

ACTN3 rs1815739, PPARA rs4253778 and IL-6 rs1800795 gene polymorphisms and aerobic performance in kickboxing athletes

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Abstract

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Aim: The purpose of this study was to determine the *ACTN3* rs1815739, *PPARA* rs4253778, and *IL-6* rs1800795 gene polymorphisms and aerobic performance indicators—namely Yo-Yo IRT1 distance (m) and estimated $\dot{V}O_{2\max}$ ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)—between elite and sub-elite kickboxing athletes, and to identify which parameter best differentiates performance levels. **Methods:** The study sample consisted of 30 voluntarily participating male kickboxers. Genetic profiling and aerobic performance testing were conducted to obtain the data. Chi-square and independent t-tests were utilized to evaluate group differences. **Results:** The analysis revealed no statistically significant differences between elite and sub-elite athletes in terms of the *ACTN3* rs1815739, *PPARA* rs4253778, and *IL-6* rs1800795 gene polymorphisms or the aerobic performance parameters ($p > 0.05$). **Conclusion:** *ACTN3* rs1815739, *PPARA* rs4253778, and *IL-6* rs1800795 gene polymorphisms, as well as the measured aerobic parameters, do not appear to be distinguishing factors between elite and sub-elite kickboxing athletes in the examined cohort. Future studies with larger sample sizes and additional genetic and performance-related variables are recommended to better understand the determinants of athletic performance in this sport.

Introduction

Kickboxing is a full-contact sport characterized by the use of hand, foot, knee, and elbow strikes exchanged between two opponents (Buse, 2009). A typical kickboxing bout consists of 3 to 12 rounds lasting 2 to 4 minutes, with rest breaks of 1 to 2 minutes (Silva et al., 2011). Athletes usually compete in five to seven matches, and each match is limited to duration of approximately 8 minutes. Kickboxing athletes must have a high level of physical and physiological endurance, as they have to perform a large number of movements in each match (Crisafulli et al., 2009). Kickboxing is a dynamic and high-intensity sport that requires the correct execution of techniques, speed, and power to be successful (Buse, 2009). However, due to short rest periods between rounds and limited recovery

time, aerobic metabolism plays an important role in this process. A well-developed aerobic capacity for effective recovery and sustained performance throughout the match gives the kickboxer the advantage of performing repetitive high-intensity movements and maintaining optimal performance until the final round (Crisafulli et al., 2009).

Physiological characteristics of athletes are evaluated by various tests. Conditioning components include parameters such as endurance, strength, flexibility, speed, balance, coordination, and reaction. Like most combat sports, kickboxing requires a combination of multiple performance components such as strength, aerobic capacity, and speed along with technical skills. However, it cannot be said that a single physical characteristic is dominant in this sport (Silva et al., 2011; Franchini et al., 2011).

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At the genetic level, some polymorphisms affecting performance in athletes have been identified. One of these genes, the *ACTN3* gene, encodes the alpha-actinin-3 protein found in fast muscle fibers and plays an important role, especially in sprint and power sports. The *ACTN3* rs1815739 polymorphism causes the cessation of alpha-actinin-3 production in homozygous carriers of the T allele (TT genotype), which may affect muscle function. It has been suggested that the TT genotype confers an advantage in endurance, and the CC genotype in power/speed sports (Dogan et al., 2022).

The *PPARA* gene encodes for the peroxisome proliferator-activated receptor alpha (PPAR- α), a protein that plays a key role in fatty acid metabolism and contributes significantly to cellular energy production. The *PPARA* rs4253778 G/C polymorphism may affect endurance performance; the G allele has been shown to contribute to aerobic performance by increasing oxidative capacity (Ulucan et al., 2020).

The *IL-6* gene produces the interleukin-6 (IL-6) protein, which is released by skeletal muscles during physical activity and contributes to both inflammatory processes and metabolic regulation. The *IL-6* rs1800795 (-174G/C) polymorphism may affect *IL-6* expression levels. The G allele has been associated with higher levels of *IL-6* and has been linked to a greater inflammatory response to exercise. This may be important for recovery and endurance performance (Ön et al., 2025).

