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Research Article

Investigation of the putative functional relevance of the *IL-6* 3'UTR genetic variants with athletic phenotype in Turkish triathletes

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Abstract: Previous research suggests that genetic variants in the interleukin-6 (*IL-6*) gene contribute to sport-related traits and athletic performance. We aimed to identify sequence variants in the *IL-6* gene region comprising the 3' untranslated region (UTR) in the Turkish triathletes and sedentary individuals and assessed their putative roles in tendency to athletic phenotype. Sequence variants were identified in the Turkish triathletes (n = 47) and sedentary individuals (n = 46) by Sanger sequencing. Allele/genotype frequencies and linkage disequilibrium (LD) patterns were calculated by the Haploview program. The functional significance of the detected variants was analyzed using *in silico* prediction tools. Four single nucleotide variants (rs13306435, rs747302620, rs2069849, rs13306436) were detected in saliva samples of the participants by sequencing the target region. Notably, rs13306436-3'UTR/*IL-6* was only seen in the triathletes, while the exonic rs747302620 was observed in only sedentary group. Also, rs13306436G>A causes loss/gain sites for binding multiple miRNAs that may be associated with athletic performance. Our findings indicate that the 3'UTR/*IL-6* may have functional relevance in determining sports talent. Future comprehensive studies focusing on the *IL-6* gene in athletes may pave the way for not only determining the athletic status of the individuals but also have implications for translational medicine.

Özet: Önceki araştırmalar, interlökin-6 (*IL-6*) geni varyantlarının spor ile ilgili özelliklere ve atletik performansa katkı sağladığını ileri sürmektedir. Bu çalışmada, Türk triatletler ve sedanter bireylerde *IL-6* geninin 3' translasyon olmayan bölgelerinde (UTR) dizi varyantlarını tanımlamayı ve bunların atletik fenotipe yatkınlıktaki varsayılan rollerini değerlendirmeyi amaçladık. Türk triatletlerde (n = 47) ve sedanter bireylerde (n = 46) dizi varyantları Sanger dizileme ile tanımlanmıştır. Alel/genotip frekansları ve bağlantı dengesizliği (LD) örüntüleri Haploview programı ile hesaplanmıştır. Tespit edilen varyantların fonksiyonel önemleri *in silico* tahmin araçları kullanılarak analiz edilmiştir. Hedef gen bölgesinin dizilenmesi sonucunda, katılımcıların tükürük örneklerinde dört tek nükleotid varyantı (rs13306435, rs747302620, rs2069849, rs13306436) tespit edilmiştir. rs13306436-3'UTR/*IL-6* sadece triatletlerde görülürken, ekzonik rs747302620 sadece sedanter grupta gözlenmiştir. Ayrıca, rs13306436G>A, miRNA'ların bağlanabileceği kayıp/kazanç bölgeleri yaratarak atletil performans ile ilişkili olabilir. Bulgularımız, 3'UTR/*IL-6*'nın sporcu yeteneğini belirlemede işlevsel bir öneme sahip olabileceğini göstermektedir. Sporcularda *IL-6* genine odaklı yapılacak gelecekteki kapsamlı çalışmalar, yalnızca bireylerin atletik durumlarının belirlenmesine değil, aynı zamanda translasyonel tıp için de çıkarımlara yol açabilir.

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Introduction

Human athletic performance is determined by combinations of intrinsic and extrinsic factors such as strength, endurance, psychology, diets, epigenetic and genetic factors (de la Iglesia *et al.* 2020, Ginevičienė *et al.* 2022). Recently, the contribution of genetic factors to athletic performance has been widely studied and the genetic heritability of exercise-related traits has been estimated to range from 50 to 68% (Konopka *et al.* 2023). Thus, genetic studies related to athletic performance have progressively increased in the last years leading to the emergence of a new field called sporomics, which aims to elucidate the determinants of athletic success using

