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Mitochondrial DNA in human hair shafts – existence of intra-individual differences?

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Abstract The sequences of the hypervariable region 1 (HV1) of the mitochondrial DNA control region from multiple hair shafts from 10 unrelated individuals were compared to determine the frequency of differences in hairs from one individual. The extraction method described herein showed an average success rate of 67% for all 150 hair shafts tested in HV1. The mtDNA sequences from the hair shafts matched the sequences from the corresponding blood and saliva samples taken from the same donor and no evidence of heteroplasmy was found. The results emphasize the reliability of DNA extraction and mtDNA typing from human hair shafts for forensic purposes.

Key words Mitochondrial DNA · Hypervariable region 1 · DNA extraction · Human hair shafts · Heteroplasmy

Introduction

Sequencing of the mitochondrial DNA (mtDNA) control region is an effective method for the forensic comparison of samples containing insufficient DNA such as telogenic hairs, bones and old and degraded material (Higuchi et al. 1988; Hopgood et al. 1992; Sullivan et al. 1992; Holland et al. 1993; Wilson et al. 1995b; Ivanov et al. 1997). Especially the analysis of hair shafts without roots has gained increasing importance (Wilson et al. 1995a). Hairs found at the scene are usually shed hairs with only telogenic or no recognizable roots which contain only little if any nuclear DNA (Higuchi et al. 1988). Because of the much higher number of templates the analysis of mtDNA offers a better chance of DNA typing.

Several studies have dealt with the analysis of hair roots as a source of mtDNA (Howell et al. 1996; Bendall et al. 1997; Wilson et al. 1997; Parson et al. 1998) but relatively little information is available about the experiences with mtDNA from single hair shafts (Higuchi et al. 1988; Wilson et al. 1995a,b, 1997).

Heteroplasmy in the control region, a phenomenon in which two or more mtDNA populations occur in a single individual, is relatively common and has been reported in connection with identification cases, in association with several diseases and in evolutionary studies (Wallace 1992; Gill et al. 1994; Comas et al. 1995; Bendall et al. 1996; Marchington et al. 1996; Hühne et al. 1998; Parsons et al. 1998). A heteroplasmic point mutation was recently found in hair shafts from a single individual with a homoplasmic blood sample (Sullivan et al. 1996). Bendall et al. (1997) described one individual with variable levels of a heteroplasmic point mutation in different hair roots. The question whether mtDNA sequences of different hairs and other biological samples from one individual are comparable is therefore widely discussed (Wilson et al. 1995a,b; Sullivan et al. 1996).

The aim of this study was to elaborate a protocol for mtDNA extraction from hair shafts and to compare the sequences of the hypervariable region 1 (HV1) obtained from different hairs and those from blood and saliva of one individual.

Material and methods

Hair shafts, blood and saliva samples were from ten unrelated German volunteers (7 female and 3 male). A total of 15 hairs from each individual were taken from 3 different head areas (forehead, temple, back of the head) and cut 0.5 cm above the scalp. Morphological parameters such as length, diameter and colour of each hair and hair treatment (shampoo, hair spray, colouring) were noted. All hairs were used immediately for processing.

The single hair shafts were rinsed in ethanol (70%) in a sterile test tube for at least 30 min followed by sterile distilled H_2O for 30 min.

The extraction procedure consists of breaking the disulfide bonds with dithiothreitol (DTT) coupled with protein digestion with proteinase K. The hair shaft material of each hair was cut into small pieces, submerged in the extraction mixture and digested as follows: DTT (0.8 M), reaction buffer (750 mM TrisHCL pH 9.0, 200 mM $(NH_4)_2SO_4$, 0.1% Tween25), proteinase K (20 mg/ml) in 400 µl at 56 °C. The incubation time for digestion was completed

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when no hair fragment remained visible (4–7 days). During incubation, proteinase K (20 mg/ml) was added every second day to ensure that enough active enzyme was present.

