

## Forensic identification of a murderer by typing volunteers of a small village of Northern Italy

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**Abstract.** In this paper, we report the real case of a murder, committed in a small village (about 1000 people) in Northern Italy, whose culprit was identified through the paternity test. The corpse of an elderly female (75 years old) was found in her house. The cause of death was determined to be a violent blow. There were also signs of sexual assault to the body. The investigators sent much evidence to our lab: it also included some stains, presumably consistent with semen, collected from the victim's pubic hair. Microscopic analysis of the semen residues permitted the identification of spermatozoa with tails attached; the subsequent DNA typing allowed us to obtain a complete male profile for 17 STRs. The investigators focused their attention on some men with criminal records of sexual crimes who were suspected of the murder, but they were all excluded by DNA profiling. However, the profiles we obtained from those people showed a characteristic similarity for many alleles/loci, due to possible inbreeding. Hence, we suggested to both the prosecutor and the investigators that the people ranging 18–65 years of age living in the village be invited to give a sample of their saliva. We received a total of 400 saliva samples, which underwent genetic analysis. All donors but one were excluded. Sample 47 in fact suggested a possible parentage linkage (father–child) with the murderer, showing at least one identical allele for any STR locus analysed, with the profile obtained from semen. Due to these results, the investigators took more information and established that the donor numbered as sample 47 had a 19-year-old son, who had not been typed because he was serving in the military. Immediately, the investigators collected and sent an oral swab of the suspect to our lab. Before the analytical results, he confessed to the crime: his DNA profiling was recognized as identical to that of the semen collected from victim. This is the first example in Italy of parental test used to identify a murderer. Moreover, this is the first report on DNA typing, of the entire population of a small Italian village used for crime investigation. © 2003 Published by Elsevier B.V.

*Keywords:* Reverse paternity analysis; DNA typing; STRs; Probability of paternity

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## **1. Materials and methods**

Stains which were likely due to semen were analyzed by the Sema™ Kit [1] for the detection of the seminal vesicle specific antigen and then submitted to microscopic observation. DNA was isolated from cigarette butts, seminal stains and reference saliva samples from 400 donors, using Qiagen automated extraction [2] and then quantified by slot-blot hybridization [3] with the chemi-luminescent signals recorded by GeneGnome CCD imaging systems [4]. For the amplification and typing we used the AmpFISTR Identifier PCR Amplification Kit [5] and the Powerplex 16.2 PCR Amplification Kit [6]. Electrophoresis was carried out on ABI Prism 310 Genetic Analyzer. Data were analysed by Gene Scan v.3.1 analytical software.

## **2. Reverse paternity and parental analysis**

All the STRs profiles obtained were accurately studied and compared to the one from the sperm to look for the presence of a consistent number of same genotype and/or identical alleles, in order to find out any possible relative of the killer. All the allele/genotype counts were hand made. Reverse paternity analysis was conducted using the Program “Pater version 2.0” [7].

## **3. Results**

A mixed profile was obtained from the sperm evidence, in which the main contributor was an unknown male, and the minor being the victim. In order to obtain the male profile which could be interpreted as best as possible, multiple PCR mixtures were conducted at different dilution of the extracts obtained from the semen traces. All DNA profiles obtained from the cigarette butts did not match the one from the sperm. No other matches were achieved from the first group of felons suspected of the crime, but it was clear and interesting that there was some similarity with the sperm STRs profile. Based on these considerations, after a meeting to which both the prosecutor and the investigators were invited, it was decided that to try to identify the perpetrator, it might be useful to obtain saliva voluntary samples from all males living in the town and compare the genetic data, with the unknown profile obtained from the sperm. All profiles were accurately studied to detect a sufficient number of alleles/loci matches. After almost 300 reference saliva samples tested, two individuals corresponding to two brothers showed a characteristic genetic similarity with the sperm profile, consistent with two loci and nine alleles in common. At this point, looking at the donor list, we decided to analyze a middle-aged man with the same surname of the two brothers, corresponding to their uncle. The profile we obtained from the latter, compared to the killer’s sperm, showed at least the same allele for each of the 17 markers tested, with a likelihood ratio [8] 225,213 odds equal to a probability of paternity of 99.9995559%, hence leading to the hypothesis that he could be the killer’s father. The day after we presented our results, his son, when visited by the investigators, immediately confessed to the murder and gave a saliva sample that, once analyzed, showed to be fully compatible with the one extracted from the sperm left on the victim’s body.

#### **4. Discussion**

A cruel murder without any apparent reason was solved only by a large amount of DNA analyses of male individuals living in the town where the murder took place. After dedicated steps for typing the mixed stains which were consistent with both male and female material left on the victim's body and long and complex work of typing hundreds of reference samples coupled with an exhausting allele count, some hypothetical parental linkages could be achieved. The results obtained from DNA analyses, while exculpating the first group of individuals suspected to be the guilty parties, led to the identification of the alleged father of the killer, given the number of common genotypes/alleles of the DNA profiles obtained. According to this hypothesis, the only son of this man supposed to be the killer's father, was then captured and confessed to the murder, giving a saliva sample which confirmed a complete match with the profile achieved from the semen traces. Once more, this case highlights the importance of very close cooperation between investigators and forensic scientists whose skill, together with an appropriate and affordable analytical strategy, are able to make a fundamental contribution to the truth.

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