different omic layers (Bongiovanni *et al.* 2019, Appel *et al.* 2021, Semenova *et al.* 2023). Almost 250 gene regions have so far been found to be associated with a tendency to exercise-related traits and athletic ability (Varillas-Delgado *et al.* 2022, Konopka *et al.* 2023, Semenova *et al.* 2023). However, multiple single nucleotide variants (SNVs) in the *COL61A, IL-6, 5-HTT, MAO-A, BDKRB2, NOS3, PPAR-A, MCT1, HIF1A1,* and *AMPD1* genes have been suggested to be associated with athletic performance in triathletes (Domingo *et al.* 2012, Grealy *et al.* 2015, Saunders *et al.* 2015, Corak *et al.* 2017, Akkoç *et al.* 2020). Yet, more research is needed to elucidate the genetic architecture of the triathletes that may contribute to their talent and well-being for sports performance.

The interleukin-6 (*IL-6)* gene, located in chromosome 7, encodes a pleiotropic cytokine involved in immune regulation, and its regulations have been shown to contribute to distinct pathologies (Ataie-Kachoie *et al.* 2013, Hirano 2021, Kishimoto & Kang 2022). *IL-6* is known to have an essential function in anti-inflammatory processes in skeletal muscle and also plays an active role in muscle repair and hypertrophy after exercise (Rosa Neto *et al.* 2009, Pedersen, 2013). Thus, plasma levels of *IL-6* are observed to be increased during acute exercise, and exercise duration is the primary mediator of the *IL-6* concentrations (Nash *et al.* 2023). Given the idea that *IL-6* production favors the tendency to physical activity and considering its important role in metabolic processes during exercise, studies in sports genetics have focused on single nucleotide polymorphisms (SNPs) in the *IL-6* gene (Akkoç *et al.* 2020, Ben-Zaken, *et al.* 2022, Nash *et al.* 2023). The most studied SNP located in the *IL-6* gene is the rs1800795G>C (c.-174C > G), which is located in the 5' untranslated region (UTR) (Eider *et al.* 2013, Fuku *et al.* 2019, Pickering *et al.* 2019, Moreland *et al.* 2022, Semenova *et al.* 2023). The IL6/rs1800795-G allele has been reported to be associated with high *IL-6* expression and athletic performance in previous studies (Bennermo *et al.* 2004, Kazancı *et al.* 2023). However, future investigations are warranted to fully assess the roles of *IL-6* sequence variants in sports genetics. The 3′UTRs play an important role in regulating of gene expression, mRNA stability, and protein function. Nevertheless, SNPs in the 3'UTRs of the genes may be located in the regulatory sequences that disrupt or enhance miRNA-mRNA interactions. In this regard, our study aimed to resequence a part of the exon 5 of the *IL-6* gene comprising 3′UTR in Turkish triathletes and assess the functional importance of the detected variants using bioinformatic tools.

Materials and Methods

Samples

Saliva samples (2 mL, in saliva collection tubes) collected from 93 volunteers aged 18 or above, including 47 triathletes (38 Males, 9 Females) from the Gelibolu and Balıkesir Avlu Triathlon races organized by the Triathlon Federation in 2022, and 46 sedentary individuals (12 Males, 34 Females) selected from the general population were included in the study. All triathletes who participated in the study were classified as elite status based on their previous performance (1st, 2nd, or 3rd place winners) in international and/or national triathlon races. Collected saliva samples were stored at -20°C until DNA isolation.

DNA Isolation

Genomic DNA was isolated from 500ul of each of the saliva samples using the Saliva DNA Extraction Kit (Hibrigen, Türkiye) by an extra spin-column purification step (Thermo Fisher Scientific, Darmstadt, Germany). Proteinase K treatment (3 hours at 56°C) was applied to all samples before DNA isolation. The NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Darmstadt, Germany) and Qubit 4.0 (Thermo Fisher Scientific, Darmstadt, Germany) were used to assess the DNA concentration and quality.

