Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

# The Determination Of The Relationship Between $Vo_{2max}$ and the Angiotensin-Converting Enzyme Gene (*ACE*) rs1799752 Polymorphisms in the Turkish National Ice Hockey Sports Team

Canan Sercan Dogan[1*], Tolga Polat[2], Orkun Akkoç[3], Muhammet İrfan Kurudirek[4], Korkut Ulucan[5]

[1] Marmara University, Institute of Pure and Applied Sciences, Department of Biology, 34722, Göztepe, İstanbul, Turkey
[2] Uskudar University, Medical Genetics and Molecular Diagnostic Laboratory, Üsküdar 34662, İstanbul, Turkey
[3] Istanbul University - Cerrahpasa , Faculty of Sports Science, Department of Moving and Training Science, 34320, Istanbul, Turkey
[4] Artvin Coruh University, Physical Education & Sports, Sports Management Department, Artvin, Turkey
[5] Marmara University, Faculty of Dentistry, Department of Medical Biology and Genetics, 34854, Istanbul, Turkey

**ARTICLE INFO**

***Original paper***

***Article history:***
*Received: January 12, 2022*
*Accepted: April 01, 2022*
*Published: June 30, 2022*

***Keywords:***

*rs1799752, ACE, $VO_{2max}$, ice hockey, maximal oxygen consumption. sports genetics*

**ABSTRACT**

In the present study, the effect of *ACE* rs1799752 polymorphism on maximal oxygen consumption ($VO_{2max}$) in ice hockey players was analyzed. For this reason, 21 male National Ice Hockey players, aged between 18-25, were recruited for the study. The conventional polymerase chain reaction (PCR) was used on the genotype rs1799752 polymorphism. The $VO_{2max}$ values were calculated by using the 20m Shuttle Run tests. The numbers and percentages of the II, ID and DD genotypes were 9 (%43), 7 (%33), and 5 (%24), respectively. The allelic distribution for I and D alleles was found to be 25 (60%) and 17 (40%), respectively. The mean $VO_{2max}$ of all the athletes was calculated as 47.52 ml. The mean $VO_{2max}$ of the II, ID, and DD genotypes were 49.74ml, 47.34 ml, and 46.43 ml, respectively. We found that the oxygen utilization capacity increased from the DD genotype to the II genotype. However, this increase was not statistically significant ($p > 0.05$). In order to confirm our findings, it is recommended that larger prospective studies depending on the effect of the relevant polymorphisms needed to be carried out.

**Doi:** http://dx.doi.org/10.14715/cmb/2022.68.10.14 

## Introduction

Athletic capacity is considered to be determined by biological, psychological, and socioeconomic factors. The two significant factors that determine the athletic performance of an athlete are genetic endowment and environmental factors. Biological factors such as gender, age, anatomical structure, intelligence, state of the locomotor system, psychological status, autonomic nervous system, functions of the secretory glands, metabolism, energy utilization mechanisms, organ systems, neuromuscular transmission rate, cardiovascular system, and especially the genetic endowment of the players are the other important factors that are crucial for athletic performance. Also, factors such as nutrition and mental support as epigenetic factors play crucial roles in athletic performance. All the genes that code for these biological factors are important for an athlete (1).

The angiotensin-converting enzyme (*ACE*) InDel polymorphism, is among the most widely examined polymorphism in terms of performance-related traits and achieving elite athlete status. The variants of the gene have been reported in multiple studies to be a determinant of endurance in athletes (2). This gene is located in chromosome 17q23 and consists of 26 exons and 25 intronic regions. It comprises a polymorphism caused by a deletion (D) or an insertion (I) of a 287 bp Alu sequence in intron 16 (3). To date, studies have shown that individuals with the DD genotype have higher tissue and plasma ACE concentrations than individuals with the ID and II genotypes (4). In the studies that compared athletes and sedentary individuals, it was suggested that individuals with the DD genotype are much more successful in sprinter activities such as short-distance running, long jump, high jump, and short-distance swimming (5). On the other hand, individuals with the II genotype have lower ACE serum concentrations and have been found to be more successful in disciplines that require endurance such as middle- and long-distance running, race walking, and skiing (6). However, there are controversial results claiming that the same results are not always achieved (7).

