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Heteroplasmic substitutions in the mitochondrial DNA control region in mother and child samples

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Abstract The sequences of the two hypervariable regions of the mitochondrial DNA control region (HV1 and HV2) from close maternal relatives (mother-child pairs) were compared to determine the frequency of mutations between two generations. A total of 68 blood samples were sequenced only in HV1 and 86 were analysed for HV1 and HV2. The intergenerational comparison led to the identification of six heteroplasmic point mutations affecting the children only. In one case a heteroplasmy of the maternal sequence was resolved to homoplasmy in the corresponding sequence of the child.

Key words Mitochondrial DNA · Hypervariable region Heteroplasmic point mutation · Length heteroplasmy

Introduction

With two non-coding hypervariable segments HV1 and HV2, the control region (CR) is the most variable region of the human mitochondrial DNA (mtDNA) (Anderson et al. 1981). MtDNA shows a maternal mode of inheritance and a rapid rate of sequence evolution. Knowledge on the frequencies of new mutations in maternal lineages is of central importance for the application of mtDNA in forensic identity investigations (Stoneking et al. 1991; Piercy et al. 1993).

Heteroplasmy (two or more populations of mtDNA in a single individual) in the control region is expected to be more widespread than has been reported (Gill et al. 1994; Comas et al. 1995; Bendall et al. 1996). For example, a single heteroplasmic polymorphism in the control region was found in the remains of Tsar Nicholas II (Gill et al. 1994). Furthermore, heteroplasmy has been described in association with several diseases (Wallace 1992; Marchington et al. 1996).

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Several studies have estimated the substitution rates for mitochondrial DNA in maternal relatives (Bendall et al. 1996; Howell et al. 1996; Parsons et al. 1997). Recently the mutation rate is under considerable debate (Macaulay et al. 1997) since Parsons et al. (1997) reported a roughly 20 times higher mtDNA mutation rate in contrast to previous phylogenetic studies (Vigilant et al. 1991).

The aim of this study was to analyse the mtDNA sequences of the two CR hypervariable segments from close maternal relatives i.e. mother and child to determine intergenerational differences between mother and offspring.

Material and methods

Blood samples from 154 German Caucasians were obtained from routine paternity cases and DNA was extracted using the QIAamp Blood Kit (Qiagen) following the manufacturers instructions.

Amplification and sequencing

Amplification of the HV1 and 2 regions and subsequent cycle sequencing using ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq FS DNA polymerase were performed as described previously (Pfeiffer et al. 1998). In HV1 the positions 16024–16365 and in HV2 the positions 73–340 were analysed and compared to the reference sequence (Anderson et al. 1981).

In order to rule out artefacts, amplification and sequencing were carried out in both directions and those mother-child pairs showing heteroplasmic substitutions and length heteroplasmy were repeated.

Characterization of heteroplasmy

A nucleotide position was considered heteroplasmic if a secondary peak of more than about 40% was present, which was confirmed in the reverse sequencing reaction and which was specific to either the mother or the child.

The proportions of two nucleotides at a given heteroplasmic position were determined by gravimetric integration of the peaks printed in the sequence hardcopy. Firstly, an internal reference peak was chosen which preceded the heteroplasmic position. Secondly, the "usual" ratio of the reference peak to the position in question was determined (by weighing) in 5–10 different non-heteroplasmic sequences. The error was incidentally found to be less than 5%.

Table 1 Sequences of seven mother and child samples in HV1 and HV2 (a letter indicates a substitution relative to the reference sequence, – no base in the reference sequence, * only HV1 was performed, T/C heteroplasmy). The exact number of the insertions in the poly-C tract is unclear, therefore at positions 16193.1 and 16193.2 the maximal number of C-insertions is given

Baseposition			HV1																	HV2						
			16069	16093	16126	16129	16145	16189	16192	16193.1	16193.2	16205	16231	16256	26261	16270	16304	16309	16316	73	146	263	309.1	309.2	310	315.1
Reference sequence			C	T	T	G	G	T	C	–	–	C	T	C	C	C	T	A	A	A	T	A	–	–	T	–
1	# 1.14	mother																			C	G	C			C
	# 1.13	child						T/C													C	G	C			C
2	# 1.50	mother		C				C		C	C											G	C			C
	# 1.49	child		C				C		C	C											G	C		T/C	C
3	# 1.56	mother															C					G	C			C
	# 1.55	child															C					G	C		T/C	C
4	# 5.14	mother							T					T		T				G		G	C	C	T/C	C
	# 5.13	child							T					T		T				G		G	C			C
5	# 5.20	mother																				G				C
	# 5.19	child																A/G				G				C
6*	# 80.45	mother	T		C		A						C		T											
	# 80.46	child	T		C		A					C/T	C		T											
7*	# 83.34	mother		C		A													G							
	# 83.35	child		C		A		T/C		C									G							

Thirdly, the heteroplasmic peak was related to the reference peak to determine how much it was diminished.