PCR Amplification and Sequencing

Primers covering the 3'UTR of the *IL-6* gene (NM_000600.5) were designed using the NCBI primer design tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). Primers targeting the fragment in the 5th exon region (600bp) covering 3'UTR (F: AGCATCCCTCCACTGCAAAG, R: TGGTGGCAGTGACAAGAAAC) were used for PCR amplification and Sanger Sequencing. For amplification of the desired fragment 2.5 µl 10X PCR buffer, 2 µl $MgCl_2$, 0.5 µl 20 mM dNTP, 0.6 µl from each primer (10 µM), 2-5 µl DNA template, and 0.15 µl AmpliTaq Gold Taq Polymerase (AmpliTaq Polymerase, ThermoFisher) were used in the final volume of 25 µl. PCR conditions are given in Supplementary Material Table S1. After amplification, PCR products were visualized and confirmed in 1.5% agarose gel electrophoresis. Sanger sequencing was performed in Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, Darmstadt, Germany).

Statistical and Bioinformatics Analysis

Haploview software was used to calculate the Hardy-Weinberg Equilibrium (HWE) *p*-value and linkage disequilibrium (LD) patterns of the SNVs. The Chi-square test was conducted using Haploview software (Barrett *et al.* 2005). Sequence chromatograms were analyzed by using the Sequencher (Gene Codes, Ann Harbor, MI) and Uniprogen software. We also used the LDlink online tool to assess the LD pattern and genotype distributions of the variants in the worldwide populations (https://ldlink.nih.gov) (Machiela & Chanock 2015). The regulatory impact of the SNVs was assessed from RegulomeDB (Boyle *et al.* 2012), while SIFT, MutationTaster, and Polyphen databases were used to assess their possible effects on protein function (Ng & Henikoff 2003, Adzhubei *et al.* 2010, Schwarz *et al.* 2014). We used the miRNASNP database (http://bioinfo.life.hust.edu.cn) to predict the potential impacts of the SNVs for miRNA bindings (Liu *et al.* 2021). A *p*-value of less than 0.05 was considered a statistically significant result.

Results

Variant Detection

A total of four variants (rs13306435, rs747302620, rs2069849, rs13306436) with minor allele frequency 0.005-0.022 were identified in the total sample (n = 93) (Table 1). Genotype distributions were found to follow HWE ($p > 0.05$) (Table 1).

We identified only one variant (rs13306436) located in the 3'UTR, and three were located in the coding region of the exon 5. The heterozygote (GA) genotype was observed for rs13306436 (3'UTR variant) in only three triathletes (MAF = 0.016) (Fig. 1) while coding variant rs747302620 was observed in only sedentary individuals (MAF = 0.011) (Tables 1, 2).

The distribution of the alleles and genotypes was not statistically significant, yet a marginal *p*-value (0.08) was observed for rs13306436-A when comparing the two groups (Table 2). The distribution of the detected variants in populations sequenced in 1000 Genome Project was also assessed (the data retrieved from the LDlink online tool is presented in Fig. 2 and Supplementary Material Table S2). The coding rs747302620 was not reported in 1000 Genome Project data, so not included in Fig. 2. Strikingly, 3'UTR variant rs13306436-A was rarely detected in populations of Asian descent (MAF ≤ 0.043) and the A-allele was not reported in remaining worldwide populations. The LD patterns of the variants were analyzed and no significant LD was found in any group (Supplementary Material Fig. S1).

