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ORIGINAL ARTICLE – OPEN ACCESS

Identification of a Novel Mitochondrial DNA Sequence Variation within the Human Mitochondrial DNA Control Region in a Population of Aegean Population

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ABSTRACT

Objective: Environmental impacts, history as well as established cultural regulations, and many more other factors had shaped broadly diverse structure of human genetic patterns. A control region of human mtDNA has served as a good genetic determination marker in many fields such as evolutionary studies and forensic genetics. The allelic variations of mtDNA have been studied in many populations including Turkish populations. Previous studies in Turkish populations lacked large cohorts. In this study, non-coding hypervariable regions of human mitochondrial DNA with extended population of Turkish individuals from Aegean region have been investigated.

Materials and Methods: To detect sequence variants in human mtDNA control region, 100 unrelated Turkish subjects were examined.

Results: The outcomes revealed 13 variable sites in hypervariable region I (HVRI) and 20 variable sites in hypervariable region II (HVRII). Polymorphisms within HVRI were detected at the positions of 16173 (C>A) and 16175 (A>G) with the allelic variation frequencies of 53% and 60%, respectively. A novel nucleotide transversion from cytosine to adenine at 16173 position was detected. Only 35% of subjects were aligned with the Cambridge Reference Sequence for the poly-cytosine tract that locates between 303 and 309 nucleotides, whereas 60% of individuals had 8 and 15% of them had 10 cytosine polynucleotides. 263G and 73G polymorphisms were evaluated with higher frequencies for the HVSII region.

Conclusion: Overall, results indicate that the determination of genotype distributions and allelic variations frequencies of human mitochondrial genome are significant to characterize admixture populations from different ethnic origins.

Keywords: mtDNA, polymorphism, Turkish, hypervariable region

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INTRODUCTION

Double-stranded human mitochondrial DNA (mtDNA) is a circular molecule of 16.6 kb in length containing 37 gene-coding and 1.1 kb non-coding regions known as control region (CR) (1). Highly variable regions within the CR or alternatively known as displacement loop (D-loop) of human mtDNA consist of three segments: hypervariable segment I (HVSI) located between the nucleotide positions 16024 and 16383; hypervariable segment II (HVSII) located between the nucleotide positions 57 and 372; hypervariable segment III (HVSIII) located between the nucleotide positions 438 and 574 (NC_0122920.1) (2, 3). Since then, sequence variations of the mtDNA CR have served as a good genetic determination marker in many fields such as population-based and evolutionary studies as well as forensic genetics (3).

Priority of many mtDNA studies was the determination of the sequences within the CR that consists of two hypervariable segments: HVSI located between 16024 and 16383 nucleotide positions, and HVSII residing the nucleotide positions between 73 and 500, respectively (4).

The allelic variations of HVRI and HVRII have been studied in many populations including Turkish populations (5–12). Mitochondrial D-loop region has been examined in 75 Turkish people from different regions of Anatolia by Mergen et al. (12). In this study, non-coding hypervariable regions of human mitochondrial DNA in 100 unrelated Turkish individuals from Aegean region of Anatolian peninsula have been investigated, and a novel polymorphism was determined.

MATERIALS and METHODS

Individuals

This study included 100 unrelated (30 females and 70 males) healthy local Turkish volunteers residing in Denizli, which is located at the Aegean region of Anatolian peninsula. The study protocol was approved by the ethics committee of our university, and all subjects gave written informed consent to participate in the study.

Genotyping and Determination the Allele Sequence of HVRI and HVRII Regions

The genomic DNA of each volunteer was extracted from EDTA-treated whole blood using the traditional phenol chloroform extraction protocol. The PCR amplification was performed on a conventional thermal cycler using two primer sets by Sullivan et al. (13) as follows: L15997 (59-CACCATTAGCACCCAAAGCT-39) and H16401 (59-TGATTTCACGGAGGATGGTG-39) for HVRI; L29 (59-GGTCTATCACCCTATTAACCAC-39) and H408 (59-CTGTTAAAAGTGCATACCGCCA-39) for HVRII. Determination of PCR products first run on agarose gels and visualized by ethidium bromide staining and subsequent UV transillumination, then genotyping of sequence allele marker sites was performed using capillary electrophoresis system (Beckman Coulter, CEQ8000 Analysis System, California, USA).

Sequenced human mitochondrial DNA HVRI and HVRII products of each participant were aligned and matched with the complete Cambridge Reference Sequence (rCRS; GenBank accession no. NC_012920.1) (14); however, determined allelic variants were classified and evaluated using MITOMAP (15) and MtDNAprofiler (16).

