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Association of mitochondrial DNA haplogroups with elite athletic status in Iranian population

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ABSTRACT

Increasing evidences suggest that certain variations in the control region of mitochondrial DNA (mtDNA) are associated with physical performance levels of individuals. The aim of the present study was to assess whether mitochondrial haplogroups are associated with elite athlete status in Iranian population. The mtDNA haplogroups of 100 Iranian athletes and 100 healthy, non-athletic individuals were genotyped. Significant differences were observed for haplogroups U and J, which were under- and over-represented in the elite athletes group. The results show that mtDNA haplogroups and their related phenotypic expression correlate with the physical performance.

1. Introduction

Human beings share 99.9% similarity in their genome sequence. The 0.1% sequence variation, which is mostly simple single nucleotide polymorphism (SNP), is mainly responsible for differences between individuals and their unique characteristics (Sripichai and Fucharoen, 2007). The genetic variations are mostly located in noncoding regions; nevertheless, they make a significant contribution to phenotypic variations, presumably through their effects on the genome protection and regulation of gene expression and protein synthesis (Vernot et al., 2012).

Many specific and complex phenotypic traits such as maximum rate of O_2 consumption, heart response to exercise, skeletal muscle strength, neuromuscular coordination and energy supply of aerobic metabolism form a quantitative, complex, polygenic phenotype known as elite athletic performance. Each of these traits is influenced by various environmental and genetic factors (Ahmetov et al., 2009; Bouchard, 2011; Eynon et al., 2011b; Puthucheary et al., 2011). Hence, myriad of genetic and non-genetic variations affect the athletic performance.

Some of the important phenotypes that are associated with physiological cell health and the ability of human body to attain an elite athlete status result from variations in mitochondrial organization and genome. Mitochondria are the center of energy production in cells, which is completely dependent on oxygen for aerobic respiration. Additionally, mitochondria have key roles in regulating cell death and survival, stem cell differentiation, muscle health, aging, and physiological adaptation to enduring exercise. Several pathophysiological conditions result from mutations in the mitochondrial genome or impairment of its functions (Eynon et al., 2011a; Yu et al., 2013; Russell et al., 2014; Collu-Marchese et al., 2015).

Small circular double stranded mtDNA, which is part of almost all eukaryotic cells' genome, is quantitatively different in different cells of the same strains. Although, the size of mtDNA is small compared to the nuclear genome, the mtDNA is more stable over time, with no recombination, higher mutation rate, and only maternal inheritance.

Mitochondrial genome contains two main regions: a major coding region encoding for 13 essential components of the biochemical pathway for energy production (Chen and Butow, 2005), as well as 22 tRNA and 2 rRNA that are used by the mitochondrial translational machinery system (Yoon and Koob, 2003). The non-coding region, known as the control region, contains a displacement loop (D-loop) and associated promoters. This region is a highly polymorphic stretch of DNA with polymorphism concentrated in 3 hypervariable regions: HVR I, HVR II, and HVR III. Neutral polymorphisms throughout the mitochondrial genome are used to describe classes of specific genotypes; the

Abbreviations: mtDNA, mitochondrial DNA; SNP, single nucleotide polymorphism; D-loop, displacement loop; PCR, polymerase chain reaction; rCRS, revised Cambridge Reference Sequence; ETC, electron transport chain; ROS, reactive oxygen species; VO_{2max}, maximal oxygen uptake.

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haplogroups. Each mitochondrial haplogroups, designated by certain specific polymorphisms, evolved from the same maternal ancestor (Ienco et al., 2011). Examining the sequence of HVR regions is sufficient to determine an individual's haplogroup with a reasonable accuracy. Mitochondrial haplogroups are mainly used for investigating historical origins and migration of human populations, but several recent studies have suggested a potential relationship between the haplogroups and different phenotypes including neurodegenerative diseases, aging and physiological responses to environmental conditions (Arning et al., 2010; Coto et al., 2011; Courtenay et al., 2012; Nishimura and Watanuki, 2014).

The aim of the present study is to assess the frequency of mitochondrial haplogroups in a representative group of elite Iranian athletes, and to establish whether the mtDNA variations are associated with the elite athletic performance in this population.

2. Materials and methods

2.1. Subjects

The present study included 100 elite Iranian athletes who had represented Iran in world championships (Table 1). A total of 100 twenty to thirty years old healthy Iranian subjects (50 male and 50 female students), with no history of cardiovascular diseases, and no regular training for athletics or competitive physical exercises, were used as the control population. All participants as well as at least their two previous generations were Caucasian and born in Iran. No familial relationship was existing inside and between athlete and control groups. A written informed consent was obtained from all the individuals participating in the survey and the study was conducted in accordance with the World Medical Declaration of Helsinki and ethical standards in sport and exercise science and research (Harriss and Atkinson, 2013). The procedure followed in the study was approved by ethical committee of Ministry of Science, Research and Technology of Iran.

