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ACTN3 R577X POLYMORPHISM AND ANAEROBIC PERFORMANCE IN ULTIMATE FRISBEE PLAYERS: A PRELIMINARY STUDY

ORIGINAL ARTICLE

ABSTRACT

Purpose: ACTN3 R577X polymorphism is a frequently studied gene polymorphism associated with athletic performance. Studies have demonstrated a strong association between the 577RR genotype and sprint and power-based sports. Ultimate Frisbee (UF) is a physically demanding sport requiring aerobic and anaerobic skills. This study aimed to evaluate the relationship between the ACTN3 R577X polymorphism and the anaerobic power capabilities of UF players.

Methods: The study included 30 UF players in the study group (mean age ± SD 21.03 ± 2.04 years) and 30 volunteers in the control group (mean age ± SD 22.17 ± 1.39 years). Anaerobic power was assessed using vertical jump, running-based anaerobic sprint (RAST), triple hop, and closed kinetic chain upper extremity tests. Blood samples were genotyped using real-time polymerase chain reaction. RR, RX, and XX represent homozygous dominant, heterozygous dominant, and recessive genotypes, respectively.

Results: Fatigue Index (FI) data from RAST test results was the only variable that differed between study and control groups (Study Group: 6.02 ± 3.52 vs. Control Group: 4.17 ± 1.71 watts/sec, p = 0.012). There was no statistically significant difference between the study and control groups in vertical jump, triple hop, and closed kinetic chain upper extremity test results. No statistically significant difference was found in anaerobic performance tests among the genotype groups in UF players.

Conclusion: In this study conducted with limited sample size, the anaerobic performance of UF players was not found to be associated with ACTN3 R577X polymorphism. However, performing the same screening in larger sample groups in future studies may yield more efficient results.

Keywords: ACTN3, Athletic Performance, Genomics, Ultimate Frisbee

ULTIMATE FRISBEE OYUNCULARINDA ACTN3 R577X POLİMORFİZMİ VE ANAEROBİK PERFORMANS: BİR ÖN ÇALIŞMA

ARAŞTIRMA MAKALESİ

ÖZ

Amaç: ACTN3 R577X polimorfizmi, atletik performansla ilişkili olarak sıklıkla çalışılan bir gen polimorfizmidir. Çok sayıda çalışma, 577RR genotipi ile sprint ve güce dayalı sporlar arasında güçlü bir ilişki olduğunu göstermiştir. Ultimate Frisbee (UF) hem aerobik hem de anaerobik beceriler gerektiren fiziksel olarak zorlayıcı bir sporudur. Bu çalışmanın amacı, ACTN3 R577X polimorfizmi ile UF oyuncularının anaerobik güç yetenekleri arasındaki ilişkiyi değerlendirmektir.

Yöntem: Çalışmaya çalışma grubunda 30 UF oyuncusu (ortalama yaş ± SD 21,03 ± 2,04 yıl) ve kontrol grubunda 30 gönüllü (ortalama yaş ± SD 22,17 ± 1,39 yıl) dahil edildi. Anaerobik güç dikey sıçrama, koşuya dayalı anaerobik sprint (RAST), üçlü sıçrama ve kapalı kinetik zincir üst ekstremite testleri kullanılarak değerlendirilmiştir. Kan örnekleri gerçek zamanlı polimeraz zincir reaksiyonu kullanılarak genotiplendirilmiştir. RR, RX ve XX sırasıyla homozigot dominant, heterozigot dominant ve resesif genotipleri temsil etmektedir.

Sonuçlar: RAST testi sonuçlarından elde edilen Yorgunluk İndeksi (FI) verileri çalışma ve kontrol grupları arasında farklılık gösteren tek değişkendi (Çalışma Grubu: 6,02 ± 3,52 vs Kontrol Grubu: 4,17 ± 1,71 watt/sn, p = 0,012). Dikey sıçrama, üçlü sıçrama ve kapalı kinetik zincir üst ekstremite test sonuçlarında çalışma ve kontrol grupları arasında istatistiksel olarak anlamlı bir fark bulunmamıştır. UF oyuncularının genotip grupları arasında anaerobik performans testlerinde istatistiksel olarak anlamlı bir fark bulunmamıştır.

