

## Genetic identification with heteroplasmic variations in maternally related individuals in forensic cases

Ayşen TEZEL\*, Ömer ÇETİNKAYA, Burçak GÜZELTEPE, Nurgül KILIÇ

Gendarmerie Criminal Laboratories, Ankara, Turkey

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**Abstract:** Mitochondrial DNA (mtDNA), inherited from the mother, is used in a large variety of areas. In order to evaluate the connection between an incident and the victim or suspect in the analysis of criminal cases, DNA was obtained from buccal swabs sampled from 21 volunteers related through the mother's generation. Using Seqscape (v2.5.5) in an ABI 3100x1 capillary electrophoresis system, mtDNA sequence analyses were performed and compared to the revised Cambridge Reference Sequence. Individual mtDNA variations were detected. In three individuals, point heteroplasmy was observed. The results were evaluated according to the mtDNA typing guidelines established by the International Society for Forensic Genetics DNA Commission. This study can play an important role as a guide in cases of genomic DNA degradation or DNA absence, such as in disaster victim identification, where the genetic identification of an individual is necessary.

**Key words:** mtDNA, heteroplasmy, control region, variations, forensic

### 1. Introduction

Mitochondrial DNA (mtDNA) sequence analysis may be used as a tool for forensic identification (Salas et al., 2001). The identification of victims of mass disasters or murders, whose bodies have remained undiscovered for many years, is one of the most challenging cases in forensic science. mtDNA analysis can be used effectively in cases where the victims and their living descendants are separated by many generations.

Interindividual variation in human mtDNA is concentrated in the control region (D-loop region). Within the control region, there are two hypervariable regions, HV1 (16024–16365) and HV2 (73–340). These two regions are the primary targets for analysis in forensic identity testing (Melton, 2004). Most individuals have a single mtDNA control region sequence type. However, individuals occasionally carry two sequences that differ at one or two nucleotide positions, a situation referred to as heteroplasmy. These variations may differ among siblings or from mother to offspring (Lutz et al., 2000). Two types of heteroplasmy are known: point-based and length-based heteroplasmy. Point heteroplasmy (PH) occurs when a person has one nucleotide in some cells and another nucleotide in other cells at a particular base position. Length heteroplasmy (LH), most commonly seen in HV2, is represented by multiple populations of

mtDNA containing polycytosine stretches (C-stretches) of various lengths. Heteroplasmic point mutations clearly present a challenge for the accurate genetic identification of individuals (Melton, 2004). For example, the same PH in other identical sequences was used to increase the significance of a match when identifying members of the Romanov royal family (Gill et al., 1994; Ivanov et al., 1996; Coble et al., 2009; Rogaev et al., 2009). Heteroplasmy was observed in samples from the royal family, which contributed significantly to the identification of the parents. On the other hand, Irwin et al. (2009) analyzed over 5000 human samples from multiple populations worldwide with heteroplasmy in the human mtDNA control region. They showed that the frequency of heteroplasmy differs among particular populations, reflecting variable mutation rates among different mtDNA lineages and/or artifacts within particular population groups. Although different types of mtDNA can be found in tissue samples from the same individual, some individuals are heteroplasmic while others are not (Carracedo et al., 2000). There is only one copy of each nuclear chromosome in a single egg, but there are approximately 100,000 copies of the mtDNA genome (Chen et al., 1995).

Our aim in this study was to use mtDNA sequence analysis for determining the mother's generation that an individual belongs to in reference samples taken from

\* Correspondence: aysentezel@hotmail.com

close maternal relatives for use in criminal cases. In our study, samples from close maternal relatives that included varying sequences were analyzed and evaluated. Buccal cells from 21 volunteers were investigated. We also studied the correlation between nucleotide positions displaying heteroplasmy and mutational hot spots. The implications of these results for forensic studies are discussed.

