








Investigation of Vitamin D Receptor Gene rs731236 Polymorphism in Turkish Rugby Players

Türk Ragbi Oyuncularında D Vitamini Reseptör Geni rs731236 Polimorfizminin Arařtırılması

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Abstract

Vitamin D is an important molecule responsible for the development, contraction and regeneration of muscles, and activates its biological functions by means of its receptors, the vitamin D receptor (VDR), which is a member of the nuclear receptor group. In the present study, we aimed to analyze the rs731236 distribution in Turkish rugby players. A total of 22 male players and 30 sedentary individuals (as a control group) participated in the study. Following DNA isolation, rs731236 polymorphisms of VDR was determined by real-time polymerase chain reaction (RT – PCR). Statistical analysis was carried out by chi-square test. CC, CT and TT genotype and percentage distributions for rs731236 polymorphism in players

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were as 5 (23%), 10 (45%) and 7 (32%), respectively. Allelic distributions were found to be 20 (45%) for C allele, and 24 (55%) for T allele. For the control group; CC, CT and TT genotypes and percentages were as 4 (13%), 17 (57%) and 9 (30%), respectively. C allele was counted as 25 (42%) and 35 (58%) for controls. When we compare athletes and control groups in the terms of genotypes, we detected no statistically significant difference ($p= 0.12$). For the allele comparison, we detected no significant difference between groups ($p= 0.76$). Determination of gene combinations in athletes is of great importance for carrying out of the optimal training programs. Therefore, we aimed to determine the distribution of the alleles related with athletic performance on successful athletes. In our cohort, the CT genotype and T allele were more intensely determined in players.

Keywords: VDR, polymorphism, athletes, rugby, athletic performance

Öz

D vitamini kasların gelişmesinden, kasılmasından ve yenilenmesinden sorumlu önemli bir moleküldür ve nükleer reseptör grubunun bir üyesi olan vitamin D reseptörü (VDR) reseptörleri aracılığıyla biyolojik fonksiyonlarını aktive eder. Bu çalışmada, Türk rugby oyuncularında rs731236 dağılımını analiz etmeyi amaçladık. Çalışmaya toplam 22 erkek oyuncu ve 30 sedanter birey (kontrol grubu olarak) katıldı. DNA izolasyonunu takiben, VDR'nin rs731236 polimorfizmleri gerçek zamanlı polimeraz zincir reaksiyonu (Rt-PCR) ile belirlendi. İstatistiksel analiz ki-kare testi ile yapıldı. Sporcularda rs731236 polimorfizmi için CC, CT ve TT genotip ve yüzde dağılımları sırasıyla 5 (%23), 10 (%45) ve 7 (%32) olarak bulundu. Alel dağılımları C aleli için 20 (%45) ve T aleli için 24 (%55) olarak bulundu. Kontrol grubu için; CC, CT ve TT genotipleri ve yüzdeleri sırasıyla 4 (%13), 17 (%57) ve 9 (%30)'dur. C aleli kontrol grubu için 25 (%42) ve 35 (%58) olarak belirlendi. Sporcular ve kontrol grubunu genotip açısından karşılaştırdığımızda istatistiksel olarak anlamlı bir fark tespit edilmedi ($p= 0,12$). Alel karşılaştırması için gruplar arasında anlamlı fark tespit edilmemiştir ($p= 0,76$). Sporcularda gen kombinasyonlarının belirlenmesi optimal antrenman programlarının yürütülmesi için büyük önem taşımaktadır. Bu nedenle atletik performansla ilgili allelerin başarılı sporcular üzerindeki dağılımını belirlemeyi amaçladık. Bizim kohortumuzda CT genotipi ve T aleli oyuncularında daha yoğun olarak belirlendi.

Anahtar Kelimeler: VDR, polimorfizm, sporcu, ragbi, atletik performans

INTRODUCTION

Sport is a physical and cognitive contest in which multiple factors effect. In both individual or team sports, individuals' athletic performance is very important and the limit of the player's ability/capacity is influenced by several factors, including physical, physiological, environmental and mental factors (Ulucan, 2016; Eken et al., 2021). All factors are directly or indirectly related with genetic endowment (Wang et al., 2018). This why the intrigue of association between sports and genes or continues to fascinate both athletes and researchers, despite current findings.

In the terms of athletic performance, muscle metabolism is expected to be optimal. Rugby, unlike other sports, is a team sport that significantly needs to be well-structured and according to the positions in terms of anthropometric diversity and physiological differences of players (Heffernan et al., 2015). Therefore, analyzing athletes' performance and defining physical boundaries is an important step to uncovered a successful game. In the simplest term, while Props (tight and loosehead, number 1 & 2) need lifting strength and endurance to scrum, Lock players (number 4 & 5) key characteristic is height and mobility. On the other hand, while the expectation from Flankers (number 6 & 7) is no fear and handling ability along with mental strength and fearlessness, Fly half (Number 10) are

required to have precise and accurate kicking ability, with excellent communication skills. When we look at the marker-assisted strategy studies done specifically on rugby athletes, there are some studies that have been conducted with the genes, which are significant in the pool of sports-related genes.

