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Distribution of Methylenetetrahydrofolate Reductase rs1801133 Polymorphism in a Turkish Professional Cyclist Cohort

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ABSTRACT

Background. The determination of the genetic endowment of athletic performance in sports is an important step in developing personal training sessions or nutritional supplements for success in sports. Information about the genetic parameters responsible for these metabolisms will help sport's scientist to develop new insights for better performance. Muscle metabolism is one of the key points in better personal athletic performance. **Objectives.** The aim of this study is to analyze the distribution of the methylenetetrahydrofolate reductase enzyme (MTHFR) rs1801133 (C677T) genotype and allele distribution in a Turkish professional cyclist cohort. **Methods.** There were 25 Turkish cyclists enrolled in the study. Peripheral blood used for DNA isolation and the conventional polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methodology were used for genotyping. **Results.** There were 14 (56%), 10 (40%) and 1 (4%) cyclist who had CC, CT, and TT genotypes, respectively. C allele was counted as 38 (76%), and T alleles as 12 (24%). 9 (50%) of the male cyclist had CC, 8 (44.4%) had CT and only 1 had TT (5.6%) genotypes. C allele was counted as 26 (72.2%), and the T allele as 10 (27.8%) in the male cyclists. In the females, the respective genotypes for CC and CT were 5 (71.4%) and 2 (28.6%). C allele was counted as 12 (85.7%) and T allele as 2 (14.3%). **Conclusion.** In our cohort, both of the two genders, the CC genotype and C allele were found to be higher when compared to the other genotypes and T allele. Larger prospective studies focusing on the influence of MTHFR rs1801133 polymorphism in athletic performance are required for confirmation of our findings.

KEY WORDS: *Athletic Performance, Epigenetic, Folic Acid, Sport Genomic, Supplement.*

INTRODUCTION

Athletic performance is considered to be the combination of inherited genetic endowment and subsequent environmental factors. Current genetic research on athletic performance focuses on the polymorphisms that contribute significantly to the performance of athletes. Individual characteristics such as endurance, strength, power, muscle coordination, and motivation are all affected by genetic factors

(1). Sport genetics studies include a whole range of studies in the areas of identifying genes that influence athletic performance, clarifying the mechanisms of action, and determining their susceptibility to athletic performance. Not only in individual sports, but also in team sports, for improved success, it is important to organize training and nutrition programs, which are under the control of

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genetic makeup (2). To have more information about the physical work capacities, such as biochemical, physiologic, and morphologic parameters, genomic data will provide valuable information for sport's scientists to optimize individual performance, training, adaptation.

There are several different cycling types, high-performance road, cross-country biking (XCMB), Downhill Mountain biking (DHMB) and bicycle motocross (BMX). Although they differ in cycling types, the cyclists' performance depends on both physiological and biomechanical parameters. Bicycle activities require long-term durability, quick power, and a maximum power for short and long distances. Biomechanical variables such as pedal force effectiveness and some morphological structures of cyclists such as muscle volume and muscle fiber type have effect on muscle performance and therefore, effect on cycling performance (3).

Methylenetetrahydrofolate reductase (MTHFR) is one of the major regulatory enzymes in a single carbon metabolism, which includes purine and pyrimidine (thymidine) synthesis. This enzyme converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-methyl-THF; a co-substrate for remethylation of homocysteine to methionine), which involves the forms of vitamin folate (4). The enzyme is coded by the *MTHFR* gene, which has been localized at 1p36.3, and consists of 11 exons (5). There are two common polymorphisms in the *MTHFR*; 677C>T (rs1801133) transition leads to the conversion of Alanine into Valin (A222V) and 1298A>C (rs1801131) the transversion leads from Glutamine to Alanine (Q429A) alteration (6). Both variants are related to reduced enzyme activity, leading to increased 5,10-methylenetetrahydrofolate and decreased 5-methyl-THF and therefore, effecting the DNA methylation metabolism.

Physical exercise stimulates adaptive changes that lead to a muscle phenotype with improved performance. Also, intense exercise causes DNA damage in professional athletes. DNA hypomethylation induces muscle growth and activation of factors that determine the differentiation of myoblasts, which promotes muscle mass (7). For cyclists, genetic variants suitable for the metabolism for explosive power phenotype and genetic variants suitable for the

endurance capacities are expected. As reduced enzyme activity disturbs DNA methylation by effecting regulatory the mechanisms controlling genes involved in muscle hypertrophy, we aimed to analyze *MTHFR* C677T rs1801133 polymorphism in professional Turkish cyclists.

