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CASE REPORT

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Canine STR analyses in forensic practice

Observation of a possible mutation in a dog hair

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Abstract In a case of the death of a 7-year-old boy, the police investigations revealed a possible dog attack contrary to the witness testimonies. DNA investigations were carried out from hairs, saliva and bloodstains with 10 canine-specific STR loci by the use of fluorescently labelled multiplex PCR and the ABI PRISM 310 genetic analyzer. The analysis of one hair sample revealed one allele deviation from the profile of the putative Rottweiler perpetrator possibly caused by a mutation. The PCR fragments in question at the PEZ20 locus were sequenced and compared with the alleles detected in the Hungarian canine population and identified on a repeat number basis. The allele frequencies were determined based on typing of 242 genetically independent canine individuals from 72 breeds. The results suggested that two of the canine individuals could be the perpetrators.

Keywords Animal identification · Canine · Hair · Saliva · Multiplex STR profiling · Mutation

Introduction

Hungary is a country with a well known and worldwide respected cynophilic and cynologic tradition and a country with a significantly high canine population. However, during the last years several attacks on humans were recorded and at least 12 fatal attacks were observed during the last year with the victims being children as well as adults.

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The genetic mapping of farm animals [1], domestic animals [2] or wild animals [3] is related to knowledge on polymorphic STR loci, which could solve cases of animal identification in an analogue way to human identification in the forensic practice [4, 5, 6]. During the analyses of biological remains of different origin for forensic purposes, special aspects such as mixtures, uniform terminology, mutation probabilities etc., must be taken into consideration [7, 8].

This work is part of a project with the main aim to establish a canine identification system in the Hungarian forensic practice with the application of commercially available canine-specific STR markers in different types of samples e.g. saliva, hair etc. [9, 10]. According to recent population studies the degree of polymorphism of the 10 microsatellite markers [8] seems to be sufficient for canine identification. These studies are complemented by the construction of allelic ladders controlled by sequencing and by the availability of data concerning the allele frequencies in the local canine populations.

Case report

In March 2000 the corpse of an unidentified male child was found in a sports establishment in Budapest. The victim was identified as the 7-year-old son of the partner of the security guard of the property. According to their testimonies and the initial crime scene investigation, the police suspected a case of sexual assault followed by homicide. The clothes of the victim were collected for further laboratory examination.

The forensic post-mortem examination revealed injuries caused by biting [11]. The cause of death was traumatic shock and the approximate time of death was estimated to be the morning of the same day when the body was found. The primary suspicion involved the Rottweiler and German shepherd guard dogs owned by the security guard as perpetrators, but they were found in their kennel when the police arrived at the crime scene. Toothprints, blood and hair samples, the stomach content and the collars were collected for further laboratory examinations.

The SEM analyses identified the marks of the German shepherd's left canine teeth on the surface of the jaws of the victim. The examination of the stomach content was negative, but the DNA analyses of the biological remains (a minute amount of blood on the German shepherd dog's collar, animal hairs and traces of

Table 1 Detected sizes of amplified fragments at PEZ1, FHC2054, FHC2010, PEZ5, and PEZ20 loci for the crime samples and suspected dogs (*Mean* the average size of fragments in the population samples, *N*=484, *nt* nucleotide)