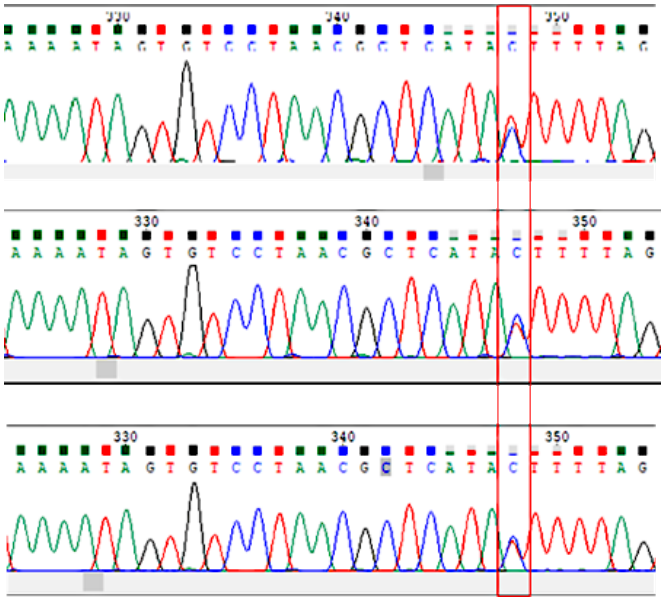

Fig. 1. The chromatograph depicts sequence variant (3'UTR-rs13306436G>A) detected in 3 triathletes.

Table 1. Allele and genotype frequencies of the identified SNVs in the samples (n = 93).

RefSNP ID	Alleles	Chr loc.[a] (GRCh38.p14)	Location	HW-p[b]	Allel Freq[c]	Genotype (n, %)	1000G[d] European MAF	ExAC[e] Global MAF	GnomAD[f] Exomes Global MAF
rs13306435	T:A	22731420	Exon5 (Asp162Glu)	1.0	T: 98.4%, A: 1.6%	TT (n = 89, 89%) TA (n = 3, 3%) AA (n = 0, 0%)	0.017	0.025562	n/a
rs747302620	A:C	22731430	Exon5 (Thr166Pro)	1.0	A: 99.5%, C: 0.5%	AA (n = 92, 92%) AC (n = 1, 1%) CC (n = 0, 0%)	n/a	0.000008	0.000004
rs2069849	C:T	22731537	Exon5 (Phe201Leu)	1.0	C: 97.8%, T: 2.2%	CC (n = 89, 89%) CT (n = 4, 4%) TT (n = 0, 0%)	0.022	0.046119	0.043579
rs13306436	G:A	22731677	3' UTR	1.0	G: 98.4%, A: 1.6%	AA (n = 90, 90%) AG (n = 3, 3%) GG (n = 0, 0%)	0	n/a	0.000699

a: Chromosomal location, b: Hardy-Weinberg *p*-value, c: Allele frequency, d: 1000 Genome project, e: The exome aggregation consortium, f: The genome aggregation database, n/a: not applicable.

Table 2. Allele and genotype frequencies of the identified SNVs in triathletes and sedanter individuals.

RefSNP ID	Associated Allele	Total MAF	Allele Frequency		Genotypes	
			Triathletes (n = 47)	Sedanter (n = 46)	Triathletes (n = 47)	Sedanter (n = 46)
rs13306435 T > A	A	0.016	T: 98.9%, A: 1.1%	T: 97.8%, A: 2.2%	TT (n = 46) TA (n = 1) AA (n = 0) n/a (n = 0) x2/*p*-value: 0.385/0.5351	TT (n = 43) TA (n = 2) AA (n = 0) n/a (n = 1)
rs747302620 A > C	C	0.005	A: 100%, C: 0%	A: 98.9%, C: 1.1%	AA (n = 47) AC (n = 0) CC (n = 0) n/a (n = 0) x2/*p*-value: 1.027/0.3108	AA (n = 45) AC (n = 1) CC (n = 0) n/a (n = 0)
rs2069849 C > T	T	0.022	C: 96.8%, T: 3.2%	C: 98.9%, T: 1.1%	CC (n = 44) CT (n = 3) TT (n = 0) n/a (n = 0) x2/*p*-value: 0.979/0.3225	CC (n = 45) CT (n = 1) TT (n = 0) n/a (n = 0)
rs13306436 G > A	A	0.016	G: 96.8%, A: 3.2%	G: 100%, A: 0%	GG (n = 44) GA (n = 3) AA (n = 0) n/a (n = 0) x2/*p*-value: 2.984/0.0841	GG (n = 46) GA (n = 0) AA (n = 0) n/a (n = 0)

