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ASSOCIATION BETWEEN *MCT1* GENE POLYMORPHISM (rs1049434) WITH THE ATHLETIC PERFORMANCE OF ELITE TRACK AND FIELD ATHLETES

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Abstract: Monocarboxylate transporter 1 (MCT1; SLC16A1) is a proton-dependent cotransporter/exchanger, located on the apical membrane of cells. MCT1 is able to transport several monocarboxylates including lactate, pyruvate and acetate, which makes this protein critical in terms of the athletic performances. The rs1049434 polymorphism in the *MCT1* gene was frequently associated with the performance of the athletes in different populations. The present study aims to decipher any possible association of the rs1049434 polymorphism with the personal best of elite track and field athletes. A total of sixty elite athletes (31 sprint/power and 29 endurance) and twenty control/sedentary with the ages of 18-35 voluntarily participated in the study. The International Association of Athletics Federations (IAAF) score scale was used to determine the performance levels of the personal best (PB) of the athletes. Whole exome sequencing (WES) was performed by the genomic DNA isolated from blood of the participants. Sport type, sex and PB were chosen as the parameters to compare within and between the groups by linear regression models. The sex was not the criterion that was significantly different between or within the groups. Although PB scores were not significant within the sprint/power group, it was significant within the endurance group by the codominant ($p=0.044$), dominant ($p=0.016$) and over-dominant ($p=0.048$) models. The rs1049434 polymorphism in the *MCT1* gene may be linked to the PB of the endurance athletes. However, other genetic alterations should be regarded to conclude the effect of this polymorphism. The multi-factorial genetic background that could associate with the athletic performance is still under investigation in our research group. Nevertheless, further studies with more participants are needed.

Key Words: Athletics, athlete, runner, rs1049434, polymorphism, *MCT1*, *SLC16A1*

ELİT ATLETLERDE *MCT1* GEN POLİMORFİZMİNİN (rs1049434) ATLETİK PERFORMANS İLE İLİŞKİSİNİN ARAŞTIRILMASI

Öz: Monokarboksilat taşıyıcı/değiştiricidir. MCT1 laktat, pirüvat ve asetat gibi monokarboksilatların transportunda görev alır ve bu durum bu proteini atletik performans açısından önemli kılar. *MCT1* genindeki rs1049434 polimorfizmi, farklı popülasyonlarda atletlerin performansı ile sıkça ilişkilendirilmiştir. Sunulan çalışma, Türk popülasyonunda, elit atletlerde, atletik performans ile rs1049434 polimorfizmi arasındaki olası ilişkiyi açığa çıkartmayı amaçlamaktadır. Çalışmaya, yaşları 18-35 arası değişen ve 31 sprint/güç ve 29 dayanıklılık olmak üzere altmış elit atlet ve yirmi kontrol dahil edilmiştir. Atletlerin performans seviyeleri, kişisel en iyi dereceleri (PB) aracılığıyla Uluslararası Atletizm Federasyonu (IAAF) skor ölçeğine göre belirlenmiştir. Katılımcılardan elde edilen periferik kandan izole edilen DNA'dan tüm ekzom dizileme (WES) çalışılmıştır. Spor türü, cinsiyet ve PB, doğrusal regresyon modelleriyle gruplar içinde ve arasında analiz edilmiştir. Cinsiyet, gruplar içinde ve arasında istatistiksel olarak anlamlı bir fark tespit edilmemiştir. Sprint/güç grubunda PB anlamlı olmasa da dayanıklılık grubunda kodominant ($p=0.044$), dominant ($p=0.016$) ve over-dominant ($p=0.048$) modelleri ile anlamlı sonuçlar olduğu saptanmıştır. *MCT1* genindeki rs1049434 polimorfizmi, dayanıklılık sınıfındaki atletlerin PB düzeyleriyle ilişkili olabilir. Ancak, diğer olası genetik faktörler dikkate alınmalıdır. Atletik performansı etkileyebilecek multifaktöriyel genetik arkaplan grubumuzca devam ettirilen çalışmalar arasındadır. Yine de daha fazla katılımcı ile ileri çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Atletizm, sporcu, koşucu, rs1049434, polimorfizm, *MCT1*, *SLC16A1*

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INTRODUCTION

Skeletal muscle produces huge amount of energy by anaerobic glycolysis during the excessive exercise (Bishop et al., 2007; Bulgay et al., 2021). When the exercise is continued, the production of lactic acid (LA) increases in blood and muscles (Bishop et al., 2007; Knuttgen, 2007). When the levels of LA in the muscle increase, acidity affects the activity of some enzymes, resulting in decrease in the energy production (Fedotovskaya et al., 2014). Hence, elevated LA has been underlined to influence the athletic performance negatively (Goran et al., 2009). Moreover, the elimination of LA quickly may contribute the athletic performance. Genetic backgrounds and training routines of the athletes have been figured out to be a reason for the differentiated lactate transport capacity (Pilgaard et al., 1994; Bugay et al., 2021). Thus, it may be fundamental to decipher the genetic background of the athletes to be able to point out the lactate capacity-related mechanisms.

