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Original Article

Mitochondrial DNA (8389-8865 base pairs) mutation search in Turkish population

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Abstract

Aim: STR loci in nuclear DNA (nDNA) is generally used for identification and identity determination in forensic sciences. However, it is not always possible to achieve successful results from these loci. Especially in biological samples containing nDNA that are degraded in quantity and quality, there might be difficulties to analyze. In such cases, it may be more reliable to use mitochondrial DNA (mtDNA) analysis. Because there is only one nDNA in the cell, while there are many copies of mtDNA. Therefore, it is advantageous to work with. HVI, HVII and HVIII regions in mtDNA are used for identification purposes in forensic sciences. However, the discrimination power of these regions are limited. As an alternative to these regions, studying the coded region will increase the discrimination power. For this reason, the aim of our study is to distinguish the points showing polymorphism in the region between 8389-8865 base pairs as an alternative to the HVI, HVII and HVIII regions. In addition, it will be optimized for routine use in biological samples that do not contain enough nDNA with the regions where the polymorphism rate is determined. **Materials and Methods:** In our study, blood samples were taken from 150 volunteers from the Turkish population who were not related. The region in question was reproduced in accordance with the method of PCR Tzen et al. The obtained sequences were compared with the Cambridge Reference Sequence. **Results:** The most common polymorphisms were found to be 8860G, 8697A, 8847delC, 8691delA. The discrimination power of the studied region was calculated to be 0.923641, the probability of random matching was 0.076359 and the genetic diversity was calculated as 0.9303827. **Conclusion:** In line with these data, it was determined that we could distinguish between individuals with the region we examined and that we could increase the discrimination power by analyzing this region together with the HVI, HVII, HVIII regions.

Keywords: Mitochondrial DNA, identification, forensic genetics, polymorphisms

INTRODUCTION

Polymorphic STR loci in nDNA are commonly used for identification and lineage determination in forensic sciences. However, biological samples that come to the laboratory often contain degraded nDNA in terms of both quantity and quality, rendering traditional STR studies inadequate or unsuccessful. In such cases, mtDNA, which shows maternal inheritance, is used due to its hundreds of copies in each cell and high mutation rate. MtDNA is particularly advantageous when working with bone, teeth, hair, and other materials with highly degraded samples [1]. Because of these features, mtDNA is utilized in forensic science to determine migration routes, in evolution studies, and also to research various diseases [2].

The D-loop region in mtDNA is commonly examined in forensic sciences, as it is polymorphic and does not encode any genes [3]. In identification and lineage studies, the HVI and HVII segments of this region are typically analyzed to obtain information. However, this information is limited and has low discrimination power [4].

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To increase the power of discrimination, researchers investigate the coding regions of mtDNA, which have a lower number of bases and may have links to diseases [5].

Population studies are crucial in forensic sciences to determine the distribution of alleles or genotypes in a given population. This information is essential for forensic identification and object determination. However, in the Turkish population, there is a lack of mutation and single nucleotide polymorphism (SNP) information in the region between bases 8389-8865 of mtDNA. This region may have potential forensic value, but without information on the population's variation, it cannot be utilized effectively. Therefore, to benefit from this region, it is necessary to conduct a population study to determine the distribution of mtDNA mutations and SNPs in Turkey. It is important to note that mutation rates can vary significantly among different populations, and it is imperative to identify mutations specific to the Turkish population to ensure accurate forensic identification. The aim of this study is to sequence the mtDNA coding region between base pairs 8389-8865 and identify any mutations specific to the Turkish population. This will provide valuable information for forensic scientists in population identification and object determination. By utilizing mtDNA polymorphism, forensic scientists can determine whether the Turkish population's mutation profile is favorable for forensic identification purposes. Conducting population studies in this area is essential for accurate forensic identification, and identifying mutations specific to the Turkish population is crucial in utilizing this region effectively.

MATERIAL AND METHOD

One hundred and fifty individuals aged over 18 years, who had no consanguineous relationship, and had given their informed consent, were randomly selected to participate in this study. Blood samples were collected from the participants and stored at -20°C until the analysis. The study was conducted with the approval of the Istanbul University Cerrahpasa Medical Faculty Clinical Research Ethics Committee (permission dated 02.06.2011, No. 19245), and all participants signed an informed consent.

