*, 2018).

When evaluated from the perspective of sports science, the issue of genetics and sporting performance is primarily among the new topics that biologists and exercise scientists are actively exploring (Eroglu & Zileli, 2015). The

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early detection of athletic talents, directing individuals towards the right sports, and establishing appropriate training plans at the competitive level are crucial factors for success (Ma *et al.*, 2013). Genetic factors, particularly those playing a significant role in the achievement of maximum success for soccer players, push the boundaries of science. Numerous studies focusing on the investigation of inter-player differences in soccer indicate that genetically, certain factors contribute significantly (Sunje *et al.*, 2021). Especially in determining the differences between professional and amateur soccer players, physical performance tests in the context of sports genetics hold great importance (Massidda *et al.*, 2012).

In contemporary soccer, the genetic factor, which is among the internal factors influencing sporting performance, is observed to contribute approximately 60-65 % of the performance variable that constitutes physical capacity (Clarkson et al., 2005; Abrantes et al., 204)). Studies indicate that genetic factors play a significant role in many functional aspects related to sporting performance (McAuley et al., 2021). The genetic factor directly influences essential aspects of sporting performance, such as muscle type distribution, musculoskeletal structure, lung capacity in metabolic terms, and the ergonomic balance of using energy, which is crucial in soccer (Gayagay et al., 1998; Myerson et al., 1999). The genetic factor is also seen to affect features related to the ergonomic use of energy, such as muscle enzyme activity, contraction speed, reaction time, and anaerobic endurance (Clarkson et al., 2005). Studies suggest that the participation of sports genetics is a fundamental reality for success in activities within soccer, emphasizing the role of sports genetics in these activities (Cooper et al., 2010; Clos et al., 2019).

Upon reviewing the literature, it is evident that one of the forefront genes investigated in the genetic aspect of sporting performance in soccer is alpha-actinin-3 (ACTN3) (Ulucan, 2016; Eroglu et al., 2018; Zileli et al., 2023). From a genetic perspective, alpha-actinin-3 gene, which examines sporting performance, has four different isoforms of the protein: ACTN1, ACTN2, ACTN3, and ACTN4 (Otey & Carpen, 2004). The alpha-actinin protein involved in studying sporting performance is a protein specific to skeletal muscles responsible for fast muscle contractions in glycolytic type and type-II X muscle fibers, and is associated with sporting performance (Ulucan et al., 2015). The gene encoding alpha-actinin is located on the long arm of chromosome 11 (11q13.1). The alpha-actinin gene consists of 22 exons, containing 901 amino acids, and the alphaactinin protein is present in the Z lines of the sarcomeres (Döring et al., 2010). ACTN3 protein encoded by this gene actively participates in muscle contraction, binding of actin

filaments, and intracellular signal transmission. Research has focused on the rs1815739 polymorphism resulting from the C>T transition in the 16th exon of the *ACTN3* gene for determining power/sprint and endurance characteristics in athletes. This amino acid change leads to the formation of a stop codon (X) instead of the codon encoding arginine (R) at the 577th amino acid position of the resulting protein (Eken *et al.*, 2021).

When referring to ACTN3, the characteristics associated with this gene indicate its connection to sporting performance. ACTN3 gene, which is one of the genes highly influential on sporting performance and the first structural gene based on sporting performance traits (Charbonneau et al., 2008), is also linked to skeletal muscle. ACTN3 gene is a major component of the skeletal muscle Z disk in fasttwitch muscle fibers, and it interacts with multiple metabolic proteins (Seto et al., 2013). The ACTN3 gene, crucial for the formation of speed and powerful contractions, is located on the long arm of the 11th chromosome (11q13.1), particularly needed in explosive sports (Ulucan & Göle, 2014). The ACTN3 protein is found in the Z lines of sarcomeres associated with skeletal muscles, playing a significant role in sporting activities dependent on muscle strength, especially (Ulucan et al., 2009).