Aerobic capacity and upper body anaerobic power seem to be the most important physiological factors of success in ice hockey. Ice hockey is played with high intensity and requires high speed, agility, muscular strength, and also depends on the players endurance and anaerobic/aerobic capacity (8). Maximal $O_2$ consumption ($VO_{2max}$) is a significant clinical and physiological parameter indicating endurance performance. To maintain a stable training adaptation, physiological parameters such as $VO_{2max}$ give important information to trainers about the athletes' status. Therefore, genotypes such as *ACE* InDel affecting the $VO_{2max}$ is of great interest for sport scientists (2).

In this study, we aimed to compare the possible effects

* Corresponding author. Email: cananscan@outlook.com

of the *ACE* rs1799752 polymorphism on the aerobic capacity of male National Ice Hockey players.

## Materials and Methods

### Participants

21 male Turkish National Ice Hockey players volunteered for the study. The study was approved by the Uskudar University Ethical Committee (61351342-/ 2019-35) and was performed in accordance with the principles of the Declaration of Helsinki seventh revision (9). All the players were informed of the experimental procedure and any potential ethical implications, and all the participants provided written informed consent. The athletes' age, weight, success (medal status), parental consanguinity, presence of any genetically transmitted diseases, doping bans, and ancestry were obtained before being invited to the study.

### Phenotyping

The maximal oxygen consumption capacity test was performed with 20 m Shuttle Run tests. The team wore sportswear and they performed warm-up exercises for 30 minutes before starting the Shuttle Run tests. The shuttle run tests were performed as previously described (10). The number of repetition runs was recorded and the estimated oxygen use was determined using the following formula:

$Y = –24.4 + 6.0 X$ ($Y = VO_{2max}$ ml/kg /min, X = running speed), as previously described.

### Genotyping

The genomic DNA isolation from the buccal epithelial of the athletes was performed with a commercial kit (The Invitrogen extraction kit, USA) by using the manufacturer's guidelines. The purity of the samples was calculated by the vOD260/OD280 ratio. The genotyping process was carried out by using conventional PCR amplification. Forward 5'-CTGGAGACCACTCCC ATCCTTTCT-3' and reverse 5'-GATGTGGCC ATCACATTCGTCAGAT- 3' primers were used for the amplification. The final volume of the PCR mixture was 50 µL and contained 1 U Taq DNA polymerase, 50–100 ng genomic DNA, 1 mM deoxynucleotide triphosphate (dNTP), 50 mM KCl, 1.5 mM MgCl2, 10 mM Tris–HCl, pH 8.0, and 1 mM of each primer. The denaturation at 94°C for five minutes was initiated by annealing at 53°C for one minute and an extension at 72°C for one minute. The amplification was finalized with 30 cycles: followed by a final extension at 72°C for seven minutes. The amplicons were run by electrophoresis on a 2,5% agarose gel and visualized under UV light after the ethidium bromide staining. The electrophoresis was found to have three possible types: a 477-bp band (II genotype), a 190-bp band (DD genotype), or both 477 and 190-bp bands (I/D genotype). The amplicons had 190 bp in the existence of the D allele and 477-bp fragment with the existence of the I allele.

### Statistical analysis

G Power analysis was carried out to determine the number of subjects. A priori power analysis was conducted to determine the sample size, which was calculated to be 15 participants with the desired level of power of 0.80, a significance level of 0.05 and an effect size of 0.60 to detect strong correlation. The adjusted sample size after taking into consideration of a 30% dropout rate was 21 participants. The statistical analysis was performed by using the SPSS 21 (Statistical Package for Social Sciences) software program. The descriptive statistics of age, height, weight, body mass index (BMI) and VO2max were presented as a minimum, maximum, and mean. The normality was tested using the Kolmogorov-Smirnov test. Visual methods such as histograms and probability plots were also used to determine the normality. To compare the VO2max results and genotypes, we used the one-way analysis of variance (ANOVA). $p<0,05$ was accepted as statistically significant.