Results and discussion

The complete sequence of the 342bp segment in HV1 was determined for 154 blood samples (77 lineages). A 268bp fragment in HV2 was analysed in 86 of these samples (43 lineages). New mutations between mother and child samples in the form of homoplasmic substitutions were not observed.

Differences in the sequences between mother and corresponding child samples occurred in seven cases in the form of heteroplasmic substitutions. In one of these cases the heteroplasmy of the maternal sequence was resolved to homoplasmy in the child. The heteroplasmic sites were mostly pyrimidine transitions (Table 1). The heteroplasmic proportions of both nucleotides are shown in Table 2.

Table 2 Proportions of both nucleotides in the seven observed heteroplasmic samples in %

			Baseposition	Heteroplasmic substitutions	Proportions of both nucleotides in %
1	# 1.13	child	16189	T/C	72/28
2	# 1.49	child	310	T/C	80/20
3	# 1.55	child	310	T/C	60/40
4	# 5.14	mother	310	T/C	40/60
5	# 5.19	child	16309	A/G	80/20
6	# 80.46	child	16205	C/T	82/18
7	# 83.35	child	16189	T/C	43/57

A heteroplasmic point mutation was found in HV1 in four children compared to the mother (Table 1; Fig. 1a, b). One of these sequences (sample 1) revealed heteroplasmy for T and C at position 16189, a site with a high substitution rate (Bendall and Sykes 1995). In another child sample (sample 7) this heteroplasmic mutation was additionally accompanied by a heteroplasmic length variation caused by at least one cytosin-insertion (16193.1) in the polycytosine C-tract which produced a characteristic unclear sequence in the nucleotides following this tract. In both cases the corresponding mother samples were homoplasmic for T at position 16189 with no C-insertion. Length heteroplasmy associated with a substitution at position 16189 in the HV1 C-stretch region has been described by Bendall and Sykes (1995) and is expected to be a common variant in population screening. In our investigation, this polymorphism was observed in 15% of all mother-child pairs.

In HV2 three heteroplasmic point mutations were found at position 310 consisting of T and C. This heteroplasmy is rather common (Marchington et al. 1997) and occurred in 16% in our database of 100 unrelated individuals. In sample 4 the sequence of the mother showed heteroplasmy for T and C at position 310 and at least two C-insertions (309.1, 309.2) in the HV2 polycytosine stretch (Fig. 1c). Similar to insertions affecting the HV1 C-tract, this sequence alteration also results in a length heteroplasmy and