Tartışma: Kısıtlı örneklem sayısı ile yapılan bu çalışmada, UF oyuncularının anaerobik performansı ACTN3 R577X polimorfizmi ile ilişkili bulunmamıştır. Ancak gelecek çalışmalarda aynı taramanın daha büyük örneklem gruplarında yapılması daha verimli sonuçlar verebilir.

Anahtar kelimeler: ACTN3, Atletik Performans, Genomik, Ultimate Frizbi

INTRODUCTION

Athletic performance is affected by genetic predisposition along with external factors like exercise and diet (1). Genetic contributions may explain about 66% of an athlete's condition variability (2). Furthermore, performance in sports is affected by a broad range of phenotypic traits resulting from a combination of many biological, physiological, and biochemical mechanisms (3, 4). Mutations, single nucleotide polymorphisms, DNA polymorphisms, and rare mutations are genetic indicators correlated with athletic traits such as muscular strength, endurance, and power (5). A growing body of evidence suggests significant genetic influences on sports performance, strength, endurance, and speed (5, 6).

Specific genes and gene sequence variants have been linked to athletic performance, but most studies have not been sufficiently repeated. Two exceptions are the α-actinin-3 (ACTN3) R577X polymorphism and the angiotensin-1 converting enzyme insertion/deletion (ACE I/D) polymorphism, which was tested with a variety of experimental approaches in several populations (7-9). It is recognized that ACTN3 R577X and ACE I/D polymorphism variations may play an essential role in achieving superior athletic performance (8, 10, 11). Based on studies on genetics and athletic performance, the ACE gene is closely associated with the endurance phenotype, and the ACTN3 gene with the strength phenotype (1, 5, 9, 10).

ACTN3 is involved in encoding the protein of α-actinin-3, which is produced specifically for type-II myofibrils, which itself has an essential function in producing fast twitches and powerful contractions (5, 10, 12-15). A nonsense polymorphism has been stated in the ACTN3 (rs1815739), the outcome of a substitution of a protein of arginine (R) residue with a premature stop-codon (X), which results in no α-actinin-3 protein detectable in muscle fibers (14). Nearly 18 percent of Caucasians have homozygous stop codon, so there is a complete deficiency in α-actinin-3 (5, 13). Several studies have found that the ACTN3 RR genotype is more overrepresented in sprint or power-oriented athletes than in controls. On the contrary, the XX genotype of the ACTN3 was also found to be overrepresented in endurance-based athletes (1, 9, 10).

Ultimate Frisbee (UF) is a high-paced team sport that involves limited physical contact and is played using a flying disc. It combines elements of soccer, basketball, rugby, and American football. UF is known for requiring a significant amount of high-intensity running, which can lead to fatigue during and after a game (16). Two teams with seven players compete in a 110-by-37-meter area with 23-meter-deep end zones on each side (17). The objective is to score by reaching the opposing end zone with the disc (18). In UF, the field size is akin to a soccer field but different from soccer, with seven players on each team instead of eleven, leading to significant physical demands. Studies have indicated that the expansive field and limited players can elevate the game's intensity (16, 17). Other studies have reported that in comparable sports, such as soccer, the workload increases due to high-paced running during the last 15 minutes of a match, and this temporary fatigue may negatively affect sprint performance (16, 19).

Studies conducted contributed to our understanding of the basic mechanisms of the UF; it consists of frequent running, change of direction, rapid acceleration, deceleration, jumping, and sprinting (16, 19). Moreover, according to a study, the UF could be described as a very energetically demanding sport with both aerobic and anaerobic characteristics. During an average match, it has been reported that players perform high and low-intensity running and sprints of relatively short duration and cover distances ranging from 4000 meters to 5000 meters (16, 20). In addition, it was reported that players spent approximately 42% of match time above >90% HRmax. Fatigue also plays a significant role in performance, with high-intensity running required throughout and specifically in the final parts of the game. It was reported that running distances were also compared between halves during the UF match, and a decrease in high-intensity running distance was found at the end of both halves. The authors thought the decline in the running distance was associated with increased fatigue and decreased anaerobic capacity and emphasized the importance of anaerobic performance

for UF sport (16). According to another study's results, blood lactate levels' findings also support the importance of the anaerobic performance capacity of UF athletes (19).