After boiling for 5 min a series of organic solvent extractions based on the method described by Sambrook et al. (1989) was performed with phenol/chloroform (1:1) followed by chloroform/isoamylalcohol (24:1) in a final volume of 800 µl. The 400 µl aqueous layer containing the DNA was transferred immediately into Microcon-100 filtration tubes (Amicon, Beverly, Mass.). DNA was recovered according to the manufacturers protocol and made up to a final volume of 30 µl with sterile distilled H_2O.

Extraction of saliva samples was performed with Chelex 100 (Biorad) according to Walsh et al. (1991). Blood samples were extracted using the QIAamp Blood Kit (QIAGEN) following the manufacturers instructions.

Extracted DNA was routinely used for PCR immediately but if necessary samples were stored at –20 °C and 5–10 µl of extracted DNA was used for amplification.

Amplification and sequencing

Amplification of the hypervariable region 1 (HV1) 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). Sequencing primers were identical to those used for amplification. MtDNA sequences were automatically analysed by the ABI Prism Sequencing Analysis Software (Version 1.2.0) and checked manually. The positions 16024–16365 of HV1 were compared to the reference sequence (Anderson et al. 1981).

DNA extracted from blood and saliva was amplified and sequenced separately. Post-amplification purification was performed with the QIAquick PCR purification kit (QIAGEN) according to the manufacturers protocol.

In order to rule out artefacts, amplification and sequencing was carried out in both directions. The basecall was confirmed when forward and reverse sequence showed complementary peaks.

To minimize the possibility of contamination, DNA extraction, amplification and sequencing were performed in different laboratories and dedicated reagents and equipment were used for mtDNA analysis. Positive and negative controls were performed for every step of the procedure and if a negative control gave a signal, all samples were rejected. Samples with unclear interpretation of results were repeated.

Results and discussion

From 10 volunteers, 15 hair shafts without roots were obtained from 3 different head areas, amplified and sequenced. The average success rate of all 150 hair shafts tested was 67%. This result is in good agreement with the study of Wilson et al. (1995b) who reported an average success rate of 71% in 24 head hairs with different cosmetic treatment.

Dark hairs with larger diameters showed a tendency towards higher success rates in comparison to fine blond hairs (Table 1) although no statistical significance was found. Hair length was not associated with the success rate because short hairs (e.g. samples PH9, PH10) showed similar or higher success rates when compared to longer hairs (e.g. samples PH1, PH3, PH7) (Table 1). This result might be due to a higher content of melanin in longer hairs, a pigment known to be a strong PCR inhibitor (Wilson et al. 1995a) or it could be that mtDNA which is

Table 1 Colour, average length, average diameter and success rate in HV1 of the 15 hairs from each individual (PH1–10)

Person	PH1	PH2	PH3	PH4	PH5	PH6	PH7	PH8	PH9	PH10
Colour	red	brown	dark brown	light brown	blond	dark brown	blond	light brown	grey	reddish brown
Average lenght cm	15	10	14	11	8	12	15	9	5	5
Average diameter mm	0.07	0.09	0.08	0.06	0.06	0.09	0.07	0.08	0.08	0.1
Success rate HV1 %	53	93	87	53	33	87	53	53	60	100

Table 2 Sequences of 10 unrelated individuals in HV1 (A letter indicates a substitution relative to the reference sequence, – no base in the reference sequence. The exact number of insertions in the poly-C tract is undecidable, therefore at positions 16193.1 the maximal number of C-insertions is given.)

Base position	16051	16069	16126	16145	16153	16162	16163	16183	16186	16189	16193.1	16218	16223	16224	16231	16255	16261	16278	16292	16294	16300	16304	16311
Reference	A	C	T	G	G	A	A	A	C	T	–	C	C	T	T	G	C	C	C	C	A	T	T
Sample																							
PH1	G					G																	
PH2			C					C		C	C								T	T			
PH3																							C
PH4														C									C
PH5			C				G		T	C										T	G		
PH6		T	C																				
PH7	G					G																	
PH8								C		C	C		T			A		T					
PH9					A																	C	
PH10		T	C	A								T			C		T						

