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MITOCHONDRIAL HAPLOGROUP DISTRIBUTION IN TURKISH ELITE ATHLETES

ELİT TÜRK SPORCULARINDA MİTOKONDRİYAL HAPLOGRUP DAĞILIMI

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Elit Türk Sporcularında Mitokondriyal Haplogrup Dağılımı

ÖZ

Bir bireyin atletik yeteneği genetik ve çevresel faktörlerin etkisindedir. Bazı mitokondriyal haplogrup ve polimorfizmlerin insan performansı ile ilişkisi gösterilmiştir. Bu çalışmanın amacı; mitokondriyal haplogrupların frekans dağılımının elit Türk güreşçiler ve sporcu olmayanlar arasında bir ilişkinin olup olmadığını değerlendirmektir. mtDNA kontrol bölgesindeki hiperdeğişken bölge I dizilemesi 52 elit Türk sporcu ve 74 sağlıklı atlet olmayan bireyden oluşan 126 kişide yapıldı. Elit Türk güreşçiler ve sporcu olmayanların dizileri revize edilmiş Cambridge Referans Dizisi ile karşılaştırıldı. Mitokondriyal haplogruplar insan mtDNA veri tabanı kullanılarak atandı ve elit Türk güreşçilerinde 15 major mitokondriyal haplogrup tespit edildi. Türk güreşçileri atlet olmayan grupla kıyaslandığında; elit Türk güreşçilerinde en yaygın haplogrupların H, U ve K haplogrupları olduğu belirlendi. Elit Türk güreşçiler ile atlet olmayanların mitokondriyal haplogrup frekansları Fisher exact testi kullanılarak karşılaştırıldı. Elit Türk güreşçiler ve sporcu olmayanlar arasında istatistiksel olarak anlamlı bir fark bulunmadı ($p = 0.4186$). Buna karşın, atletik olmayan gruba göre elit Türk güreşçiler arasında K ve U3 haplogruplarında önemli farklılıklar tespit edildi ($p < 0.05$).

Anahtar Kelimeler: Elit sporcu, atletik performans, mtDNA, mitokondriyal haplogrup, Türk populasyonu

Mitochondrial Haplogroup Distribution in Elite Turkish Athletes

ABSTRACT

An individual's athletic ability is influenced by genetic and environmental factors. Some mitochondrial haplogroups and polymorphisms have been shown to be associated with human performance. The aim of this study is to asses whether the frequency distribution of mitochondrial haplogroups is an association between elite Turkish wrestlers and non-athletes. Sequencing of the hypervariable region I in the mtDNA control region was performed in 126 individuals, consisting of 52 elite Turkish athletes and 74 healthy non-athletes. The sequences in the elite Turkish wrestlers and non-athletes were compared with the revised Cambridge Reference Sequence. The mitochondrial haplogroups were assigned with the human mtDNA database and 15 major mitochondrial haplogroups were identified in elite Turkish wrestlers. It was determined that the most common haplogroups in elite Turkish wrestlers compared with non-athlete group are H, U and K haplogroups. The mtDNA haplogroup frequencies of elite Turkish wrestlers and non-athletes were compared using Fisher's exact test. No statistically significant difference was found between elite Turkish wrestlers and non-athletes ($p=0.4186$). However, significant differences were determined in the K and U3 haplogroups among elite Turkish wrestlers compared to the non-athletic group ($p < 0.05$).

Key Words: Elite athlete, athletic perfomance, mtDNA, mitochondrial haplogroup, Turkish population

INTRODUCTION

The human physical performance is a polygenic trait in which genetic and environmental factors are involved[1]. The human physical traits such as muscle strength, muscle size and response to training would be determine an individual's athletic ability[2,3].

Several studies clarified the interrelation between genetic and environmental factors using the twin method on the athlete status. It is estimated that heritability of athletic status is 66% in dizygotic twin pairs (DZ) who had attended sporting competitions[4]. About 200 genetic variations associated with physical performance and health-related fitness phenotypes were identified. 20 polymorphisms of those were found to be related with performance phenotypes[5].