Studies on kickboxing in the literature include physical profile and flexibility (Tatlıbal et al., 2022), body composition (Rydzik et al., 2021), *DRD2* and *ANKK1* genes (Michałowska-Sawczyn et al., 2021), *BDNF* gene (Niewcizas et al., 2021), *ADRA2A* gene (Eken et al., 2021), mental characteristics (Ivanchenko et al., 2020), technical-tactical analysis (Ouergui et al., 2013), mental training (Slimani et al., 2014), and time-motion analysis (Ouergui et al., 2014a). In order to deepen the existing knowledge and contribute to the development of the most appropriate training programs, there is a need to examine the physical and physiological characteristics of kickboxers from different angles according to the level of competition (Slimani et al., 2017).

We could not detect enough study in which genetic factors and aerobic characteristics have been compared together. This study aimed to determine the link between *ACTN3* rs1815739, *PPARA* rs4253778, and *IL-6* rs1800795 polymorphisms and aerobic performance

metrics (Yo-Yo IRT1 and VO_2max), to assess intergroup differences among elite and sub-elite kickboxers, and to determine the most discriminative performance parameter.

Methods

Participants

A total of thirty male kickboxers participated in this study, including 15 elite (national-level) athletes (mean age: 21.53 ± 1.30 years; height: 1.78 ± 0.05 m; body mass: 74.46 ± 11.43 kg; body fat: $14.73 \pm 4.88\%$) and 15 sub-elite athletes (mean age: 20.27 ± 1.28 years; height: 1.75 ± 0.07 m; body mass: 68.69 ± 10.57 kg; body fat: $14.25 \pm 6.97\%$).

The study protocol was reviewed and approved by the University Ethics Committee (approval no: 2021/14-61351342). The study procedures complied with the ethical standards outlined in the Declaration of Helsinki (revised version II). Each participant received a detailed written explanation of the experimental protocol and voluntarily signed informed consent forms.

Procedure

Buccal epithelial cell samples were obtained from all athletes in a designated area for genetic analysis. Following sample collection, participants completed the Yo-Yo Intermittent Recovery Test Level 1 (Yo-Yo IRT1) in an indoor facility. Participants were instructed to refrain from strenuous physical activity for at least 24 hours prior to testing, avoid caffeine and alcohol intake for a minimum of 12 hours, and maintain their habitual sleep routines. Furthermore, participants were required to consume their last meal at least 2–3 hours before testing and to maintain adequate hydration status. All testing sessions were conducted at the same time of day to minimize circadian variation effects. A standardized warm-up routine consisting of 5 minutes of jogging, 3 minutes of dynamic stretching, and 7 minutes of specific technical drills was performed under the supervision of a certified coach (adapted from Diker et al., 2023).

Genotyping

Genomic DNA was isolated from buccal cell samples using a PureLink DNA Extraction Kit (Invitrogen, Thermo Fisher Scientific, USA) according to the manufacturer's guidelines. The concentration with an average of 20 ng of DNA of the isolates were included for the study (The Invitrogen Qubit 4 Fluorometer, Thermo Fisher Scientific, Inc., Waltham,

MA, USA). All the genotyping procedures were carried out by Real-Time PCR (StepOnePlus, Thermo Fisher Scientific, USA) and TaqMan Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA; catalog no. A30866).

Each reaction (10 µl total volume) contained 5 µl Genotyping Master Mix, 3.5 µl nuclease-free water (Thermo Fisher Scientific, USA), 0.5 µl genotyping assay (Catalog no: 4351379; for *ACTN3* rs1815739 polymorphism C___590093_1_ assay was used; for *PPARA* rs4253778 C___2985251_20 and for *IL-6* rs1800795 C___1839697_20 assays were used), and 1 µl of DNA template. The probe and primer sequences employed in the assay are presented in Table 1.

Yo-Yo IRT1

Participants' aerobic endurance was evaluated through the Yo-Yo Intermittent Recovery Test, Level 1 (Yo-Yo IRT1), a field-based assessment widely acknowledged for its reliability and validity in estimating intermittent exercise performance, particularly in team sports (Krustrup et al., 2003). Although laboratory evaluations are often considered the gold standard for determining physiological capacity, field-based protocols such as the Yo-Yo IRT1 provide a practical and sport-specific alternative for assessing aerobic fitness in athletes (Krustrup et al., 2005).