## Results and Discussion

The mean average of age (years), height (m), weight (kg), BMI (kg/m2) and $VO_{2max}$ (kg/ml/min) values for the players according to the genotypes are summarized in Table 1. The average height of the players with the DD genotype was higher than the players with the ID and II genotypes. For the weight and BMI values, the players with the II genotypes were higher than the DD and ID genotypes.

All the players were successfully genotyped. The percentage of the ACE genotype in the players was 9 (43%), 7 (%33), and 5 (24%) for the DD, ID, and II genotypes, respectively. In addition, the allele frequency was 25 (60%) and 17 (40%) for the D and I alleles, respectively (Table 2).

When we compared the genotypes and $VO_{2max}$ values, increasing values were detected from the DD genotype to the II genotype; DD (46.43 ± 8.77 kg/ml/min), ID (47.34 ± 4.83 kg/ml/min) and II (49.74 ± 4.31 kg/ml/min). However, this increase depending on the genotypes showed no statistically significant difference ($p =0,686$) (Table 3).

Taking into account the relationship between the *ACE* InDel polymorphism, the II genotype is considered to be desirable in endurance athletes, including ice hockey players. We hypothesized that well-trained players with the II genotype would be characterized by a significantly greater $VO_2$max than those with the ID or DD genotypes. To test

**Table 1.** Body composition characteristics of the three ACE genotype groups of the Turkish ice hockey national sports team.

| Genotype | Age (year) | Height (m) | Weight (kg) | BMI (kg/m²) | $VO_{2max}$ (kg/ml/dk) |
|---|---|---|---|---|---|
| **DD** | 19,00±3,39 | 1,81±0,07 | 73,33±12,57 | 22,34±3,08 | 46,43±8,77 |
| **ID** | 19,50±3,51 | 1,77±0,05 | 68,17±6,04 | 21,89±2,36 | 47,34±4,83 |
| **II** | 18,40±3,78 | 1,77±0,05 | 76,78±14,86 | 24,62±4,87 | 49,74±4,31 |

**Table 2.** Genotype and allelic distribution of the ACE InDel polymorphisms in ice hockey player cohort.

| | Genotype Frequency | | | Allele Frequency | |
|---|---|---|---|---|---|
| **Players (N=21)** | **DD** | **ID** | **II** | **D** | **I** |
| **Number(Percentage)** | 9 (43%) | 7 (33%) | 5 (24%) | 25 (60%) | 17 (40%) |

**Table 3.** There were no noteworthy correlations between ACE activity and $VO_{2max}$ measures. N: number $VO_{2max}$ : maximal oxygen consumption.

| Genotypes | N | $VO_{2max}$ Mean (kg/ml/min) | Minimum | Maximum | F | P |
|---|---|---|---|---|---|---|
| **DD** | 9 | 46,43±8,77 | 26,4 | 57,5 | | |
| **ID** | 7 | 47,34± 4,83 | 38,5 | 51,9 | 0,385 | 0,686 |
| **II** | 5 | 49,74 ±4,31 | 43,7 | 54,5 | | |
| **Total** | 21 | 47,52± 6,57 | 26,4 | 57,5 | | |

our hypothesis, we calculated the maximum oxygen intake values with the *ACE* InDel genotype in 21 male National Ice Hockey players. The present study is the first to determine the possible association between the $VO_{2max}$ and the *ACE* rs1799752 polymorphism in the National Hockey Sports Team. In our cohort, we report that the $VO_{2max}$ was higher in the players with the II genotypes, and lowest in the players with the DD genotypes. Nevertheless, this decrease was not statistically significant in the ice hockey player cohort.