The DNA isolation from the collected blood samples was performed using the Invitrogen Purelink Genomic DNA Mini Kit® (Thermo Fisher Scientific) which is based on silica extraction technology. DNA amounts of the obtained isolates were determined by fluorometric method with Qubit ™ dsDNA HS Assay Kit (Thermo Fisher Scientific). Tzen et al. (2001) method was modified and samples were multiplied using TT20173-F (5′-CCCCTCTAGAGCCCATAAAGC-3′) and TT29174-R (5′-GTGCATGAGTAGGTGGCCTGC-3′) primers in the first phase of PCR amplification. For the PCR reaction, 5.0 µL 1X PCR buffer, 2.0 µL 1.5 mM MgCl2, 0.2 µL 1U AmpliTaq Gold DNA polymerase (Applied Biosystems), 4.0 µL 0.125 mM dNTP mixture, and 1.0 µL 1 µM for each primer were used. DNA samples were diluted to 1-2 ng and added to the PCR mixture. It was completed with distilled water with a total volume of 50 µL. PCR was performed on 9700 (Applied Biosystems) device. PCR cycle; denaturation at 95° C was set to 32 cycles and 10 minutes at 72° C for 10 minutes denaturation, 1 minute at 94° C, 1 minute at 56 ° C, and 1 minute at 72° C. PCR products include primers and dNTPs that are not bound outside the target region. Escherichia coli Exonuclease I (Exo I) and Shrimp Alkaline Phosphatase (SAP-Shrimp Alkaline Phosphatase) were used to remove non-reactive dNTP and primers after PCR. For this process, 0.65 µL (1U/µL) SAP and 0.35 µL (5U/µL) Exo I were added on each 3.75 µL PCR product. The mixture was heated at 37° C for 90 minutes and at 80° C for 20 minutes.

BigDye Terminator v3.1 Cycle Sequencing Kit was used in the second phase of PCR amplification (Thermo Fisher Scientific). The second PCR mixture was prepared on a cold mold in order not to be affected by heat. For the PCR reaction, a mixture containing 4.0 µL BigDye Terminator v3.1 ready reaction mixture, 2.0 µL 5X sequencing buffer and 1.5 µL sequencing primer per sample was prepared. 1 µL of purified PCR product was added for the sample to this mixture. As a positive control, 1 µL -M13 control primer and pGEM®-3Zf (+) double chain control DNA and 1 µL distilled water were used as negative control. The total volume was completed with 20 µL of distilled water. PCR cycle; denaturation was set at room temperature for 10 seconds at 96° C, 5 seconds at 50° C, and 4 minutes at 60° C. Zymogen Sequencing Clean Up Kit was used to remove unbound fluorescently labeled ddNTPs in the environment for sequencing of the second PCR products (Zymo Research).

Electrophoresis of PCR products was performed on the ABI Prism 310 Genetic Analyzer (Applied Biosystems) using Data Collection Software 3.0. Data analysis was done with GeneScan Analysis Software 3.1.2 (Applied Biosystems), Seqscape, Finch TV, Sequencher 4.10.1. Polymorphic points were determined by comparing mtDNA profiles obtained from samples with Cambridge Reference sequence preloaded in SeqScape program.

RESULTS

To assess the repeatability of the method, five samples were randomly selected and re-analyzed by the same person at different times. The results showed that the same genotype was obtained each time, indicating high repeatability. To determine the polymorphic points, all samples were compared with the Cambridge Reference Sequence. The analysis revealed that the most common mutation detected in the samples was 8860a> G, (found in 88 individuals). Other mutations included 8697g> A (found in 19 individuals), 8847delC (found in 8 individuals), and 8691delA (found in 13 individuals). Additionally, 12 individuals had the same genotype as the Cambridge Reference Sequence. Table 1 presents the common mutation results and comparisons with German and two different populations of Taiwan for the Turkish population [4,6,7]. Haplotypes were grouped according to the polymorphisms detected in our study. People with the same genotype as the Cambridge Reference Sequence were not included in this grouping. Discrimination power and random match probabilities

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values were calculated.