Soccer is a highly competitive sport, a characteristic that is closely tied to the intricate nature of its requirements, encompassing technical, tactical, psychological, and physiological domains (mendez *et al.*, 2011). Key physiological factors in soccer involve a combination of endurance and speed (Rampini *et al.*, 2006; Chen *et al.*, 2019), such as repeated short sprints (Oliva Lozano *et al.*, 2020). Additionally, modern soccer has increased demands for strength and power. Heritability studies on sport-related traits suggest that genetic factors play a significant role in determining endurance, speed-power, and strength abilities (Guilherme *et al.*, 2021). This genetic influence may be, at least in part, attributed to the specificity of muscle fibers genetics (Lewis *et al.*, 2015).

Various physical ability for soccer players, strength correlate with soccer sprints (Booysen *et al.*, 2016) and agility ability (Harper *et al.*, 2021) and differs by soccerplaying leagues (Coelho *et al.*, 2016). Consequently, the force applied at varying speeds and agility levels can typically elucidate the required force-velocity predisposition of athletes (Santiaogo *et al.*, 2008; Secérovic *et al.*, 2017), serving as potential indicators for the agility and jumping performance of soccer players (Coratella *et al.*, 2018). In terms of the force-velocity and agility profile, soccer performance is characterized by elements such as high-speed running and sprinting (Gissis *et al.*, 2006), acceleration and deceleration, as well as change of direction performance (Modriç *et al.*, 2019). As there is no distinct correlation observed between playing leagues and conditioning specificity, it remains uncertain whether the force-velocity specificity is genetically predetermined for soccer positions. Conversely, certain genetic markers have been linked to the metabolic traits of soccer attackers (Pimenta *et al.*, 2013).

Over the recent years, numerous candidate gene studies have explored the impact of various genetic polymorphisms on the speed, power, and agility performance of soccer players (Clos et al., 2021). Several studies have been conducted on the impact of the ACTN3 rs1815739 polymorphism on the rate of change in the sporting performance of professional soccer players (Secerovic et al., 2017). The research suggests that elite soccer players with CC (RR) and TT (XX) genotypes exhibit specific sensitivity to parameters related to the musculoskeletal system, strength, direction change, sudden acceleration, and agility (Rodas et al., 2021). It indicates that such attributes provide players with greater advantages within the game, leading to increased physical variations on the field and subsequent superiority in gameplay (Petr et al., 2022). Despite claims that genetic factors confer certain advantages to elite athletes, there is ongoing debate about whether these attributes result from genetic factors or acquired physical characteristics (Andrzejewski et al., 2015).

Based on the information above, highlighting the differences between professional and amateur league soccer players holds significance for the development of soccer. The aim of this study is twofold: (1) to identify differences in certain anaerobic parameters (10 m sprint, 30 m sprint, anaerobic power, and Illinois agility tests) between professional and amateur soccer players, and (2) to determine whether there is a difference in the *ACTN3* gene polymorphism between professional and amateur soccer players. Ultimately, the goal is to reveal which parameters contribute to the differentiation in this regard. We are confident that our examination of various leagues will make a valuable contribution to the existing literature, and the outcomes will lay the groundwork for future studies in the same domain.

MATERIAL AND METHOD

This study performed a cross-sectional study with genotyping, sprint speed, agility ability and power measurement of *ACTN3* at two different angular. Prior to the measurements, participants did not engage in any training sessions or other physically demanding activities. Samples were collected before the participants' general warm-up routine, which included 5 to 10 minutes of aerobic exercise

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(free jog), followed by 5 to 10 minutes of individual static stretching and 5 to 10 minutes of dynamic stretching involving hops and dynamic lunges. Subsequently, following the warm-up, all players underwent sprint speed testing (10-30m), followed by the ilionis agility test, with a 3-minute rest interval between individual attempts. Recently, 6 X 35m interspersed with 10 seconds of passive recovery repeated sprint test has been conducted.