2.2. Saliva sample collection and DNA isolation

Saliva samples were collected from participants for DNA isolation. DNA extraction was performed using the Saliva DNA collection, preservation and isolation kit (RU35700, NorgenBiotek Corp; Ontario, Canada) according to the manufacturer's instructions.

2.3. mtDNA haplogroup genotyping

Polymerase chain reaction (PCR) was used to amplify mitochondrial HVR I and II for DNA sequencing using *AccuPower® Pfu* PCR PreMix (Bioneer, Korea). The primers were F15975 (5′-CTC CAC CAT TAG CAC CCA AA-3′) and R389 (5′-CTG GTT AGG CTG GTG TTA GG-3′). A fragment of 983 bp was amplified and the PCR primer pairs were subsequently used for the sequencing. The purified PCR products were sent to Macrogen for sequencing (Macrogen Inc., Korea) with both forward and reverse primers. T to C polymorphism in position 166 (166 T > C) of some PCR samples caused 10 C nucleotide placed together consecutively; the sequencing was disrupted at that point. In this case, to complete the sequencing, two more primers, F16524 (5′-AAG CCT AAA TAG CCC ACA CG-3′) and R042 (5′-AGA GCT CCC GTG AGT GGT TA-3′) were used. Fifteen percent of the samples were sequenced twice to verify the results. Sequences were compared with the Revised Cambridge Reference Sequence (rCRS) (GenBank NC_012920) using MAFFT, a web-based multiple sequence alignment program (http://mafft.cbrc.jp/alignment/server/index.html) and the variations were identified. The haplogroups were classified according to human mtDNA database, PhyloTree mtDNA tree Build 16 (19 Feb 2014) (http://www.phylotree.org/). Alternatively, the sequences were analyzed for classifying mtDNA using specific web-based programs such as mtDNA manager (http://mtmanager.yonsei.ac.kr/search_sample.php), Haplogrep (http://haplogrep.uibk.ac.at/), MitoTool (http://www.mitotool.org/dloopRSRS.html), and MitoWeb (http://www.mitomap.org/bin/view.pl/MITOMASTER/).

Table 1
Participant characteristics of elite Iranian sport players.

Sport	Number	Sex
Weightlifting	16	Male
Wrestling	12	Male
Basketball	25	Male & female
Taekwondo	20	Male & female
Volleyball	16	Male
Track and field	2	Female
Karate	2	Female
Canoe	7	Female

2.4. Statistical analysis

Haplogroups frequencies between control and elite athletes were compared using the test of difference between proportions. The prevalence of each haplogroup, which is considered as an attitude, was compared between the two test populations to determine whether the difference between two proportions is significant. The null hypothesis H_0 was defined as "the two population proportions were equal ($p_1 = p_2$)". The test statistical significance level was considered to be 0.05.

3. Results and discussion

Mitochondria contain a polymorphic, double-stranded DNA, extensively used for drawing phylogenetic tree and analyzing historical migration of human populations. Mutations in mtDNA during the history of human evolution shaped the current human mtDNA variations cumulatively and usually non-pathogenically (Santos et al., 2008). It is hypothesized that mutations in mtDNA allow adaptation to changes in climate and diet. Hence, mtDNA variations, referred to as haplogroups, show striking geographical and topographical dispersion patterns (Mishmar et al., 2003; Sun et al., 2007; Shen et al., 2014). Main mtDNA haplogroups, also known as macrohaplogroups, make up the internal branches of the human phylogenetic tree. They are classified using the letters A to Z. The main haplogroups indicate ancient mutations. Each of the haplogroups has many sub-branches and relegated terminal branches, classified by a subsequent set of numbers and letters and refer to more recent mutations.

The mitochondrial haplogroups were found to associate with many human phenotypes including variations in cell metabolism, aging (Courtenay et al., 2012), metabolic and degenerative diseases (Nishigaki et al., 2010; Ridge et al., 2012), and cancers (Wallace, 2012). Here, thirteen main mitochondrial haplogroups in the Iranian elite athletes and control subjects were analyzed. The results indicate that 98% of the mitochondrial lineages found belong to branches of western Eurasian origin (HV, JT, UK, I, W, and N haplogroup), i.e., descendants of the macrohaplogroup N (Fig. 1). This haplogroup is the ancestor of almost all the haplogroups found in Europeans, as well as most people from the Middle East and Caucasians, and sometimes characterized as pan-Eurasian (Comas et al., 2004). The most frequent haplogroups in the elite athletes were H (25%), J (16%), and U (14%), whereas in the control group, the most frequent haplogroups were U (26%), H (22%), and T (12%). The two populations had similar frequency for most of the detected haplogroups. Significant differences were observed for haplogroups U and J ($p < 0.05$) (Fig. 2).

Derenko et al. (2013) presented a large scale analysis of complete mitochondrial genome of 352 Iranians, mostly from three major ethnic groups (Indo-European-speaking Persians and Turkic-speaking Qashqais and Azeris) (Derenko et al., 2013). The frequency of