Several studies dealing with angiotensin-converting enzyme (ACE) and α-actinin-3 (ACTN3) known as candidate performance genes have shown significant associations between genetic polymorphisms, and power and/or endurance athlete status[6,7].

The mitochondria undertake crucial roles in the generation of energy and contain their own genomic DNA. Mitochondrial DNA (mtDNA) consists of sequences in coding region, and the non-coding region that is called the control region (CR)[8]. The control region serves as the origin of replication for the heavy strand (H-strand), and like the promoters for transcription of the light- and heavy-strands[9,10].

Mitochondria have features as maternal inheritance and high copy number of mtDNA[11]. The mutation rate of mtDNA are much higher than those of nuclear DNA owing to lack of efficient DNA repair mechanisms[12].

mtDNA variations in control region have been associated with metabolic and neurological diseases, various cancers, and extensively used for identification of individuals in anthropology and forensic science[13,14,15].

Over the past decades, a large number of studies suggested numerous associations between mtDNA and physical ability in various sport fields[16]. 155 genetic markers within all chromosomes and mtDNA were shown association with elite athlete status[17]. A study in Kenya revealed that L0 and L3 mtDNA haplogroups of international athletes in distance running displayed statistically significant differences[18].

While earlier studies on athletic performance focused mainly on one genetic polymorphisms, recent studies apply a total genotype score (TGS) model to determine "optimal" polygenic profile based on published performance-associated polymorphisms in elite athlete status[19,20,21]. Many studies demonstrated that polygenic profile similarity within population would limit the probability of finding the "perfect" athletic performance[22,23].

There are insufficient studies about sports and genetic in Turkey[24,25]. The purpose of this study was to determine whether the frequencies of mitochondrial haplogroups differ between the elite Turkish wrestlers and non-athletes.

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MATERIAL AND METHOD

Participants

The elite Turkish wrestlers and non-athletes were selected from individuals residing and born in Turkey (126). The male Turkish wrestlers were chosen from the athletes who participated in both national- and international-level competitions. Blood samples were obtained from 52 elite Turkish wrestlers, and 74 maternally unrelated non-athletes randomly selected (control group).

Extraction and Amplification

DNA was extracted by phenol-chloroform method[26]. A total of 126 DNA samples were amplified using primers L15997: 5'- CACCATTAGCACCCAAAGCT-3' and H16401: 5'-TGATTTCACGGAGGATGGTG-3' comprising HVI (hypervariable region I). PCR amplification was performed for 30 cycles, each of which consisted of 94oC for 30 s, 56^{o}C for 30 s and 72^{o}C for 90 s[27]. PCR products were separated with a 2% agarose gel electrophoresis and visualized products stained by ethidium bromide on an ultraviolet light. The purification of PCR products were performed with QIAquick PCR purification kit (Qiagen, Hilden, Germany).

DNA Sequencing

The sequencing was performed by using BigDye™ Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, CA, USA) with the same primer set of the PCR reaction. The reaction condition for PCR was 30 cycles of 94°C for 30 s, 56°C for 30 s and 60°C for 4 min. The sequences were analyzed with 3130xl Genetic Analyzer (Applied Biosystems, CA, USA). In some cases, to ensure high quality data, both forward and reverse primers were used.

Haplogroup Classification

The mtDNA sequence reads were aligned to the revised Cambridge Reference Sequence (rCRS)[28]. Cambridge reference sequences (no mutations) and polymorphisms on sequence reads were determined using Clustal Omega for multiple sequence alignment[29]. The mtDNA sequences of each subject were assigned to haplogroups with the MITOMAP (http://www.mitomap.org/MITOMAP)[30].

Statistical Analyses

Mitochondrial haplogroup frequencies were calculated in the elite Turkish wrestlers and non-athletes. mtDNA haplogroup frequencies between elite athletes and non-athletes were compared by using Fisher's exact test by the R-project. $p < 0.05$ was considered statistically significant.

