Int J Legal Med (1999) 112 : 287–290

ORIGINAL ARTICLE

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Mitochondrial DNA typing from human axillary, pubic and head hair shafts – success rates and sequence comparisons

Received: 18 January 1999 / Accepted: 15 February 1999

Abstract The analysis of mitochondrial DNA (mtDNA) from shed hairs has gained high importance in forensic casework since telogen hairs are one of the most common types of evidence left at the crime scene. In this systematic study of hair shafts from 20 individuals, the correlation of mtDNA recovery with hair morphology (length, diameter, volume, colour), with sex, and with body localisation (head, armpit, pubis) was investigated. The highest average success rate of hypervariable region 1 (HV 1) sequencing was found in head hair shafts (75%) followed by pubic (66%) and axillary hair shafts (52%). No statistically significant correlation between morphological parameters or sex and the success rate of sequencing was found. MtDNA sequences of buccal cells, head, pubic and axillary hair shafts did not show intraindividual differences. Heteroplasmic base positions were observed neither in the hair shafts nor in control samples of buccal cells.

Key words Mitochondrial DNA · Hypervariable region 1 · Head, axillary and pubic hair · Shafts · Heteroplasmy

Introduction

Shed hairs are usually telogen hairs and contain only little if any nuclear DNA (Higuchi et al. 1988; Allen et al. 1998). In contrast to nuclear DNA, mtDNA occurs in high copy numbers per cell (Bodenhagen and Clayton 1974) and therefore allows the analysis of single hair shafts even after long time intervals since shedding. The question whether mtDNA sequences derived from different hairs and other biological samples from one individual can vary and to which extent is still unanswered. The discussion began when the first heteroplasmic point mutation was described in hair shafts from an individual with a homoplasmic blood sample (Sullivan et al. 1996). Our group has recently studied mtDNA sequences of 150 head hair shafts from different head areas of 10 volunteers and compared the sequences with the corresponding blood and saliva samples (Hühne et al. 1999), with no deviations between corresponding samples. None of the individuals tested was heteroplasmic in blood or saliva. Bendall et al. (1997) found various levels of heteroplasmy among single hair roots in one individual with a heteroplasmic point mutation at np 16256 detected in blood and buccal cells. The hair roots were taken from two different skull areas and from the thumb side of the left wrist.

The intention of this study was to expand our investigations (Hühne et al. 1999) and to compare mtDNA sequences from head, pubic and axillary hair shafts to corresponding buccal cells in order to explore whether different body regions can affect mtDNA recovery and sequence structure. Furthermore our study aimed to investigate the influence of morphological parameters and the sex on mtDNA recovery from hair shafts.

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Material and methods

Head, axillary and pubic hair shafts as well as control samples of buccal cells were collected from 20 unrelated volunteers. The number of sampled hairs per body region varied between 4 and 15 (Table 1).

The hair shafts were cut off 5 mm above the skin surface. The colour, length and diameter of each hair were documented. Because of the oval cross section of mainly axillary hairs, diameters were found to vary even within one hair from one individual, so an average diameter from the longest and the shortest measurements was calculated. The volume was calculated as $V = \pi r^2 l$, where r was the half of the calculated average diameter and l was the length of each hair.

The single hair shafts were washed in ethanol (70%) for 30 min followed by a further wash step in sterile distilled H_2O for 30 min. Extraction, amplification and subsequent cycle sequencing of the hypervariable region 1 (HV1) using the ABI (Applied Biosystems) PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq FS DNA polymerase (Perkin Elmer) was performed as described previously (Hühne et al. 1999; Pfeiffer et al.

Table 1 Sex of the donor, calculated average diameter and average volume of the hair shafts, colour and average success rate of mtDNA sequencing results of head, pubic and axillary hair shafts from 20 volunteers (n = number of investigated hair shafts, sex: f = female, m = male, colour: 1 = red, 2 = brown, 3 = blond, 4 = grey, 5 = black)