Locus	$Saliva_I$	$Saliva_{II}$	$Saliva_{III}$	$Saliva_{IV}$	$Saliva_V$	$Saliva_{VI}$	Hair	Dog_1	Dog_2	$Mean_{nt}$	SD_{nt}
PEZ1	–	–	–	–	107.82	107.71	107.90	–	107.97	107.77	0.08
PEZ1	115.96	115.85	115.82	115.85	115.98	115.90	116.12	115.95	116.09	115.94	0.06
PEZ1	120.11	120.11	120.11	120.00	120.16	120.14	–	120.22	–	120.15	0.01
FHC2054	146.46	146.37	146.34	146.25	146.38	146.34	–	146.41	–	146.36	0.03
FHC2054	–	–	–	–	150.49	150.45	150.49	–	150.55	150.47	0.03
FHC2010	220.27	220.27	220.24	220.24	220.38	220.37	220.42	220.28	220.40	220.38	0.01
FHC2010	232.48	232.48	232.40	232.40	232.50	232.41	232.50	232.43	232.44	232.46	0.06
PEZ5	98.34	98.22	98.23	98.33	98.81	98.53	98.85	98.38	98.88	98.67	0.20
PEZ5	–	–	–	–	107.11	106.91	107.12	–	107.15	107.01	0.14
PEZ20	171.42	171.49	171.37	171.44	171.53	171.60	171.70	171.46	171.56	171.57	0.05
PEZ20	–	–	–	–	–	–	175.34	–	–	175.36	0.15
PEZ12	266.15	266.28	266.29	266.28	265.88	266.08	–	266.09	–	266.04	0.28
PEZ12	–	–	–	–	269.73	269.92	269.73	–	269.60	269.92	0.21
PEZ12	277.73	277.85	277.85	277.87	277.43	277.61	–	277.66	–	277.61	0.21
PEZ12	–	–	–	–	294.92	294.99	294.83	–	294.86	294.97	0.17
PEZ3	–	–	–	–	117.80	117.71	117.81	–	117.85	117.79	0.05
PEZ3	120.79	120.79	120.80	120.68	120.87	120.83	–	120.88	–	120.81	0.06
PEZ3	127.03	126.91	126.96	126.84	126.97	126.93	126.99	127.07	127.06	126.97	0.07
PEZ6	168.30	168.35	168.23	168.28	168.25	168.28	–	168.26	–	168.25	0.06
PEZ6	–	–	–	–	172.12	172.04	172.17	–	172.06	172.08	0.07
PEZ6	–	–	–	–	176.05	176.00	176.05	–	176.00	176.08	0.38
PEZ6	182.70	182.82	182.70	182.82	182.72	182.81	–	182.74	–	182.83	0.39
PEZ8	–	–	–	–	220.67	220.66	220.67	–	220.68	221.02	0.40
PEZ8	224.64	224.64	224.63	224.63	224.72	224.70	–	224.65	–	224.71	0.39
PEZ8	–	–	–	–	229.86	229.80	229.81	–	229.81	229.58	0.36
PEZ8	232.85	232.85	232.78	232.78	232.87	232.83	–	232.79	–	232.79	0.28
FHC2079	–	–	–	–	265.76	265.82	265.71	–	265.75	266.01	0.35
FHC2079	270.45	270.45	270.45	270.45	270.00	270.18	–	270.48	–	270.27	0.27
FHC2079	–	–	–	–	281.89	281.93	281.97	–	281.95	282.15	0.36

suspected canine saliva on the victim's coat) did not suggest any other perpetrator apart from the two suspected guard dogs. The security guard and the woman were subsequently charged with negligant conduct resulting in death brought about by the execution of a job-related function, and deception of the criminal justice authorities by providing false witness statements.

Materials and methods

Hairs of animal and human origin were collected from the victim's coat and after the initial optical (UV) examination six suspected saliva spots were collected. A spot of blood was found on the dog's collar and was also collected. Blood and hair samples from both alleged perpetrator animals were used for comparison. The samples of genetically independent individuals (*n*=242) of 72 pure breeds were used to construct an allelic ladder for the PEZ20 locus and statistical analysis. One catagen dog hair was selected by microscopy for DNA analysis. The DNA extraction and the canine DNA analyses were carried out as previously described [8]. After quantification, (QuantiBlot Human DNA Quant kit) the human DNA was coamplified by the use of AmpFlSTR Profiler Plus and Cofiler PCR Amplification kits (Applied Biosystems). The fluorescence-based automated detection of the PCR products by ABI PRISM 310 Genetic Analyzer was performed according to the manufacturer's instructions.

The monoplex amplification of the PEZ20 locus was performed with 5'-cctaaattagag-gtctaacc and 5'-gtaagcggga-atgtgctcctc primers, and the following PCR conditions: 1 ng DNA, 0.2 μM each primer, 2.5 mM $MgCl_2$, 200 μM each dNTP, 1×PCR buffer II, 5 U TaqGold (Applied Biosystems) in a 50 -μl reaction mix, with cycle conditions of 95°C for 11 min, followed by 32 cycles of 94°C for 30 s, 56°C for 30 s, 72°C for 30 s and finally 72°C for 10 min, in a GeneAmp PCR System 2400. The forward and reverse sequencing reactions were performed by the application of the ABI PRISM BigDye Terminator Cycle Sequencing kit and an ABI PRISM 377 DNA sequencer. The determination of the fragment sizes of the constructed allelic ladder was carried out using the ABI PRISM 310 Genetic Analyzer and CXR 60-400 (Promega Corp.) as well as GeneScan 500 ROX and GeneScan 400HD ROX (Applied Biosystems) internal sizing standards.