n/a; genotypes not determined

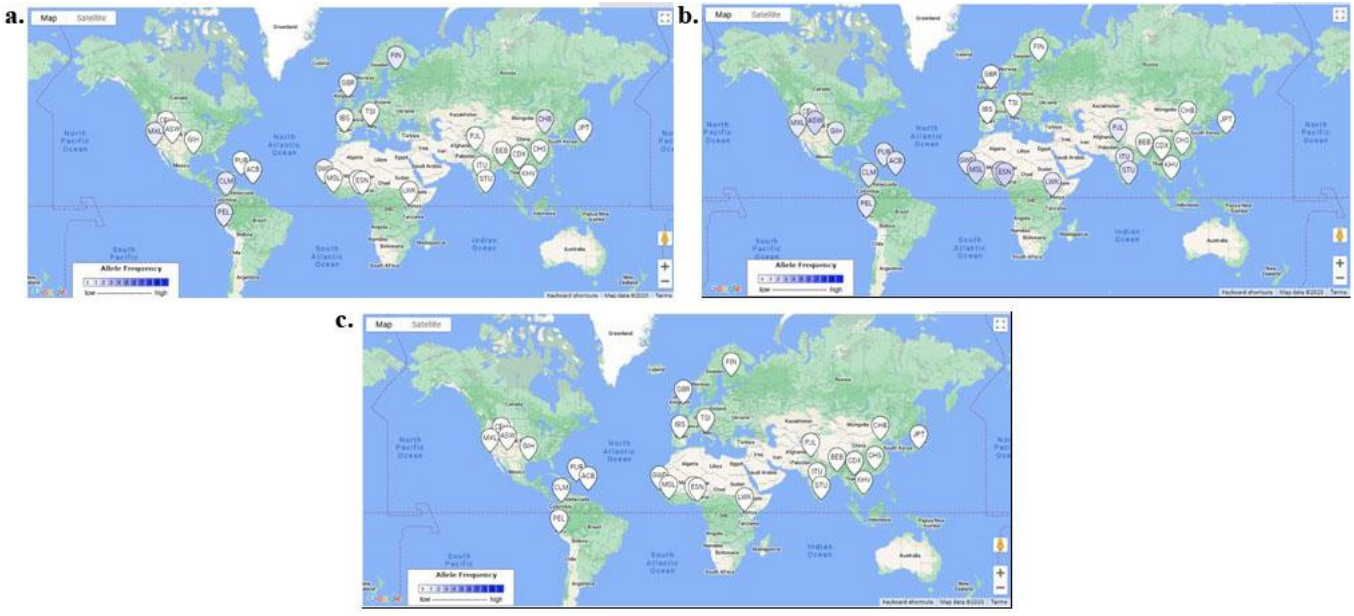

Fig. 2. Allele frequency distribution of **a.** rs13306435, **b.** rs2069849, **c.** rs13306436

In silico Functional Analysis of the Identified Variants

Genetic Changes that Affect the Protein

We identified three variants in the coding region of the exon-5 of which two were missense variants causing amino acid replacement [rs13306435 (p.Asp162Glu) and rs747302620 (p.Thr166Pro)] and one [rs2069849 (p.Phe125=)] was a synonymous variant. The results of the MutationTaster, SIFT, and Polyphen databases indicate that rs13306435 does not have a detrimental effect on protein, yet it is likely to be a regulatory variant by affecting the binding of regulatory proteins (RegulomeDB score=2a) (Table 3). Meanwhile, rs747302620 and rs2069849 had a RegulomeDB score of 4, indicating their possible regulatory role by residing in the transcription factor binding region (Table 3). Distributions of the identified variants in two groups are given in Table 2.

