CASE REPORT

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Paternity Analysis when the Putative Father is Missing: First Case in Chile*

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ABSTRACT: Genetic marker analysis is a powerful tool for solving paternity-related problems when the putative father is missing. This report describes the first time this approach was employed in Chile to solve such a problem. In the case presented, the alleged father was missing as a result of the political detentions that took place in Chile during 1973. It was not possible to obtain any biological sample from him because he was missing. Thus, the case was resolved by means of genetic marker analysis of the alleged father's close relatives.

KEYWORDS: forensic science, paternity analysis, DNA typing, alleged father missing, D1S80, D12S1090, D3S1744, D18S849

To solve paternity problems, one often relies on genetic marker analyses of the persons involved (1,2). The incorporation of DNA polymorphism typing into paternity analysis in the last decade represents a major advance since it allows one to achieve a high probability of exclusion. These polymorphisms are very informative genetic markers even when the alleged parents are close relatives (3).

Special cases of paternity analyses are those in which the putative father is unavailable for testing and his genotype must be deduced from that of his close relatives.

This report focuses on a case in which the alleged father was missing and his son wanted to establish paternity. Consequently, it was necessary to rely on genetic information provided by relatives of the alleged father.

Case Report

This case was sent to the Servicio Médico Legal of Chile by the Court of Appeals of the country to determine if the citizen ER was the son of EE. In Chile, the last name of a child is determined by his father's last name.

At the time of this writing Mr. EE was still missing as a consequence of the "disappearances" that occurred in Chile following the 1973 military coup. In Chile, during the military government, "disappearance" was frequently an euphemism for imprisonment in the case of leftist opponents to the government. After the democratic elections of 1989, many of these "disappearances" were reported. Among the disappearances reported was that of Mr. EE who was one of the leaders of a leftist revolutionary movement. At the time of Mr. EE's detention (Sept. 1973) Mrs. GW was his legitimate wife. They had a son and Mrs. GW was expecting their second child. However, when this second child was born, his mother decided, to protect him by giving him a different last name. Thus, the newborn was registered in the National Register of Births as ER.

Later, when Mrs. GW was interrogated by the military, she stated that she and her husband had been separated and the son was actually the result of her union with another man: Mr. R. In reality, Mr. R had not been romantically involved with Mrs. GW. Mr. R had only recognized the child as his own for humanitarian reasons.

Subsequently, Mrs. GW and her two children moved to Cuba, where they rebuilt their lives. In Cuba, however, her second child was known by his real last name: E. Accordingly, all his legal documents were registered using E, not R. Unfortunately, when he wanted to return to Chile, more than 20 years later, this created a great deal of confusion because Chilean records only acknowledged the existence of ER; EE did not exist in any Chilean records.

Prompted by this situation, his mother (Mrs. GW), initiated a number of legal proceedings in Chile to re-establish his identity as the son of EE, namely, to change the Birth Certificate to EE from ER. As part of this process, the Court of Appeals of Santiago ordered a DNA study to verify the affiliation of the citizen known in Chile as ER.

Materials and Methods

DNA was prepared from blood samples obtained by venipuncture from the child whose identity needed to be clarified, his mother, his alleged full brother, and his alleged paternal grandparents. The DNA was extracted by the organic method described by Budowle et al. (4).

The D1S80 typing by PCR (5) employed the primers and the protocol described by Kasai et al. (6). Amplification was carried out in a Perkin-Elmer DNA Thermal Cycler 9600 using 5-20 ng of template DNA. The amplified products were resolved by electrophoresis on vertical polyacrilamide gel (0.4 mm thick) and silver staining (7).

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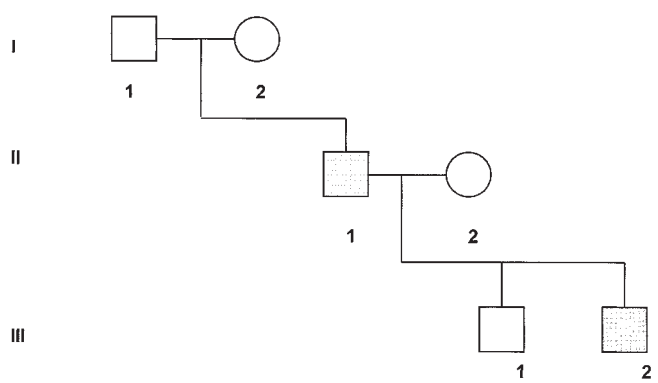
The D12S1090, D3S1744, and D18S849 typing was performed using the "Quick-Type Multiplex I" kit from Lifecodes Corp. (Stamford, CT). Amplification was carried out in a Perkin-Elmer DNA Thermal Cycler 9600 following the manufacturer's recommendations using 5 ng of template DNA. The fragments were resolved by electrophoresis on denaturing (urea) polyacrylamide gel (0.4 mm thick) and silver staining (7).