The estimated maximal oxygen uptake (VO_{2max}) of each participant was estimated from the total distance achieved in the test using the predictive equation proposed by Bangsbo et al. (2008):

$$VO_{2max} \text{ (ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}) = [\text{Yo-Yo IRT1 distance (m)} \times 0.0084] + 36.4$$

Data Analyses

The statistical evaluations were conducted using IBM SPSS Statistics software (version 23.0, Chicago, IL, USA). The normality of data distribution was verified through the Shapiro–Wilk test, and Levene's test was employed to examine the equality of variances. For genetic data, Chi-Square analysis was utilized to

compare genotype distributions between groups, while Independent-Samples T-Tests were applied to assess group differences in performance-related variables. Statistical significance was defined at a p-value threshold of less than 0.05.

Results

ACTN3 (Table 2), *PPARA* (Table 3), *IL-6* (Table 4) genotype and performance [Yo-Yo IRT1 (m), estimated VO_{2max} (ml·kg⁻¹·min⁻¹)] (Table 5) findings of elite and sub-elite kickboxing athletes are shown below.

As shown in Table 2, among the 15 elite kickboxing athletes, 8 athletes had the CT genotype (53.34%), 5 had CC (33.33%), and 2 had TT genotype (13.33%). In contrast, among the 15 sub-elite kickboxing athletes, 6 had CT genotype (40.00%), 3 had CC (20.00%), and 6 had TT genotype (40.00%). Although a higher frequency of the CT genotype was detected in the elite group and a higher TT genotype frequency in the sub-elite group, the difference in genotype distribution between the groups was not statistically significant ($p=0.25$). This indicates that *ACTN3* rs1815739 genotype distribution is not significantly associated with competitive level in kickboxing athletes.

For *PPARA* rs4253778 polymorphism, among the 15 elite kickboxing athletes, 12 individuals had CC genotype (80.00%), 3 had CG (20.00%). We detected no GG genotype (0.00%) in elite group. In the sub-elite group, consisting of 15 athletes, 9 had CC genotype (60.00%), 5 CG (33.33%), and one had GG genotype (6.67%). While the CC genotype was predominant in both groups, a slightly higher frequency of the CG and GG genotypes was observed in sub-elite athletes compared to elite athletes. However, these differences in *PPARA* rs4253778 genotype distribution was not statistically significant ($p=0.38$), indicating no association between genotype and athletic classification level in this cohort.

Table 1

Sequences of the TaqMan probe used for genotyping the *ACTN3* rs1815739, *PPARA* rs4253778, and *IL-6* rs1800795 polymorphisms.

		5'- 3' Sequence
<i>ACTN3</i> rs1815739	VIC/FAM	CAAGGCAACACTGCCCCGAGGCTGAC[T/C]GAGAGCGAGGTGCCATCATGGGCAT
<i>PPARA</i> rs4253778	VIC/FAM	ACACTTGAAGCTTGATATCTAGTTT[G/C]GATTCAAAGCTTCATTTCCCATAT
<i>IL-6</i> rs1800795	VIC/FAM	ACTTTTCCCCCTAGTTGTGTCTTGC[C/G]ATGCTAAAGGACGTCACATTGCACA

Table 2
ACTN3 rs1815739 polymorphism genotype distribution.

	ACTN3 rs1815739 Genotype			n	p
	CT	CC	TT		
Elite Kickboxing Athletes	8 53.34%	5 33.33%	2 13.33%	15	0.25
Sub-elite Kickboxing Athlete	6 40.00%	3 20.00%	6 40.00%	15	

Table 3
PPARA rs4253778 polymorphism genotype distribution.

	PPARA rs4253778 Genotype			n	p
	CC	CG	GG		
Elite Kickboxing Athletes	12 80.00%	3 20.00%	0 0.00%	15	0.38
Sub-elite Kickboxing Athlete	9 60.00%	5 33.33%	1 6.67%	15	

Table 4
IL-6 rs1800795 polymorphism genotype distribution.

Variance	IL-6 rs1800795 Genotype			n	p
	CG	CC	GG		
Elite Kickboxing Athletes	5 33.33%	2 13.33%	8 53.34%	15	0.73
Sub-elite Kickboxing Athlete	7 46.67%	2 13.33%	6 40.00%	15	

Table 4 summarizes *IL-6* rs1800795 polymorphism distribution in our cohort. 5 of the elite athletes had CG genotype (33.33%), 2 had CC genotype (13.33%), and 8 had GG genotype (53.34%). In the sub-elite group, 7 had CG genotype (46.67%), 2 had CC (13.33%), and 6 had GG genotype (40.00%). Although the GG genotype appeared more frequently in the elite group, and the CG genotype more frequently in the sub-elite group. Genotype distribution was also not statistically significant ($p=0.73$) between two groups.