RESULTS

Genotyping of Sequence Variants

The polymorphic sites of sequenced the CRs (HVRI and HVRII) of human mtDNA are shown in Tables 1 and 2, respectively. The integrated results of 100 unrelated subjects revealed 13 variable sites in HVRI and 20 variable sites in HVRII. Moreover, a novel variant of nucleotide transversion from cytosine (C) to adenine (A) at the position 16173 was detected (Fig. 1). The most frequently observed position of nucleotide substitutions within HVRI was nucleotide transversion from cytosine (C) to adenine (A) at 16173, a transition of adenine (A) to guanine (G) at 16175, and a transition of cytosine (C) to guanine (G) at 16176 (Fig. 1) with the calculated frequencies of 53%, 60%, and 82%, respectively (Table 1). Interestingly, the two most frequently determined positions of nucleotide substitutions with the measured frequency of 100% were a transversion of adenine (A) to guanine (G) at 73 and a transition of adenine (A) to guanine (G) (Table 2). A transversion of thymine (T) to cytosine (C) at the position of 152 in HVRII was determined the third most frequent variation with the frequency of 22%.

In the poly-cytosine tract between the positions 303 and 309, only 35% of individuals showed seven cytosine matches as similar as the Cambridge Reference Sequence, whereas most other individuals with the frequency of 60% had eight cytosines and the rest 15% owned ten cytosines (Fig. 2). The other microsatellite in the position placed between 311 and 315, studied subjects showed five cytosines as hosting in the Cambridge Reference Sequence.

DISCUSSION

Analyzing human mitochondrial DNA could be helpful to trace down and gain clues about the individual's shared haplotype with their ancestral proportions and present populations especially in admixed societies such as Anatolia.

In the literature, relevant studies were aimed to investigate human mtDNA allelic variation in the Turkish population (10, 11); however, these studies lacked good sample size (12). Other study by Mergen et al. (12) examined two hypervariable regions of the mtDNA in a larger cohort constituting individuals well distributed in different geographical regions of Anatolian peninsula. In the study, 75 unrelated Turkish subjects from all around the Anatolia were examined for allelic sequence polymorphism of the CR of human mtDNA. An expanded study by Serin et al. (17) examined the mtDNA CR of 224 subjects from Southeastern Turkey. A nucleotide substitution of cytosine (C) to thymine (T) at the 16223 base position has been observed with the frequencies of 20%, 11.7%, and 14.2% in southern, northern, and western Anatolia, respectively (12, 17). Moreover, a heteroplasmic transversion position C16176G has been observed in four Southern Eastern Turkish subjects (17). However, they did not detect variations at the nucleotide positions 16173 and 16175.

Nevertheless, in this study, 100 unrelated Turkish individuals who were the residents of Denizli province of Agean coast of Anatolia were used to analyze possible population-specific nucleotide sequence variation of the CR (HVRI and HVRII) of human mtDNA. The most frequently observed nucleotide substitutions were a novel variant showing a nucleotide transversion from cytosine (C) to adenine (A) at the position 16173, a nucleotide transition from adenine (A) to guanine (G) at the position of 16175, and a nucleotide substitution from cytosine (C) to guanine (G) at the position of 16176 with the frequencies or 53%, 60%, and 82%, respectively.

The availability of nucleotide substitutions that are detected in this study has been checked on MITOMAP Database (http://www.mitomap.org/MITOMAP) and Uppsala University's Human Mi-

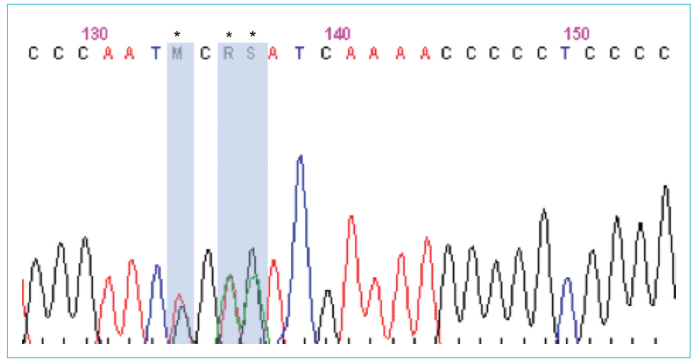

Figure 1. Most frequently detected variations 16173 (C > A at 16173, A > G at 16175, and C > G at 16176) in HVRI of the mitochondrial DNA by sequencing (variants have been indicated with "*" sign)