In the cellular level, one of the proteins involved in the lactate transport capacity is monocarboxylate transporter1 (MCT1; SLC16A1). MCT1 protein is responsible for the transport of several monocarboxylates including lactate, pyruvate and acetate. Therefore, MCT1 protein is critical in terms of the athletic performances (Ahmetov, & Fedotovskaya, 2015). Particularly rs1049434 polymorphism (1470T>A) in the *MCT1* gene has widely been studied in the literature. Merezhinskaya et al. (2000) defined the rs1049434 polymorphism for the first time by emphasizing that less lactate transport was seen in the individuals with allele T (Merezhinskaya et al., 2000). In another study, allele T was linked to the increased lactate accumulation in blood under high-density resistance training (Cupeiro et al., 2010). Similarly, allele T was associated with increased lactate levels in athletes (Fedotovskaya et al., 2014). In addition, the A/A genotype of the *MCT1* polymorphism was shown to be over-represented in wrestlers compared to controls and associated with lower blood lactate concentrations after 30-s Wingate Anaerobic test (WAnT) and during intermittent sprint tests in Japanese wrestlers (Kikuchi et al., 2017). The association studies have also been carried out for football players in Italy (Massidda et al., 2021), Spanish individuals (Cupeiro et al., 2012) and triathletes (Piscina-Viudez et al., 2021), Polish elite sprint/power athletes (Sawczuk et al., 2015), and Brazilian and European endurance athletes (Guilherme et al., 2021) by focusing on diverse parameters. Still, similar studies should be conducted to totally explore the association of this polymorphism with sports parameters in a population-specific manner.

The present study aims to evaluate the rs1049434 polymorphism in the athletics athletes via sprint/power and endurance athletes in the presence of control group. The allele and genotype frequencies and the association of the polymorphism with the parameters, sex and personal best (PB) scores were determined between and within the groups. To our knowledge, this is the first study to investigate the rs1049434 polymorphism in Turkish athletes. Thus, codominant, dominant and recessive genetic models were assessed to determine differences amongst athlete phenotypes (sprint/power and endurance). We suggest that the genetic findings assessing the sprint/power and endurance disciplines in the athletics branches can guide further research. We hypothesized that the athletes with T/T genotype would present lower score on PB for sprint/power while higher score for endurance abilities.

METHOD

Participants

The study involved sixty elite athletes (sprint/power: 11 females (35.5%) and 20 males (64.5%); endurance: 10 females (34.5%) and 19 males (65.5%) licensed in different clubs and affiliated to the Turkish Athletics Federation (M Age (year) = 25.07, SD = 4.80; M Length (cm) = 174.97, SD = 7.89; M Body weight (kg) 72.50, SD = 22.40; M Sport experience (year) = 9.40, SD = 4.80; M Personal-best (PB) = 1005.63, SD = 94.55). The number of the controls (non-athletes) were 20 (6 females (30.0%) and 14 males (70.0%); M Age (year) = 23.51, SD = 7.13) and they were healthy unrelated citizens of Turkey without any competitive sports experience. The informed voluntary consent and demographic information forms were applied for the athletes and controls groups before the measurements.

Athletic Performance/Personal Bests

The International Association of Athletics Federations (IAAF; World Athletics) score scale was used to determine the performance levels of the athletes depending on their personal best (Spiriev, 2014). For instance, the IAAF score scale of a male athlete who runs 100 meters in 10.05 sec is 1189 while that of a marathon runner who complete the race in 2 h 20 min 11 sec is 997. Thus, the performance scale of the marathon runner is less than that of 100 m runner. The IAAF scales are useful for the determination of performances of the athletes from diverse athletics events and genders.

Whole Exome Sequencing

Total genomic DNA was isolated from peripheral venous blood of the participants for further genetic screenings using DNeasy Blood and Tissue Kit (Qiagen, Germany) according to the supplier's instructions. The quality of the isolated DNA was verified using 1% agarose gel electrophoresis and concentration was determined by NanoDrop (NanoDrop 1000 Spectrophotometer; Thermo Scientific, USA).

Whole Exome Sequencing (WES) was performed after library preparation by Twist Human Comprehensive Exome Panel (Twist Biosciences, USA) according to the instructions of the supplier. Briefly, DNA was fragmented enzymatically, size selection was carried out and hybridization was applied using Twist Hybridization probes and Dynabeads™ MyOne™ Streptavidin T1 (Invitrogen, USA), and the library was enriched by polymerase chain reaction (PCR). The concentration and size of the libraries were determined and sequences were performed using Illumina NextSeq500 according to manufacturer's standard protocol.