Sex	Head hair shafts					Pubic hair shafts					Axillary hair shafts				
	n	Volume mm^3	Average diameter mm	Colour	Average success %	*n*	Volume mm^3	Average diameter mm	Colour	Average success %	*n*	Volume mm^3	Average diameter mm	Colour	Average success %
f	15	0.58	0.07	1	53	4	0.35	0.1	1	50					
m	15	0.45	0.08	2	53	4	0.68	0.12	2	100	4	0.32	0.11	2	100
f	15	0.76	0.09	2	87	4	0.58	0.14	2	0	4	0.06	0.05	2	0
m	4	0.25	0.1	2	100	4	0.22	0.09	2	100	4	0.02	0.03	2	100
m	4	0.2	0.08	2	100	4	1.57	0.16	2	100	4	0.34	0.1	2	25
m	15	0.39	0.1	2	100	4	0.37	0.12	2	0	4	0.2	0.1	2	0
m	4	0.49	0.1	2	25	4	0.6	0.12	2	25	4	0.15	0.07	3	0
f	15	0.31	0.06	2	53	4	0.42	0.1	2	75					
f	15	0.7	0.08	2	87	4	0.33	0.1	2	75					
f	15	0.64	0.09	2	93	4	0.47	0.1	2	75					
f	4	0.78	0.08	2	100	4	0.24	0.1	2	100					
f	4	0.42	0.07	2	50	4	0.1	0.08	3	50					
m	4	0.15	0.07	3	100	4	0.93	0.13	2	100	4	0.26	0.09	2	0
f	15	0.23	0.06	3	33	4	0.2	0.08	3	75	4	0.15	0.09	3	75
f	15	0.58	0.07	3	53	4	0.33	0.1	3	0	4	0.09	0.06	3	0
f	4	0.21	0.05	3	75	4	0.77	0.12	3	75	4	0.03	0.06	3	100
m	4	0.11	0.06	3	100	4	0.16	0.06	3	100	4	0.03	0.06	3	100
f	4	0.5	0.09	3	100	4	0.53	0.1	3	75	4	0.03	0.08	3	100
m	15	0.25	0.08	4	60	4	0.64	0.13	2	100	4	0.12	0.06	2	75
m	4	0.42	0.1	5	75	4	0.5	0.1	2	50	4	0.15	0.06	2	50

1999). Buccal cells were extracted using Chelex (Walsh et al. 1991). MtDNA sequences were analysed by two investigators independently using the Sequence Navigator software (Version 1.0.1 ABI). Each mtDNA sequence was confirmed by the forward and the reverse sequencing reaction. The nucleotide positions 16024–16365 were compared to the reference sequence (Anderson et al. 1981). A sequencing reaction was considered successful only when the forward and the reverse sequencing reactions without exception showed complementary peaks.

Results and discussion

The average success rate in mtDNA sequencing was the highest for head hair shafts (75%) followed by pubic (66%) and axillary (52%) hair shafts (Table 1). The results are comparable to those of Wilson et al. (1995b) who reported an average success rate of 71% in 24 head hair shafts.

No significant correlation was found between the average success rate in mtDNA sequencing and the sex of the donor, the average length, the average diameter and the volume (Table 1). Head hairs from males were on average shorter but with a larger diameter than in females and the average success rate in males (79%) was higher than in females (71%). Furthermore, the average diameter of head, pubic and axillary hair shafts (Table 1, Fig. 1 a-c) and also the colour (Table 1) showed no correlation with the success rate.

The degree of PCR inhibition caused by melanin as described by Yoshii et al. (1992) and Wilson et al. (1995a) has not been investigated in this study. To overcome the inhibitory effect of melanin, as demonstrated by Giambernardi et al. (1998), bovine serum albumin (BSA) was added to our PCR reaction mix.

The average human scalp contains about 100 000–150 000 hair follicles in 3 stages of development. The active growth phase (anagen) of head hairs lasts between 2 and 8 years. During this phase the turnover of the matrix cells is the highest of all human tissues with the exception of bone marrow (Linch et al. 1998). Therefore a high mitochondrial activity is required. Preparations for a cessation of activity are made during the hair breakdown phase (catagen) where the programmed cell death (apoptosis) results in double strand cleavage of nuclear DNA in the hair roots. During the resting phase (telogen), when the activity has ceased, the hairs are naturally shed (Linch et al. 1998). Approximately 80% of head hairs are in the anagen phase, less than 1% in the catagen phase and approximately 20% in the telogene phase (Petraco et al. 1988). Presumably the success rate of mtDNA recovery from head hair shafts is the highest in anagen hairs. The telogen phase of pubic and axial hairs lasts longer than the periods of growth (Linch et al. 1998), therefore less than 50% of pubic and axillary human hairs are in the anagen growth stage. In our study the hair shafts were cut randomly without determination of growth stages. The established success rate for head hairs (75%) and the similar results of Wilson et al. (1995b) could be explained by different growth phases, which can also be one of the rea-

