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CASE REPORT

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Forensic mtDNA hair analysis excludes a dog from having caused a traffic accident

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Abstract A dog was suspected of having caused a traffic accident. Three hair fragments were recovered from the damaged car and subjected to DNA sequence analysis of the canine mitochondrial D-loop control region. The results were compared to saliva and hair samples from the alleged dog, as well as to control hair samples from four unrelated dogs of different breeds. Two sequence types exhibiting five nucleotide differences in a 377 bp fragment were identified among the four controls. Whereas the evidence hair fragment was identical to the type 1 control sequence, the alleged dog shared the type 2 control sequence except for one position. Thus the dog could be excluded as the origin of the hair fragment. As canine mtDNA appears to exhibit only limited polymorphism, mitochondrial D-loop sequence comparison is currently only suitable for exclusions.

Key words Mitochondrial DNA · Hair · Dog · D-loop · PCR · Identification

Introduction

The police report plainly stated that "after the collision, the dog left the scene of accident without proving his identity ...". However, the dog allegedly left behind three hair fragments of 1–2 cm length at the damaged front end of the car. The driver had a supposition about the "perpetrator's" identity, and filed a lawsuit for damages. He assumed that it was one of two German shepherd dogs belonging to a local shop owner, as this animal had been treated for minor injuries shortly after the accident. Subsequently, our laboratory was contacted by the judge of the county court about the possibility to identify the dog based on the hair fragments from the car. Shortly after we received this request, a report was published about the ancestry of the domestic dog based on comparisons of mitochondrial D-loop control region DNA sequences [1]. Comparative mtDNA analysis of the D-loop hypervariable regions I and II is highly successful for hair typing [2] as well as in human identification cases, e.g. from bone samples [3, 4]. We investigated the dog case using direct mtDNA sequence analysis from the hair fragments as well as from control saliva and hair samples from the "alleged" dog.

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Materials and methods

The collection of control saliva and hair samples was ordered by the investigating judge and was performed by a veterinarian. Furthermore, hair samples from four "voluntary" dog donors of different breeds served as additional controls and were used to establish the PCR and sequencing conditions. DNA extraction from hair was carried out by proteinase K digestion and phenol extraction followed by concentration in Centricon-30 spin dialysis tubes (Amicon, Beverly, MA, USA). As the reference sequence for dog mtDNA, the EMBL database entry MICFTANDR (accession no. X97343, last update June 12, 1996; Rothuizen J, de Gouw H, van der Vugt HHJ, Plas MA, Nijman IJ, van Wolferen M, Hellebrekers LJ, Bos JA, Dorrestein GM, Hoelzel AR, Lenstra JA. Variation in the mitochondrial tandem repeat in dogs and bears. Utrecht, The Netherlands) containing 1220 bp of the D-loop region was used. Primers L16462 and H222 (numbering according to their approximate location in the human mtDNA sequence [5]) as described in ref. [1] were used to amplify a 377 bp fragment, which is equivalent to positions 289–666 of the Rothuizen dog sequence. PCR conditions were as follows: initial denaturation for 3 min at 94 °C, then denaturation at 94 °C for 45 s, annealing at 52 °C for 30 s, extension at 72 °C for 3 min, and a final extension for 10 min at 72 °C (using a Perkin Elmer TC-1 thermocycler). For DNA extracted from the control hairs and saliva samples, 30 cycles were carried out, whereas for DNA extracted from the evidence hair fragments, the number was increased to 40 cycles. Samples without DNA were included in all experiments to serve as controls for contamination monitoring. DNA sequencing was carried out with an ABI Prism 310 automated sequencer and BigDye Terminator sequencing reagents (ABI-Perkin Elmer, Weiterstadt, Germany).

Table 1 Observed nucleotide differences between evidence hair, alleged dog, and control dogs relative to EMBL database reference sequence (access. no. X97343)

Rothuizen et al.		Evidence hair	Alleged dog	Control dogs (n = 4)	
Position	Sequence			Type 1	Type 2
343	T	C	T	C	T
349	C	G	G	G	G
357	T	T	C	T	T
455	T/C	T	C	T	C
481	G	–*	G	–*	G
498	C	T	C	T	C
546	A	G	A	G	A

* Single base deletion

Results and discussion

Among the four dog control samples, two typical mtDNA sequences were obtained which differed from each other at 5 positions (see Table 1). Control type 2 was identical to the Rothuizen sequence except for a C to G exchange at pos. 349, which was also shared by control type 1. From the three hair fragments obtained from the damaged car, only the longest fragment was suitable for reproducible PCR and sequence analysis. The evidence hair was identical to type 1 control sequence thus confirming the canine species origin of this hair. However, the alleged dog shared all variable positions (except for the T/C exchange at pos. 357) with the type 2 control sequence. Thus it could be excluded that the hair from the damaged car originated from this individual animal. After learning about the outcome of this investigation, the plaintiff withdrew the suit and the case was subsequently dismissed.

In another recent publication about mtDNA variation among dogs, it has been demonstrated that the extent of polymorphism is not as pronounced as in humans [6]. Among 102 domestic dogs of 52 breeds, only 19 different sequence variants with relative frequencies between 1 and 21% were found by analyzing a 257 bp PCR fragment approx. equivalent to the human HV1 region (and represented in positions 1–230 of the Rothuizen sequence except for the first 27 bp). Furthermore, it was observed that identical sequences were shared between dogs from the same or different breeds, as has also been found in our limited case study. Apparently, the high degree of inbreeding especially in pedigree dogs is only in part consistent with the restricted mtDNA heterogeneity, which may also reflect the approaches in conventional dog breeding. Thus forensic analysis of dog mtDNA sequences is suitable at present only for cases of exclusion, whereas more extensive studies are required to establish the molecular and biostatistical basis to prove a match.

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References

1. Vila C, Savolainen P, Malsonado JE, Amorim IR, Rice JE, Honeycutt RL, Crandall KA, Lundeberg J, Wayne RK (1997) Multiple and ancient origins of the domestic dog. Science 276: 1687–1679
2. Vigilant L, Pennington R, Harpending H, Kocher TD, Wilson AC (1989) Mitochondrial DNA sequences in single hairs from a southern African population. Proc Natl Acad Sci USA 86:9350–9354
3. Hagelberg E, Sykes B (1989) Ancient bone DNA amplified. Nature 342:485–488
4. Ivanov PL, Wadhams MJ, Robym RK, Holland MM, Weedn VW, Parsons TJ (1996) Mitochondrial DNA sequence heteroplasmy in the Grand Duke of Russia Georgij Romanov establishes the authenticity of the remains of Tsar Nicholas II. Nat Genet 12:417–420
5. Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG (1981) Sequence and organisation of the human mitochondrial genome. Nature 290:457–465
6. Savolainen P, Rosen B, Holmberg A, Leitner T, Uhlen M, Lundeberg J (1997) Sequence analysis of domestic dog mitochondrial DNA for forensic use. J Forensic Sci 42:593–600