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# Occurrence of heteroplasmy in related individuals

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**Abstract**

The aim of this study was to analyse the mtDNA sequences of the two hypervariable segments HV1 and HV2 from close maternal relatives, i.e., mother–child and sibling–sibling, by direct sequencing in C.E.
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*Keywords:* mtDNA; Heteroplasmy; Diseases

## 1. Introduction

Mitochondrial DNA is only transmitted by the mother and is not subject to the mechanism of recombination [1]: mutation is thus the only possibility of genetic diversity. It is characterized by a high rate of mutations [2]: when a mutation arises, cells initially contain a mixture of wild-type and mutant mtDNAs, a condition known as heteroplasmy [3]. The percentage of mtDNA mutants may differ within the same maternal lineage; in addition, heteroplasmy may segregate to homoplasmy in a single generation [4] or a new mutation may occur and be fixed within a single transmission [5]. Knowledge of the significance of the condition of heteroplasmy is of crucial importance for forensic applications, since heteroplasmic nucleotide substitutions may be found in biological material belonging to the same individual and inside maternal lineages, affecting the correct comparison of evidence and the reconstruction of relationships. With regard to this phenomenon, studies carried out until now have shown its significantly different frequency, to the extent that further investigations must be carried out.

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## 2. Materials and methods

DNA from the fresh whole blood of 25 mother–child pairs, regarding cases of questioned paternity coming to our laboratory, and of 25 patients with hematological neoplastic pathologies and subjected to bone marrow transplant, and of their sibling donors, was extracted in 5% Chelex according to standard procedures [6]. The HV1 region was amplified and sequenced under the conditions suggested by Parson et al. [7]. For the HV2 region, the procedure of Holland et al. [8] was followed, with minor modifications. Sequencing products were analysed on an ABI Prism 310 (Applied Biosystems). In order to exclude contamination, errors in the procedures of extraction, amplification or sequencing, or errors of incorporation of Taq polymerase, not only were both strands sequenced but heteroplasmic samples were extracted twice, using different blood aliquots.

## 3. Results and discussion

Heteroplasmy is the condition where two or more mtDNA haplotypes, differing by sequence or length, are present in the same individual.

Six cases of heteroplasmy were observed in this study one in the mother–child pairs and the remaining five in three sibling pairs (Table 1). In each of the latter, one individual

Table 1
Sequencing data of mother, child and sibling pairs (1,2,3,4) revealing cases of heteroplasmy

| | | HV1 | | | | | HV2 | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | | | | | | | | | | |
| | | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | | |
| | | 0 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 5 | 5 | 9 | 0 | 0 | 2 | 6 | 0 | 0 | 1 | | |
| | | 9 | 1 | 8 | 8 | 0 | 2 | 3 | 4 | 7 | 9 | 0 | 0 | 0 | 2 | 4 | 5 | 9 | | 6 | 2 | 3 | 5 | 0 | 4 | 5 | 3 | 9 | 9 | 5 | | |
| | | 2 | 1 | 3 | 9 | 9 | 3 | 4 | 2 | 8 | 4 | 1 | 4 | 9 | 4 | 4 | 6 | 0 | | | | | | | | | | .1 | .2 | .1 | | |
| Anderson | | T | C | A | T | T | C | C | C | C | C | C | T | A | T | C | T | G | A | T | T | A | T | A | T | G | A | I | I | I | | |
| Mother | | | | C | C | | | T | | | | | | | C | | | | G | | | | C | | | | G | | | C | | |
| Child | | | | C | C | | | T | | | | | | | C | | | | G | | | | C | | T/C | | G | | | C | | |
| 1 DON | | T/C | | | | | T | | | T | T | | | G | | | | A | G | C | C | | C | G | | | G | | | C | | |
| 1 REC | Th.M. | C/T | | | | | T | | | T | T | | | G | | | | A | G | C | C | | C | G | | | G | | | C | | |
| 2 DON | | | | | C | | | | | | | | | | | | C | | | | | | | | | | G | C | C | C | | |
| 2 REC | Th.M. | | | | C | | | | | | | | | | | | C | | | | | | | | | | G | C | | C | | |
| 3 DON | | | A/G | | | C/T | | | | | | C | | | | | | | | | | | | | | | G | C | | C | | |
| 3 REC | LNHL | | G/A | | | C/T | | | | | | C | | | | | | | | | | | | | | | G | | | C | | |
| 4 DON | | | | C | C | T/C | T | | T | T | | | | | T | | | G | | | G | C | G | | | A | G | C | | C | | |
| 4 REC | ANLL | | | C | C | | T | | T | T | | T | | | T | | | G | | | G | C | G | | | A | G | C | | C | | |

DON = donor. REC = recipient. Th.M. = Thalassemia major. LNHL = lymphoblastic non-Hodgkin lymphoma. ANLL = acute non-lymphatic leukemia.