Participants: The study sample comprises three professional teams aiming for championship in the Turkey soccer Second and Third Leagues during the 2021-2022 season (2nd League, n=20, 3^{rd} League, n=25, and 3rd League, n=26), and three amateur teams (n=62) striving for championship in the Regional Amateur League (Three Different Regional Amateur Temas, n = 20+20+22). Professional league players engaged in an average of 9.7 ± 2.8 hours of training per week throughout the season, while amateur team players conducted an average of 8.7 ± 1.9 hours of weekly training. Both groups played approximately one official match per week, totaling 30 official matches throughout the season. The age, anthropometric characteristics, training age, and weekly training hours of professional and amateur soccer players are presented in Table II. All participants included in this research are of white race and possess the characteristic of being national soccer players. The study protocol was approved by the Ethical Committee of Usküdar University (2021/14-61351342), and the study adheres to the principles of the Declaration of Helsinki II. Written informed consent, outlining the study's procedures and objectives, was obtained from all participants. This research received support from Gümüshane University Scientific Research Organization (GÜBAP2902-21.A0310.02.01). Throughout the entire experiment, the rights and confidentiality of the participants were protected, and genetic information was solely used for the purposes outlined in this study.

Table I. Study's Design.

Enrollment				
Volunteered Soccer Players				
Assessed for eligibility (n=133)				
Not Meeting Inclusion Criteria				
Injury history or limited physical strength,				
Smoking habit and Pharmacological ergogenic aids,				
On time for the test injuries, sickness or drop-out				
Consent to Participate				
Test Visit				
Volu nteering Consent				
Randomization & Counterbalanced Design				
Allocations				
Participants (n=133)				
Allocated Professional Players =71				
Allocated Regional Amateur Players = 62				
Body Composition Measurements = 08:00-12:00				
Running Base Anaerobic Sprint Ability Test (RAST) = 14:00-17:00				
End of Study and Analysis Assessment				

Looking at Table II, it can be observed that the average age of professional soccer players is 23.66 ± 4.11 years, height is 1.79 ± 6.99 cm, body weight is 76.02 ± 6.76 kg, body fat percentage is 11.59 ± 3.11 %, experience is 14.27 ± 0.12 years, and weekly training duration is 9.7 ± 2.8 min. On the other hand, amateur soccer players have an average age of 23.63 ± 3.77 years, height of 1.81 ± 5.77 cm, body weight of 76.36 ± 7.53 kg, body fat percentage of 15.60 ± 4.65 %, experience of 11.33 ± 0.48 years, and weekly training duration of 8.7 ± 1.9 min.

Table II. Demographic data of the soccer players participating in the study players participating in the study.

		n	X	sd
Age (year)	Professional	71	23.66	4.11
Age (year)	Amateur	62	23.63	3.77
Longhth (am)	Professional	71	1.79	6.99
Lenghth (cm)	Amateur	62	1.81	5.77
Waight (1m)	Professional	71	76.06	6.76
Weight (kg)	Amateur	62	76.36	7.53
Body Fat Percentage (%)	Professional	71	11.59	3.11
Body Pat Percentage (%)	Amateur	62	15.60	4.65
	Professional	71	14.27	0.12
Experience (years)	Amateur	62	11.33	0.48
Training (min/week ⁻¹)	Professional	71	9.7	2.8
manning (min/week)	Amateur	62	8.7	1.9

Sample Collection and Genotyping. DNA samples were obtained by collecting buccal epithelial samples using a swab between January and February 2021. A researcher visited the training facilities of each soccer team to explain the purpose, benefits, and risks of the research and to obtain written consent from the participants volunteering for the study. During this process, health personnel at the clubs (Nurse, First Aid Specialist, club masseur) assisted the researcher. After the samples were collected, they were cooled at 4 °C and sent to the laboratory. In the subsequent stage at the laboratory, genomic DNA extraction from the samples was performed using the QIACube equipment (QIAGEN, Venlo, Netherlands) through an automated extraction method to obtain a solution with a DNA concentration of 25 ± 40 ng/mL. The solution was stored at -20°C until genotyped.

Genotyping of the *ACTN3* rs1815739 polymorphism was performed using Real-Time PCR on a StepOnePlus (Thermo Fisher Scientific, Inc.) instrument and Taqman Genotyping Mater Mix genotyping kits according to the manufacturers' protocols (Thermo Fisher Scientific, Inc.). For a total reaction volume of 10µl, 5µl Genotyping Master Mix (Applied Biosystems, Foster City, CA), 3.5µl nuclease-free H_2O (Thermofisher, USA), 0.5µl *ACTN3* genotyping assay (Applied Biosystems) and 1µl DNA were used. All analyzes without a clear genotype assignment were repeated. If assignment of a genotype failed, the participant was excluded from analysis.