As presented in Table 5, the mean Yo-Yo IRT1 performance of elite kickboxing athletes was 728.00 ± 260.20 meters, while sub-elite athletes achieved a

slightly higher average of 773.33 ± 298.44 meters. Similarly, the average VO_{2max} of elite athletes was 42.51 ± 2.18 $ml \cdot kg^{-1} \cdot min^{-1}$, compared to 42.90 ± 2.51 $ml \cdot kg^{-1} \cdot min^{-1}$ in the sub-elite group. Although the sub-elite athletes demonstrated marginally higher scores in both aerobic performance parameters, the differences between the two groups were not statistically significant for either Yo-Yo IRT1 distance ($t = -0.44$, $p = 0.66$) or VO_{2max} values ($t = -0.44$, $p = 0.66$). These results suggest that aerobic performance, as measured by these two parameters, does not significantly distinguish between elite and sub-elite kickboxing athletes in this sample.

Table 5
The average Yo-Yo IRT1 (m), estimated VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$), standard deviation (sd), t and p values of elite and sub-elite kickboxing athletes (n: 15).

		Mean	SD	t	p
Yo-Yo IRT1 (m)	Elite Kickboxing Athletes	728.00	260.20	-0.44	0.66
	Sub-elite Kickboxing Athletes	773.33	298.44		
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	Elite Kickboxing Athletes	42.51	2.18	-0.44	0.66
	Sub-elite Kickboxing Athletes	42.90	2.51		

SD: Standard deviation.

Discussion

Kickboxing is a high-intensity and versatile combat sport that requires athletes to use their technical, physical and mental competencies simultaneously. This sport has a competitive structure in which many physical and mental components, such as speed, strength, agility, endurance, and strategic thinking, are displayed simultaneously. During competitions, athletes perform intense series of attacks and defenses followed by short rest breaks. Therefore, kickboxing is a sport in which not only anaerobic bursts are important, but also a high level of aerobic capacity. Effective recovery, sustaining repetitive high-intensity movements, and maintaining performance until the last round of the match are directly related to a well-developed cardiovascular system (Crisafulli et al., 2009; Silva et al., 2011).

Sports performance is influenced by environmental factors such as training frequency, nutrition, and psychological endurance, as well as the genetic makeup of individuals. Recent studies have identified many single nucleotide polymorphisms (SNPs) affecting athletic capacity and revealed that these genetic variations cause individuals to differ in parameters such as endurance, strength, recovery time, and even motivation. In this context, with the increase in genetic research in sports sciences, it has become important to identify genetic variations that may contribute to performance, especially in combat sports. Several meta-analyses and population-based studies have emphasized the importance of genetic predisposition in martial arts disciplines, highlighting specific gene variants such as *ACTN3*, *PPARA*, and *IL-6* for their potential influence on speed, endurance, and recovery ability (Youn et al., 2021; Bosnyák et al., 2015).

The aim of this study was to examine the differences in *ACTN3* rs1815739, *PPARA* rs4253778 and *IL-6* rs1800795 gene polymorphisms and aerobic performance [Yo-Yo IRT1 (m), estimated VO_2max ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)] between elite and sub-elite kickboxing athletes.

We detected no statistically significant differences between elite and sub-elite kickboxing athletes in terms of *ACTN3* gene (Table 1), *PPARA* gene (Table 2), *IL-6* gene (Table 3) and Yo-Yo IRT1 (m) and estimated VO_2max ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) (Table 4) parameters in our cohort.

Encoded by the *ACTN3* gene, the α -actinin-3 protein is expressed in type II (fast) muscle fibers and contributes significantly to explosive strength and

speed-oriented activities. The TT genotype resulting from the rs1815739 polymorphism leads to the absence of this protein, while the CC genotype is associated with high explosive power. Tacal Aslan et al. (2024) examined the distribution of *ACTN3* genotypes across different sports branches in their study on elite and sub-elite level Turkish athletes and revealed that especially the CC genotype may be effective for power performance. Similarly, a recent meta-analysis by El Ouali et al. (2024) demonstrated that the CC genotype of *ACTN3* rs1815739 is significantly overrepresented in power and combat sport athletes compared to endurance athletes and non-athletes ($\text{OR} \geq 1.48$, $p < 0.001$), suggesting a clear association between the CC genotype and superior explosive strength.