Results

Figure 1 shows the phenotypes of the relatives of the child under investigation. To explore if there was an exclusion each locus was analyzed taking into account the alleged father's genotypes which were "rebuilt" from the genotypes of his parents and his oldest son. For instance, consider the D1S80 locus. The genotypes of the alleged father's parents are 33/31 and 24/31. Therefore, the putative father's possible genotypes are 33/24, 33/31, 31/24 and 31/31. If we take into account the oldest son's genotype (33/22), it can be concluded that the alleged father must carry the 33 allele because the 22 allele is from maternal origin. Thus, the putative father's genotype must be either 33/24 or 33/31. To complete this paternity analysis all possible genotypes of the alleged father must be considered. Analyzing all the information from the four genetic markers typed, one can conclude that there is not exclusion of paternity because for all loci studied, the allele that the child received from his biological father was present in one of the alleged father's possible genotypes.

To determine the evidence if the alleged father was the true father we computed the paternity index (8,9). Such index is a likelihood ratio that allows one to estimate the *a posteriori* probability that the man under investigation could be the biological father, based on Bayes' Theorem (when the prior probability of H1 = paternity and H2 = non paternity are assumed to be equal (10,11). The likelihood ratio L is the probability of genetic evidence given H1 divided by the probability of the evidence given H2.

$$L = \frac{\Pr(A, B, C, D, E/H1)}{\Pr(A, B, C, D, E/H2)} \quad (1)$$



PHENOTYPES

LOCUS	I-1	I-2	II-1	II-2	III-1	III-2
D1S80	33/31	24/31	?	31/22	33/22	31
D12S1090	20/22	22/29	?	12/25	12/29	12/29
D3S1744	18/21	18/21	?	18/21	18/21	18
D18S849	16	16	16	16/17	16	16

FIG. 1—*Individuals: I-1: Grandfather, I-2: Grandmother, II-1: Alleged father missing, II-2: Mother, III-1: First son, and III-2: Questionated Son.*

where

A be genotype of Grandfather
B be genotype of Grandmother
C be genotype of Mother
D be genotype of First Son of Mother
E be genotype of Second Son of Mother

The two propositions are:

H1: The two sons had the same father
H2: The two sons had different father

If we analyze n independent genetic markers, the joint paternity index is

$$L = \prod_{i=1}^{n} L_i \quad (2)$$

The *a posteriori* probability that the alleged father could be the biological father (when a priori probability of 0.5 is assumed) is:

$$P = \frac{L}{L + 1} \quad (3)$$

No typing is available for the father. Let X be the genotype of the Mother's husband. This man is the son of the Grandfather and Grandmother and he is the father of the First Son. Under H1, X is also the genotype of the father of the second son. Let Y be the genotype of the father of the Second Son under H2. Even though this man is named, he has not been typed, so will be regarded as being random. The expression (1) is most easily evaluated by arranging genotypes of individuals to be conditional on the genotypes of their parents:

$$L = \frac{\Pr(D, E/A, B, C, H1)\ \Pr(A, B, C/H1)}{\Pr(D, E/A, B, C, H2)\ \Pr(A, B, C/H2)} \quad (4)$$

$$= \frac{\Sigma_x \Pr(D,E/X, B, C, H1)\ \Pr(X/A, B, C/H1)\ \Pr(A, B, C/H1)}{\Sigma_{x,y} \Pr(D, E/X, Y, A, B, C, H2)\ \Pr(X, Y/A, B, C/H2)\ \Pr(A, B, C/H2)}$$