## 2. Materials and methods

### 2.1. Sample preparation and pedigree construction

Buccal swab samples were taken from 21 maternally related volunteers. In order to form the basis in forensic cases, a male volunteer (1.4) was accepted as the proband due to being determined as G185A. DNA was obtained from the buccal swab of 10 male and 10 female healthy volunteers. The pedigree diagram is shown in Figure 1.

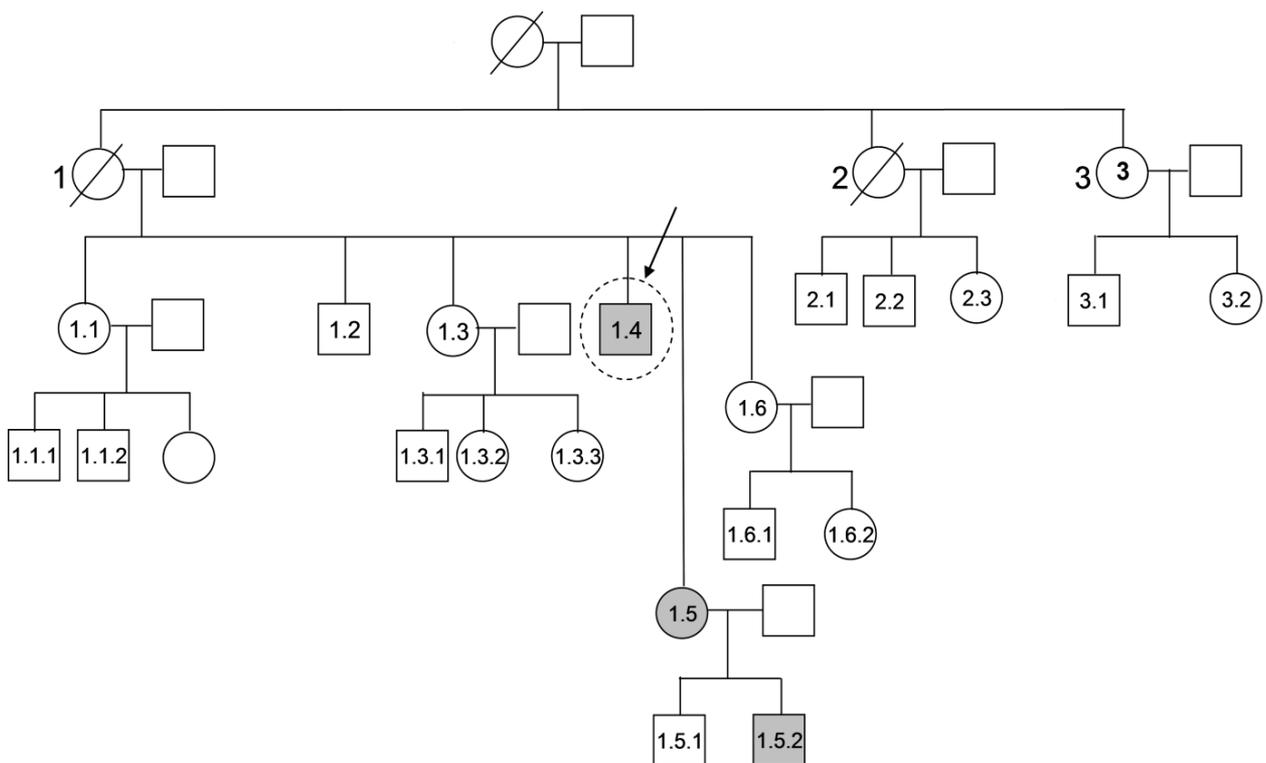
### 2.2. DNA isolation, amplification, and sequencing

The buccal swab samples were stored at 4 °C until DNA isolation. To minimize the possibility of contamination, all extractions were performed in a laminar flow cabinet on different days for each individual. Negative controls were used in all experiments. Pre- and postextraction products were examined and stored under separate laboratory conditions. DNA was extracted from buccal cells using the method described by Walsh et al. (1991). The DNA extracts were amplified using mitoSEQr RSA primers (Applied

Biosystems). The amplification products were cleaned with ExoSAP-IT (USB Corporation). The products were first detected by NuSieve agarose gel electrophoresis with ethidium bromide staining. Sequencing was conducted using a BigDye Terminator v.3.1 sequencing kit (Applied Biosystems). These products were purified using a DyeEx 2.0 Spin Kit (QIAGEN) and analyzed with fluorescence-based capillary electrophoresis, carried out using the ABI 3100xl capillary electrophoresis system with SeqScope (v.2.5) for product evaluation (Applied Biosystems). The sequencing results were confirmed with bidirectional sequencing.