MATERIALS AND METHODS

Participants. A total of 25 cyclists, aged between 19-23 years, without any genetically transmitted diseases or in their first-degree relatives, were recruited for the study. To eliminate environmental factors, we excluded the ones who had vitamin and folate restriction. All the participants had the standard daily dose of Vitamin B supplement. The study was approved by the Üsküdar University Ethics Committee, and the study procedure was in accordance with the principles of the Declaration of Helsinki II. All the subjects provided written informed consent prior to enrollment.

DNA sample collection. The DNA was isolated from the peripheral blood using a PureLink DNA isolation kit (Invitrogen, Van Allen Way Carlsbad, Calif., USA). The procedures were conducted according to the provided manufacturers' instructions.

***MTHFR* C677T genotyping.** The amplification of the polymorphic region was maintained by using the primers 5'-TGA AGG AGA AGG TGT CTG GGG GA-3' and 5'-AGG ACG GTG CGG TGA GAG TG-3'. The PCR reactions were conducted in a 50- μ l mixture containing 100 ng genomic DNA, 5 μ l 10X Taq buffer, 3 μ l 25 mM MgCl₂, 1 μ l 0.2 mM dNTP, 2 μ l 1 μ M primers and 2 units Taq DNA polymerase. The reaction was completed after 38 cycles; after the pre-denaturation at 94°C for 5 min, all the cycles had the denaturation at 94°C for 45 sec, annealing at 62°C for 45 sec, and the extension at 72°C for 45 sec. The final extension at 72°C for 10 min completed the amplification process. In order to determine the allelic types, an overnight *Hinf*I (Thermo Scientific, USA) digestion at 37°C was performed. The undigested 198-bp amplicon was determined as the wild-type and named CC genotype; whereas the mutant TT genotype was digested into 175- and 23-bp fragments. The heterozygous

individuals (CT genotypes) exhibited the 198-, 173- and 23-bp band patterns (Figure 1).



Figure 1. The agarose gel electrophoresis view of the MTHFR rs1801133 polymorphism. The first lane represents the 100 bp molecular marker, lane 2 and 4 are CC (undigested), lane 3 is CT, and lane 5 is the TT genotype.

RESULTS

There were 25 Turkish national cyclists who participated in our study. 14 cyclists had CC (56%), 10 (40%) had CT, and only 1 (4%) had TT genotypes. When the allelic distributions were examined, the C allele was counted as 38 (76%), and T alleles as 12 (24%). In our cohort, the CC genotype and C allele were dominating when compared to the other genotypes and T allele. When we examined the gender distribution, 9

(50%) of the male cyclists had CC, 8 (44.4%) had CT, and 1 had TT (5.6%) genotypes. The C allele was counted as 26 (72.2%), and the T allele as 10 (27.8%) in the male cyclists. In the females, the CC and CT genotypes were detected as 5 (71.4%), and 2 (28.6%) respectively. We detected no TT genotypes in the female cyclists. The C allele was counted as 12 (85.7%) and the T allele as 2 (14.3%). The genotype and allelic distributions for the *MTHFR* gene are summarized in Table 1.

Table 1. The numbers and percentages of the MTHFR rs1801133 genotype and alleles in the cyclists.

	MTHFR Genotype			Allele Frequency	
	CC	CT	TT	C	T
Female Cyclists	5 (71.4%)	2 (28.6%)	0 (0%)	12 (85.7%)	2 (14.3%)
Male Cyclists	9 (50%)	8(44.4%)	1 (5.6%)	26 (72.2%)	10 (27.8%)
Total	14 (56%)	10(40%)	1 (4%)	38 (76%)	12 (24%)

DISCUSSION

Muscle cells display a number of molecular processes under regular high intensity exercise. The primary mechanisms as an adaptation to regular exercise are the muscle mass increases, which are mainly supplied by the proliferative activation and myogenic differentiation of the mononuclear satellite cells that fuse with the enlarging myofibers (7). These changes in the muscle cells are considered to be under the control of the DNA methylation processes, in which the *MTHFR* plays crucial roles, especially

in the methylation process of the genes responsible for generating new adaptive proteins. Therefore, it is important to have knowledge about the genetic parameters affecting this metabolism. *MTHFR* rs1801133 is one of the most important functional polymorphism in this regard. DNA hypomethylation has recently been shown to be an important factor in exercise-induced adaptation in the skeletal muscles (8).