The *PPARA* gene encodes the PPAR- α protein, which is involved in fatty acid metabolism and plays an important role in energy production. The rs4253778 polymorphism has been specifically associated with endurance performance; the G allele has been shown to contribute to aerobic performance by increasing oxidative capacity. In some analyses in combat sports, this genotype has been reported to occur at a high frequency and may enhance endurance. Previous studies also show that the combination of *PPARA* and *IL-6* is associated with speed performance (e.g. in 1 km time records) (Kazancı et al., 2023). Additionally, Youn et al. (2021) reported that GG genotype and G allele frequencies were significantly higher among elite-level combat athletes compared to sedentary controls. Similar findings were supported by Polish studies showing the G allele to be associated with increased VO_2max and training response in combat sports (López-León et al., 2016).

The *IL-6* gene encodes the interleukin-6 protein, which is secreted from muscles during exercise and is involved in inflammation and recovery processes. The rs1800795 polymorphism can affect *IL-6* expression levels, and it has been shown that carriers of the G allele, in particular, have higher inflammatory responses after exercise. Ulucan et al. (2023) showed that *IL-6* genotypes may be determinants of sports performance in different sports branches. Moreover, the GC genotype has been associated with faster post-exercise recovery and favorable endurance traits in long-distance athletes (Kazancı et al., 2023). This genotype has also been linked to a more efficient adaptive immune response following intense physical effort.

Our findings showed that the estimated VO_2max values of elite and sub-elite kickboxing athletes (42.51

and 42.90, respectively) were close to each other but lower than those reported in the literature (Table 4). When the literature was examined in terms of estimated VO_2max , studies including athletes from Canada (4 elite) (Zabukovec et al., 1995), Tunisia (30 amateur) (Ouergui et al, 2014b), Turkish (10 national) (Salci, 2015), Serbian (16 elite, 34 sub-elite) (Ljubisavljević et al., 2015), Portugal (13 elite) (Silva et al., 2011) reported values of 61.5, 51.9, 48.5, 49.81, and 57.99 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, respectively. The values of elite male kickboxing athletes in the literature range between 48.5-61.5 $\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$. These findings emphasize that kickboxing places a strong demand on cardiovascular and respiratory functions.

Conclusion

ACTN3 rs1815739, *PPARA* rs4253778 and *IL-6* rs1800795 gene polymorphisms may have different levels of effects on athletic performance in martial arts. In large-scale meta-analyses, especially among martial arts athletes, it has been reported that *ACTN3* and *PPARA* genes are found at higher frequencies in elite level athletes and that these genotypes may be determinants of strength and endurance performance (Bosnyák et al., 2015; Ruiz et al., 2010). However, genetic studies specific to kickboxing are very limited. This situation makes the current study qualified to fill the gap in the literature.

We detected no statistically significant difference between elite and sub-elite kickboxing athletes in terms of *ACTN3* rs1815739, *PPARA* rs4253778 and *IL-6* rs1800795 gene polymorphisms and aerobic performance parameters such as Yo-Yo IRT1 and estimated VO_2max ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) ($p > 0.05$). According to these results, we can speculate that these genes and aerobic parameters are not discriminating factors in this sample. Nonetheless, given the complex physiological demands of kickboxing and the multifactorial nature of sports performance, it is recommended that future studies include larger sample sizes, consider polygenic models, and examine gene-environment interactions to gain a more comprehensive understanding of genetic contributions to athletic success in combat sports.

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Author contributions

Conception: Raif Zileli (RZ) and Korkut Ulucan (KU), design: RZ, Mehmet Söyler and KU, supervision: RZ,

funding: Serdar Bayrakdaroğlu (SB), materials: Tolga POLAT (TP), Özlem Özge YILMAZ (ÖÖY), Beste TACAL ASLAN (BTA), KU, data collection: RZ, MS, SB, Mehmet Onur Sever (MOS), analysis: RZ and Hüseyin Özkamçı (HÖ), literature review: RZ, İbrahim Can (İC), TP, ÖÖY, BTA, writing: RZ, MS, HÖ, Gürkan Diker (GD), TP, ÖÖY, critical review: RZ, MS, MOS, Sadi Ön, SB, İC, HÖ.

Ethical Approval

Üsküdar University, Ethical Committee approved the study protocols (2021/14-61351342), and the study is conducted in accordance with the principles of the Declaration of Helsinki II.

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Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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