Raw data were processed to by the Genome Analysis Toolkit (GATK)'s (Van der Auwera et al., 2013). HaplotypeCaller program to obtain Binary Alignment Map (BAM) files and subsequently produce an output Variant Call Format (VCF) file via the GRCh38/hg38 reference genome. Variants were annotated by ANNOVAR (Wang et al., 2010) and each single nucleotide polymorphisms (SNPs) were analyzed manually.

Data Analysis

The SPSS (Statistical Package for Social Sciences) for Windows 25.0 was used for data analysis. In the evaluation of the data, descriptive statistical methods (number, percentage, and mean) were used. Before performing any analysis on the data, the study determined whether they met the requirements for parametric tests. To that end, the variables were tested for normality while Kolmogorov-Smirnov and Shapiro-Wilk ($p = .200$; $.785$ respectively) were used for homogeneity of variance. As result of these tests, parametric tests were performed for

the variables distributed. Genotype and allele frequencies were calculated for the polymorphism and Hardy-Weinberg equilibrium (HWE) was assessed using the chi-square (χ^2) or Fisher's exact test. Allele and genotype frequencies and association approaches were obtained SNPStats (Sole et al., 2006) using linear regression with linear regression multiple inheritance models: co-dominant, dominant, recessive, and over-dominant. To confirm the results obtained using the linear regression models we also analyzed the data by means of the one-way analysis of covariance (ANCOVA), adjusting for sex and sports experience. Data were significant when $p < 0.05$.

Ethics Committee Approval

The study was carried out in accordance with the Declaration of Helsinki and approval was obtained from the Gazi University Non-Interventional Clinical Research Ethics Committee with the decision dated April 05, 2021 and numbered 09.

RESULTS

The present study aims to decipher any possible association of the rs1049434 polymorphism with the PB/athletic performances of a group of Turkish elite athletes in the presence of control group. The allele frequencies were 54% ($n=87$) for allele T and 46% ($n=73$) for allele A within all participants. Although the frequencies of the alleles were close to each other within athlete group (52% for allele T and 48% for allele A), they were different from each other within the control group (62% for allele T and 38% for allele A).

The analyses for the genotype frequencies showed that heterozygosity (T/A) was more in the athletes while the wild-type (T/T) was more in the control group. Nonetheless, the frequencies of the genotypes were not statistically significant between athletes and the control group. When the athletes were separated into two groups as sprint/power and endurance, there were also not any significance between the genotype ratios. The analyses to figure out whether there were any associations between sex and the genotypes within the athletes showed that there were not any significance between the sex and the genotype ratios (Data not shown).

Finally, there were not any associations between PB and genotype ratios within the sprint/power athletes (Table 1) while PB was significantly correlated with rs1049434 polymorphism according to the codominant ($p=0.044$), dominant ($p=0.016$) and over-dominant model within the endurance athletes ($p=0.048$; Table 2).

Table 1. rs1049434 association with the PB within the sprint/power athletes.

Model	Genotype	n	Mean score (PB)	Difference (95% CI)	P-value
Codominant	T/T	9	984.78 (35.95)	0.00	0.89
	T/A	15	979.07 (27.98)	-3.02 (-88.67 - 82.63)	
	A/A	7	1006.71 (26.99)	19.24 (-83.62 - 122.11)	
Dominant	T/T	9	984.78 (35.95)	0.00	0.85
	T/A-A/A	22	987.86 (20.74)	-8.03 (-90.58 - 74.53)	
Recessive	T/T-T/A	24	981.21(21.6)	0.00	0.63
	A/A	7	1006.71 (26.87)	21.18 (-64.27 - 106.62)	
Over-dominant	T/T-A/A	16	994.38 (22.87)	0.00	0.75
	T/A	15	979.07 (27.98)	-11.55 (-82.88 - 59.77)	
Log-additive	---	---	---	8.091(-41.52 - 59.33)	0.73

*Statistically significant differences ($P < 0.05$); adjusted by sports experience + sex.

Table 2. rs1049434 association with the PB within the endurance athletes.

Model	Genotype	n	Mean score (PB)	Difference (95% CI)	P-value
Codominant	T/T	6	1087.17 (35.77)	0.00	0.044*
	T/A	17	992.94 (16.81)	-86.58 (-150.33 - 22.84)	
	A/A	6	1039.83 (29.54)	-61.69 (-137.80 - 14.41)	
Dominant	T/T	6	1087.17 (35.77)	0.00	0.016*
	T/A-A/A	23	1005.17 (14.93)	-78.99 (-135.84 - 19.14)	
Recessive	T/T-T/A	23	1017.52 (17.48)	0.00	0.91
	A/A	6	1039.83 (29.54)	-3.95 (-74.35 - 66.44)	
Over-dominant	T/T-A/A	12	1063.5 (23.24)	0.00	0.048*
	T/A	17	992.94 (16.81)	-57.72 (-112.19 - -3.26)	
Log-additive	---	---	---	-32.70 (-73.11 - 7.71)	0.13

*Statistically significant differences ($P < 0.05$); adjusted by sports experience + sex.