Vitamin D is a fat-soluble sterol molecule that plays a direct or indirect role in musculoskeletal development such as muscle growth (Dawson-Hughes et al., 2017) and bone mineralization (Orr, Holt, Wilkins, & Boone, 1923). In particular, the active form of vitamin D (25OHD₃), which is 1,25(OH)₂D₃ and also known as calcitriol, which acts as a key regulator in many systems such as immune response (Murdaca et al., 2019), cardiovascular events (Wang, Manson, Song, & Sesso 2010) apoptosis and cancer mechanisms (Deeb, Trump, & Johnson, 2017) as well as the central nervous system (Häusler & Weber, 2019). It activates its biological functions by binding its receptor, vitamin D receptor (VDR). Any mutation on the pathways may affect the activity of gene regulation or change the entire system that can cause mortal diseases as previously mentioned (Kim et al., 2017). The vitamin D receptor is a member of the nuclear receptor group. The gene encoding the vitamin D receptor (VDR) is localized at 12q13.11. It is 100 kb long and has more than 100 polymorphic regions identified before (Norman et al., 2006). The rs731236 polymorphism in exon 9 of *VDR* is caused by T>C transition, due to the change ATT codon converts to ATC and encodes the isolate form (Ulucan et al., 2012).

The aim of this study is to investigate the distribution of *VDR* rs731236 polymorphism in rugby players. To date, there are no studies on Turkish rugby players in the terms of *VDR* rs731236 polymorphism. Therefore, it is aimed to determine the distribution of this gene allele in rugby players. According to the obtained data, it is aimed to be a pioneer for future studies and to contribute to the literature.

METHODS

Participants

A total of 22 male professional rugby players and 30 sedentary controls, all with Turkish ancestry, were recruited for the study.

Ethical Statement

Our study and study protocol were prepared in accordance with the Helsinki Declaration-2 (2015) guidelines and approved by Usküdar University Non-Interventional Ethics Committee (Protocol code: B.08.06,YÖK.2.ÜS.0.05.06/2013/09). The volunteers participating in the study were given detailed information about the analyzes and outputs before the study and their consent forms were obtained from them.

VDR Genotyping

DNA Isolation: Oral epithelium cells were collected by DNA collection swabs from the volunteers who participated in the study, and DNA isolation was completed by using PureLink DNA isolation kit (Invitrogen, Van Allen Way Carlsbad, CA, USA). Briefly, 20 µL proteinase K was vortexed by adding 10µL of RNAase to 200 µL of DNA isolation. After 2 min at room temperature, 200µL of binding buffer was added and homogenized with stirring. After incubation for 10 minutes in a 55 °C water bath, 200 µL of ethanol was added and vortexed for 5 seconds. It was taken to the filtered tube and centrifuged at 10000g for 1 minute. The supernatant was discarded and 500 µL of washing buffer was added to the pellet and centrifuged at 10000 g for 1.15 seconds. 80µL of elution buffer was added and incubated and centrifuged at maximum speed for 1 minute. The DNA samples obtained were stored at – 20 °C until the analysis of the respective gene regions was completed.

Genotyping of VDR rs731236

Genotyping of *VDR* rs731236 was performed from the isolated DNAs by using 7500 Fast Real-Time PCR System (Applied Biosystems). Taqman Genotyping Assays (Applied Biosystems Foster City, CA, USA) genotyping kit was used for allelic determination. C and T alleles were determined using VIC and FAM primers, respectively (Table 1). Genotyping was completed using 5 µL master mix, 3.75 µL H₂O, 0.25µL assay and 1µL (10 ng) DNA.

Table 1. VIC/FAM labelled times primer use to rs731236 polymorphism

qPZR	qPZR Sequence (5 [^] 3
VIC/FAM	GGACGCCGRGCTGAT [C/T] GAGGCCATCCAGGAC

Statistical Analysis

All data were analyzed by using SPSS 20,0 for windows (SPSS Inc., Chicago, IL, USA). Statistical analysis was conducted by using chi-square test. Relationships yielding p – values less than 0.05 (p<0.05) were considered to be significant.

RESULTS

22 rugby athlete and 30 control groups participated in the study. Genotypes and alleles for the *VDR* rs731236 polymorphism are summarized in the table. CC, CT and TT genotypes and percentages for rs731236 polymorphism were analyzed as 5 (23%), 10 (45%) and 7 (32%); respectively. C allele was counted as 20 (45%) and T allele as 24 (55%). CC, CT and TT genotypes and percentages for rs731236 polymorphism in controls were as 4 (13%), 17 (57%) and 9 (30%); respectively. For allelic distributions, C allele was counted as 25 (42%) and T allele as 35 (58%). When we compare athletes and controls in the terms of genotypes, we detected no statistically significant difference (p=0.12).