Physical Performance Testing. During the preparation period of the 2021-2022 season, two different performance tests were applied to soccer players. Performance tests were carried out by the same person and following the same procedures for each team. Both professional and regional amateur soccer players were familiar with the testing procedure as it had been part of their physical evaluation in the past. All performance tests were performed on the same day and within 24 hours following a low-intensity regeneration training day. On the test day, anthropometric data were collected and the players completed a standard warm-up consisting of twenty-five minutes of light-to-moderate jogging, lower limb dynamic stretching, and submaximal sprint and jump attempts. After this, the participants performed the following tests:

Body Composition Measurements. Measurements of height and weight were conducted in the morning. Height was assessed using a stable stadiometer (+0.1 cm, Holtain Ltd., Crosswell, UK). Research on body composition adhered to established rules and principles, aligning with the International Biological Program guidelines for the selection of measuring instruments and standardized measurement techniques. In this study, two body composition metrics were considered: body weight and body fat percentage. The assessment of body composition utilized a Tanita body fat scale (model BC-418MA), employing an indirect measurement method. This involves the transmission of a safe electrical signal through the body via electrodes located in the standalone unit. The Tanita Scale, equipped with an athletics mode, allows athletes to closely monitor their body weight, overall health, and fitness levels, including all relevant parameters. All measurements were consistently conducted by the same researcher (Gaudino et al., 2022).

Sprint Time. The assessment of maximal velocity in a straight-line sprint covering 0-10m-30m was conducted using photocells (Witty System, Microgate, Bolzano, Italy), following Vescovi's protocol designed for a 35m sprint. The starting point for each sprint was 50 cm behind the photocell line. Participants initiated the sprint from a standing position, placing their preferred foot behind the starting line, and accelerated maximally until crossing the final photocell gate at 30m. Each participant performed two maximal 30m sprints, with a minimum of 2 minutes of passive recovery between attempts. The test took place on the soccer pitch, and participants were equipped with soccer cleats. The fastest recorded time was used for subsequent statistical analysis (Vescovi, 2012).

Illinois agility test. The field's length measures 10m, with a width (distance between the start and finish points) of 5m. Within the testing area, four cones were strategically positioned at a 3.3m distance from each other, marking the start, finish, and two turning points (Fig. 1). Participants initiated the test in a prone position with hands at shoulder level. The trial commenced upon the 'go' command, prompting subjects to run at their maximum speed. The trial concluded when players crossed the finish line without displacing any cones. The best time from three trials was selected for analysis (Daneshjoo *et al.*, 2013).

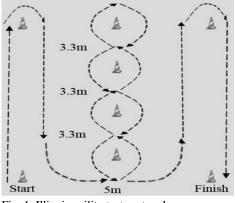


Fig. 1. Illinois agility test protocol.

Running Base Anaerobic Sprint Ability Test. The Repeated sprint ability test was performed according to a previously validated protocol (Zagatto et al., 2009). In brief, the test protocol involved six maximal straight-line sprints of 35m each, with a 10-second recovery period that included deceleration. The same equipment used for speed testing was employed for the Repeated Sprint Ability (RSA) test. Participants initiated their sprints upon hearing an audio signal from the system. Following each sprint, a brief deceleration phase was permitted, after which participants prepared for the subsequent sprint. Timing for each run was recorded using two photocells positioned precisely at 35m, and the start of each sprint (with a 10-second interval) was signaled by an audio cue from the photocell system. The anaerobic power of participants was calculated based on the least time achieved across the sprints.

Participants' rights and confidentiality were protected throughout the entire experiment, and genetic information was used only for purposes within the scope of this research.

Statistical analysis. SPSS 23 (SPSS Inc., Chicago, IL, USA) package program was used for statistical analysis of the findings obtained in the test results. Variance homogeneity of the data was done with Levence Test and normal distribution analysis was done with Shapiro-Wilk Test. Chi-Square and Mann Whitney U Test were used to analyze the parameters. Statistical significance was tested at the p<.05 level.

