Sakarya University Journal of Science

ISSN : 2147-835X
Publisher : Sakarya University

Vol. 28, No. 5, 1040-1046, 2024
DOI: https://doi.org/10.16984/saufenbilder.1500586

Research Article

Comparison of *MCM6* rs4988235 Polymorphism Allele and Genotype Frequencies in Professional Football Players and a Sedentary Control Group

Beste Tacal Aslan[1], Tolga Polat[1], Özlem Özge Yılmaz[1*], Aleyna Tanrıverdi Muhan[2], Rukiye Ziya[2], Korkut Ulucan[1]

1 Marmara University, Faculty of Dentistry, Department of Medical Biology and Genetics, İstanbul, Türkiye, btacal@gmail.com, tolga.polat@marmara.edu.tr, ozlem.ozge@marmara.edu.tr, korkutulucan@hotmail.com
[2] Marmara University, Institute of Health Sciences, Department of Basic Medical Sciences, İstanbul, Türkiye, aleynatanriverdimbg@gmail.com, rukiyeziyaa@gmail.com
*Corresponding Author

ARTICLE INFO

Keywords:
MCM6
Lactose
SNP
Football
Genetic

Article History:
Received: 13.06.2024
Accepted: 03.10.2024
Online Available: 18.10.2024

ABSTRACT

This study investigated the minichromosome maintenance 6 (*MCM6*) rs4988235 polymorphism in professional football players, comparing genotype and allele frequencies with a sedentary control group. The control group comprised 64 participants with no history of athletic activity, while the athlete group included 20 football players. DNA extraction from blood samples was performed using a PureLink DNA isolation kit, following the manufacturer's instructions. Real-Time PCR was employed to analyze the *MCM6* rs4988235 polymorphism in the isolated DNA. Statistical analysis of the acquired data was conducted using chi-square analysis via IBM SPSS 21.0 software (IBM Statistical Package for Social Sciences Corp., Armonk, NY, USA). A p-value less than 0.05 was considered statistically significant. The CC genotype was absent in all football players. Conversely, CT (10%) and TT (90%) genotypes were identified in 2 and 18 individuals, respectively. In the control group, the distribution of genotypes was as follows: CC (0%), CT (17, 26.6%), and TT (47, 73.4%). The C allele frequency was 5% (2 individuals) in football players and 13.28% (17 individuals) in the control group. The T allele frequency was 95% (38 individuals) in football players and 86.72% (111 individuals) in the control group. No statistically significant differences were observed between the football players and the control group regarding genotype ($p = 0.122$) or allele frequencies ($p = 0.149$).

1. Introduction

Lactose is a disaccharide, a molecule formed by the combination of glucose and galactose. Lactose occurs naturally in mammalian milk and is the main carbohydrate component of milk. In the small intestine, it is hydrolyzed by the enzyme lactase into glucose and galactose monosaccharides and absorbed into the bloodstream (Ingram, et al, 2009). Lactose intolerance is a pathophysiological condition that occurs when lactose cannot be digested or absorbed due to a lack of ß-galactosidase enzyme in the jejunum. This condition affects the majority of the elderly population [1].

Lactase deficiency stands as a prevalent autosomal recessive ailment characterized by a diminished activity of lactase fluorohydrolase, a pivotal enzyme responsible for lactose hydrolysis within the small intestine. In the absence of adequate enzymatic function, lactose, a disaccharide, remains unabsorbed, traversing undigested into the colon where it becomes subject to bacterial fermentation. Consequently, individuals afflicted by this condition encounter a spectrum of gastrointestinal symptoms

Cite as: B. Tacal Aslan, T. Polat, Ö. Ö. Yılmaz, A. Muhan, R. Ziya, K. Ulucan (2024). Comparison of MCM6 rs4988235 Polymorphism Allele and Genotype Frequencies in Professional Football Players and a Sedentary Control Group, *Sakarya University Journal of Science*, 28(5), 1040-1046. https://doi.org/10.16984/saufenbilder.1500586

This is an open access paper distributed under the terms and conditions of the Creative Commons Attribution-NonCommercial 4.0 International License.

including discomfort and bloating. Commonly denoted as lactose intolerance, this physiological incapacity to effectively metabolize lactose underscores the intricate interplay between genetic predisposition and biochemical mechanisms governing human digestive processes [2].