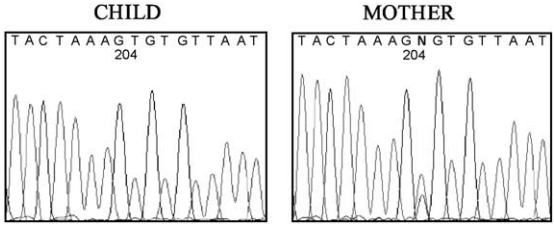


Fig. 1. Electropherograms of a mother–child pair showing sequence heteroplasmy in child's sample at position 204 in HV2 region.

(recipient) was affected by some kind of hematological neoplastic pathology (Table 1); the other subject was the brother who donated bone marrow. One heteroplasmic substitution was found in only one member of a mother–child pair at nt 204 for the HV2 region (Fig. 1). The five distinct examples of heteroplasmy identified in the analysis of sibling pairs involved one length and four sequence heteroplasmy conditions. Length polymorphism occurred in the HV2 C-stretch and the sequences differed in the number of cytosines between nt 302 and nt 310: in sample 3DON there were two different haplotypes, one of them with a C-insertion in position 309.1, absent in sibling with neoplastic pathology. One case of sequence heteroplasmy concerned a sibling pair (no. 1), and occurred at nt 16092 for the HV1 region: overlapping of one T and one C was found in both subjects, although in different proportions (Fig. 2). In another sibling pair (no. 3), three separate cases of heteroplasmy were observed: one length heteroplasmy for the HV2 region, as mentioned above, and two sequence heteroplasmies for the HV1 region at nt 16111, in which one A and one G were observed, and at nt 16209, with overlaps of one T and one C, affecting both subjects (Fig. 3). Sample 4DON showed overlapping of one T and one C at position 16209, not found in the recipient brother. This subject, instead, showed a homoplasmic transition C → T at nt 16301, not present in the donor. Lastly, sample 2DON showed a C-insertion at nt 309.2 absent in the recipient sibling.

In conclusion, we may state that this study revealed a higher percentage of heteroplasmy in analysis of blood samples from individuals affected by hematological neoplastic pathologies and in their healthy sibling donors, with respect to blood samples from mother–child pairs. In addition, we noted that the heteroplasmies observed concerned not

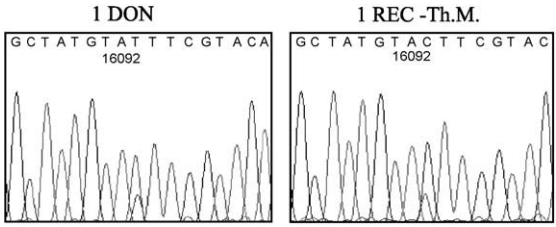


Fig. 2. Example of sequence heteroplasmy at position 16092 in HV1 region in a sibling pair (DON = donor; REC = recipient).

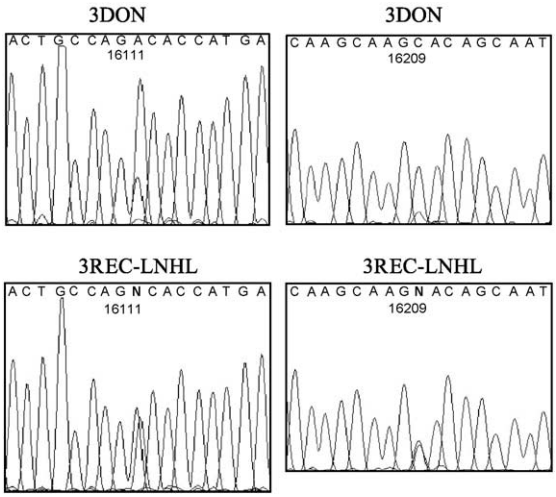


Fig. 3. Electropherograms showing two examples of sequence heteroplasmy in nucleotide positions 16111 and 16209 in HV1 region in a sibling pair (DON = donor; REC = recipient).

only the subject of the sibling pair affected by neoplastic pathology, but also the healthy donor sibling. We believe this finding requires further study, and we intend to increase the number of observations.

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