HUMAN MITOCHNODRIAL DNA HETEROPLASMIC VARIATION AMONG THIRTEEN MATERNALLY RELATED FAMILY MEMBERS

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The advantages of using mitochondrial DNA (mtDNA) for human identification are: 1. The many polymorphisms in the control (non-coding) region can be used to distinguish between non-maternally related individuals; 2. The mtDNA of maternally related individuals can be used to verify the identifications; 3. The large numbers of mitochondria/cell permit mtDNA extraction from minute or degraded samples when there is insufficient nuclear DNA. One disadvantage of using mtDNA for human identification is the possibility that a heteroplasmy (different base pairs at the same mtDNA site) may exist. At this time, it is not clear whether these hetroplasmies exist in the mtDNA in individual mitochondria, in different mitochondria in the same cell, or in mitochondria from different cells within the same tissue. The presence of one of the heteroplasmic base pairs in one tissue sample and the other heteroplasmic base pair in the second sample can result in an exclusion rather than a match. Although mitochondria are known to be maternally inherited, it is not known how individual mitochondria are passed between generations. To examine this issue, the inheritance of a mtDNA heteroplasmic site (position 16291 in the HV1 region) was examined in thirteen maternally related family members across four generations.

The family consisted of a female (generation 1E) who had three daughters (generation 2 E A, B, and D) and two sons (generation 2Γ E and F). The third generation consisted of a son (3Γ A) and a daughter (3E) from 2EA, a daughter (3EB) from 2EB, two sons (3ΓD and 3ΓD') and a daughter (3ED) from 2ED. The fourth generation consisted of one daughter (4EB) from 3EB. DNA was extracted from buccal swabs from each member of the family. In addition, DNA was extracted from hair (roots and shafts of 24 hairs, five nails and three blood samples from 2EA. Sequencing was performed with the ABI™ PRISM® 310 Genetic Analyzer using the BigDyeTerminator™and the BigDyePrimer sequencing kits.

The Anderson sequence (1) indicates that a cytosine (C) exists at position 16291. In generation 1E, 82% of the mtDNA at this site was C and 18% was T. In generation 2, four out of the five siblings had 85% to 91% C, however, one sister (2EA) had approximately 45% C and 55% T. The third generation children of 2EA increase the proportion of T to 93 and 99%. Increasing proportions of T were also noted in the third and fourth generations from 2EB (T increased from 13% to 25% to 31%). However, the trend toward more T in succeeding generations was not observed in 2ED who had 9% T (down from 18% T in 1E) and whose three children had 4%, 12%, and 16%.

Comparisons to the proportions of the C: T heteroplasmy in the buccal swab and different hairs (both roots and shafts), nails, and bloodstains from a single individual (2EA) indicated wide variation. The buccal swab was 45% C: 55% T, the hair ranged from 10% T to 100% T, the three bloodstains were all about 65% T, and the five nails ranged from about 55% to 72% T. In four out of the 24 hairs, the proportion of T in the root was significantly different from that in the shaft: whereas in the rest of the hairs the proportions were about the same. In some cases, the root had more T, and in others, the shaft had more T. In two hairs (both roots and shafts), an additional polymorphism was noted: 1. Hair #2 has a C instead of a T at position 16357, and 2. In hair #21, there was a C insertion at position 16188.1.

These results indicate that in a single individual, the proportions of the base pairs contributing to a heteroplasmy can vary widely. In addition, although mtDNA and any inherent heteroplasmies are known to pass from mother to child, the proportions of the base pairs in those heteroplasmies can show wide variations between siblings and generations.

Anderson, S., *et al.* (1981). Nature 290:457-465