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The first successful use of simple low stringency familial searching in a French criminal investigation

ABSTRACT: We describe how a very simple application of familial searching was used to resolve a decade old, high profile rape/murder in France. This was the first use of familial searching in a criminal case, using the French DNA database, which contains approximately 1,800,000 profiles. When an unknown forensic profile (18 loci) was used to search that database using CODIS at low stringency, a single match was identified. This match was the father of the man who left the semen recovered from murder victim Elodie Kulik. The match was confirmed using Y-chromosome DNA from the putative father, an STR profile from the mother, and finally a tissue sample from the exhumed body of the man who left the semen. Because of this identification, the investigators are now pursuing possible co-conspirators. This article describes the combination of methods which led to the match, resulting from open discussions between scientists and investigators.

The scientific basis for familial searching is well established, and the method is commonly used internationally to solve missing persons cases (Butler, 2005). The main principle of familial DNA searching is that relatives share alleles by descent. By searching a database for close but not exact matches of a DNA profile, likely relatives, and coincidental (not by common descent matches are obtained). Recent papers point to the problem of adventitious (coincidental matches) as a hindrance to practical implementation (Rolphs, 2012). But the proper application of likelihood of odds ratios, and the planned expansion of the CODIS core loci to 21 loci, will greatly decrease the number of profiles that will have to be investigated. In the case reported here, using an 18 loci match, a single, possible first degree male relative was identified in the 1,800,000 member French offender and arrestee database.

First used to solve crimes by U.K. forensic scientists in 2003, as of 2011 there were 157 U.K. submitted familial DNA cases, with 93 active, 42 closed or solved by other methods, 8 solved by other means but a relation was in familial results, and 13 resulted in convictions (12%) (Harmon, 2011). Familial searching has been used to solve homicides with great success since 2006 (Bieber, 2006), and recently, its use has been expanded to rape, battery and even property crimes (DenverDa website). This technique is valuable in criminal cases in which DNA profiles have been determined, but no database matches are obtained. The method is presently used in the UK, California, Colorado, and Virginia. It has been championed in the US by Colorado District Attorney Mitchell Morrissey, and was recently used for the first time under exceptional guarantees of public acceptance in California to solve the Grim Sleeper murders (Meyers et al, 2011; Miller, 2010). While it is a generally accepted method in the UK, controversy about expanded use of familial searching in the US still exists (Cassandra et al., 2011), and two US jurisdictions prohibit it by statute, Maryland and the District of Columbia. Several critical studies of the statistical underpinnings and database issues (Rohlfs et al., 2012, Reid et al., 2008), and others concerned with the ethical ramifications of familial searching (Gershaw, 2011; Murphy 2011; Greely et al., 2006) have been published. However, a bill was first introduced in the US Congress in 2011 (H. R. 3361), which may eventually lead to a national policy on familial searching (Kim, 2011). Advocates point out that the tool has the same ability to exonerate as to implicate. One US DNA exoneration has already been partially secured through familial searching which led to the true perpetrator. Daryl Hunt in North Carolina was freed in 2004 after more than 18 years in prison. One author (GH) is working with Innocence Projects in four states that have cases where the method could help identify the true perpetrators of crimes now blamed on men claiming innocence.

Familial searching consists of searching a database to identify probable biological relatives of an unknown DNA profile. Several private software companies and state crime laboratories in the US have developed and validated software that assigns probabilities to the matches. While familial STR DNA searches may find a number of potential relatives, the addition of Y-chromosome haplotypes can narrow the list of possible male relatives significantly. For this reason several jurisdictions require that a Y-Chromosome profile be included in requests for familial searches. The software can typically identify relatives to the second degree (aunts, uncles, grandparents, grandchildren, nieces, nephews, or half-siblings), but probabilities for more distant relationships can also be calculated. Using this method in conjunction with traditional investigatory techniques in a holistic approach has proven useful in the case discussed here, and elsewhere (Budowle, 2010; Gregory and Rainbow, 2011).

In the case we describe here, familial searching was used to solve a 2002 rape/murder in the Picardie region north of Paris. Since this was the first use of this technique by the investigators at the French Gendarmerie Nationale it went through a thorough judicial analysis by the Ministry of Justice. This was especially important since the method had been criticized by some French police experts and trade unionists (Ménielle, 2012).

CODIS Stringency Settings and Familial Searching

When CODIS searches are performed the stringency can be set in the CODIS software at three levels: high (perfect match on all alleles), moderate (partial match, allowing a variable number of match alleles) and low (sharing an allele at each locus). The lower the stringency, the more matches will be detected in the database, and the more potential matches will be expected in the general population.