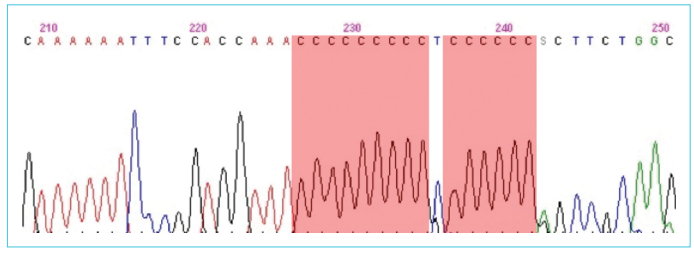

Figure 2. The sequencing results indicating (in red box) the poly-cytosine tract within the positions located on between 303and 309 and 311 and 315

Table 1. List of variable sites observed in the hypervariable region I (HVRI) of human mtDNA control region. The total amount of observed polymorphic markers and their matching comparisons with the Cambridge Reference Sequence as well as their genomic position are shown together

Ref	16051A	16064T	16076C	16081A	16137A	16149A	16173C	16175A	16176C	16214C	16242C	16248C	16249T
1		G			G	C	A	G	C	G	T	G	
2	G				G	C							
3							A			G			
4			G	G			A		G				
5							A		G		T	G	C
6							A		G				
7				G			A		G	G			
8							A		G				C
9	G						A		G				
10							A		G				
11		G					A		G				C
12							A	G	G	G			
13			G				A	G	G				
14							A	G	G				
15							A	G	G	G			
16							A	G	G				
17	G						A	G	G				
18							A	G	G				
19			G				A	G	G				
20				G						G			
21										G			
22		G					A	G					
23							A	G					
24							A	G					
25							A	G		G			
26							A		G				
27							A		G				
28			G						G				
29									G				
30									G				
31									G				
32		G							G				
33									G				
34								G	G				
35							A	G	G				
36							A	G	G				
37							A		G				
38							A		G				
39							A		G				
40			G				A		G				
41							A		G				
42									G				
43									G				
44									G				
45									G				
46									G				
47								G	G				
48								G	G				
49								G	G	G			
50								G	G				
51									G	G			
52							A		G	G			
53							A		G	G			
54							A		G	G			
55							A		G	G			
56				G							G		
57					G						G		C
58				G			A		G	G			
59							A		G	G			C
60							A		G	G			
61							A		G	G			C
62							A		G	G			
63							A		G	G			
64							A		G	G			
65									G	G			
66									G	G			
67									G	G			
68									G	G			
69									G	G			
70									G	G			
71									G	G			
72									G	G			
73									G	G			
74									G	G			
75			G						G	G			
76									G	G			
77									G	G			
78									G	G			
79									G	G			
80									G	G			
81									G	G			
82							A		G	G			
83							A		G	G			
84							A		G	G			
85							A		G	G			
86							A		G	G			
87							A		G	G			
88							A		G	G			
89							A		G	G			
90							A		G	G			
91							A		G	G			
92				G									
93											G		
94				G									
95													
96							A		G	G	G		C
97				G							G		
98					G								C
99				G									
100				G							G		

Table 2. List of variable sites observed in the hypervariable region I (HVRII) of human mtDNA control region. The total amount of observed polymorphic markers and their matching comparisons with the Cambridge Reference Sequence as well as their genomic position are shown together

Ref	073A	078C	087A	115T	118G	132C	146T	152T	153A	159T	225G	228G	236T	239T	263A	275G	279T	285A	294C	296C
1	G								G	C	A				G					
2	G														G					
3	G														G					
4	G														G				T	T
5	G														G			G		
6	G					C	C								G		G			
7	G				G		C					A			G				T	
8	G				G										G				T	
9	G					C				C					G					
10	G			C											G					
11	G	T	T	C											G					
12	G				G		C							C	G	A				
13	G														G					
14	G						C						C		G					
15	G				G										G					
16	G														G					
17	G						C								G					
18	G														G					
19	G														G					
20	G						C								G					
21	G														G					
22	G														G					
23	G					C				C					G					
24	G														G					
25	G														G					
26	G						C								G					
27	G					C									G					
28	G									C					G					
29	G														G					
30	G						C								G					
31	G					C									G					
32	G														G					
33	G									C					G					
34	G														G		G			
35	G														G					
36	G														G					
37	G									C					G					
38	G					C									G					
39	G														G					
40	G						C								G					
41	G														G					
42	G														G					
43	G						C								G					
44	G														G					
45	G														G					
46	G						C								G					
47	G														G					
48	G														G					
49	G						C								G					
50	G														G				T	