Within the global demographic structure, approximately 70% of the human population is characterized by a primary deficiency of the enzyme lactase. This prevalence rate varies in correlation with the diversity of ethnological origins and can be interpreted as a result of genetic selection due to the prevalence of dairy-based foods in the diet. Especially in communities where pastoralist cultures predominate and dairy products play a central role in the diet, as in the septentrional regions of Europe, the prevalence of primary lactase deficiency is remarkably low at two percent. According to findings in the anthropological literature, populations with a high prevalence of lactose malabsorption among adults are usually communities with agricultural and hunting traditions, which historically introduced milk consumption at a late stage and/or preferred fermented dairy products with reduced lactose content [3-6].

The Lactase gene (*LCT*, 2q21-22) encodes the lactase enzyme expressed in the intestine [7]. There are many single nucleotide polymorphisms (SNPs) associated with lactose intolerance and they occur at different frequencies in different populations [8]. A group from Finland described two polymorphisms in introns 9 and 13 of the minichromosome maintenance 6 (*MCM6*) gene [9]. One of these, -13910T (rs4988235), was the first allele identified to be associated with lactase digestive capacity [10]. SNPs known as -13910C/T or rs4988235 are located in intron 13. The single nucleotide polymorphism at position -13910 of the *LCT* gene (rs4988235) has been shown to regulate lactase expression. In addition, the T variant has been associated with an increased transcription of the LCT protein [11].

Individuals harboring at least one copy of the -13910T variant allele are deemed genetically endowed with lactase persistence, thereby retaining the ability to efficiently metabolize lactose into adulthood. This genetic predisposition confers a distinct advantage over counterparts possessing the CC genotype, characterized by markedly diminished lactase activity. Consequently, those with the -13910T variant exhibit a heightened capacity for lactose tolerance, facilitating enhanced digestion and assimilation of lactose-containing foods, in stark contrast to individuals lacking this genetic variant [12, 13].

Athletic performance is considered to be a combination of many factors. Nutrition and diet programs of athletes are one of the main factors affecting athletic performance [14]. Determination of polymorphisms affecting athletic performance among football players can play an active role in changing and regulating the nutritional habits of football players. In our study, we aimed to examine the genotype and allelic distributions of *MCM6* rs4988235 polymorphism in football players and the relationship between the distribution and the positions of the athletes.

2. General Methods

2.1. Study group

20 football players of Turkish origin from Maltepespor football club in the age range of 17-33 years, who were active and regularly training, participated in the study. In the same age range, 64 sedentary individuals were included in the study for genotype and allele analysis. The training program of the football players was reported as a minimum of 4 days (40-120 min) and one match per week. The study protocol was approved by the Üsküdar University Ethics Committee (61351342/ŞUBAT 2024-79) and was prepared in accordance with the guidelines of the Helsinki Declaration-2 (2015).

Consent Form The athletes were invited to participate in the study as part of an independent research conducted by Marmara University Faculty of Dentistry, Department of Medical Biology and Genetics. After the athletes were informed about the study and their questions were answered, the consent forms were mutually signed, first by the athletes. DNA Isolation DNA isolation was completed from peripheral blood

samples using PureLink DNA isolation kit (Invitrogen, Van Allen Way Carlsbad, CA, USA) according to the manufacturer's procedures. The DNA samples obtained were stored at -20°C until the analysis process of the relevant genes was completed.

2.2. Genotyping

Genotyping of the Minichromosome maintenance 6 (*MCM6*) rs4988235 polymorphism was performed using Taqman Genotyping Assays (Applied Biosystems Foster City, CA, USA) using Real-Time PCR device (StepOne Plus, USA) from the isolated DNA material. Genotyping procedures were completed by real-time PCR with Taq-Man probe method using 5 µL master mix, 3.75 µL H_2O, 0.25 µL assay and 1 µL (10 ng) DNA for a total of 10 µL.

2.3. Statistical analysis

Statistical analyses of the obtained data were performed with chi-square analysis using IBM SPSS 21.0 (IBM Statistical Package for Social Sciences Corp., Armonk, NY, USA). $p < 0.05$ was considered statistically significant.

3. Results and Discussion

When the genotypes were analyzed, it was found that 2 players had CT (10%) and 18 players had TT (90%) genotypes, and no player had CC genotype. C allele was found to be 2 (5%) and T allele was found to be 38 (95%) in football players. No individual in the control group was found to have CC genotype, 17 individuals were found to have CT (26.6%) and 47 individuals were found to have TT (73.4%) genotypes. In allelic distribution, C allele was found in 17 (13.28%) and T allele in 111 (86.72%) individuals. Statistically significant differences were not found between the two groups in terms of genotype ($p = 0.122$) and allelic distribution ($p = 0.149$) (Table 1).