## 3. Results

DNA obtained from 21 buccal swab samples taken from volunteers was subjected to mtDNA sequence analysis. Individual 1.4 (male) was accepted as the proband, and living volunteer relatives from the mother's generation were studied as shown in Figure 1. MtDNA sequence analyses of maternally related individuals revealed 73G, 228A, 263G, 295T, insC (315–316), 462T, 489C, 16069T, and 16126C variations in each individual. These variations were compared to the revised Cambridge Reference Sequence (rCRS) sequences (Andrews et al., 1999; Parson et al., 2007). Only a G185A point heteroplasmy was observed in 1.4 (male), 1.5 (female), and 1.5.2 (male).



**Figure 1.** Heteroplasmic variation among family members. Gray symbols indicate point heteroplasmic individuals (G185A).

Forward and reverse mtDNA sequences were evaluated for each individual. Point heteroplasmy was detected in 14.8% of individuals. Various mutations were found in 85.2% of the 21 samples. The point heteroplasmy of individual 1.4 was observed in buccal cells, as shown in Figure 2. A 295T variation observed in the mtDNA sequence of 1.6 (female) (sister of 1.4 (male)) is shown in Figure 3.

**4. Discussion**

Understanding mtDNA variations is extremely important in phylogeographic studies, studies that include populations, and medical and criminal genetics (Bodner et al., 2011). mtDNA sequence analysis is also a highly important tool for the genetic identification of individuals in criminal cases. Additionally, the study of mtDNA plays a crucial role in cases of degraded or very little nuclear DNA, such as in disaster victim identification.

In this study, the family members of the mother's lineage (10 females, 10 males) of an individual with point heteroplasmy (1.4, male) were studied in order to form a basis for analysis in criminal cases. A buccal swab sample was taken as the reference sample, since it is the tissue type with the least frequency of heteroplasmy (Bark et al., 1999). mtDNA sequence variations were observed among the 21 individuals (Table). PH was only detected in three samples, suggesting that there are different mtDNA variations in different tissues of an individual. It is also possible that this is due to the bottleneck theory (Bendall et al., 1996).

Since different mtDNA is observed when evaluating genetic identification studies, this difference should be considered when making exclusions. In these evaluations, the International Society for Forensic Genetics DNA commission criteria must be followed (Bär et al., 2000). PH can be used as a determinant for the identification method. In our study, G185A PH was observed in three individuals at position 185.

There have been studies conducted on hair samples, a tissue type in which a higher heteroplasmy level has been observed (Budowle et al., 2002). Heteroplasmy has been detected at different frequencies in hair, blood, and saliva samples in various

studies (Sullivan et al., 1996; Pfeiffer et al., 1999; Huhne et al., 1999). According to the bottleneck model, observation of mtDNA variants in different tissues of an individual, between species (Solignac et al., 1984; Laipis et al., 1988), and even between individual humans (Poulton, 1995; Bendall et al., 1996) is possible. If the heteroplasmic segregation that can form in human mtDNA lineages occurs in meiotic segregation, variation can take place in the next generation (Bendall et al., 1996).