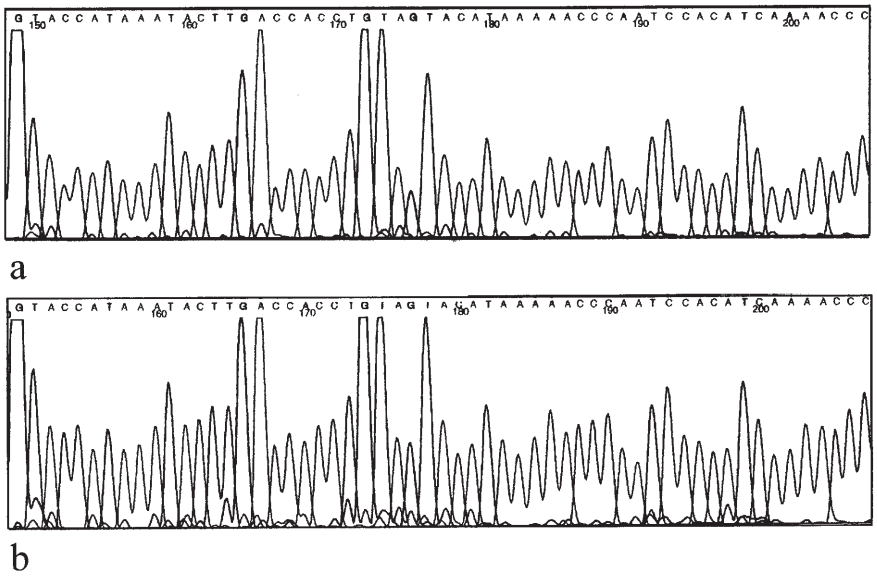

Fig. 1 Comparison of the sequencing results from blood (a) and hair shaft (b) from the donor PH4 (positions 16130–16186 in the forward strand of HV1, the reverse sequences showed similar results, sequencing primers used in both directions were the same as those used in the amplification reaction)

amenable for amplification is concentrated in the proximal part of a hair shaft.

Differences in the success rates in hairs from the three different head areas were not found. Hair treatment (e.g. colouring, shampoo) also showed no influence on the success rates.

Sequencing of the hypervariable region 1 of the mitochondrial control region from blood and saliva samples revealed nine different sequences in comparison to the reference sequence (Anderson et al. 1981) (Table 2).

The comparison of the mtDNA sequences obtained from hair shafts with the corresponding blood and saliva samples showed no differences (Fig. 1). It should be noted that some electropherograms showed minor traces of background peaks below the primary peaks (Fig. 1b), a problem recently described by Parson et al. (1998). As defined in an earlier study (Hühne et al. 1998), a nucleotide position was considered heteroplasmic if a secondary peak of more than about 40% peak height below the primary peak was present, which could be confirmed in the reverse sequencing reaction. Using this threshold, intraindividual heteroplasmic point mutations, i.e. heteroplasmic substitutions in a hair shaft in contrast to the blood or vice versa, were not observed. These results are in agreement with the studies of Wilson et al. (1995a, b) who also found no differences in the mtDNA sequences between hairs and blood in 30 individuals.

Our results suggest that mtDNA typing from human hair shafts is an applicable and reliable method for forensic purposes. The extraction protocol provides a high success rate depending on hair diameter and structure. However, the question whether it is possible to assign a hair to the corresponding blood sample is widely discussed. Bendall et al. (1997) described variable levels of a heteroplasmic point mutation in different hair roots of the same individual. Wilson et al. (1997) found a family with a heteroplasmic substitution in the blood. Telogenic hairs of the family members exhibited a wide range of nucleotide contributions at the heteroplasmic position. Reynolds (personal communication 1998) described up to six differences in hairs from one individual. Sullivan et al. (1996) found a heteroplasmic point mutation in 5 out of 12 hair shafts from a single donor and homoplasmy in the corresponding blood sample. Various explanations for the different findings might be taken into account. The heteroplasmy rate may depend on the methodology used for analysing mtDNA sequences (Parsons et al. 1997). Different definitions of the heteroplasmic phenomena leading to different interpretations and the possibility of external contamination must be considered. In the present investigation we have applied extreme caution to exclude any contamination. The positive effect of our measures is confirmed by the homogeneity of our results.

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