Table 1. Comparison of mtDNA with German and Taiwanese population in terms of mutation rates

Position	Number of persons (n=150)	Turkish population (%)	German (%)	Taiwan (%)	Taiwan (%)
8860a>G	88	58.6	64	61.2	51.2
8697g>A	19	12.6	6.1	-	-
8691delA	13	8.6	-	-	-
8847delC	8	6	-	-	-
8679delA	6	4.34	-	-	-
8828delA	5	3.62	-	-	-
8481c>T	5	3.62	-	-	-
8815delC	5	3.62	-	-	-
8817delA	5	3.62	-	-	-
8691a>C	4	2.89	-	-	-
8701a>G	3	2.17	-	52.5	39.5
8354c>T	-	-	3.3	-	-
8448t>C	-	-	2.8	-	-
8473t>C	1	0.72	2.8	-	-
8584g>A	1	0.72	-	-	14.3
8414c>T	1	0.72	-	-	8.4

The most common haplotypes are 8860a> G (36 individuals), 8697g> A 8860a> G (7 individuals), 8847delC 8860a> G (3 individuals), 8691delA (2 individuals), 8512−8513insA (2 individuals) and 8697g> A (3 individuals). In our study, a total of 38 insertion and 95 deletion-type changes were seen, and the base changes resulting from transversion are shown in Table 2.

Table 2. Details of the mutations observed in mtDNA

Mutation Type Transition		Mutation Type Transition	
Transition	**Number**	**Transversion**	**Number**
t>C	13	a>C	16
c>T	27	c>A	9
g>A	29	a>T	7
a>G	100	t>A	13
TOTAL	169	g>T	0
		t>G	4
Mutation Type	**Number**	g>C	1
Insertion	38	c>G	7
Deletion	95	**TOTAL**	57

The following values were obtained as a result of random match probability, discrimination power and genetic diversity calculation.

Xi: Haplotype number, n: Number of persons

Xi: It was calculated by dividing the number of people seen by the total person.

Probability of random matches;$P=\Sigma (Xi^2)$

0.076359

Discrimination power;$DP=1-\Sigma (Xi^2)$

=0.923641

Genetic diversity;

$$h = \frac{(1 - \Sigma X^2)n}{(n-1)}$$

It was calculated as=0.9303827.

DISCUSSION

nDNA analysis is a routinely used forensic technique that helps resolve many cases. Alternative methods, such as mtDNA sequence analysis, are used when routine analysis methods fail. When using mtDNA sequence analysis, assessments are typically made based on the polymorphism results in the HVI and HVII regions of mtDNA, but these regions have limited discriminatory power. Eight polymorphisms in the mtDNA coding region (8281–8289d, 1736, 13263, 4883, 3594, 10873, 10400, and 12705) have been studied in other regions of the world using phylogenetic analysis of the HVI and HVII regions to identify strains of different origins [8]. This study aims to identify mutations in the Turkish population by performing sequence analysis of the mtDNA coding region between base pairs 8389-8865. We suggest that this polymorphic region can increase the discriminatory power of forensic identification studies of mtDNA in the Turkish population.

The selected region has little effect on the phenotype and has been excluded from the study if associated with disease, such as point myoclonic epilepsy with ragged-red fiber (A → G(8344)) , ataxia (G → A(8363)), neurogenic muscle weakness (T → G(8993)) and retinitis pigmentosa (T → C(8356)), due to ethical considerations in forensic science research [6, 9, 10, 11].

Andrews et al. re-analyzed the Cambridge Reference Sequence and compared it to the original sequence. They found that the a > G base change at position 8860 is a very common polymorphism worldwide [12, 13]. In our study, the most common mutation was the a > G change at position 8860, with a frequency of 58.6%. This mutation was found to be present in 64% of the German population, 61.2% in one of two different studies of the Taiwanese population and 51.2% in the other [4, 6, 7].