DISCUSSION AND CONCLUSION

In the present study investigated the genotype distributions and allele frequencies of the *MCT1* rs1049434 (1470T>A) polymorphism between elite endurance, elite sprint/power athletes and matched controls. To our best knowledge, the current study is first investigation to determine whether the rs1049434 polymorphism influences PB of elite endurance and elite sprint/power athletes.

The results underlined that PB scores were not associated with the genotypic variations for sprint/power group. Nonetheless, although there was not any significance within sprint/power athletes in terms of the rs1049434 polymorphism, the athletes with A/A genotype had higher PB scores. Similar results were also available in the literature. For instance, rs1049434 polymorphism was studied in Italian footballer players and allele A was shown to be favorable for repeated sprint ability (RSA) (Massidda et al., 2021). Moreover, high levels of lactate in skeletal muscle were reported to facilitate the performances of the athletes by increasing the muscle mass and power in sprint/power athletes (Bishop et al., 2007; Girard et al., 2011). Our findings, thus, pointed out that allele A may be correlated with the sprint/power branches.

According to our results, PB scores were significantly linked to the rs1049434 polymorphism for the endurance group. The PB scores were higher in the athletes particularly with T/T genotype, rather than T/A or A/A genotype. In a study in which 1208 (318 endurance athletes and 890 non-athletes) Brazilian and 867 (315 endurance athletes and 552 non-athletes) European individuals participated, even though there was ethnical differences, the athletes with wild-type (T/T) genotype had lower lactate accumulation and higher maximal oxygen uptake (VO_2max) levels. In addition, T/T genotype was associated with endurance of the athletes (Guilherme et al., 2021), which was also reported by another study (Sawczuk et al., 2015). Hence, in addition to our results, the availability of allele T would be associated with endurance in the athletes. Still, whether this suggests that endurance athletes better tolerate the decreased lactate transport associated with the presence of the T/T genotype, or whether this polymorphism displays some advantage for endurance athletes, is currently not clear. It is important to highlight that more parameters such as environment, psychology and epigenetic mechanisms besides the PB scores should be involved in similar studies.

The ratio of the allele T was reported to be higher in Polish climbers compared to the control group. However, there were not any differences between climbers and controls for Japanese

climbers; moreover, the A/A genotype was linked to the endurance in the same study (Saito et al., 2021). In another study, allele T was associated with sprint/power rather than endurance (Sawczuk et al., 2015). The allele T was further linked to the endurance properties in a study conducted by Israeli athletes (Ben-Zaken et al., 2015). The allele and genotype frequencies may differ between diverse ethnicities because of the environmental conditions (Bulgay et al., 2021; Yıldırım et al., 2022). Therefore, the findings of the present study could be limited to Turkish population. To classify the elite athletes, genetic, environmental and epigenetic factors should separately be evaluated. Determination of these factors could promote trainers, sport scientists and athletes with clarifying proper branches and enhancing sport success. Moreover, we believe that epigenetic studies bring new information and get better knowledge, which is under progress by our research group. Before, Zelka et al. (2019) had similar results on a small Turkish athlete cohort and Akkoc et. al (2020) on Turkish ironman triathlon athletes.

In the literature, there have been limited studies whose results may be different from each other in terms of association approach with athlete performances. These different results could be a result of comparison of the athletes from diverse disciplines, studying with non-homogenous and limited groups, and limited history of the participants such as unclear ethnicity, sport experiences and epigenetics (Bulgay et al., 2020; Bulgay et al., 2022). Thus, further studies are needed by eliminating such limitations to totally explore the association of the rs1049434 polymorphism in the Turkish athletics performances.

The present study figured out that the T/T genotype was associated with the endurance in the Turkish athletes. Based on this indication, the individuals with allele T may be advised to the endurance groups. Also, the possible epigenetic effects of LA on muscle metabolism and athletic performance should be considered in future studies. It is important to consider the homogeneity and quantity of the study groups as an important detail in terms of the consistency of the genetic reflections explained as a result of the research studies conducted. However, other genetic alterations should be regarded to conclude the effect of this polymorphism. The multi-factorial genetic background that could associate with the athletic performance is still under investigation in our research group. Nevertheless, further studies with more participants are needed to clarify the effect of the rs1049434 polymorphism on athletic performance.

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Conflicts of Interest The authors declare no conflict of interest.

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