FINDINGS

The mtDNA hypervariable region I was amplified and sequenced in 52 elite Turkish wrestlers and 74 non-athlete group. The sequence reads of 126 subjects were compared with revised Cambridge Reference Sequence. Cambridge reference sequences including no polymorphisms were determined in only 2 subjects in the 52 elite Turkish wrestlers and only 6 subjects from 74 non-athletes. The identified mtDNA

variants of each subject were assigned to mitochondrial haplogroups with the human mtDNA database, e.g., MITOMAP. The frequencies of mtDNA haplogroups were calculated in 50 elite Turkish wrestlers and 68 subjects of non-athletes.

17 mitochondrial haplogroups (B, C, D, F, H, I, J, K, L, M, N, P, R, T, U, X, Z) were observed in total 118 individuals (Fig.1). The elite Turkish wrestlers had a wide variety of mtDNA haplogroups (15 kinds) when compared with non-athletic group (11 kinds).

The most common haplogroups were H, U and K in elite Turkish wrestlers. The H, U and J also were the most common haplogroups in non-athletes. No haplogroups P and R in elite Turkish wrestlers and haplogroups D, F, I, M, X and Z in non-athletes observed.

mtDNA haplogroup frequencies in elite Turkish wrestlers were compared with those in non-athlet group. No significant difference was found between elite Turkish wrestlers and non-athlet group for mitochondrial haplogroups ($p = 0.4186$). However, haplogroup K and haplogroup U3 were found to differ significantly among elite Turkish wrestlers compared to the non-athletic group ($p < 0.05$).

DISCUSSION AND RESULTS

Some studies have shown an association between mitochondrial haplogroups and athletic performance. The majority of the studies were on endurance athletes (road cyclists and endurance runners) and sprint athletes (sprinters, throwers and jumpers), but there is still little data for wrestlers[31,32]. The present study was performed to define whether the mtDNA haplogroups in HVI of mtDNA control region differ in sequence variation between the elite Turkish wrestlers and non-athletes.

The majority of mtDNA variations have no effect on mitochondrial function. Some of haplogroup-specific polymorphisms is important for QXPHOS in endurance athletes[33]. This study did not determine a significant difference in mtDNA haplogroups between elite Turkish wrestlers and the non-athletic group ($p = 0.4186$). Sprint/power performance is more related to anaerobic glycolysis than OXPHOS[34]. The frequencies of haplogroup H, U, and K were higher than those of other mitochondrial haplogroups in elite Turkish wrestlers.

The haplogroup K (8.0%) and haplogroup U3 (8.0%) were overrepresented in elite Turkish wrestlers. The present study has shown that haplogroups K and U3 differ significantly in the elite Turkish wrestlers compared with the non-athletic group ($p < 0.05$).

Haplogroups K and J were found extensively among Finnish sprinters. However, haplogroup K or subhaplogroup J2 was observed infrequent among the Finnish endurance athletes[35]. Maruszak et al. (2014)[36] determined that haplogroup K was infrequent in Polish endurance athletes than the controls. The frequency of haplogroup J was a higher in Iranian athletes consisting sprint/power athletes than the controls[37]. No haplogroup J was found in Turkish power athletes in this study. Mitochondrial haplogroup K and haplogroup J is a negative marker for physical performance in endurance athletes[35]. This results suggested that haplogroup K and J are candidates for being “uncoupling genome[38].

17 major mtDNA haplogroups in HVI were identified in both healthy non-athletes (11 kinds) and elite Turkish wrestlers, who were in different 15 mitochondrial haplogroups (Fig. 1). The study on mitochondrial haplogroups determined 12 major mitochondrial haplogroups including performance-associated haplogroups F (sprint/power athletes) and G1 (endurance/middle-power athletes) in elite Japanese athletes. This study explained that some mitochondrial haplogroups would affect physical performance[39]. Another study in Japan suggested that the major haplogroup N is decisive for anaerobic physical performance in muscle power[40].

No association was found with elite power athletic status in the present study. The enviromental factors such as training, nutrition, and socio-economic status may be effective on elite Turkish wrestlers. In the future, other polymorphisms related to strength/sprint and endurance performance in elite Turkish athletes should be investigated in mtDNA and nuclear DNA.

The findings of the haplogroup K and haplogroup U3 in the present study should be supported by studies with larger sample sizes. The association of haplogroup K and U3 with the sport performance must be replicated in other cohorts.

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