Like our findings, Kurtulus et al. (11) reported no statistically significant difference between the *ACE* InDel polymorphism and $VO_{2max}$ in wrestlers. Similar to the studies by Kurtulus et al. (2019), Holdys et al. (6), reported no statistically significant difference between the *ACE* InDel polymorphism and $VO_{2max}$ values. In their cohort, 154 male and 85 female Physical Education university students, I allele was found to have an advantageous effect on higher maximal oxygen uptake values. They also reported that individuals with the II genotype were more common in individuals practicing aerobic sports than individuals with the DD genotype in individuals training in anaerobic disciplines. However, these results showed no significant association between the I/D polymorphism in the ACE gene and $V_{O2max}$ values. A similar study reported that I allele was related to the $VO_{2max}$ values, but this relationship was not statistically significant in terms of the ACE Indel polymorphism and $VO_{2max}$ values (12).

Previously, Hagberg et al. (13) reported a significant relationship between the ACE InDel polymorphism and $VO_{2max}$ values in a group of postmenopausal women, but as this study was conducted on sedentary individuals, it was hard to speculate on the findings in terms of athletes.

According to Cerit et al. (14), the *ACE* DD genotype had an advantage in developing a short-duration aerobic performance improvement which required high-level $VO_{2max.}$ Like this study, Zhao et al. (15) reported that individuals with the *ACE* DD genotype had more $VO_{2max}$ values than those with the *ACE* II genotype.

Orysiak et al. (16) claimed that the InDel polymorphism of the *ACE* was not a $VO_{2max}$ marker. More recently, Trent et al. (17) determined that Australian Olympic rowers had a surplus of the *ACE* I alleles and the *ACE* II genotype; in the profile of a normal healthy group. These findings suggest the hypothesis that the relationship between $VO_{2max}$ and athletic performance has not been fully explained (18) although training in severe anaerobic conditions is known to significantly affect short-term performance (19).

In our study, the DD genotype and D allele were superior to the other genotypes and the I allele. In another study conducted on 21 Turkish professional footballers, the *ACE* DD, ID and II genotypes were reported as 40%, 44%, and 14%, respectively. According to these results, the authors reported that the D allele influenced athletic performance in football players. A similar study, Ulucan et al. (20) performed on 24 young basketball players and reported DD, ID, and II genotypes as 46%, 50% and 4%, respectively in this study group. When the allelic distributions were examined, they stated that the D allele was 71% and the I allele was 29%. The authors reported that the D allele positively affected the athletic performance of basketball players. In a study analyzing the distribution of the *ACE* I/D allele among professional dancers, it was reported that the ID genotype and the D allele may have a genetic advantage for the physical susceptibility of dancers (21).

## Conclusions

More research is needed to clearly analyze the existence of genetic factors that individuals are thought to be affected by the $VO_{2max}$. The main limitation in this study is the low number of volunteers. We hope that this study will be a guide for new studies in various sports.

## Acknowledgments

This work was supported by the Turkey Ice Hockey Federation. We would like to thank Halit Albayrak, Deniz İnce and Osman Gülen for their efforts in this research.

## Interest conflict

The authors declare no conflict of interest within the publication of the study.

## Disclosure statement (declaration of interest statement)

No potential conflict of interest was reported by the authors

## Authors' contributions

Author CSD, author OA and author KU have given substantial contributions to the conception or the design of the manuscript, author CSD, author MIK and author TP to acquisition, analysis of the data. The sample collection, lab working and manuscript writing was achieved by author CSD. OA, MIK and TP participated to drafting the manuscript, author KU and author OA revised it critically. All authors contributed equally to the manuscript and read and approved the final version of the manuscript.

## Funding

None.