As far as the current knowledge goes, there is a limited number of physiological studies in the literature investigating the blood lactate levels and heart rate (HR) levels of UF athletes during the game. However, as far as we know, no research has examined the link between genetic factors and athletic performance in this sport (16, 18, 19). This study aimed to assess the relationship between the ACTN3 R577X polymorphism and the anaerobic power performance of UF athletes. It was hypothesized that there was a statistically significant difference between anaerobic performance findings and genotype groups of ACTN3 R577X polymorphism among UF players.

METHODS

Design

For this cross-sectional study, physical performance tests were performed in the synthetic turf field and the university's physiotherapy and rehabilitation department laboratory. A physiotherapist carried out all performance assessments. The subjects were asked to control their intake of caffeine and alcohol two days before the test and to avoid vigorous exercise for at least the previous 48 hours. The evaluations were divided into two parts: performance tests were performed in the first part, and blood samples were taken in the second part. Each episode was held on separate days during the same week. After the performance measurement process of all participants was completed, the blood sample collection phase started. After conducting individual interviews with the participants, appropriate appointment days were scheduled, and a single blood sample was collected from each participant using one tube. The research sample was collected between January 2020 and May 2020.

Participants

The study group (SG) consisted of thirty healthy UF players who were not diagnosed with any cardiovascular disease, did not have any musculoskeletal injury, and have been playing UF for at least six months. The control group (CG) consisted of thirty healthy individuals who were not diagnosed with any cardiovascular disease, had no musculoskeletal injury, and were not a member of any competitive sports team. The primary sociodemographic data of the participants and the factors related to their health status were collected using a structured face-to-face questionnaire. This questionnaire included questions about the participants' age, height, weight, body mass index (BMI) health habits, health status, injuries, current diseases, and length of participation in the team. Voluntary participants in the two groups were determined to participate in the study using the following inclusion and exclusion criteria:

Inclusion criteria:

- Have not undergone any surgical intervention in the lower or upper extremities in the last six months
- Have not experienced any orthopedic injuries in the last six months
- Being in the age range of 18-25
- Volunteer to participate in the study

Exclusion criteria:

- Having any diagnosed cardiorespiratory disorders
- Any musculoskeletal trauma in the last six months
- Participants with other diseases that may cause clinical symptoms, such as systemic diseases,
- Not to be in the 18-25 age range
- Not volunteering to participate in the study

Ethics statement

This study adhered to the principles of the Declaration of Helsinki. Written informed consent was acquired from every participant, and the research received approval from the Ethics Committee at Yeditepe University Faculty of Medicine (Decision No: 37068608-6100-15 / 1095).

Sampling

To determine the sample size for this study, we based our calculations on the estimated effect size (d=1.64) derived from the vertical jump test values

reported in a previous study in the literature (21). Although there are no established cutoff values in the literature for the vertical jump test, the normative values for vertical jump height in the young adult age group were reported as 56.38 ± 8.89 cm in the study conducted by Patterson et al. (22). Using the estimated effect size obtained from the study's data, the alpha level (α) was 0.05, and the power was 0.95. Thus, it was accepted as the sample size of 22 participants, with a minimum of 11 subjects in each group.

Anaerobic performance testing

Four tests were used to evaluate anaerobic performance: the Vertical Jump Test, the Running-based Anaerobic Sprint Test (RAST), the Triple Hop for Distance Test, and the Closed Kinetic Chain Upper Extremity Stability Test (CKCUEST). All participants performed a standard warm-up exercise before their performance tests.