Results and discussion

The blood sample collected from the dog collar (<2 ng total human DNA, c<0.06 ng/μl DNA) gave results corresponding to the victim's genetic profile (PM: 1.08×10^{14}). The multiplex PCR amplification of the samples of canine origin (c: 1–3 ng/μl DNA, determined by monoplex PCR titration) was successful. The sizing precision of ≤0.40 nt standard deviation allows a ±0.5 nt allele size window to be set for genotyping. From the six presumed saliva spots ($Saliva_{I\text{-}VI}$) the profile of four samples matched the German shepherd (Dog_1) and the profile of two matched to the mixed profile of the German shepherd and Rottweiler (Dog_2). The microscopic examination revealed naturally

Table 2 Sequence structure. sequenced fragment length and allele frequencies (*N*=484) at PEZ20 locus

Allele designation	Fragment length (nt)	Repeat region	Allele frequency
11	170	$-(AAAT)_{11}-$	0.0021
12	174	$-(AAAT)_{12}-$	0.1097
13	178	$-(AAAT)_{13}-$	0.5570
14	182	$-(AAAT)_{14}-$ or $-(AAAT)_{13}-(AAAA)_{1}-$	0.2025
15	186	$-(AAAT)_{15}-$	0.1055
16	190	$-(AAAT)_{16}-$	0.0190
17	194	$-(AAAT)_{17}-$	0.0021
19	202	$-(AAAT)_{18}-(AAAA)_{1}-$	0.0021

shed and plucked human hairs, one morphologically identified sensory muzzle hair possibly from a Rottweiler dog, in catagen phase, and many shed hairs possibly originating from a German shepherd and a Rottweiler dog. The genetic profile of the plucked hair corresponded to the profile of the male Rottweiler in 9 loci, but was however heterozygote at the PEZ20 locus (Table 1), in which the ratio of the amplified fragments was >2:1 with regards to the peak areas in the electropherogramm.

The DNA from the hair, the two suspected individuals and the kit positive PCR control as well as all known fragments of the allelic ladder in the PEZ20 locus, were amplified with monoplex PCR. The sizes of the analogue monoplex PCR products corresponded with those amplified by the multiplex PCR. According to data collected from population studies in Hungary, an allele containing 18 repeat units had never been detected. In the case of the 184 bp control DNA fragment (from the kit) and the 202 bp fragment of the allellic ladder, instead of the $-(AAAT)_n$ (n=13 and 19) repetition, an $-(AAAT)_{n-1}-(AAAA)_1$ sequence was detected. The DNA sequences were checked by the computer program BLASTN 2.2.1 (Apr.-13-2001) [12] (Search effected via the www. interface at the NCBI, on 07. 11. 2001.). The sequences were sent to the DNA sequence databank GenBank (NCBI, Bethesda, Md, USA) and the accession IDs are AF454051 and AF454052. The heterozygote profile of the tactile hairs demonstrated alleles with deviation in the number of (AAAT) repeats. According to the sequenced fragments the construction of an allele nomenclature based on the repeat number is possible (Table 2).

There was evidence of departure from HWE ($P_{Exact\ test}$: 0.00085, H_{obs}: 0.5021, H_{exp}: 0.6266) for the locus PEZ20 (*N*=484). Due to the sampling from a mixed population this discrepancy is likely to be due to an inbreeding effect in such a genetically closed purebred subpopulation. The PM values of the matching genetic profiles were calculated by the observed fragment frequencies in the population sample and they were 2.1×10^{16} in the case of the German shepherd (10 loci) and 5.8×10^{10} in the case of the Rottweiler (9 loci). These statistics of the two pure breeds in question require extended studies including a larger number of individuals per breed. Considering the calculated likelihood ratio the observed genetic profile of the mixed saliva stains is 2.34×10^{15} times more probable given the stains originated from the known dogs rather than from two other, unrelated individuals from the Hungarian canine population.

The experts' opinion in multiple directions, as well as the detective's investigations and the fact that the genetic profiles were gained from a relatively high number saliva stains, did not indicate another unknown individual (human or animal) as the perpetrator. In such a violent case the perpetrator dog cannot avoid direct contact with the victim. Due to the fact that the dog bites always result in biological remains and the lack of an additional hypothetical perpetrator, the detected unbalanced (peak areas rate >2:1) heterozygote genotype of the tactile hair at PEZ20 locus is likely to be the result of a somatic mutation.

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