Table 3. *In silico* functional analysis of the detected variants.

RefSNP ID	Genomic Location (NG_011640.1)	Genetic Location	Amino Acid Change	MAF	RegulomeDB Score	SIFT	MT	PP2
rs13306435	g.9274T>A	Exon	D>E	0.016	2a	T	B	B
rs747302620	g.9284A>C	Exon	T>P	0.005	4	T	B	PD
rs2069849	g.9391C>T	Exon	F>F	0.022	4	T	B	PD
rs13306436	g.9531G>A	UTR	-	0.016	5	n/a	B	n/a

MAF; Minor Allele Frequency; RegulomeDB Score; 2a, TF binding + matched TF motif + matched Footprint + chromatin accessibility peak; 4, TF binding + chromatin accessibility peak, 5, TF binding or chromatin accessibility peak; SIFT; T, Tolerated; MT, MutationTaster; B, Benign; PP2, Polyphen2; PD, Probably Damaging.

Genetic Changes that Affect the Binding of Regulatory Molecules

We detected only one 3'UTR variant (rs13306436) with potential as a microRNA-associated single nucleotide polymorphism (mirSNP) and regulatory properties. The rs13306436G>A change was predicted to cause formation of new miRNA binding sites for hsa-miR-5007-3p and hsa-miR-1279 and the loss of existing miRNA binding sites for hsa-miR-539-3p, hsa-miR-5003-3p, hsa-miR-1-5p and hsa-miR-485-3p (Table 4). Meanwhile, all detected variants have been found to have a potential role in *IL-6* gene regulation according to the RegulomeDB scores (<5). The rs13306435 located in exon 5 has a RegulomeDB score of 2a implying the significance of the sequence for binding multiple regulatory proteins. Also, the 3'UTR variant (rs13306436) had a RegulomeDB score of 5, indicating its importance as a transcription factor binding site (Table 3).

Table 4. miRNA binding sites affected by 3'UTR rs13306436G>A.

miRNA	Effect	Target Score	Duplex SNP-miRNA
hsa-miR-5007-3p	Gain	21.58	3'UTR: 5' GUUGUUCUCUAUGGAGAACUAAAAAUAUGAGCGUUAGGACA 3' | | | | | | . miRNA: 3'UAAUCUCAAACCAAGUAUACUA 5'
hsa-miR-1279	Gain	25.09	3'UTR: 5' GUUCUCUAUGGAGAACUAAAAAUAUGAGCGUUAGGAC 3' | | | | | | . miRNA: 3'UCUUUCUUCGUUAUACU 5'
hsa-miR-539-3p	Loss	21.08	3'UTR: 5' GUUGUUCUCUAUGGAGAACUAAAAGUAUGAGCGUUAGGACA 3' X | | | | | . miRNA: 3' UUUCUUUAACAGGAACAUACUA 5'
hsa-miR-5003-3p	Loss	21.56	3'UTR: 5' GUUGUUCUCUAUGGAGAACUAAAAGUAUGAGCGUUAG 3' | | | | X | . miRNA: 3' GGGGUUGUUGGAUCUUUUCAU 5'
hsa-miR-1-5p	Loss	23.63	3'UTR: 5' GUUGUUCUCUAUGGAGAACUAAAAGUAUGAGCGUUAG 3' | | X | | | | . miRNA: 3' UACCCGUAUAUUUCUUCAUACA 5'
hsa-miR-485-3p	Loss	24.08	3'UTR: 5' GUUGUUCUCUAUGGAGAACUAAAAGUAUGAGCGUUAG 3' X | | | | | . miRNA: 3' UCUCUCCUCUCGGCACAUACUG 5'

Discussion

Accumulating efforts have attempted to uncover the genetic determinants causing the interindividual variations in athletic tendencies and sports performance. These studies' findings alluded that the athletes' genetic profile may not only lead them to be successful in sports but also may be associated with advantageous traits for favourable health (Varillas-Delgado *et al.* 2022). However, athletic performance is a highly heterogeneous trait and is influenced by several factors that need to be meticulously investigated. Recent technological advances facilitated the identification of multiple genetic variants associated with exercise-related traits and sporting aptitude. Yet, sports genetics studies are still emerging, and a better understanding of the molecular mechanisms contributing to talent in specific sports disciplines is necessary.