For the vertical jump test, the fingertip of the third finger of the athlete was labeled with chalk. The athlete stood near the wall, maintaining both feet on the floor, extending as high as possible with one side, and labeling the wall with the endpoint of the third finger. This first point of touch was called the point of M1. The athlete tried to jump as high as possible and left a mark by touching the point where they reached the highest point with her fingertip. This point was referred to as the M2 point. The observer calculated the distance from M2 to M1. The athletes performed the test three times. The observer calculated the distances and average distances obtained in all three jumps. This value was used to measure the power. Harman Formula was used to calculate the peak and average power. The ICC value of the vertical jump test was reported as 0.98 based on measurements in healthy athletes (23).

The RAST protocol consists of six sprints with maximal effort on a 35-meter line with 10-second intervals for recovery. The times of the six sprints were recorded separately. The anaerobic power (calculated as watts) performance of each run was estimated from the following equivalence: Power = (force x speed) or (body mass x distance2) / time3) (24). The Fatigue Index (FI) value was calculated when the minimum power was subtracted from the maximum power and divided by the duration of a total of 6 sprints. Anaerobic Capacity (AC) was calculated when the power outputs of 6 sprints were added. The RAST test helps assess anaerobic power output (ICC = 0.88) (25).

The parallel tape was placed on the floor about 15 cm wide for the triple hop for the distance test. The athlete was asked to stand on the extremity to be tested just behind the starting line. Throughout the straight line, the athlete was asked to jump three times consecutively with maximum effort on the dominant leg. The test was conducted three times, and the recorded result was the mean of the total distance. The Triple hop for distance test has been reported to be a reliable alternative for assessing the power elicited by the lower extremity (ICC = 0.92) (26).

To perform the CKCUEST, a non-slip surface, two pieces of athletic tape, and a chronometer were used. Lines were formed with two athletic bands spaced 0.9 m apart. The athlete was asked to take a standard push-up position with their hands next to the lines. Then, the athlete was asked to move their hands from one line to the other line as quickly as possible. The number of touches to lines in 15 seconds was recorded. The average sum of the number of touches obtained in three trials was calculated. The score and the power can be determined by the following formulas: Score = the average number of touches / Height (inch); Power = (68% of body weight x the average number of touches) / 15. The ICC value of the CKCUEST test was reported as 0.927 (27).

Blood sample collection and genetic analysis

All venous blood samples from the participants were collected in 5 ml tubes containing Ethylenediaminetetraacetic acid (EDTA), ensuring their safety. These peripheral venous blood samples were stored at -80 °C, maintaining their integrity. The tubes were equipped with EDTA to prevent blood clotting. DNA isolation was performed using the iPrep pure link system from Invitrogen, a division of Thermo Fisher Scientific Inc. DNA extraction was automated using the iPrep DNA extraction robot (Invitrogen, Carlsbad, California, USA) and the blood genomic DNA isolation kit with iPrep. The purity and concentration of DNA were assessed using

NanoDrop (Invitrogen, Carlsbad, California, USA).

The allele and genotype frequencies were assessed for Hardy-Weinberg equilibrium using chi-squared tests. Genotyping analysis was performed using the 7500 Fast Real-Time Polymerase Chain Reaction (Applied Biosystems, Foster City, CA) system. Molecular beacons were employed in the Real-Time PCR to identify the SNPs. These molecules give fluorescent light when hybridization with a supplementary target region. As supplementary target areas are present, molecular beacons open due to the connecting DNA sequences to supplement target regions. Fluorescent signals can be easily detected. Molecular beacons used in Real-Time PCR have two different wavelengths for allele-specific detection: wildtype and mutant alleles (28). The R577X polymorphism of ACTN3 was specified using PCR. We conducted genotyping to isolate a specific segment of the gene and analyze the ACTN3 R577X polymorphism. The targeted genetic region for genotyping was rs1815739 within the ACTN3 gene. The conditions for the Real-Time PCR were set with an initial 10-minute incubation at 95°C, followed by denaturation at 92°C for 15 seconds in each cycle and annealing/elongation at 60°C for 1 minute in each cycle. The denaturation and annealing/elongation steps were repeated for 40 cycles.