RESULTS

All the players were successfully genotyped. *ACTN3* rs1815739, 10m sprint (sec), 30m sprint (sec), Illinois Agility (sec), Power (watt), Relative Power (watt/kg) findings of professional soccer players and regional amateur league soccer players are presented below.

Looking at Table III, in professional soccer players, CC genotype was 15 (21.13 %) soccer players, CT genotype was 21 (29.58 %) soccer players and TT genotype was 35 (49.29 %), and in amateur players, CC genotype was 6 (9.68 %) soccer players and CT genotype was 28 (45.16 %) soccer player and TT genotype was determined as 28 (45.16 %). When the data in our study is evaluated, while no statistically significant difference could be detected in the *ACTN3* rs1815739 gene polymorphism between the groups (p>0.05), it is seen that the TT allele is dominant in professionals, and the CC and TT alleles are dominant and close to each other in amateurs.

Looking at Table IV, the average speed test 10m results of professional soccer players is 1.56 ± 0.14 sec; While the average of 30m results was 3.85 ± 0.22 seconds, the average of amateur soccer players' speed test 10m results was 1.64 ± 0.13 seconds; The average of 30m results was determined as 4.47 ± 0.51 seconds, and a significant difference was found between both groups of players in favor of professional soccer players' [Players]. While the average of professional soccer players' [Players].

Table III. ACTN3 rs1815739 gene interaction between professional and amateur soccer players.

	Genotype		p Value	Allelic Frequency		p Value	
	CC	СТ	ТТ		С	Т	
Professional	15	21	35		51	91	
Percentage	21.13 %	29.58 %	49.29 %	0.0801	35.92 %	64.08 %	0.5305
Control group	6	28	28		40	84	
Percentage	9.68 %	45.16 %	45.16 %		32.26 %	67.74 %	

Significance was evaluated at at least p<0.05. Comparison with the control group was made using the χ^2 test.

was 15.47 ± 0.60 , respectively, the amateur soccer players' average Illinois agility test results were found to be 15.84 ± 2.03 , respectively, and no significant difference was detected between both groups of players (p>0.05). The average anaerobic power (watt) results of professional soccer players was 1227.48 ± 253.16 ; While the average relative power

(watt/kg) results was 16.13 ± 2.91 , the average power (watt) results of amateur soccer players was 829.68 ± 279.78 ; The average relative power (watt/kg) results were determined as 10.88 ± 3.58 , and a significant difference was detected between both groups of players in favor of professional soccer players (p<0.05).

Table IV. The avarage 10 m sprint (sn), 30 m sprint (sn), illinois agility (sn), power (watt), relative power (watt/kg) standart deviation (sd), t and p values of professional and regional amateur soccer players.

		n	X	sd	Z	р
10m sprint (sec)	Professional	71	1.56	0.14	-3.27	0.01*
	Amateur	62	1.64	0.13		
30m sprint (sec)	Professional	71	3.85	0.22	-7.04	0.01*
	Amateur	62	4.47	0.51		
Illinois Agility (sec)	Professional	71	15.47	0.60	-1.66	0.09
	Amateur	62	15.84	2.03		
Anaerobic Power (watts)	Professional	71	1227.48	253.16	-6.66	0.01*
	Amateur	62	829.68	279.78		
Relative Power (watt/kg)	Professional	71	16.13	2.91	-7.04	0.01*
	Amateur	62	10.88	3.58		

DISCUSSION

The current study aimed to achieve two primary objectives: (1) to ascertain the association between the *ACTN3* rs1815739 polymorphism and variations in physical performance in response to diverse soccer leagues, assessed through 10m-30m sprints and Ilionis ability tests, as well as power parameters; and (2) to compare *ACTN3* genotypic distributions among players from different soccer leagues.

In soccer, speed and power play pivotal roles in distinguishing an exceptional athlete from an average one. With this premise, assessing the genetic parameters of maximum power in the lower limbs provides a non-invasive and indirect method for identifying predictors of speed and agility capabilities (Maciejewska-Skrendo et al., 2020). Especially in soccer based on modern technology, the triangle of speed-agility-explosive power; It has been characterized in a modernized way in terms of sudden speed, constant speed, accelerated direction changes, effective bilateral fights for strength and, accordingly, the realization of agility with explosive power of different intensities (Massidda et al., 2020). In their study, Gineviciene et al. (2014), stated that the sportive performance cycle is a combination of an innate genetic characteristic of the soccer player and educational factors acquired through training, and accordingly, achievements such as explosive power, power, strength and agility are determined by the genetic characteristics of the athletes (Gineviciene et al., 2014). Literature evidence also states that certain genes are directly related to athletic performance, especially closely related to power, speed and power factors (Ma *et al.*, 2013; Nazarov *et al.*, 2001).