Table 1. *MCM6* rs4988235 genotype and allelic distribution comparison

MCM6	**Genotype Distribution**			**p**	**Allelic Distribution**		**p**
rs4988235	**TT**	**CT**	**CC**		**C**	**T**	
Athlete (n=20)	18	2	-	0.122	2	38	0.149
%	%90	%10	-		%5	%95	
Control (n=64)	47	17	-		17	111	
%	%73.4	%26.6	-		%13.28	%86.72	

Chi-Square Test; $p<0.05$

The table shows *MCM6* rs4988235 genotype distribution and allele frequency. This gene is associated with lactose intolerance. It can be interpreted that those with TT genotype are lactose tolerant, those with CT genotype are partially tolerant and those with CC genotype are prone to intolerance.

According to the table, 18 of the 20 people in the football player group had TT genotype and 2 had CT genotype. In other words, lactose tolerance is very high in this group. In the control group, 47 of 64 people had TT and 17 had CT genotype. In other words, lactose tolerance is also high in this group, but lower than in the football player group.

The difference between genotype distribution and allele frequency is not statistically significant. P values are greater than 0.05. This means that there is no significant difference in *MCM6* rs4988235 genotype and allele frequency between football players and control groups.

Table 2 includes the physical characteristics and genotypes of football players in different positions, such as age, height, weight and Body Mass Index (BMI).

According to these data, it can be said that the team is generally young and has a physically fit structure. BMI values are generally in a healthy range. In terms of genotype, it appears that only two players have the 'TC' genotype and the others have the 'TT' genotype.

In addition to environmental factors such as nutrition, genetic predispositions play an important role in the development of athletes [15]. Since an individual's dietary and supplementation strategies can significantly affect their physical performance, personalized nutrition aims to optimize health, body composition and exercise performance by targeting dietary recommendations in sport populations according to the individual's genetic profile [16]. In lactose-sensitive individuals, lactose digestion can cause many abnormal symptoms. In this case, a hydrogen test or invasive biopsy sample is taken to make a diagnosis. Genetic testing can provide highly sensitive lactose intolerance results noninvasively [17].

Raz et al. [18] conducted a study in the Israeli population with 439 participants. The results showed a significant association between *LCT* rs4988235 (-13910C/T) ethnicity and genotype. The prevalence of the CC (*LCT* rs4988235) genotype associated with adult hypolactasia was 97%, 93%, 83% and 82% among Bedouin Arabs and Iraqi, Ashkenazi and Moroccan Jews, respectively. A significant correlation was found in determining the genotype prevalence in Jews and it was recommended to adjust dietary recommendations accordingly.

Table 2. Position, age, height, weight, body mass ındex (BMI) and genotype distribution of football players

Footballers	Location	Age	Length (cm)	Weight (kg)	BMI $(kg/m)^{-2}$	Genotype
1	Goalkeeper	19	193	81	21,7	TT
2	Right Back	22	178	73	23	TT
3	Right Back	32	164	60	22,3	TT
4	Left Back	32	171	73	25	TT
5	Left Back	20	174	69	22,8	TT
6	Center Back	21	186	79	22,8	TT
7	Center Back	21	186	82	23,7	TT
8	DM	21	182	77	23,2	TT
9	DM	21	185	78	22,8	TT
10	DM	22	185	73	21,3	TT
11	CM	25	171	70	23,9	TT
12	CM	20	175	72	23,5	TT
13	CM	21	176	65	21	TT
14	CM	21	180	74	22,8	TT
15	Right Wing	27	180	73	22,5	TT
16	Right Wing	20	174	72	23,8	TT
17	Left Wing	20	180	73	22,5	CT
18	Left Wing	21	179	74	23,1	CT
19	Center Forward	21	186	82	23,7	TT
20	Center Forward	17	187	80	22,9	TT
		Age $\bar{x} \pm SD$	Length $\bar{x} \pm SD$	Weight $\bar{x} \pm SD$	BMI $\bar{x} \pm SD$	
		22.20 ± 3.91	178.65 ± 6.84	73.60 ± 5.48	23.02 ± 0.87	

BMI=Body Mass Index; CM=Central Midfielder; DM=Defensive Midfielder

In a study of 151 volunteers, Adler et al. [19] found genotypes associated with the LP phenotype in 74 (41.0%) and the frequency of the T allele of the *LCT* gene was 24.8%. Tomasz et al. [20] investigated the frequency of *LCT*-13910C>T polymorphism in 223 volunteers from Poland. The *LCT* rs4988235 T allele (lactase persistence) was found to be present in 51% of individuals sampled from the Polish population. All data from populations show the relationship between genetic variants of lactose tolerance and lactose intolerance.