In the present study, we analyzed 25 professional cyclists, some of whom represent at National levels. 56% of our athletes had CC

genotype, 40% had CT and 4% had TT genotypes. C allele was 76% and T allele 24%. In both the male and female cyclists, the CC genotypes and C alleles were higher than the other genotypes (CT and TT) and T allele. When we consider the general population, the TT genotype and T allele are relatively underrepresented. Teruzzi et al. (2011) analyzed 77 elite athletes and compared them to 54 sedentary controls. They reported that 63.5% of the elite athletes had a CT genotype, 26.9% had CC, and the remaining had TT genotypes. In an early study, Dinc et al. (2016) analyzed 48 Turkish soccer players that played in some of the A2 teams of the Turkcell Super League and Bank Asya Major League in Turkey and compared them to 48 sedentary individuals in terms of the *MTHFR* rs1801133 polymorphism and in some anthropometric and biochemical blood parameters. In their cohort, they identified a significant difference between *MTHFR* rs1801133 polymorphic alleles and the analyzed parameters; the soccer players were found to have lower levels of triglyceride, total cholesterol and LDL cholesterol levels, and higher levels of folate than the sedentary individuals. They also reported that the participants who had TT genotypes had a lower level of Vitamin B12 and folate and a higher level of Hcy than the participants carrying CC and CT genotypes. However, they did not compare the allelic differences between the soccer players and the sedentary individuals. When we compared the genotype results with these previous studies, there were some differences in terms of the genotype percentages. Thus, the genotype results had some differences within the different populations and within the same populations. This fact may explain our different results when compared to the earlier ones.

Banting et al. (2015) analyzed five different SNPs, including the *MTHFR* rs1801133 in male Polish (108 endurance, 100 power/ sprint and 440 sedentary) and Russian (70 endurance, 107 power/ sprint and 104 sedentary) cohorts. In the Polish samples, in the endurance athletes, the percentages were 49.40, and 11 for the CC, CT, and TT genotypes, respectively. For power/ sprint athletes, the respective genotype percentages for CC, CT and TT genotypes were 51, 40 and 9. Our results were in agreement with the Polish cohort, indicating the dominance of the CC genotype and

C allele. A Russian cohort from the same study for endurance athletes showed similar results to ours; their respective genotype percentages for CC, CT, and TT were 51, 43 and 6. However, in the power/ sprint athletes, the genotype percentages for CC, CT, and TT were 41, 49 and 10, respectively. In their Polish and Russian cohort, they observed no statistically significant difference between the genotypes of athletes and the sedentary individuals.

Other functional polymorphism in the *MTHFR* is the A>C transversion located at position 1298 (A1298C, rs1801131). This variation leads to a change of glutamate into alanine at the C-terminal regulatory domain of *MTHFR* protein, and confer reduced enzyme activity (11). Zarebska et al. (2013) analyzed the distribution of the 1298A>C variant in 302 Polish and 842 Russian athletes, for which they divided the athletes into four groups: endurance, strength/endurance, sprint/strength, and strength/endurance. They also enrolled 1540 control participants onto the study and concluded that not only the C677T, but also the A1298C polymorphism is an important biomarker in athletic performance. They found that the C allele seems to be beneficial in sprint-strength and strength activities when compared to other athlete groups and control subjects.

CONCLUSION

It is a well-known fact that individuals have different responses to similar training: from a lack of adaptive response to extreme overload. Studies to date have shown that individuals having the same genotype for a given DNA respond to the same exercises much more similarly to those with different genotypes, indicating that genes have crucial roles in the determination of individual differences to physical activities. In addition, some genetic parameters have important roles in the recovery metabolism of muscles during the resting times after intensive exercise. Having information about them will enable coaches to set up individual improved training sessions depending on the genomic variations.

One of the major limitations of our study was the number of subjects. In the present study, we had 25 cyclists enrolled for the study. To have more precise results, we should have more samples for the genotype. Our second limitation

was the lack of biochemical results such as the homocysteine and methionine levels. These parameters may be affected by other environmental factors, for example, stress, nutrition, or endocrinal conditions. To date, it is very clear that DNA methylation does not solely control exercise-induced gene expression (13) as there are other complex cellular transductional network systems which modify the DNA methylation (14). However, despite these limitations, this initial report supplies information about the allelic distribution of C677T polymorphism in Professional Turkish cyclists.

APPLICABLE REMARKS

- Determining the *MTHFR* C677T genotype will be helpful for trainers, coaches, and cyclists when setting up training and recovery time sessions. Also, having information about *MTHFR* C677T will be helpful for folic acid or multivitamin supplementation.

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