Statistical analysis

The data underwent analysis through SPSS for Windows (Version 25.0; IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as mean ± standard deviation (SD). The normality of variables was assessed using the Shapiro-Wilk test. The Mann-Whitney U test was chosen for non-normally distributed data, while the independent t-test was applied to normally distributed data. Comparison of performance values between genotype groups in the study and control groups was evaluated using one-way or multivariate analysis of variance (ANOVA) with the post hoc Tukey test. The threshold for statistical significance was established at $p<0.05$. Allelic and genotype frequencies were analyzed using Fisher's exact or chi-squared (x2) test. The x2 test was employed to assess the conformity of genotypic frequencies with Hardy-Weinberg expectations (29).

RESULTS

Physical features of participants

This study involved 30 athletes (21 males, 9 females; 21.03 ± 2.04 years) from Yeditepe University UF Team and 30 healthy individuals (19 males, 11 females; 22.17 ± 1.39 years) from Yeditepe University students. While there was no significant difference in height, weight, and BMI between the SG and CG, a statistically significant difference in age ($p < 0.05$) was evident between the two groups (Table 1).

Table 1. Physical Features of Participants (mean ± SD)

	SG (n = 30) (mean ± SD)	CG (n = 30) (mean ± SD)
Age (years)	21.03 ± 2.04*	22.17 ± 1.39
Height (cm)	175.87 ± 9.08	174.80 ± 8.74
Weight (kg)	69.97 ± 11.13	69.60 ± 12.93
BMI (kg/m^2)	22.54 ± 2.73	22.56 ± 2.27

Note: Independent samples t-test was used to compare variables showing normal distribution data between independent groups. *p < 0.05, compared with CG.

BMI: body mass index, SG: study group, CG: control group.

Genotype distributions ACTN3 R577X among groups

According to the distribution of genotype characteristics among the SG and the CG, the RR (n=12), RX (n=13), and XX (n=5) were determined. In addition, RR (n=12), RX (n=11), and XX (n=7) were determined in the control group (Table 2). Based on the chi-squared test outcomes, genotype frequencies in both groups were found to conform to Hardy-Weinberg equilibrium ($p > 0.05$).

Table 2. Genotype Distribution of Genotypes

	SG (n=30)	CG (n=30)
XX	17%	23%
RX	43%	37%
RR	40%	40%
HWE-*p value*	0.647	0.178

Note: The genotype frequencies determined according to Hardy-Weinberg equilibrium by chi-squared tests.

SG: study group, CG: control group, RR: homozygous dominant, RX: heterozygous dominant, XX: homozygous recessive, HWE: hardy-weinberg equilibrium.

Intergroup comparison of the anaerobic performance test results

The Independent Samples t-test was used to compare the anaerobic performance variables between the SG and CG. No statistically significant differences were found between the groups in vertical jump tests, closed kinetic chain upper extremity tests, and triple hop test results ($p > 0.05$). The FI was the only variable that showed a statistically significant difference between the groups ($p < 0.05$) (Table 3).

Table 3. Intergroup Comparison of the Anaerobic Performance Test Results

	SG (n = 30) (mean ± SD)	CG (n = 30) (mean ± SD)
VTJ distance (cm)	45.89 ± 11.58	41.64 ± 9.66
PAP (watts)	7187.82 ± 970.04	6905.21 ± 945.67
AAP (watts)	1189.24 ± 429.07	1090.63 ± 447.62
PPO (watts)	506.53 ± 171.04	460.58 ± 154.96
APO (watts)	385.34 ± 120.31	384.65 ± 131.58
MPO (watts)	285.52 ± 105.47	309.93 ± 111.46
RPPO (watts)	7.14 ± 1.91	6.58 ± 1.54
FI (watts/sec)	6.02 ± 3.52*	4.17 ± 1.71
AC (watts)	2312.05 ± 721.91	2307.94 ± 789.50
CKCUEST Touch (number of touches)	27.90 ± 4.44	26.63 ± 4.27
CKCUEST Score (touches/inch)	0.40 ± 0.06	0.39 ± 0.05
CKCUEST Power (watts)	89.01 ± 21.40	85.11 ± 24.89
Triple Hop Distance (cm)	536.15 ± 90.40	530.34 ± 87.51

Note: Independent samples t-test was used to compare variables showing normal distribution data between independent groups. *$p < 0.05$, compared with CG.