For the allele comparison, we detected no significant difference between groups ($p=0.76$). Table 2 summarizes the genotype, allele comparison of our cohort and their statistical analysis

Table 2. Comparison of the VDR rs731236 polymorphism of the study cohort

	VDR Genotype			p-value	Allele Frequency		
	CC	CT	TT		C	T	p-value
Players (n=22)	5	10	7	0.12	20	24	0.76
Percentage	23%	45%	32%		45%	55%	
Controls(n=30)	4	17	9		25	35	
Percentage	13%	57%	30%		42%	58%	

DISCUSSION and CONCLUSION

Rugby is a high-impact sport that is played with two teams of 15 players, one with two halves of 40 minutes each. Therefore, optimal athletic performance should be supplied for successful players. In the formation and development of athletic performance, muscle metabolism is expected to be optimal. The effect of genetic variations on metabolism on and cellular basis of activities, such as exercise, is important a lot through article crucial, therefore optimal to more favourable forms are expected to be included in the genotypes of athletes (Ulucan, Göle, Altindas, & Güney, 2013).

Recent studies demonstrated the significance of genetic parameters in athletic performance and physical fitness. In the studies carried out to date, more than 200 gene variants were associated with athletic performance, some related with structural and, some with functional factors like mediators or enzymes (Arica et al., 2018). The comparison works between environmental and genetic factors to determine the athletic performance and which have more effect on sports exercise metabolism has not been fully identified and warrants further research is a point of research (Bıyık et al., 2018).

CT genotype and T allele were more dominant compared with other genotypes and C allele in terms of VDR rs731236 polymorphism in our cohort. According to the best of our knowledge, this report is the first which investigates reflects the genotype distribution of rs731236 polymorphism in Turkish rugby players.

Rabon-Stith et al. (2005) stated that rs2228570 polymorphisms influences bone mineral density of endurance training in healthy individuals. In the same study, they reported that they were not associated with aerobic training in a similar study. In another study, the effect of *Apa1* and *Bsm1* polymorphisms on muscle endurance was stated the same effect is not associated with *Taq1* polymorphism (Wang et al., 2006).

Bahat et al. (2010) reported that extensor strength of the knee was associated with rs1544410, but no significant association was found in rs2228570 and rs731236 haplotypes. In the study conducted with rs2228570 polymorphism on young Italian male footballers, they found the CC genotype as 52%, CT genotype as 34% and TT genotype as 14%, and demonstrated that the obtained genotype

results may be an important marker in football susceptibility (Micheli et al., 2011). Eken et al. 2018 analyzed *Fok1* (rs 2228570) and *Bsm1* (rs1544410) polymorphisms in 27 athletes and stated that CC genotype and C allele for *Fok1* polymorphism and AG genotype and A allele for *Bsm 1* were superior to other genotypes and alleles in the given cohort.

Studies investigating the role of rs731236 and athletic performance are not enough. Some previous studies in the literature reported that the *VDR* rs2228570 polymorphism increases the risk of stress fractures in athletes who exercise heavily, and the genetic variant in question should be analyzed to create healthy training programs. Varley et al. (2018) reported the important role of *VDR* rs10735810 and rs731236 in the pathophysiology of stress fractures in a study with 518 elite athletes. They stated that homozygotes of the rare allele of rs731236 polymorphism showed an association with increased stress fracture risk when compared to those homozygotes for the common allele combined with heterozygotes. This demonstrates the important functions of rs731236 in athletic performance, considering the role of this polymorphism in stress fracture condition. Another study on 385 Israeli soldiers, 17 genes, including *VDR* gene, were analyzed and it was determined that *VDR* could create the risk of stress fracture (Yanovich et al., 2012). Polymorphisms in different genes, including *VDR*, can impact muscle strength and alter responses to training stimuli. Lazary et al. (2013) conducted a study on 788 Hungarian school children and analyzed three functional SNPs (rs4516035, rs1544410 and rs731236) in their cohort. They constructed a haplogroup of SNPs (rs1544410-rs731236-rs10783215), and reported that haplogroups were significantly associated with increased grip strength of the dominant hand. As a result of their study, they concluded that *VDR* polymorphisms are significantly associated with different muscle-related phenotypes.

Genes are very important in determining athletic performance. As athletic performance involves multifactorial elements, analyzing the gene groups instead of single genes may provide precise results. In the rugby cohort, we detected no statistically significant difference in the terms of genotype and in the terms of alleles. The low numbers of the players and lacking the information of serum 25-hidroxy D vitamin levels may be considered as the major limitations of the present study. However, we believe that the effect of *VDR* gene on athletic performance should be determined with similar studies involving a greater number of subjects.

Conflict of interest: There is no conflict of interest between the authors regarding the publication of this article.

Authors' contributions: Design of the study: 1. Author % 40, 2. Author % 10, 3. Author % 10, 4. Author % 5, 5. Author % 5, 6. Author % 15, 7. Author 15 contributed. All authors have read and approved the final manuscript

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