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Mitochondrial DNA polymorphism in 103 unrelated Caucasian Danes

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Abstract. We describe a high-quality database containing data from the hypervariable regions HV1 and HV2 on the mitochondrial control region of 103 unrelated Caucasian Danes. Each sample was amplified in duplicates and sequenced twice in both the forward and reverse direction with primer and terminator chemistry, respectively. The data were analysed with the quality assessment program Phred. A total of 94 unique sequences were observed. Most of the observed mutations were T to C or C to T transitions compared to the Cambridge Reference Sequence (CRS).

Keywords: DNA sequencing; Typing; MtDNA; Forensic genetics; Database

1. Introduction

Sequencing of the hypervariable regions HV1 and HV2 of mitochondrial DNA is commonly used in forensic casework. The statistical interpretation of the results depends on the population frequency of a particular haplotype in the population of interest. Thus, it is important to establish a database representing the different haplotypes present in the given population. Although sequencing of the mitochondrial control region has been performed routinely for years, recent reports indicate that some databases used also in the forensic genetics setting may include errors [1]. Recognising the problems reported concerning the validity of population data, we have established a high-quality database consisting of 103 Danes of Caucasian origin.

2. Materials and methods

Blood samples from 103 unrelated Caucasian Danes were each divided in two subsamples. DNA was extracted from each of the subsamples using either Chelex 100 or phenol/chloroform. HV1 and HV2 were amplified separately in two steps for each of the subsamples. In the first amplification, the primers L-15997/H-16401 and L-48/H-408 were used. In the second amplification, the primers L-15997(-21)-M13/H-16401-M13Rev and L-48(-21)-M13/H-408-M13Rev were used [2]. The first sample was sequenced twice

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(forward and reverse) with the BigDye Primer chemistry, and the second sample was sequenced twice (forward and reverse) with the BigDyeTerminator Cycle Sequencing chemistry. Electrophoresis of the extension products was performed on either ABI 377 or ABI 310 (Applied Biosystems). If length heteroplasmy was detected, the sample was further sequenced as decribed by Rasmussen et al. [2]. Sequences were compared to the Cambridge Reference Sequence (CRS) [3] using the Sequencher (GeneCodes) software, and a quality score was obtained with the Phred (CodonCode) software. Base calls with Phred values above 20 were considered as being of sufficient quality. The final assignment at each base position was done when concordant results were obtained from both forward *and* reverse sequencing with one of the subsamples and forward *or* reverse with the other subsample, i.e., with at least three different combinations of the two chemistries and the two sequencing directions. This threefold consensus strategy combined with the use of Phred quality scores for each base call ensured a high quality of the base positions included in the database.

3. Results and discussion

The sequence data showed 94 different haplotypes in the 103 samples examined. One haplotype was present three times, and eight haplotypes were present twice. Each of the remaining haplotypes was observed only once. When comparing each of the sequences in the database to CRS [3], 73 mutations were observed in HV1 and 37 mutations were observed in HV2. The mutations were represented with a high number of transitions, but also transversions, deletions or insertions were present. Nineteen samples showed length heteroplasmy with poly C in positions 16,184–16,193 in HV1, in positions 303–315 in HV2 or in both. Table 1 presents an overview of the different types of mutations found.

Table 1
Sequence polymorphism in the hypervariable regions HV1 and HV2 of mtDNA of 103 unrelated Danish Caucasian individuals

	HV1	HV2
Transitions		
T–C	111	85
C–T	112	31
A–G	19	184
G–A	19	17
Transversions		
C–A	4	1
G–C	2	–
A–C	5	–
T–G	1	–
G–T	–	1
Insertions		
+2A	–	1
+1T	–	3
+1C	–	154
+2C	1	6
Deletions	–	
−1A	2	–

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