SG: study group, CG: control group, VTJ: vertical jump test, PAP: peak anaerobic power output for the VTJ, AAP: average anaerobic power output for the VTJ, PPO: peak power output for the running-based anaerobic sprint test (RAST), APO: average power output for the RAST, MPO: minimum power output for the RAST, RPPO: relative peak power output for the RAST, FI: fatigue index for the RAST, AC: anaerobic capacity for the RAST, CKCUEST: closed kinetic chain upper extremity stability test.

Intragroup comparison of the anaerobic performance test results for the study group

The One-way ANOVA test was employed to compare anaerobic performance variables among the study group's RR, RX, and XX genotype groups. The statistical analysis revealed no significant difference between vertical jump test parameters groups. Similarly, the findings indicated no significant difference according to genotype groups regarding RAST parameters. Moreover, the statistical analysis showed no significant difference in CKCUEST parameters in the genotype groups. Finally, there was no significant difference between the genotype groups in triple hop distances (Table 4).

Table 4. Intragroup Comparison of the Anaerobic Performance Test Results for the Study Group (UF Players)

	RR (n = 12) (mean ± SD)	RX (n = 13) (mean ± SD)	XX (n = 5) (mean ± SD)
VTJ distance (cm)	45.19 ± 11.49	45.53 ± 13.71	48.53 ± 5.90
PAP (watts)	7172.36 ± 1083.86	7141.25 ± 1009.55	7310.01 ± 712.59
AAP (watts)	1196.14 ± 498.16	1169.96 ± 416.79	1222.83 ± 358.28
PPO (watts)	508.24 ± 179.05	494.32 ± 150.93	534.13 ± 232.70
APO (watts)	371.56 ± 131.48	390.99 ± 108.81	403.70 ± 144.21
MPO (watts)	271.20 ± 121.43	292.40 ± 96.09	307.99 ± 104.95
RPPO (watts)	7.04 ± 1.90	7.10 ± 1.82	7.49 ± 2.51
FI (watts/sec)	6.32 ± 3.09	5.59 ± 3.76	6.41 ± 4.49
AC (watts)	2229.41 ± 788.88	2345.95 ± 652.90	2422.24 ± 865.29
CKCUEST Touch (number of touches)	27.17 ± 5.76	28.62 ± 3.01	27.80 ± 4.60
CKCUEST Score (touches/inch)	0.39 ± 0.08	0.41 ± 0.04	0.40 ± 0.06
CKCUEST Power (watts)	88.17 ± 25.41	90.25 ± 17.46	87.81 ± 24.92
Triple Hop Distance (cm)	540.00 ± 104.68	539.38 ± 87.11	518.50 ± 76.71

Note: The One-Way ANOVA test was used to compare anaerobic performance variables in the study group regarding the genotype variants.

SG: study group, CG: control group, VTJ: vertical jump test, PAP: peak anaerobic power output for the VTJ, AAP: average anaerobic power output for the VTJ, PPO: peak power output for the running-based anaerobic sprint test (RAST), APO: average power output for the RAST, MPO: minimum power output for the RAST, RPPO: relative peak power output for the RAST, FI: fatigue index for the RAST, AC: anaerobic capacity for the RAST, CKCUEST: closed kinetic chain upper extremity stability test, RR: homozygous dominant, RX: heterozygous dominant, XX: homozygous recessive

DISCUSSION

This study examined the relationship between anaerobic power variables and the ACTN3 R577X polymorphism among UF players. The genotype

frequencies for RR, RX, and XX among UF players were 40%, 43%, and 17%, respectively. Moreover, the observed genotype frequencies of ACTN3 gene R577X polymorphism in all three groups were found to be consistent with Hardy-Weinberg equilibrium ($x2 = 0.208$, $p = 0.647$; $p > 0.05$ - consistent with HWE). One of the other findings was that there was no significant difference in anaerobic performance variables among the different genotype groups of the ACTN3 R577X polymorphism. Eynon et al. conducted a study involving athletes from three European countries, categorizing them into team sport athletes, endurance athletes, and sprint/power athletes (30). They compared the genotype and allele frequencies among these groups and found that team athletes were less likely to possess the 577RR allele than sprint/power athletes. This discrepancy is attributed to the differing physical demands of team sports, which are intermittent and require repeated powerful movements such as short-distance sprints and jumps. Massidda et al.'s study on Italian athletes corroborated Eynon et al.'s findings (30, 31). Therefore, the literature revealed that the ACTN3 R577X polymorphism was not associated with team sports performance (30). According to the results of our study, we similarly concluded that there was no association in UF athletes.