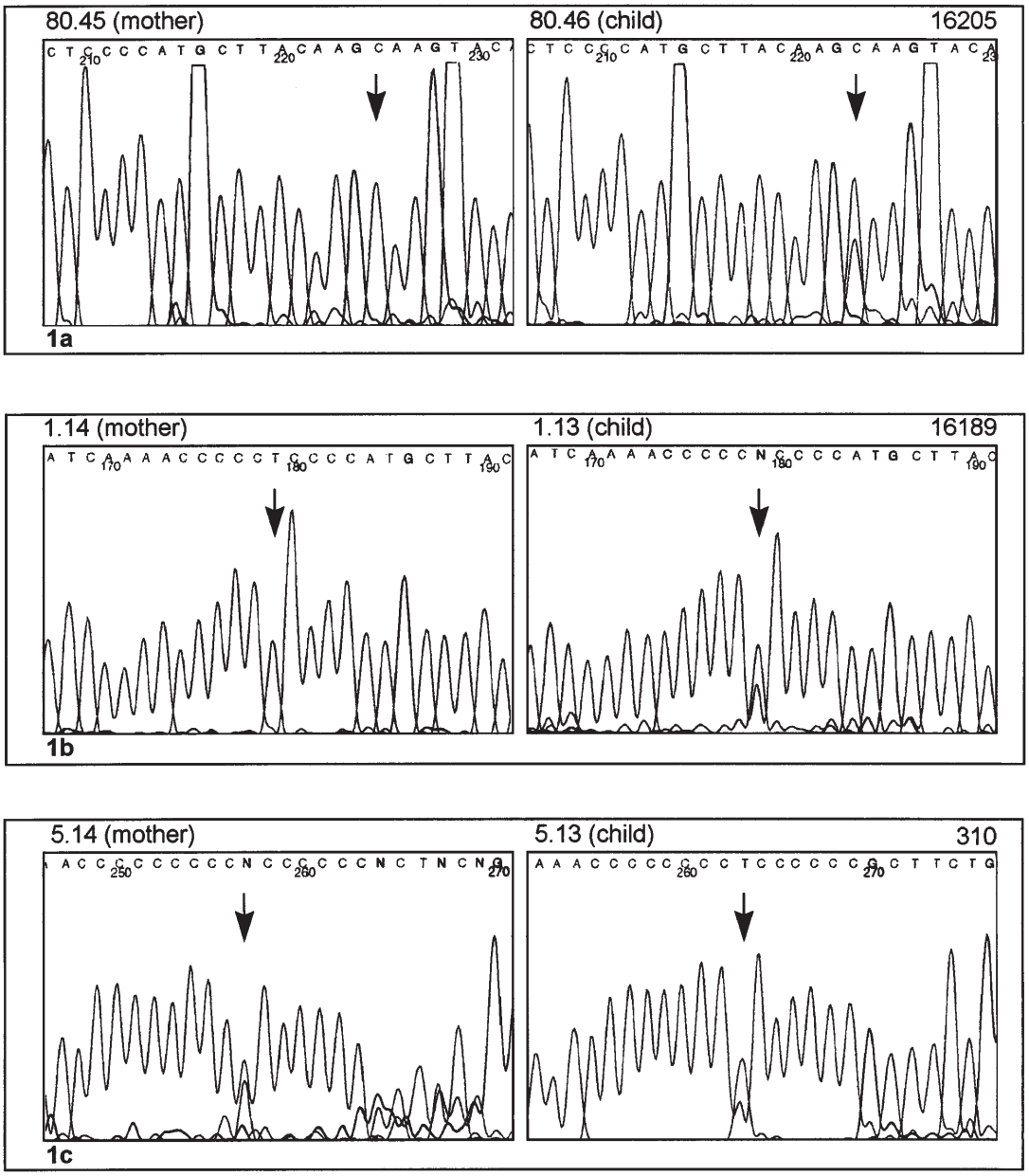

Fig. 1 a–c Electropherograms of three mother-child pairs showing heteroplasmic substitutions in two children samples (1 a, 1 b) and one mother sample (1 c). The base positions are diplayed in the upper right corner and indicated by arrows

blurring of bands after this tract as recently described by Parsons et al. (1997). In contrast, the corresponding child sequence showed a difference comprising only one cytosin-insertion and homoplasmy for T at position 310. The heteroplasmy of the mothers sequence resolved to homoplasmy in the child, a phenomenon previously described by Ivanov et al. (1996) and Parsons et al. (1997).

Child 80.46 appears to have a significant T-Peak underlying the maternal C-peak (Fig. 1). However, quantification showed that it represented only 18%. This is a caution that it is indispensable for quantification to use an internal reference peak as well as non-heteroplasmic sequences for comparison (see Material and methods).

Several studies have dealt with the question whether the differences between mother and offspring may be the consequence of the so-called bottleneck hypothesis (Hauswirth and Laipis 1982; Poulton 1995; Bendall et al. 1996; Marchington et al. 1997). It is suggested that at some stage of oogenesis selective amplification of a small number of mtDNA genomes allows a genotype present at only low levels in the mother to become established in the following generation. Therefore it is conceivable that the heteroplasmic mother in sample 4 exhibited both or more sequences whereas possibly only one sequence had been transmitted to the child.

The comparatively high frequency of heteroplasmic substitutions in our investigation is possible evidence for new mutations in a subset of mitochondria (Howell et al. 1996). However, these genetically altered mitochondria might disappear in the following generations due to the same bottleneck phenomenon.

The relatively high mutation rate reported by Parsons et al. (1997) might be explained if both homoplasmic and heteroplasmic substitutions were taken into account. Therefore we recommend to indicate the type and the location of the substitution to obtain comparable results for phylogenetic investigations.

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