## References

1. Ulucan K, Sercan C, Bıyıklı T. Distribution of Angiotensin-1 Converting Enzyme Insertion/Deletion and α-Actinin-3 Codon 577 Polymorphisms in Turkish Male Soccer Players. GEG 2015;7:1-4.
2. Gineviciene V, Utkus A, Pranckeviciene E, Semenova EA, Hall ECR, Ahmetov II. Perspectives in Sports Genomics. Biomed

2022;10:298.
3. Sercan C, Ulucan K, Eken BF, et al. Spor genetiği ve ACE gen ilişkisi. İÜBSBD 2016;3(2):26 – 34.
4. Akkoç O, Sercan C, Kırandı Ö, Erol M, Kapıcı S, Kayhan RF, Akkoç T, Ulucan K. Determination of the distribution angiotensin-converting enzyme (ACE I/D) and alpha-actinin-3 (ACTN-3 R577X) among elite sprinters and middle-long distance runners in Turkey. Prog Nutr 2020;22(2).
5. Ulucan K, Göle S, Altindas N, Güney AI. Preliminary findings of alphaactinin-3 gene distribution in Turkish elite wind surfers. BJMG 2013;16(1):69- 72.
6. Holdys J, KrysCiak J, Stanisławski D, Gronek, P. ACE I/D Gene Polymorphism in Athletes of Various Sports Disciplines. Hum Mov 2011;12(3):223–231.
7. Kim CH, Cho JY, Jeon JY, Koh YG.ACE DD genotype is unfavorable to Korean short-term muscle power athletes. Int J Sports Med 2010;31:65–71.
8. Ransdell LB, Murray T A. Physical profile of elite female ice hockey players from the USA. J. Strength Cond Res 2011;25(9):2358-2363.
9. World Medical Association General Assembly. Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. Fortaleza, Brazil. Copyright, World Medical Association. All Rights Reserved. Retrived from: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-researchinvolving-human-subjects/. 2013
10. Léger A, Mercier D, Gadoury C, Lambert J, The multistage 20 metre shuttle run test for aerobic fitness. J Sports Sci Summer 2007;6(2):93-101.
11. Kurtuluş M, Günay M, ÇelenkÇ, et al. Elit Türk Güreşçilerinin Anjiotensin I-Dönüştürücü Enzim Gen Polimorfizmi Ve VO2 max Düzeyleri İle Atletik Performans Arasındaki İlişkinin Belirlenmesi. Spormetre 2019;17(4):275-284.
12. Rankinen T, Wolfarth B, Simoneau JA,et al., No association between the angiotensin-converting enzyme ID polymorphism and elite endurance athlete status. J Appl Physiol 2000;88:1571–1575.
13. Hagberg JM, Robert EF, Steve D, et al., V O2max is associated with ACE genotype in postmenopausal women. J Appl Physiol 1998;85(5):1842–184.
14. Cerit M, Çolakoğlu M, Erdoğan M, Berdeli A, et al., Relationship between ACE genotype and short duration aerobic performance development. Eur J Appl Physiol 2006;98:461-465.
15. Zhao B, Moochhala SM, Tham S.Relationship between angiotensin-converting enzyme ID polymorphism and (VO2max ) of Chinese males. Life Sc 2003;73(20):2625–30.
16. Orysiak J, Zmijewski P, Klusiewicz A, et. al., The association between ACE genevariation and aerobic capacity in winter endurance disciplines, Biol Sport 2013;30(4):249-253.
17. Trent RJ, Gayagay G, Yu B, Hambly B, S.Elite endurance athletes and the ACE gene 1 allele: the role of genetic factors in athletic performance. Am J Hum 1998;103(1):48-50.
18. Di Prampero PE., Factors limiting maximal performance in humans. Eur J Appl Physiol 2003;90:420-429.
19. Roberts AD, Billeter R, Howald H. Anaerobic muscle enzyme changes after interval training. Int J Sports Med 1982;3(1):18-21
20. Ulucan K, Çam N, Sercan C, et. al., Genç Basketbolcularda Anjiotensin Dönüştürücü Enzim (ACE I/D) ve Alfa- Aktinin-3 (ACTN3 R577X) Gen Polimorfizimlerinin Belirlenmesi İçin Pilot Bir Çalışma. SBD 2015;26(2):44-50.
21. Biyik B, Kapici S, Sercan C, et al., Angiontensin converting enzyme insertion/deletion polymorphism of Turkish professional hip-hop and latin dancers, PJSS 2018;9(3):49-54