Ref	073A	078C	087A	115T	118G	132C	146T	152T	153A	159T	225G	228G	236T	239T	263A	275G	279T	285A	294C	296C
51	G														G					
52	G														G					
53	G														G					
54	G														G					
55	G									C					G					
56	G														G					
57	G														G					
58	G														G					
59	G														G					
60	G														G					
61	G														G					
62	G														G					
63	G														G					
64	G														G					
65	G						C								G					
66	G														G					
67	G														G					
68	G									C					G					
69	G														G					
70	G						C								G					
71	G														G					
72	G						C								G					
73	G														G					
74	G						C								G					
75	G														G					
76	G														G					
77	G						C								G					
78	G														G					
79	G														G					
80	G														G					
81	G									C					G					
82	G														G					
83	G														G					
84	G														G					
85	G														G					
86	G														G					
87	G						C								G					
88	G														G					
89	G														G					
90	G						C								G					
91	G														G					
92	G														G					
93	G														G					
94	G						C								G					
95	G														G					
96	G														G					
97	G						C								G					
98	G														G					
99	G						C								G					
100	G														G					

tochondrial Genome Database (HMGD) (http://www.mtdb.igp.uu.se); however, a C16173T single nucleotide polymorphism is the most frequent variant according to the databases. Despite its high frequency, a novel C16173T variation that was detected in the Turkish population previously has not been shown in the HMGD, shown on the MITOMAP Database (18–20), surprisingly. This could be because the variant is region-specific; therefore it is common in the region, but it does not represent the general Turkish population. Additionally, nucleotide transversion from adenine to guanine at the position 16175 was only determined in a Japanese individual (21). Lastly, a detected nucleotide transversion of cytosine to guanine transversion at the position 16176 was determined in two individuals from populations of Georgia and Jordan (22, 23) and four Southern Eastern Turkish individuals (17). Identifying the global mtDNA variations would help to understand for human evolution studies better; therefore, scientists examined maternal inherited mitochondrial molecule in the population of 53 different origins (21).

The sequence analyses in Caucasoid populations indicated guanine to adenine single nucleotide polymorphism at 263 nucleotide position of HVRII region; as specified by Calafell et al. adenine nucleotide at the position of 263 is defined as the reference sequence for HVRII (10). Additionally, another adenine to guanine nucleotide transition was observed at 73 sequence position. The 146C, 150T, 152C, 195C, and 199C polymorphisms were defined as the most common polymorphisms within the HVSII region of human mtDNA in the Turkish population (12). Recently, Guney et al. determined the allelic frequencies of 263G, 750G, 1438G, 4769G, 8860G, and 15326G variations were greater than 95% among in the Turkish population (24). Both variations at nucleotide sequence positions of 263G and 73G were detected with the highest frequencies (100%) in this study. Therefore, the comparison of this study's outcomes with relevant literature show a great diversity of the studied population. The main reason of this variety is the admixture genetic makeup of Anatolian peninsula as well as studied subjects of this study.

Recent study has indicated that mtDNA U haplogroup has more predisposition to benign and malign thyroid entities, and eight mtSNPs were shown to be associated with benign to malign thyroid tumor in Turkish population as well as they indicated that the D310 poly-C sequence might have a potential diagnostic biomarker for thyroid nodules (25). Therefore, these accumulated data with detected differences contribute to human population and evolutionary studies as well as giving valuable markers. Moreover, the results of this evaluation provide exhaustive clues concerning the genetic makeup of Turkish population of Anatolia, which is geographically located between the continents of Asia and Europe. Collectively, the outcome of different studies including our study reveals that the allelic distributions of maternal lineages show variations in different regions or different origins among Turkish people living in Anatolia peninsula Nevertheless, to determine complete genotype distributions within the Turkish population with different origins/regions require further investigations.

In conclusion, taking together the databases from different populations will increase thriving role of mtDNA typing. Thus, data of this study indicated a significant contribution to the literature.

Ethics Committee Approval: The study protocol was approved by the ethics committee of Pamukkale University Faculty of Medicine (Approval date: 07.04.2017 and Aproval Number: 60116787-020/26151).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – AK; Design – AK, SGT, MCE; Supervision- AK; Resource – AK; Materials – AK; Data Collection and/or Processing – AK, SGT, MCE.; Analysis and/or Interpretation – MCE; Literature Search – MCE; Writing – MCE; Critical Reviews – AK, SGT, MCE.

Conflict of Interest: The authors have no conflicts of interest to declare.

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