For identification in criminal cases, when the sequence analysis in the control area of the mtDNA (HV1 and HV2) is evaluated, there can be mtDNA sequence variations between mother and child due to heteroplasmy. In this case, if possible, different tissue types should be examined (Asari et al., 2008). Hypervariable areas in human

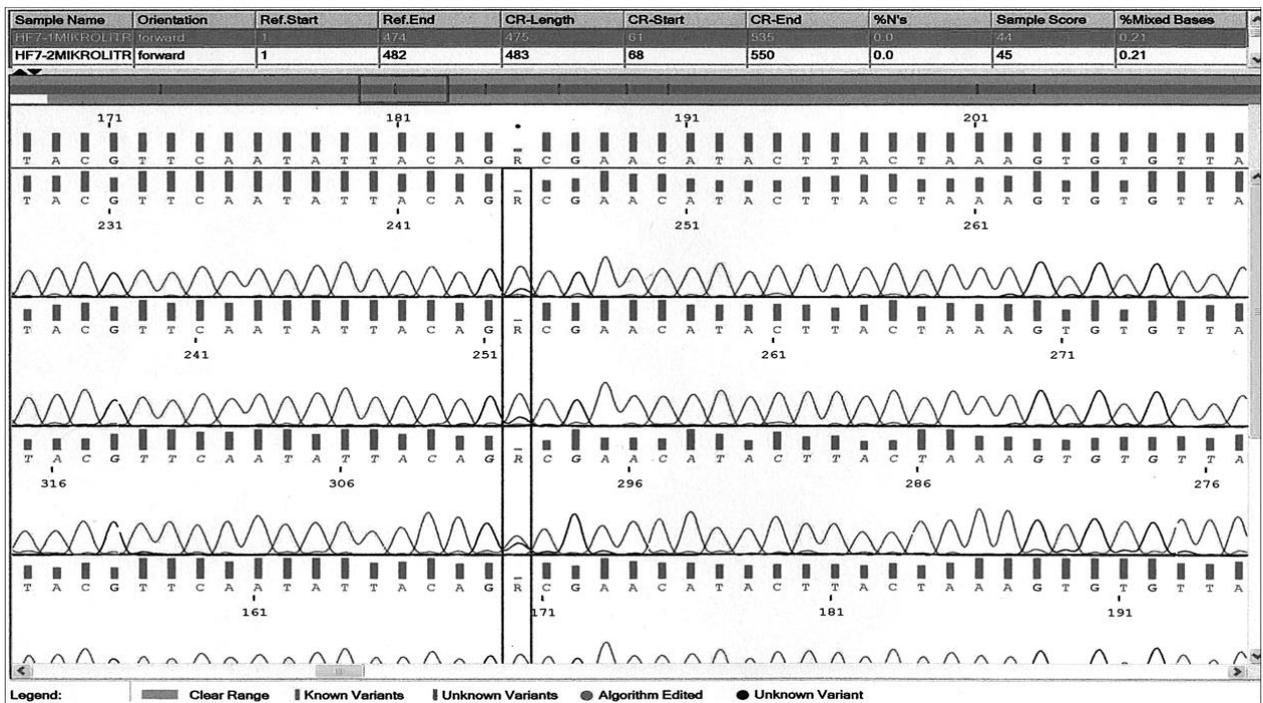


Figure 2. G185A point heteroplasmy in the buccal cells of individual 1.4.