When the studies on soccer players at different league levels, such as professional and amateur, are examined, it is seen that there are limited studies investigating physical performance tests in addition to genotype evaluation. In this study, ACTN3 rs1815739 polymorphism was evaluated as a genetic marker. Genotype evaluation was made in professional and amateur soccer players and the difference between professional and amateur performance variables was investigated. As a physical test; sprint speed test, Illinois agility test and anaerobic power parameters were evaluated. Is there a difference in the ACTN3 genotype parameter in the research? Do field test parameters, which are physical performance tests, differ? The questions were emphasized (Tables III and IV). Melián Ortiz et al. (2021) stated in their study that polymorphisms in the a-actin-3 (ACTN3) gene are genetic markers for individual characteristics that affect athletic performance such as strength and agility.

Evaluation of ACTN3 rs1815739 polymorphism.

In the study conducted in professional soccer players; CT allele (21.13 %), CC allele (29.58 %), TT allele (49.29 %) in amateur soccer players; It was determined that there were CT allele (9.68 %), CC allele (45.16 %), and TT allele (45.16 %) (Table III). While the TT allele is dominant in professional soccer players, both the CC and TT alleles are dominant in amateur soccer players. When the literature was examined, the expression "speed gene" was used for the *ACTN3* gene (Chan *et al.*, 2008; Berman & North, 2010). However, when evaluated in terms of league level, there are contradictory results.

In contrast to our study findings, a study reported that the CT allele of the ACTN3 genotype was significantly high (26.53 %) in elite athletes (Murtagh *et al.*, 2020). Similarly, in another study, Yang et al. (2003) stated that ACTN3 genotype was associated with speed and power phenotypes and that elite athletes had significantly higher CT allele frequencies than amateur athletes. On the other hand, Roth et al. (2008) also reported that the findings of these studies were found many times in speed, power and strength elite athletes, but the findings were not conclusive. Studies conducted on professional soccer players also indicate that the CC genotype is higher in the ACTN3 rs1815739 polymorphism (Lippi et al., 2010; Pimenta et al., 2013). In a study involving Spanish elite and amateur athletes, it was statistically significant that elite athletes had CT genotype (46 %), CC genotype (30.08 %), amateurs had CT (53 %) and CC genotype (39.12 %) and both genotype values were not very high. It has also been reported that there is no difference (Varillas-Delgado et al., 2021). On the other hand, Coelho et al. (2019) stated that in their study where they examined the connection between physical performance and ACTN3 rs1815739 polymorphism in 138 Brazilian soccer players, no significant relationship was detected in terms of genotype-phenotype. In a study conducted with elite and intermediate level athletes, it was found that elite athletes had CC genotype (26%), CT genotype (25%), intermediate level athletes had CC genotype (48.22 %), and CT genotype(13.35 %). It was reported that no significant difference was detected (Varillas Delgado et al., 2020).

Supporting our study findings, in a meta-analysis study on *ACTN3* rs1815739 polymorphism, it was reported that professional athletes had higher CT and TT genotypes than amateur athletes (López-Valenciano *et al.*, 2020). Similarly, in a study conducted by Jacob *et al.* (2019) with sub-elite soccer players, CT, CC, TT genotypes were found to be 11.01 %, 44.05 % and 48.32 %, respectively. It is seen that all three genotypes are similar to the amateur soccer player genotype values in this study.

In parallel with the data obtained from professional soccer players in our study, a study conducted on elite soccer players found that the C and T alleles were at higher frequencies in elite soccer players (Santiago *et al.*, 2008).