The ACTN3 R577X polymorphism has shown a significant correlation with athletic performance in various sports disciplines, including team sports like football, handball, and ice hockey; sprint/power sports such as sprinting and weightlifting; and endurance sports such as rowing, endurance road cycling, and marathon running (5, 30, 32, 33). No studies have examined genetic polymorphism in UF players, making it challenging to compare genotype distributions. Previous studies have explored various physiological parameters in UF (16, 18, 19). A study demonstrated that UF players cover approximately 4000 to 5000 meters in a typical 50-minute match. Additionally, it was reported that players spent around 42% of the match time above 90% of their maximum heart rate (HRmax). Based on this information, the study concluded that UF is a high-intensity intermittent sport characterized by extensive running, sprinting, and a substantial cardiovascular load (16). Another study evaluating HR, perceived exertion, and blood lactate levels in UF players during a match found that HR values were vigorous (mean±SD = 94.3±5.1 %HRmax) according to ACSM guidelines (19). Borg's scale indicated RPE values of 13.85±2.11, classifying them as moderate to high exertion levels. These results suggest that UF athletes likely performed consistently above their anaerobic threshold, increasing reliance on the anaerobic glycolytic energy system to sustain exercise intensity. This hypothesis is further supported by the athletes' average blood lactate levels of 4.7 to 8.3 mmol·L-1 during the match, exceeding the general anaerobic threshold indicator of 4 mmol·L-1. Considering the lactate levels, the importance of anaerobic capacity in UF sports is recommended in the literature. We employed valid and reliable tests to ensure the practical assessment of anaerobic performance (23, 25-27).

Numerous studies have associated the 577RR genotype with sprint and power-type disciplines (30, 32-34). Conversely, the XX genotype is more prevalent among endurance athletes (10, 35). However, these findings remain a subject of ongoing debate and further investigation. Importantly, although our study could not demonstrate a relationship with anaerobic performance, we found that the genotype distributions were consistent with the team sport studies conducted by Eynon and Massidda et al. In both our research and the studies conducted by Eynon and Massidda et al., the most prevalent genotype among team athletes was RX (40-54%), followed by RR (33-43%) and XX (13-20%) (30, 31). These findings underscore the significance of incorporating anaerobic training into the training regimens of UF team players. Kobayashi et al. studied the ACTN3 R577X polymorphism, examining Japanese athletes' bone mineral density (BMD) and maximal anaerobic power output (36). They concluded that athletes with the RR genotype exhibited higher BMD and maximal anaerobic power output than other genotypes. This suggests that individuals with the RR genotype may have more favorable training responses. These findings could inform the regulation of training programs and determine optimal training strategies through genotyping, potentially enhancing sports performance. Although not the current study's primary aim, various parameters related to anaerobic performance, such as muscle architecture, bone mineral density,

and metabolic pathway efficiency, may also be considered. Other possible genetic factors and polymorphisms influencing athletic performance should also be considered. Consequently, taking these factors into consideration, a multifactorial study design could be adopted for similar future research (37, 38).

This study has several limitations that should be acknowledged. First, the small sample size limits the generalizability of the findings. Second, this study did not evaluate other physiological factors influencing performance. Third, the study did not assess the effects of other potential variables on anaerobic performance; these variables may impact an athlete's anaerobic capacity. Given these limitations, future research should adopt a multifactorial approach, incorporating a broader range of variables to provide a more comprehensive understanding of the factors influencing anaerobic performance in UF players.

In conclusion, the authors believed that this study provided relevant findings that underscored the significance of incorporating anaerobic training into the training regimens of UF team players.

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