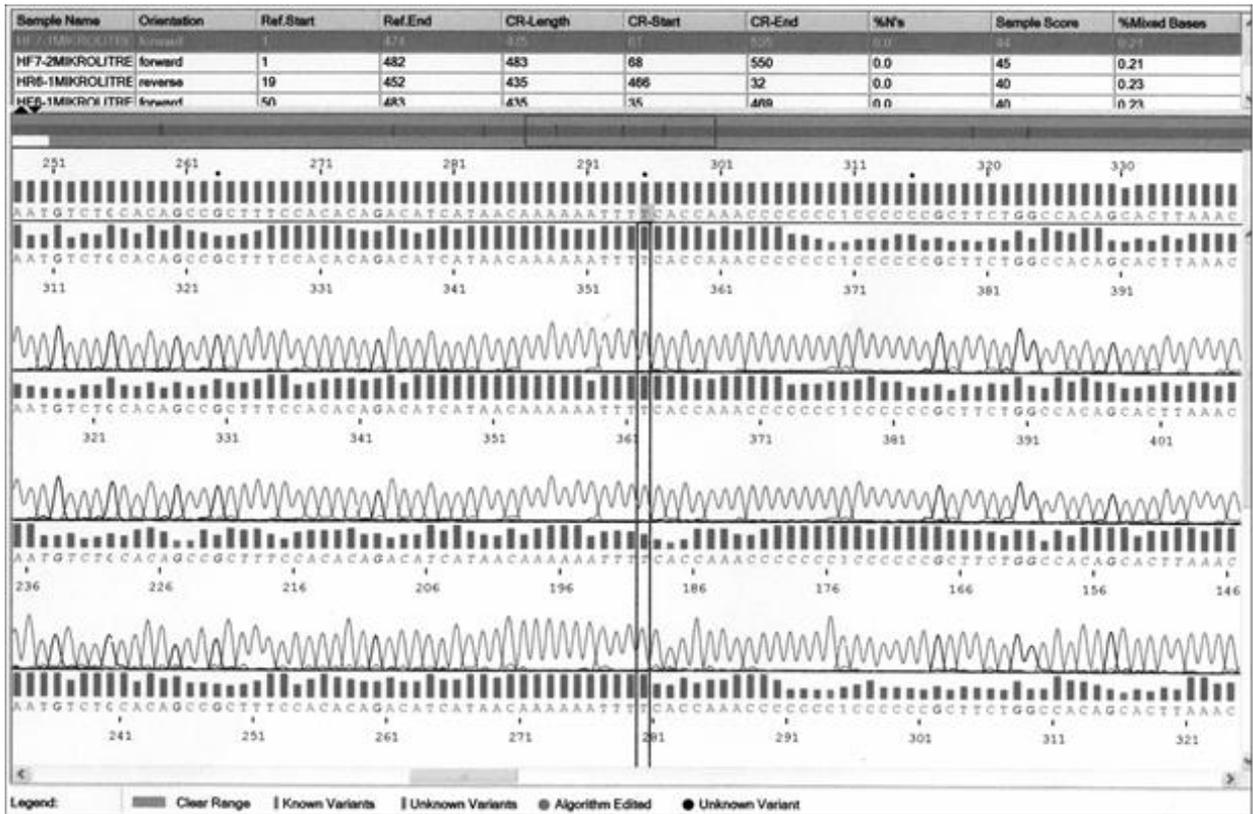


Figure 3. Variation at position 295 in the buccal cells of individual 1.6.

mtDNA (HV1, HV2, and HV3) are hot spots in terms of both somatic and germline mutations. These areas are considered to be open to new mutations (Stoneking, 2000). The variations detected in the mtDNA control area of the 21 individuals in this study are not considered to be

Table. Observed nucleotide variations between individuals and rCRS.

(Region) Base position	r(CRS)	Samples
(HV2) 73	A	G
(HV2) 185	G	A (PH)
(HV2) 228	G	A
(HV2) 263	A	G
(HV2) 295	C	T
(HV2) 315-316	C	insC
(HV2) 462	C	T
(HV2) 489	T	C
(HV1) 16069	C	T
(HV1) 16126	T	C

hot spot variations according to the studies of Stoneking (2000) and Irwin (2009). The detected mtDNA variations occur naturally; they are not related to age, sex, disease, or haplogroup origin (de Camargo, 2011; Goto et al., 2011).

In this study, it was shown that all the individuals from the mother's generation had the same mtDNA variations. Although PH was seen in 3 of the 21 individuals, all individuals carried the same maternal mtDNA sequence. Different tissue types should be examined when differences are found.

In conclusion, this study offers guidance for disaster victim identification and genetic identification by using the mtDNA sequence of the mother's generation when there is no genomic DNA, degraded DNA, or no DNA at all. Consequently, this study is useful for analysis in criminal cases.

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