The 8697g> A mutation is the second most common mutation with a frequency of 12.6%. In the German population, this mutation has a frequency of 6.1%. Other common polymorphisms include 8691delA 8.6%, 8847delC 6%, 8679delA 4.34%, 8828delA 3.62%, 8481c> T 3.48%, 8815delC 3.62%, 8817delA 3.62%, 8691a> C 2.89%, 8701a> G 2.17%, and 8473t> C, 8584g> A, 8414c> T 0.72% each.

The 8701a> G mutation was found to have a frequency of 52.5% in one study in Taiwan and 39.5% in another. This ratio is significantly higher than the Turkish population (2.17%). Similarly, the frequency of the 8473t> C mutation in the German population was much higher than the Turkish population, with rate of 2.8%. The frequency of the 8584g> C, and 8414c> T mutations in the Taiwan population was much higher than the Turkish population, with rates of 14.3%, and 8.4%, respectively.

Furthermore, while the 8691delA, 8847delC, 8679delA, 8828delA,

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8481c> T, 8815delC, 8817delA, and 8691a> C mutations were observed in the Turkish population, they were not found in the German and Taiwanese populations. On the other hand, the 8354c>T and 8448t>mutations were found to have rates of 3.3% and 2.8%, respectively, in the German population, but were not observed in the Turkish and Taiwanese populations.

In summary, the frequency of mutations varies among different populations. Therefore, it is important to study the prevalence of genetic mutations in specific populations in order to better understand the genetic diversity of these populations.

In this study, a total of 169 transition type mutations, 57 transversion type mutations, 38 insertion type mutations, and 95 deletion type mutations were identified. Notably, no transition type mutations showing a g>T change were found in this study. These findings suggest that there is significant diversity in terms of mutation types present in the Turkish population.

While analyzing mtDNA, researchers have discovered instances of heteroplasmy. Although the current study did not detect any heteroplasmy, it's important to note that different tissues from the same individual may exhibit heteroplasmy when analyzed [14]. As such, it's crucial to take heteroplasmy into account when comparing two DNA samples for forensic identification purposes. However, evaluating DNA samples in cases of heteroplasmy can be challenging. Based on our analysis, the probability of two unrelated individuals in the Turkish population having the same genotype in the region we studied was calculated to be 0.076359. This low ratio suggests that the likelihood of match chance is low. However, the discrimination power of the region we examined alone is not sufficient for forensic purposes and has been calculated to be 0.923641. To increase the discrimination power, it is necessary to evaluate polymorphic regions such as HVI and HVII in the mtDNA, expanding the analysis to include 9021 bases unrelated to diseases. A population study in the United States showed that mtDNA examination increased the discrimination power for the US Caucasus and US Hispanic populations. To further improve discrimination power, we suggest examining regions coded in individuals with similar HVI and HVII regions, as well as exploring highly informative polymorphic clusters in published or unpublished sequence data [15]. In a recent population study conducted in the Liaoning region of China, 317 unrelated individuals were analyzed using mtDNA HVI, HVII, and coded regions. The study revealed that the Liaoning Han population is distantly related to the Tibet group but relatively close to the Miao group. The gene diversity (0.9997±0.0003), polymorphism information content (0.99668), and probability of random matching (0.00332) were also highly informative [16].

Other studies have shown that mtDNA analysis can yield successful results in both population studies and ancient bone samples. For instance, SNP points and control region HVI determined in the coding region, in combination with HVII regions, were used to analyze small samples from Joseon kingdom tombs in Korea dating back to the 1300s [17].

CONCLUSION

Our study and other research highlight the importance of mtDNA analysis in identifying similarities and differences between populations when nDNA is insufficient, such as in determining lineage in biological materials. In future studies of Turkish populations, increasing the sample size and collecting samples from different regions can yield even more valuable insights.

Conflict of interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

The authors declare that they have received no financial support for the study.

Ethical approval

The study was conducted with the approval of the Istanbul University Cerrahpasa Medical Faculty Clinical Research Ethics Committee (permission dated 02.06.2011, No. 19245), and all participants signed an informed consent.

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