The stringency parameter addresses two major concerns in searches using suboptimal profiles. The first is the ambiguities introduced through sub-optimal profiles which may include confounding factors such as potential mixtures, and low levels of DNA with potential dropped out alleles. The second problem that can be overcome by lowering the stringency is possible errors in analysis and interpretation, resulting in mis-assigned alleles. It was also clear early on that one could use a moderate or low stringency search to identify relatives of the query profile in the database. Since, one inherits an allele at each locus from each parent, by setting the CODIS stringency low (only a single allele at each locus required for a match), one could identify all children or parents of the query profile. In a similar manner, the stringency may be adjusted to identify possible relatives of any degree, though the number of coincidental (not related by descent) matches increases as the stringency is dropped.

It is important to recognize that a match should always be stated in terms of a probability statistic in relation to a given database. Even high stringency DNA matches in CODIS result in probabilistic statements based on how common a profile is expected in a given population. A perfect high stringency match in CODIS consists of a match on all examined loci (for both alleles) between a forensic profile from a crime scene and that from a convicted offender in the database, and no coincidental 13 locus match between unrelated individuals has been reported. Familial searching is based on the same statistical foundations upon which all genetic match probabilities depend. In the French system, CODIS searches in criminal cases are normally performed at high stringency, with only a difference of only one allele allowed when comparing forensic and database profiles.

Confirming familial relationships using lineage markers

Since the Y-chromosome is passed from father to son without change (except for infrequent mutations), Y chromosome STR analysis is used in many jurisdictions to confirm familiar relationships among men. Mitochondrial DNA analysis can similarly be used to confirm maternal lineage, since mitochondria are passed from mother to child intact. In California, Y-chromosome profiles are required before familial searches will be approved. In the case described here, Y-chromosome profiles were used to demonstrate the family relationship between the familial hit (father), and non-database suspect (son).

Results and Discussion: A French application of familial DNA searching along with forensic intelligence

Familial DNA searching was applied to a 10-year old French murder case last year. The case concerned a high profile rape and murder perpetrated in 2002 in Picardie, a region located about 60 km north of Paris, France. After a car crash, the victim made an 26-second phone call to 911, in which the operator could only hear her screaming, while at least two people were talking in the background. The victim's car was found the following morning. Her body was located the day after, in a waste disposal for grass, 1 mile from her car. She had been raped and strangled to death, and there were obvious signs that the perpetrators tried to burn the body. The case attracted national attention from both the media and justice authorities, but languished without significant leads. The crime took place at night in a rural environment, and no one came forward to provide accurate information. Semen was recovered at the crime scene.

STR analysis were conducted on the semen with two different analysis kits (Powerplex 16® from Promega and Identifiler® from Applied Biosystems) giving an 18 loci genetic profile belonging to an unknown male. This information led to a court order for testing, which was followed by, an unsuccessful mass male sampling that was carried out in the area around the crime scene. Over the years, more than 5300 DNA samples were tested. No matches to the genetic profile were found in the French CODIS system, nor in other European DNA databases, even though a monthly request was sent to Interpol to perform a search in all countries using the CODIS system. This frustration led the investigators to search for an alternative approach.

In 2007 Emmanuel Pham-Hoai was a Gendarmerie lieutenant assigned to the Gendarmerie forensic laboratory (Institut de recherche criminelle de la Gendarmerie nationale-IRCGN) and student working on his Master of Science degree in molecular biology at Cergy-Pontoise University (CPU). Dr. Greg Hampikian met Pham-Hoai when he visited the IRCGN and CPU to discuss the forensic technology collaborations, and new developments in DNA testing. The two began a research collaboration comparing the forensic DNA techniques used different international laboratories. In 2010, Pham-Hoai, now a captain, was assigned as head of the investigation into Elodie Kulik's murder. He was invited by a high ranking Gendarmerie officer serving at the Ministry of Justice to be trained in forensic intelligence techniques being developed at the Gendarmerie Criminal Department in Bordeaux (Crispino, 2006, 2009; Crispino et al., 2009). The Kulik case continued to be investigated without significant progress, and Pham-Hoai was charged with researching new scientific approaches to analyze the forensic evidence.

In October of 2010, Science published a news article "Familial DNA Testing Scores A Win in Serial Killer Case" (Miller, October 2010), which seemed to hold promise in the Kulik case. Pham-Hoai contacted Dr. Hampikian in order to seek his advice about the Science paper, and find out about the state of familial DNA searching in the US and UK where Hampikian was working on cases. He emailed the case facts to Hampikian, who happened to be in a familial DNA search workshop at the International Symposium on Human Identification (ISHI 2010) meeting. Dr. Hampikian discussed the case with panel members, and advised Pham-Hoi that software was available at both the California and Colorado law enforcement laboratories, as well as several private companies, that could identify potential relatives of the semen donor in this case. They discussed the potential barriers to such an approach, and Hampikian spoke with representatives from Denver Colorado District Attorney Mitch Morrissey's office, and the scientists from California who worked on the Grimm Sleeper who were presenting at meeting. The scientists from Colorado were willing to share their software and provide training, and Pham-Hoai began inquiries with the French Justice Department. Hampikian also explained to Pham-Hoai that if permission to use the familial DNA software was not approved, there was a simpler, though less broadly applicable method that could be used by setting the CODIS search parameters for low stringency. In this way, the father of the unknown profile could be identified. The father would necessarily share an allele at every locus, and a brother might also possible be identified. It would not help for more distant relatives, but it was worth a shot. Since such searches were already a part of the French protocols for missing persons cases, no new software would have to be validated.