Mc Auley *et al.* (2021) suggested and articulated gene variants that could be beneficial in evaluating soccer players

from different leagues, especially for multiple gene polymorphisms, in their study on soccer. Sarmento *et al.*, (2020) emphasized the necessity of investigating the human genom along with various biological variables and highlighted the presence of individually reduced soccer player genomes in team sports like soccer, according to the results obtained in their studies. They also stated that all of these would significantly contribute to determining differences in athletic performance.

Consequently, it appears that the *ACTN3* rs1815739 CT, CC and TT genotypes obtained from the study do not differentiate between professional and amateur soccer players. When comparing the findings from the study with existing literature, both parallels an contrasts are noted. Further research is warranted to elucidate these contradictory results in the literature.

Evaluation of Sprint and Agility Performance.

While the 10 m speed test average of the professional soccer players participating in the study was 1.56±0.14 sec, the 30 m speed test average was 3.85±0.22 sec, the RAST Power average was 1227.48±253.16 watts, the Relative power average was 16.13±2.91 watt/kg, while the 10 m speed test average of amateur soccer players was it was determined as 1.64 ± 0.013 sec, 30 m speed test average as 4.47 ± 0.51 sec, RAST Power average as 829.68±279.78 watts, Relative power average as 10.88±3.58 watt/kg (Table III). Considering the analysis results, a significant difference was found between the groups (p < 0.05). In other words, it has been observed that professional soccer players have better data than amateur soccer players in all parameters. While the average Illinois agility test of professional soccer players participating in the study was 15.47±0.60 seconds, it was found to be 15.84 ± 2.03 seconds for amateur soccer players (Table IV). Considering the analysis results, no significant difference was found between the groups (p>0.05). In other words, it can be said that amateur soccer players and professional soccer players have similar data.

When the literature is examined; In their study by Arnason *et al.* (2004); They determined that the average of the 10 m speed test of professional soccer players was 1.63 ± 0.08 sec, the average of the 30 m speed test was 3.18 ± 0.16 sec, the average of the 10 m speed test of amateur soccer players was 1.64 ± 0.09 sec, and the average of the 30 m speed test was 4.17 ± 0.18 sec. This study is similar to our findings. In their study, Sunje *et al.* (2021) compared some physical parameters of professional and amateur soccer players. In this study, they reported that the 30-meter sprint time was 4.15 ± 0.12 seconds in professional soccer players and 4.33 ± 0.15 seconds in amateur soccer players. In another study, Izzo *et al.* (2018), tracked the movement profiles of amateur and professional soccer players during the matches they played with a GPS device. In the study, the highest speed parameter that amateur soccer players could achieve was recorded as 28 km/h, while this parameter was recorded as 31 km/h in professional soccer players. In another study, Cometti *et al.* (2001), examined the sprint performances of French soccer players.

In the study, it was shown that both the 10-meter sprint and 30-meter sprint performances of 1st League soccer players $(1.80\pm0.06-4.22\pm0.19 \text{ respectively})$ were better than those of 2nd League $(1.81\pm0.05-4.24\pm0.149 \text{ respectively})$ and amateur soccer players $(1.85\pm0.07-4.29\pm0.14$ respectively). When looking at different studies on sprint speed, Sander *et al.* (2013), stated it as 4.36 ± 0.54 sec in German amateur soccer players, Aguiar *et al.* (2008), stated it as 4.23 ± 0.25 sec in amateur Portuguese soccer players, and Sampaio *et al.* (2007), stated it as 4.88 ± 0.10 sec in amateur doccer players. When looking at sprint speed data in general, some differences are seen, although they do not change the general judgment. It is thought that this difference arises from the fact that the leagues to which players belong vary in terms of physical quality.

Rampinini *et al.* (2009), found in their study that the average repetitive sprint performance of elite soccer players was lower than that of amateur soccer players (7.17 \pm 0.09-7.41 \pm 0.19 s; p<0.001), and their lactate responses after repetitive sprint performance were also lower (5.7 \pm 1.5-8.2 \pm 2.2 mmol/L). In their study, Abrantes *et al.* (2004), examined Portuguese League soccer players in terms of repeated sprint performance. The findings showed that the average repetitive sprint performances of the 1st League players were significantly different compared to the 2nd League players and semi-professional league players (p<0.001).