In this study, we seek to determine the regulatory variants located in the 3'UTR region of the *IL-6* gene in triathletes, which may be associated with their athletic status. Thus, 47 triathletes and 46 sedentary individuals participated in the study and the *IL-6* gene region encompassing 3'UTR was sequenced by Sanger sequencing. Three coding SNVs and one 3'UTR SNV (rs13306436) were identified of which rs13306436G>A (MAF = 0.016) was detected in only triathletes. We also compared the MAF distributions of the identified variant to the MAF determined in the large-scale sequencing projects (Table 1 and Supplementary Material Table S2). Our results for two coding SNVs (rs13306435 and rs2069849) were similar to 1000 Genome Project results obtained in populations of European descent. The MAF of the other coding SNV (rs747302620) was not reported in the 1000 Genome project (Supplementary Material Table S2), and it was rarely detected in the exome sequencing projects (ExAC Global MAF = 8E-6 and gnomAD Exomes Global MAF = 4E-6) **(**Table 1). The MAF value of the 3'UTR variant rs13306436 in 1000 Genome Global was 0.0048, and it was not detected in populations of European descent (Supplementary Material Table S2). However, rs13306436G>A change was rarely seen in Asian populations (MAF = 0.0051-0.0433). Although three triathletes were heterozygotes for rs13306436G>A in our study, none of the sedanters in our sample carried the A-allele. A small population size may explain this but still, our results need further consideration as the A allele may lead favourable phenotype for athletic status and thus be observed in only triathletes.

The 3'UTR of the genes is known to comprise functional sequences that are targets for regulatory molecules, including miRNAs (Mayr, 2019). Nevertheless, suggesting evidence implies the substantial role of 3'UTRs for the tendency to physical activity as SNPs located in the 3'UTR region of the multiple genes were reported to be associated with athletic performance (O'Connell *et al.* 2014, Grealy *et al.* 2015, Saunders *et al.* 2015, Heffernan *et al.* 2017, Rivera *et al.* 2020). Recently, miRNAs associated with exercise-related traits have gained attention, and several miRNAs were shown to be differentially expressed during acute or chronic exercise in athletes, which may ease exercise-induced pathologies and lead to their athletic success (de Gonzalo-Calvo *et al.* 2015, Li *et al.* 2018, Massart *et al.* 2021, Zhou *et al.* 2020, Kotewitsch *et al.* 2024). Thus, a better understanding of the roles of the miRSNPs in sport-related genes is important for athlete health and talent identification. Our analyses revealed that the 3'UTR variant (rs13306436G>A) found in only triathlete group is located in miRNA binding sites of hsa-miR-1-5p, hsa-miR-485-3p, hsa-miR-539-3p, hsa-miR-5003-3p, hsa-miR-1279 and hsa-miR-5007-3p (Table 4). The biomarker potentials of hsa-miR-1-5p, hsa-miR-485-3p, hsa-miR-539-3p, hsa-miR-5003-3p, and hsa-miR-1279 were extensively studied in the literature, yet limited evidence exists for the functional relevance of hsa-miR-5007-3p in human diseases and traits (Yang *et al.* 2015, Montalbo *et al.* 2018, Hu *et al.* 2019, Chen *et al.* 2022, Jing *et al.* 2023, Ryu *et al.* 2023, Yue *et al.* 2023). However, our results obtained from the miRNASNP database show that the G>A change disrupts the binding site of hsa-miR-1-5p, hsa-miR-485-3p, hsa-miR-539-3p, and hsa-miR-5003-3p while creating putative binding sites for hsa-miR-1279 and hsa-miR-5007-3p. Previously, hsa-miR-1-5p has been suggested as a muscle-specific/muscle-enriched miRNA (myomiR) due to its crucial role in myogenesis, and its expression has been shown to increase after acute exercise (Meurer *et al.* 2016, Silva *et al.* 2017, Siracusa *et al.* 2018). Meanwhile, the dysregulation of circulating hsa-miR-485-3p was also observed during exercise training, suggesting its potential role in exercise adoption (Silva *et al.* 2017).