The Gendarmerie officer began direct contacts with the author of the *Science* article, and the Denver District Attorney's office. He then advised the officer in charge of the CID in Bordeaux about possible implementation. An official request was sent to the French CODIS (*comité technique en charge du* FNAEG) technical board to check the feasibility of a familial DNA search, as the technique had never been used in a French criminal case, while Hampikian researched international applications of familial searching.

From November 2010 to November 2011 discussions concerning the legal and technical matters regarding the request were processed, and further studies were performed in order to satisfy legal requirements. Eventually, the use of special familial searching software was not approved, but a

low stringency search for direct relatives (matching one allele at each examined locus) was considered.

In November 2011, the Director of the French criminal department of Justice granted authorization to perform the first low stringency familial DNA search of convicted offenders and arrestees using the French CODIS system. French law specifically allows such low stringency searches of the CODIS missing persons database in order to match a relative to unidentified remains, but the law does not address the use of low stringency or familial searches in criminal cases. As this was a high profile rape and murder case, extra care was taken to secure support for the procedure.

At that time of the search, the French CODIS held approximately 1,800,000 genetic profiles from convicted offenders and arrestees. The total French population was about 65,000,000 people, with less than 3% of the French population represented in the French DNA database. Current French law requires DNA labs to provide at least 16 loci results for any analyzed DNA profile. Powerplex 16® and Identifiler® each address 16 loci, with 14 loci in common (French criminal procedure law code, article A.38). Each kit also amplifies alleles at two unique loci specific of each kit. This means that by using those two kits for one DNA sample, an 18 loci result can be obtained. French law authorizes the registration of up to 18 loci in a single profile (Legifrance.gouv.fr).

In the case described here, the following low stringency match results were obtained in December 2011:

- ▲ 18 loci : 1 individual
- ▲ 16 loci : 292 individuals

The 18 loci low stringency match was called X1, a man who had been incarcerated at the time of the crime. The crime scene evidence, and the profile of X1 had both been analyzed 10 years earlier using both Powerplex 16® and Identifiler®, resulting in 18 loci genetic profiles. The remarkable single match at 18 loci was soon confirmed with a Y-STR analysis. Since this was not a direct match, and could have been adventitious, complimentary forensic intelligence was required. This was especially true in this first use of familial searching in a French criminal case. The single match at 18 loci was fortuitous as it spared investigators from following up with any of the 292 individuals who matched shared one allele at each locus with the forensic profile.

The Y-STR from those two genetic profiles matched perfectly, supporting with a strong likelihood that the source of the trace was a male of X1's family in direct patrilineal lineage (Butler, 2003; Gusmao, 2006). A study of his genealogy through traditional investigative means, including public records, was conducted in order to identify male members of the family whose ages indicated that they may have contributed the forensic semen sample.

The family of X1 originated from, and lived close to, the crime scene. Within the male lineage, X1's father was identified, along with his two sons. Because of his advanced age, X1's father was eliminated, as was one of the sons who was a young child when the murder occurred. That left only the older son, who had died a few months after the crime at age 23. The genetic evidence, and his age led detectives to identify him as the primary suspect. His death shortly after the crime, explained why he was not identified during the mass DNA screening, which occurred after his death.

While these facts were compelling evidence, the secrecy of the investigation required forensic intelligence that could be gathered unobtrusively. Investigators immediately considered exhumation, but decided that it would potentially alert his accomplices of the investigation. Instead, investigators obtained a DNA sample from the mother of the deceased suspect. Her profile contained at each locus the allele in the evidence sample not supplied by X1, the suspect's father.

With all parental alleles accounted for, the detectives decided to exhume the body, but only after obtaining permission to investigate and follow up on the identified the social contacts of the deceased son, and designing a plan for working with the press.

Finally, on January 16th, 2012, the Prosecutor of the jurisdiction publicly announced that 10 years after the murder of Elodie Kulik, a suspect had been identified thanks to a new method of DNA analysis. While there was significant controversy about the familial search approach, the concordance between the exhumed profile and the evidence put to rest any question of about the efficacy of low stringency familial searching in this case. The investigation has now shifted to identifying the accomplices recorded on the 911 tape.

The French DNA Database and Future Use of Familial Searching

From the perspective of French law and the French code of criminal procedure, an offender entered into the French CODIS system has a diminished expectation of privacy once he or she has been convicted. When condemned by a judge, the DNA profile of an offender remains in the database for 40 years. The French DNA database currently contains DNA from suspects and condemned individuals, and French law allows suspects, once they have been cleared by the investigation, to petition the prosecutor to remove their genetic profile. This paper describes the first use of familial searching in France, and although the French Department of Justice approved its use in this case, many significant civil rights questions remain to be settled concerning its future use.

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