In our study, we aimed to determine the minichromosome maintenance 6 (*MCM6*) rs4988235 polymorphism in football players. None of the football players had the CC allele. It was found that 2 were in CT (10%) and 18 were in TT (90%) genotypes. C allele was found to be 2 (5%) and T allele was found to be 38 (95%) in

football players. No individual in the control group was found to have CC allele. 17 individuals had CT (26.6%) and 47 individuals had TT (73.4%) genotypes. In allelic distribution, C allele was found to be 17 (13.28%) and T allele was found to be 111 (86.72%). There was not a statistically significant difference between the two groups in terms of genotype (p=0.122) and allelic distribution (p=0.149).

In conclusion, this study addressed the combination of genetic predispositions and nutritional factors in athletes, specifically focusing on the minichromosome maintenance 6 (*MCM6*) rs4988235 polymorphism. When the genotype and allele distributions in football players were compared with the control group, no statistically significant differences were found. However, this study emphasizes that there are insufficient and diversified studies of this polymorphism in athletes in the literature and may contribute to increasing the sample size by providing a reference for future studies.

4. Conclusion

These findings highlight the importance of personalized nutrition strategies based on athletes' genetic profiles. Creating appropriate nutrition programs for athletes can help them optimize their performance. Furthermore, the increased use of genetic analyses in the sports community may contribute to the athletic performance of teams and individual athletes. Researchers may conduct additional studies, such as larger sample sizes, more comprehensive analysis of genetic and environmental factors, to better understand the results and more accurately assess the impact of genetic factors on athletic performance. This study can be considered as a fundamental step to understand the effects of genetic factors on traits such as lactose tolerance in athletes and to develop sport-specific nutritional strategies.

Article Information Form

Funding
The author (s) has no received any financial support for the research, authorship or publication of this study.

Authors' Contribution
The authors contributed equally to the study.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest or common interest has been declared by the authors.

The Declaration of Ethics Committee Approval
This study protocol was approved by the Üsküdar University Ethics Committee (61351342/ŞUBAT 2024-79) and was prepared in accordance with the guidelines of the Helsinki Declaration-2 (2015).

The Declaration of Research and Publication Ethics
The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

Copyright Statement
Authors own the copyright of their work published in the journal and their work is published under the CC BY-NC 4.0 license.

References

[1] S. Suri, V. Kumar, R. Prased, B. Tanwar, A. Goyal, Y. Kaur, Y. Gat, A. Kumar, J. Kaur, D. Singh, "Considerations for development of lactose-free food", Journal of Nutrition & Intermediary Metabolism, vol. 15, p. 27-34, 2019.

[2] T. Kleinbielen, L. Palencia-Madrid, C. Garcia-Ibarbia, F. Ortiz, J. A. Riancho, M. M. de Pancorbo, "Association of LCT-13910C>T polymorphism and hip fracture in a cohort of older adult population from Northern Spain", Gene, vol. 783, p. 145560, 2021.

[3] S. B. Matthews, J. P. Waud, A. G. Roberts, A. K. Campbell, "Systemic lactose intolerance: A new perspective on an old problem", Postgraduate Medical Journal, vol. 81, 953, pp. 167-173, 2005.

[4] M. B. Heyman, "Lactose intolerance in infants, children, and adolescents", Pediatrics, vol. 118, 3, pp. 1279-1286, 2006.

[5] S. A. Tishkoff, F. A. Reed, A. Ranciaro, B. F. Voight, B. C. C. Babbitt, J. S. Silverman, K. Powell, H. M. Mortensen, J. B. Hirbo, M. Osman, M. Ibrahim, S. A. Omar, G. Lema, T. B. Nyambo, J. Ghori, S. Bumpstead, J. K. Pritchard, G. A. Wray, P. Deloukas, "Convergent adaptation of human lactase persistence in Africa and Europe", Nature Genetics, vol. 39, 1, pp. 31-40, 2007.

