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Evaluation of mtDNA stability across the maternal line: A study on three generations in two families

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ABSTRACT

Sometimes deficiency paternity testing can not be resolved with routine STR analysis and nuclear DNA fails to give conclusive results. In these cases, mitochondrial DNA (mtDNA) analyses have been proved more effective. In general, mtDNA transmission is stable across many generations; therefore the mtDNA typing allows to assess maternal relationships, even in deficiency cases. To evaluate the consistency of mtDNA, we compared HVI and HVII regions in three generations along the maternal line (great-grandmother, grandmother, mother, daughter or son) in two families, confirming the useful of this marker in such cases.

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1. Introduction

Mitochondrial DNA (mtDNA) has provided forensic scientists with a valuable tool for determining the source of DNA recovered from damaged, degraded or very small biological samples. MtDNA is a small circular genome located in the mitochondria, which are located outside of cell nucleus. Most human cells contain hundreds of copies of mtDNA genomes, as opposed to two copies of the DNA that is located in the nucleus. This high copy number increases the likelihood of recovering sufficient DNA from compromised DNA samples, and for this reason, mtDNA can play an important role in missing persons investigations, mass disasters and other forensic investigations involving samples with limited biological material. Additionally, mtDNA is maternally inherited, then the mtDNA hypervariable regions (HV) are well suited for forensic identification using a maternal relative as reference sample [1], even if the unknown and reference sample are separated by many generations. If a kinship test is performed and the result is not conclusive, mtDNA testing can be considered as a supplemental test to verify if individuals are linked on their maternal line. All individuals (both male and female) who have descended from the same maternal lineage will have the same mtDNA profile. In general, mtDNA transmission is stable across many generations; therefore the mtDNA typing allows to assess maternal relationships, even in deficiency cases. In order to evaluate the consistency of mtDNA we investigated uniparental mtDNA inheritance in three generations along the maternal line (great-grandmother, grandmother, mother, daughter or son, Fig. 1) in two families.

2. Materials and methods

The study was conducted on DNA samples of two families (indicated as "A" and "B") across the maternal line, covering three generations. DNA was extracted from oral swabs using Chelex® 100 procedure (BIORAD, Richmond, CA, USA). The two family trees were first confirmed by genomic STRs analyses using AmpFlSTR® Identifiler® Plus (Applied Biosystems, Foster City, CA, USA) and the probability of paternity was calculated using the software Familias v.3.1.8 [2]. Beside, all samples were amplified by multiplex PCR-based direct sequencing, using primers reported in literature [3,4]. The purified samples were directly placed on Applied Biosystems 3500 Genetic Analyzer (Applied Biosystems). All sequences were aligned using the Sequencing Analysis v6.0 software (Applied Biosystems) and compared to the revised Cambridge Reference Sequence (rCRS).

3. Results

The members of the two-family trees were typed (Fig. 1). Using software Familias v.3.1.8, the probability of relatedness was between 98,17% (relation 2–7) and 74,29% (relation 1–7) for family A and between 99,53% (relation 1–4) and 80,73% (relation 1–5) for

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Table 1
Deviations from rCRS identified for each individual.

Individuals from family A	HVI	HVII
1	T16093C, T16126C, A16163G, C16186T, T16189C, C16294T	A73G, A263G, 309.1C, 315.1C
2	T16093C, T16126C, A16163G, C16186T, T16189C, C16294T	A73G, A263G, 309.1C, 315.1C
3	T16093C, T16126C, A16163G, C16186T, T16189C, C16294T	A73G, A263G, 309.1C, 315.1C
4	T16093C, T16126C, A16163G, C16186T, T16189C, C16294T	A73G, A263G, 309.1C, 315.1C
5	T16093C, T16126C, A16163G, C16186T, T16189C, C16294T	A73G, A263G, 309.1C, 315.1C
6	T16093C, T16126C, A16163G, C16186T, T16189C, C16294T	A73G, A263G, 309.1C, 315.1C
7	T16093C, T16126C, A16163G, C16186T, T16189C, C16294T	A73G, A263G, 309.1C, 315.1C
Individuals from family B	HVI	HVII
1	T16224C, T16311C, T16362C	A73G, A263G, 315.1C
2	T16224C, T16311C, T16362C	A73G, A263G, 315.1C
3	T16224C, T16311C, T16362C	A73G, A263G, 315.1C
4	T16224C, T16311C, T16362C	A73G, A263G, 315.1C
5	T16224C, T16311C, T16362C	A73G, A263G, 315.1C
6	T16224C, T16311C, T16362C	A73G, A263G, 315.1C

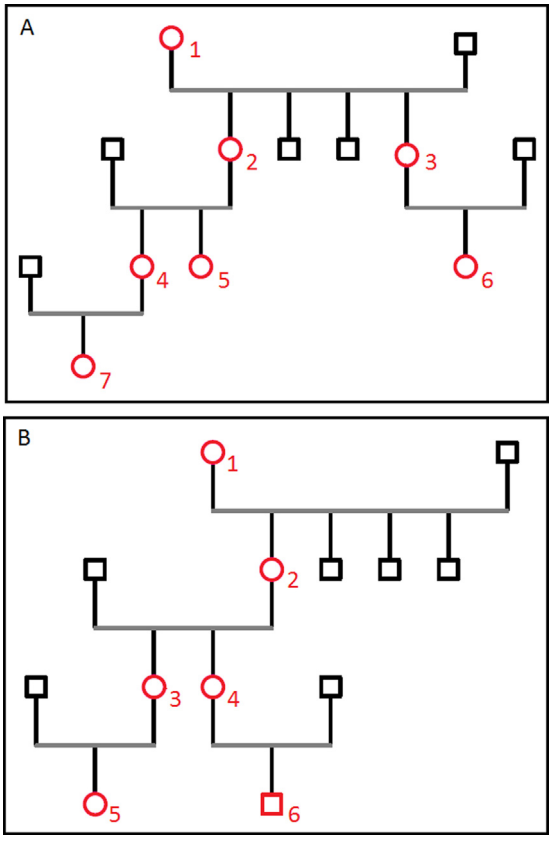

Fig. 1. The two family trees (A and B). In red the typed individuals, covering three generations along the maternal line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

family B (Fig. 1). The complete sequence of HVI and HVII regions were determined; both strands were analysed and compared to ensure the results. All sequences were compared to the revised Cambridge Reference Sequence (rCRS). As shown in Table 1, all the individuals belonging to the same family showed the same haplotype. In family A, deviations from rCRS were found in the HVI region at positions 16093 (T → C), 16126 (T → C), 16163 (A → G), 16186 (C → T), 16189 (T → C) and 16294 (C → T). In HVII region the deviations at positions 73 (A → G), 263 (A → G) and two cytosine insertions (309.1C and 315.1C) were also found. Family B showed variations at position 73 (A → G), 263 (A → G), an insertion 315.1C, 16224 (T → C), 16311 (T → C) and 16362 (T → C).

4. Discussion and conclusions

The obtained data confirm that sometimes in deficiency paternity testing STR analysis fails to give conclusive results. In these cases, mitochondrial DNA analyses have been proved more effective. The feature of maternal inheritance can be useful to support or refute the identity of samples by comparison with reference samples from known maternal relatives. Although considering the small sample size (only two families investigated), our results suggest that three-generations maternal relatives share identical mtDNA sequences, without neomutation from one generation to another. These data confirm the importance and the utility of mtDNA, allowing comparison of family members who share a common matrilinear ancestry and providing the basis for identification and maternal relationship both in biological evidence and in living individuals.

Conflict of interest

None.

Role of funding

None.

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