In a study conducted by Michailidis (2018), it was shown that the 30 m sprint and agility performances of semiprofessional soccer players were better than amateur soccer players (p<0.001). In another study examining the physiological parameters of elite, subelite and recreational soccer players, it was found that the vertical jump, isometric strength and 10 m sprint transition performances of elite soocer players were significantly different from the subelite and recreational groups (Gissis *et al.*, 2006). In the literature, especially in studies evaluating anaerobic performance, we see that elite soccer players are clearly superior to subelite and amateur soccer players from past to present. It is thought that this difference is due to the fact that high-intensity sessions are included more in the training of elite soccer players. In light of this information, in our study it was thought that anaerobic performance would be a distinguishing parameter in the context of the physical quality of the soccer player.

LIMITATIONS. The results obtained from this study present some limitations that need to be addressed in order to improve the genetic factors and athletic performance of professional and amateur soccer players. Although the study was conducted on a homogeneous sample of professional and amateur soccer players, the sample size was relatively low and professional and amateur soccer players were not subjected to the same training, competition and diet protocols. Therefore, caution should be exercised in drawing definitive conclusions regarding soccer performance of the *ACTN3* genotype and whether there is a difference between professional and amateur soccer players.

CONCLUSIONS

Genetics has a clear and undoubted impact on both sports performance and exercise adaptation. One of the best-studied genes in this context is *ACTN3*, which has been reliably shown to affect speed-power and strength phenotypes. In summary, the study showed that there were professional and amateur players with different genotypes of the *ACTN3* rs1815739 polymorphism. On the other hand, it should be stated that there are differences and similarities in terms of physical performance values of professional and amateur soccer players. Therefore, in our study, limited to our sample group, it can be said that gene polymorphism is not a differentiating factor between professional and amateur soccer players, but speed (10 m and 30 m) and anaerobic power parameters are the differentiating factors.

SUGGESTIONS. Future studies with larger samples and genetic markers should be conducted to confirm the results of this study. Thus, examining the interaction of genes and field tests in soccer players is important in that it includes the study of the polygenic profile of the players, as in previous studies conducted with different types of athletes.

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RESUMEN: Los objetivos de este estudio fueron: 1º identificar diferencias en ciertos parámetros anaeróbicos (sprint

de 10 m, sprint de 30 m, potencia anaeróbica y pruebas de agilidad de Illinois) entre jugadores de fútbol profesionales y amateurs, y 2º determinar si existe una diferencia en el polimorfismo del gen ACTN3 entre jugadores de fútbol profesionales y aficionados. En definitiva, el objetivo fue revelar qué parámetros contribuyen a la diferenciación en estos dos aspectos. En la investigación participaron un total de 133 jugadores de fútbol voluntarios, incluidos 71 profesionales y 62 aficionados. La extracción de ADN de las células epiteliales orales se realizó utilizando un kit comercial para determinar los antecedentes genéticos de los atletas y se realizó una PCR en tiempo real para el genotipado. El análisis estadístico de los hallazgos obtenidos a partir de los resultados de las pruebas se realizó utilizando el programa de paquete SPSS 23 (SPSS Inc., Chicago, IL, EE. UU.). La homogeneidad de la varianza de los datos se evaluó mediante la prueba de Levene y los análisis de distribución normal se realizaron mediante la prueba de Shapiro-Wilk. Para el análisis de parámetros se emplearon las pruebas de Chi-cuadrado y U de Mann-Whitney. El nivel de significancia se fijó en p<0,05. La evaluación de los datos en nuestro estudio no reveló diferencias estadísticamente significativas en el polimorfismo del gen ACTN3 rs1815739 entre los grupos (p>0,05). Sin embargo, existe una diferencia estadísticamente significativa en los parámetros anaeróbicos (sprint de 10 m, sprint de 30 m y potencia anaeróbica) excepto para la prueba de Illinois (p<0,05). En conclusión, nuestro estudio encontró que el polimorfismo genético no es un factor diferenciador entre jugadores de fútbol profesionales y amateurs, pero sí los parámetros de velocidad (10 m y 30 m) y potencia anaeróbica.

PALABRAS CLAVE: Gen; Fuerza; Polimorfismo; Pique.

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