[6] M. C. E. Lomer, G. C. Parkes, J. D. Sanderson, "Review article: Lactose intolerance in clinical practice – myths and realities", Alimentary Pharmacology & Therapeutics, vol. 27, 2, pp. 93-103, 2008.

[7] P. P. Apostol, M. Cristea, M. Ivan, M. Pelin, "Testing The Mcm6 13910-C/T And 22018-G/A Polymorphisms In Samples From Three Different Populations", Journal of Hygienic Engineering and Design, vol. 37, 2021.

[8] E. L. Chin, L. Huang, Y. Y. Bouzid, C. P. Kirschke, B. Durbin-Johnson, L. M. Baldiviez, E. L. Bonnel, N. L. Keim, I. Korf, C. B. Stephensen, D. G. Lemay, "Association of lactase persistence genotypes (rs4988235) and ethnicity with dairy intake in a healthy US population", Nutrients, vol. 11, 8, p. 1860, 2019.

[9] M. A. Arroyo, A. C. P. Lopes, V. B. Piatto, J. V. Maniglia, "Perspectives for Early Genetic Screening of Lactose Intolerance: -13910C/T Polymorphism Tracing in the MCM6 Gene", Open Biology, vol. 3, pp. 66-71, 2010.

[10] C. J. Ingram, C. A. Mulcare, Y. Itan, M. G. Thomas, D. M. Swallow, "Lactose digestion and the evolutionary genetics of lactase persistence", Human Genetics, vol. 124, pp. 579-591, 2009.

[11] L. C. Olds, E. Sibley, "Lactase persistence DNA variant enhances lactase promoter activity in vitro: functional role as a cis regulatory element", Human Molecular Genetics, vol. 12, 18, pp. 2333-2340, 2003.

[12] O. Alharbi, A. El-Sohemy, "Lactose intolerance (LCT-13910C>T) genotype is associated with plasma 25-hydroxyvitamin D concentrations in Caucasians: a Mendelian randomization study", The Journal of Nutrition, vol. 147, 6, pp. 1063-1069, 2017.

[13] R. H. Lewinsky, T. G. Jensen, J. Møller, A. Stensballe, J. Olsen, J. T. Troelsen, "T-13910 DNA variant associated with lactase persistence interacts with Oct-1 and stimulates lactase promoter activity in vitro", Human Molecular Genetics, vol. 14, 24, pp. 3945-3953, 2005.

[14] B. F. Eken, H. Gezmiş, C. Sercan, S. Kapıcı, Ö. M. Chousein, D. Kıraç, S. Akyüz, K. Ulucan, "Analysis of Vitamin D Receptor Gene Fok1 (Rs2228570) and Bsm1 (Rs1544410) Polymorphisms in Turkish Athletes", Istanbul Gelisim University Journal of Health Sciences, 6, pp. 561-572, 2018.

[15] K. Ulucan, S. Yalcin, B. Akbas, F. Uyumaz, M. Konuk, "Analysis of Solute Carrier Family 6 Member 4 Gene promoter polymorphism in young Turkish basketball players", The Journal of Neurobehavioral Sciences, 1, 2, 2014.

[16] N. S. Guest, J. Horne, S. M. Vanderhout, A. El-Sohemy, "Sport nutrigenomics: personalized nutrition for athletic performance", Frontiers in Nutrition, vol. 6, p. 8, 2019.

[17] P. Ridefelt, L. D. Håkansson, "Lactose intolerance: lactose tolerance testing

versus genotyping", Scandinavian Journal of Gastroenterology, vol. 40, 7, pp. 822-826, 2005.

[18] M. Raz, Y. Sharon, B. Yerushalmi, R. Birk, "Frequency of LCT-13910C/T and LCT-22018G/A single nucleotide polymorphisms associated with adult-type hypolactasia/lactase persistence among Israelis of different ethnic groups", Gene, vol. 519, 1, pp. 67-70, 2013.

[19] Raz, M., Sharon, Y., Yerushalmi, B., & Birk, R. (2013). Frequency of LCT-13910C/T and LCT-22018G/A single nucleotide polymorphisms associated with adult-type hypolactasia/lactase persistence among Israelis of different ethnic groups. Gene, 519(1), 67–70.

[20] T. Płoszaj, K. Jędrychowska-Dańska, H. Witas, "Frequency of lactase persistence genotype in a healthy Polish population," Open Life Sciences, vol. 6, 2, pp. 176-179, 2011.