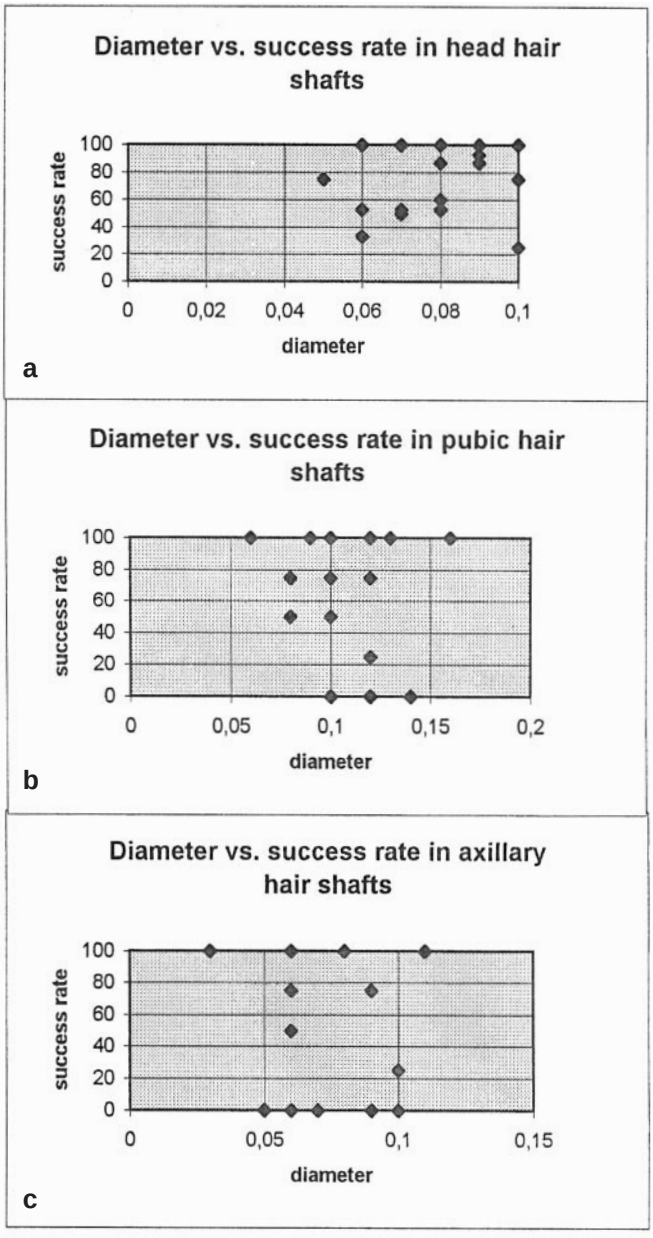

Fig. 1 a–c Relationship between the average diameter of head, pubic and axillary hair shafts (mm) and the success rate of mtDNA HV1 sequencing (%)

sons for the higher success rate in mtDNA recovery from head hairs. On the other hand telogen hairs are the most common evidential material in forensic casework and if the success rate mainly depends on the growth stage, the probability of obtaining a sequence structure would be lower than the average obtained in this study. From casework in our laboratory with telogenic hairs exclusively, i.e. 9 cases with 96 hairs, we obtained an average success rate of approximately 75%. Therefore, further facts must also be considered as having an influence on the varying success rate.

Axillary and pubic hairs grow up to 0.37 mm per day initially and can reach up to 70 mm in length whereas head hairs grow with about the same speed and, uncut, can grow to more than 100 cm long (Balabanova and Wolf 1989). Therefore, the mitochondrial activity can be expected to be higher in head hairs than in axillary and pubic hairs which can result in a higher number of mitochondria in head hairs.

The occurrence of the heteroplasmy phenomenon in the control region has been reported in an identification case first by Ivanov et al. (1996). Jazin et al. (1996) found similar levels of heteroplasmy in two brain regions from the same individual which was homoplasmic in blood and they concluded that heteroplasmy seems to be more frequent in non-mitotic tissues. For detection of heteroplasmic base positions, sequencing methods with expanded high-resolution sequencing efforts are recommended (Bendall et al. 1996). These methods are time-consuming and not usually applied in routine casework. In an earlier study (Hühne et al. 1998) we characterized levels of heteroplasmy by gravimetric integration of the peaks printed in the sequence hardcopy. 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 reproduceable. In this study, using the earlier postulated definition and the same sequencing method, heteroplasmy was neither observed in buccal cells nor in head, axillary and pubic hair shafts from the 20 subjects investigated. However, a heteroplasmy discordance between the mtDNA haplotypes derived from blood and single hairs seems to occur very rarely. In those exceptional cases expanded high-resolution methods such as denaturating gradient gel electrophoresis (DGGE) can be additionally used to compare heteroplasmy levels in the questioned tissues (Jazin et al. 1996).

Our results demonstrate that the specific body area from which hairs are taken affects the mtDNA recovery, i.e. the sequencing success rate, but not the mtDNA sequence structure. Furthermore, intra-individual discordances in the HV1-sequence structure between hair shafts and buccal cells seem to occur only in exceptional cases and do not endanger the routine case work with hair shafts in forensic laboratories.

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