

Forensic identification of two murderers by DNA multi-reverse parental analysis

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Abstract. In this paper, we present a murder case committed by three men in a small town in northern Italy in which two of the murderers were identified by means of a multi-reverse parental analysis approach. Four different complete male profiles for 17 STRs were obtained from the bloodstains and other evidence collected at the crime scene, one of which belonged to the victim. Two of the three unknown profiles revealed similar characteristics for many alleles/loci tested, which could be explained by a possible parental linkage. The investigators focused their attention on some families in gypsy communities with records of robberies. All donors were excluded except for two individuals whose profiles were very similar to the two unknown DNA profiles obtained from the crime scene. We then decided to type the close relatives of these two individuals. Among the relatives typed, four of them appeared to belong to the parents of the two unknown men involved in the murder. As a consequence, one murderer was arrested after a few weeks, the other more than 2 years. © 2003 Published by Elsevier B.V.

Keywords: Reverse parental analysis; DNA typing; STRs

1. Materials and methods

DNA was extracted and purified from bloodstains, cigarette butts and saliva samples of about 100 reference samples, using Qiagen automated extraction [1] and subsequently quantified by slot-blot hybridization [2] with the chemiluminescent signals recorded by GeneGnome CCD imaging systems [3]. For the amplification and typing, we used the *AmpFISTR Identifiler PCR Amplification Kit* [4] and the *Powerplex 16.2 PCR Amplification Kit* [5]. Electrophoresis was carried out on an ABI Prism 310 Genetic Analyzer. Data were analysed by Gene Scan v.3.1 analytical software.

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2. Reverse paternity and parental analysis

All the STRs profiles obtained from evidence collected and from reference samples were studied and compared to each other, to look for the presence of a consistent number of same genotypes and/or identical alleles, in order to identify any possible killer's relative. All the allele/genotype counts were hand-made. Reverse paternity analysis was conducted using the Program "Pater version 2.0" [6].

3. Results and discussion

Two of the bloodstains collected at the crime scene were found to belong to two unknown individuals and, with respect to the numbers of same genotypes (two) and identical alleles (nine) of their profiles, a possible parental linkage was suggested. All the remaining evidence came from the victim, excluding one cigarette butt, which showed a third unknown profile, suspected to belong to the third perpetrator. According to the elements found during the preliminary investigation as well as from the genetic results we achieved, the investigators concentrated their attention on some individuals of a gypsy community. Many reference saliva samples were then collected and delivered to our lab, to be submitted for DNA typing. Among the profiles we obtained, two of them, which belonged to two cousins of the same family group, showed a very interesting similarity with those of the two bloodstains found at the crime scene.

As shown in Table 1, this unusual similarity consisted in 8 matching loci plus 8 identical alleles between the first individual (first cousin) and one of the two bloodstains and 5 matching loci plus 12 identical alleles, between the second individual (second cousin) and the second bloodstain, pointing out clear and strong evidence of parental linkage which indicated that the two unknown profiles, could come from the same family group. According to these results and looking at the family trees we asked to study in the meantime, we suggested to the investigators to collect more appropriate samples of reference saliva, especially from the two related families of the two cousins previously mentioned. The genetic results obtained were then subjected to statistical calculations [7] (as shown in Table 2), which strongly supported the hypothesis that among the family samples typed, there were the four parents (father and mother) of the two unknown individuals who left the bloodstains at the crime scene.

As a consequence of these results, we then typed all saliva samples of the available sons of the two families considered, but no complete matches were achieved indicating that the

Table 1
Common genotype/allele between the two unknown profile and the I°/II° cousins

	I° unknown profile	II° unknown profile
<i>Common genotype</i>		
I° cousin	2	8
II° cousin	5	2
<i>Common allele</i>		
I° cousin	10	8
II° cousin	12	8

Table 2
Likelihood ratio (LR) and probability of paternity (*P*, %) values

Samples considered for statistical analyses			No. of STRs-typed	<i>P</i> (%)	LR (odds)
I° father	I° mother	I° unknown blood profile	17	99.99999999999916%	1.19951*10 ¹⁴
II° father	II° mother	II° unknown blood profile	17	99.99999999999980%	5.99221*10 ¹⁴

only two missing sons should be the two killers. The statistical calculations for these family groups were made according to the reverse parental approach. The 2 suspects at large were then found and arrested and asked to give a sample of their saliva, which was submitted to DNA analysis, providing a full match for all 17 STRs typed. These results definitively proved that they were the two criminals who had left the bloodstains at the crime scene after a violent fight with the victim. All this confirmed the validity of our initial hypothesis and showed it was fundamental to apply the multi reverse parental analysis approach to identify the culprits of a murder, stressing once more the huge power of DNA in the forensic field.

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