IL-6 is a key molecule of the cytokine signaling pathway and is released from active skeletal muscles during exercise while maintaining muscle energy homeostasis (Catoire & Kersten, 2015, Nash *et al.* 2023). *IL-6* acts as a myokine overproduced during muscle contraction and boosts exercise performance by allowing training adaptations (Trinh *et al.* 2021, Leuchtmann *et al.* 2022). The role of *IL-6* in exercise physiology has been widely investigated in previous studies and certain SNVs in the *IL-6* gene were repetitively studied in athletes from different sports disciplines and ethnic populations. The results of a recent study conducted in Turkey demonstrated that *IL-6*/rs1800795G>C was found more frequently (MAF = 0.19) in Ironman triathlon athletes (n =10) (Akkoç *et al.* 2020). The functional IL6/rs1800795-C allele has also been associated with athletic performance in different studies recruited distinct athlete groups and was suggested to have a role in mechanisms related muscle repair (Yamin *et al.* 2008, Ben-Zaken *et al.* 2015, Cenikli *et al.* 2016, Ben-Zaken *et al.* 2017, Akkoç *et al.* 2020, Sofu, 2020, Kazanci *et al.* 2021, Tuna *et al.* 2022). Meanwhile, *IL-6*/rs2228145A>C was also proposed to influence interindividual differences in physical activity levels by fortifying the *IL-6* and soluble fragment of the *IL-6* receptor (s*IL-6*R) complex formation (Nash *et al.* 2023). The underlying mechanisms related to associations of *IL-6* SNVs with athletic talent indeed depend on the functional effects of SNVs in the *IL-6* gene, therefore a deeper understanding of the *IL-6* variations possibly promote

athletic success is highly important (Ben-Zaken *et al.* 2017, Nash *et al.* 2023). To the best of our knowledge, the 3'UTR of the *IL-6* has not been sequenced in triathletes before, and thus, our results yield a novel perspective on the contribution of the *IL-6* in sports genetics. However, our study has some limitiations. First, our sample size can be too small for detecting rare and low frequency variants with possible functional roles so they might have been missed in our analyses. Also, the effects of the variants on gene expression were not evaluated which can be uncovered by further research. Nevertheless, elucidating the miRSNP potential and functional relevance of 3'UTR rs13306436 in athletic predisposition deserves further attention and comprehensive investigations.

In conclusion, our study provides suggestive evidence for the possible functional implications of the 3'UTR region of the *IL-6* in athletic tendency, and future studies are needed to ensure the prominent role of *IL-6* in the tendency to physical activity.

Ethics Committee Approval: Ethics committee approval was received for this study from the Institutional Review Board (IRB) of the Bursa Uludag University by the number 2022-1/18. All participants were informed about the study and participated after signing informed consent forms. Sample collections in the race areas were accomplished with the approval and permission of the Turkish Triathlon Federation.

Data Sharing Statement: All data are available within the study and in the Supplementary Material.

Author Contributions: Concept: D.P., Design: D.P., H.F.N., Execution: D.P., H.F.N., F.A.B., Material supplying: D.P., H.F.N., Data acquisition: D.P., H.F.N., Data analysis/interpretation: D.P., H.F.N., F.A.B., Writing: D.P., H.F.N., F.A.B., Critical review: D.P., F.A.B.

Conflict of Interest: The authors have no conflicts of interest to declare.

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