$$= \frac{\Sigma_x \Pr(D,E/X, C, H1)\ \Pr(X/A, B)\ \Pr(A)\ \Pr(B)\ \Pr(C)}{\Sigma_{x,y} \Pr(D/X, C, H2)\ \Pr(E/Y, C, H2)\ \Pr(X/A, B)\ \Pr(Y)\ \Pr(A)\ \Pr(B)\ \Pr(C)}$$

$$= \frac{\Sigma_x \Pr(D,E/X, C, H1)\ \Pr(X/A, B)}{\Sigma_{x,y} \Pr(D/X, C, H2)\ \Pr(E/Y, C/H2)\ \Pr(X/A, B)\ \Pr(Y)}$$

$$= \frac{\Sigma_x \Pr(D, E/X, C, H1)\ \Pr(X/A, B)}{[\Sigma_x \Pr(D/X, C, H2)\ \Pr(X/A, B)]\ [\Sigma_y \Pr(E/Y, C, H2)\ \Pr(Y)]}$$

This development has assumed independence of A, B, C, X, Y.

For the four loci typed, Table 1 shows the computation of the paternity index (L). Based on gene frequencies of the Chilean population for these alleles (Table 2), an overall likelihood ratio of 234.38 is obtained (12), which corresponds to an *a posteriori* probability of 0.99575 that the alleged father could be the biological father.

The Court of Appeals accepted this analysis as evidence that the person known in Chile as ER was actually the son of EE and consequently proceeded to change the National Register of Births.

Discussion

DNA polymorphisms are very informative genetic markers and they allow one to achieve a high probability of exclusion even

TABLE 1—*Computation of the paternity index for the four loci analyzed.*

		Under H1:		
X	33, 31	20, 29 or 22, 29	18, 18 or 18, 21	16, 16
Pr(D, E/X, C, H1)	1/16	1/16 or 1/16	1/4 or 1/8	1/4
Pr(X/A, B)	1/4	1/4 or 1/4	1/4 or 1/2	1
Σ_xPr(D, E/X, C, H1)Pr(X/A, B)	1/64	1/32	1/8	1/4
		Under H2:		
X	33, 31 or 33, 24	20, 29 or 22, 29	18, 18 or 18, 21 or 21, 21	16, 16
Pr(D/X, C, H2)	1/4 or 1/4	1/4 or 1/4	1/2 or 1/2 or 1/2	1/2
Pr(X/A, B)	1/4 or 1/4	1/4 or 1/4	1/4 or 1/2 or 1/4	1
Σ_xPr(D/X, C, H2)Pr(X/A, B)	1/8	1/8	1/2	1/2
		Under H2:		
Y	33, 31 or 31, $\overline{31}$	29, 29 or 29, $\overline{29}$	18, 18 or 18, $\overline{18}$	16, 16 or 16, $\overline{16}$
Pr(E/Y, C, H2)	1/2 or 1/4	1/2 or 1/4	1/2 or 1/4	1/2 or 1/4
Pr(Y)	p^2_{31} or $2p_{31}p_{\overline{31}}$	p^2_{29} or $2p_{29}p_{\overline{29}}$	p^2_{18} or $2p_{18}p_{\overline{18}}$	p^2_{16} or $2p_{16}p_{\overline{16}}$
Σ_yPr(E/Y, C, H2)Pr(Y)	$p_{31}/2$	$p_{29}/2$	$p_{18}/2$	$p_{16}/2$
L	$1/(4p_{31}) = 2.27$	$1/(2p_{29}) = 23.81$	$1/(2p_{18}) = 1.47$	$1/(4p_{16}) = 2.95$

TABLE 2—*Gene frequencies of involved alleles in the Chilean population.*

D1S80	D12S1090	D3S1744	D18S849
31 = 0.11	29 = 0.012	18 = 0.341	16 = 0.339

when the alleged father is missing. The case reported here demonstrates the usefulness of locus specific DNA analysis to solve paternity studies when the putative father is absent. In these cases, the alleged father's genotype can be reconstructed from the genetic information available from his first degree relatives. Multilocus DNA probe typing do not allow rebuilding a genotype of a missing person based on his relative's genotypes.

Other cases of missing putative parents have been reported in Argentina (13). In that country, those cases were solved by gathering information from the grandparents of the children whose parents were missing.

The case reported in this paper was the first of this type investigated in Chile. Also, this was the first time in Chile that a Birth Register was changed as a result of biological evidence.

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