STRs, Y-STRs, Amp-FLP, SLPs and MLP Identified the Victim of a Suicide

Andrea Sala[1], Nancy Iannucci[1], Gustavo Penacino[1], Oscar Chiappetti[2], María Pivas[2], Graciela Pross Laporte[2], Carlos Falcón[2] and Daniel Corach[1].
[1]Servicio de Huellas Digitales Genéticas and Cátedra de Genética y Biología Molecular-Fac. Farmacia y Bioquímica Buenos Aires University, Junín 956, 1113-Buenos Aires Argentina
[2]Poder Judicial de la Provincia de Entre Ríos, Argentina

INTRODUCTION

At present, identification of a person alive or dead is a routine process that may involve a wide range of technical approaches. Information provided by dactiloscopy, odontology, radiology and/or tomography might lead to unbiased identification, especially if previous information is available, although DNA typing technology is the most precise and robust mean for identification purposes. By comparing genetic profiles of closely related persons it is possible to establish biological kinship. Some cases in which politically relevant figures are concerned usually require an overwhelming effort to convince the public opinion of the identity of that person. Even though objective and confidential demonstration of an individual's identity may require routinely about ten to twelve STR and four VNTR typing, in this case a much higher number of genetic markers were used in order to provide comparable data for all second labs that may be required to perform the test. The aim of this paper is to present the results obtained during the DNA-based identification of the remains of a powerful and wealthy executive thought to be in close contact with the Argentine establishment.

THE STORY

A photojournalist was found dead and burned in a sand-pit in southeastern Buenos Aires province. His last job was about the social and economical life of a powerful man, owner of most of the mail service of Argentina and several other businesses (including customs control), unknown before the journalistic investigation. After staking out the mogul's home, the photographer snapped a picture of the businessman. He was widely quoted as saying "taking a photograph of me is like shooting me in the head". Months later, at dawn on Jan 25, 1997, the photographer was abducted. A fisherman found the smoldering wreckage of his car in a ditch, his charred body on the front seat. He had been handcuffed, shot in the temple, doused with kerosene and set ablaze. After about seventeen months of investigations, two hundred and sixty six policemen dismissed, protests and several arrests, the judge found a possible connection between the homicide and the businessman. The judge required him to declare last March. His absence from the trial resulted in a police search for him across the country.

On May 20th, as police surrounded his house in Entre Ríos Province, the nation's most powerful businessman apparently put a 12-bore shotgun into his mouth and pulled the trigger. He was hastily buried in Buenos Aires. A judge ordered the family not to cremate the body. During the autopsy direct recognition (by relatives), dactiloscopic, radiographic, tomographic and odontological procedures identified the corpse. However, social and political pressures forced the judge to order a DNA analysis in order to confirm the identity of the body because most of the people did not believe that the owner of six hundred million U.S. dollars committed suicide.

EXPERIMENTAL STRATEGY

The investigation required three steps. First, to certify the genetic identity of all the tissues received. Second, to trace patrilineage by Y-STRs in both cadaveric material and putative sons. Third, to compare the genetic profiles of the putative sons, their mother and the cadaveric material in order to determine biological kinship between the remains and the offspring.

MATERIALS AND METHOD

Tissue Samples

DNA was extracted from seven different body tissue samples including muscle, spleen, lung, kidney, liver, hair and blood. Blood sample of putative sons and their mother were used as DNA source. In all cases DNA extraction protocol was the CTAB method. Samples from cadaveric materials were purified by Microcon 100 (Amicon, USA).

AMPLIFICATIONS

CTT, FFv and Silver STR™ III triplexes were amplified according with Promega protocol (Protocol Number 10, *GenePrint® STR Systems Technical Manual*,

Rev. 11/97). Amplicons were electrophoresed in 6% acryl-bisacrilamide (38:2) denaturing gels, at 60 Watts fixed. Profile detection was carried out by silver staining (*DNA Silver Staining System*-Promega).

HUMFABP, D6S366 and Y chromosome specific STRs were amplified in the presence of α^{32} PdATP. After electrophoresis gels were transferred to Whatman 3MM paper and exposed to radiographic film (Kodak X-Omat) from two hours to overnight. D1S80 were performed as previously described (1).

Genomic DNA restricted with Hae III were southern blotted and analyzed with seven chemiluminescent single locus probes (SLPs) including LH-1, PH-30, MS-1, CEB-42 (Life Technologies, USA), YNH-24, TBQ-7 and EFD-52 (Promega Corp. USA). Finally, the multilocus probe (MLP) 33.15 (Cellmark) was used on the same membrane in accordance with conventional procedures (2).

DATA ANALYSIS

Matching probability was estimated in accordance with NRCII report recommendations (3). Paternity index was calculated assuming a prior probability of 50% using Essen Moller equation. Calculations were based on local frequency distributions (4,5) for all the markers employed, except for CEB42, where the American Caucasian database from Life Technologies was used. Combined Y-chromosome specific STRs were computed as a haplotype and its frequency was obtained from our local databases (6). Analysis of 33.15 was performed as previously described (7).

RESULTS

To ensure the precision of the results for the ongoing investigation, all techniques and systems routinely available in our lab were employed. Over 600 gr. of diverse body tissue was received for analysis. In order to determine the genetic identity of all the different tissues supplied, each DNA sample was analyzed independently. Matching probability (MP) for each system and cumulative MP for all hypervariable markers are presented in Table 1. Table 2 depicts total MP obtained. Observed genetic identity in all DNA samples investigated allowed us to confirm that all of them belonged to a single individual.

By comparing Y-STRs profiles of the putative sons and the body samples it was possible to trace the patrilineage. Genetic identity of all the Y microsatellites confirmed that the putative sons belonged to the same patrilineage as the corpse whose tissues were investigated. Although seven Y-STRs systems were typified, only five of them were considered in the statistical calculations. Since the haplotype detected in the samples investigated had not been previously observed in our population database a frequency of 0.03 was assigned (Jeffreys, personal communication).

By means of eleven autosomal STRs the initial results for the paternity test were obtained. Only three triplexes CTT, FFv and SilverSTR™ III, gave a Paternity Indice (PI) equivalent to four single locus probes.

Some SLPs profiles obtained with the body tissues showed band shift, in particular in those restriction bands with low molecular weight suggesting the presence of contaminants. However, no band shift was observed in the DNA sample extracted from the blood obtained from the corpse. DNA extracted from hair was only typed by means of STRs. Tables 3 and 4 summarized PI and cumulative PI in all single locus markers with respect to one of the children analyzed. Similar PIs were obtained with respect to the other one. A rare genotype profile of D1S80 (18-21) was observed in the body samples. Its frequency in our database is 0.02. The second variant was shared with one of the putative son, with a PI=16. After seven SLPs assays, the membrane was finally probed with one multilocus probe: 33.15. Over 15 individual-specific bands were counted. Paternity indices of $3x10^3$ and $1.7x10^5$ were attained for the putative sons.

Cumulative paternity indices of the man whose remains were investigated regarding their putative sons were in the order of 10^{13}.

DISCUSSION

In some instances identification can only be accomplished by DNA typing approaches, especially in those cases in which skeletonized, putrified or fragmented human remains are the materials to be investigated (8, 9, 10, 11, 12). In other cases, in which abundant odontological, radiological, tomographical and dactiloscopical identificatory information has been accumulated, molecular identification might be redundant. The present case may be included in the second category. However, since the victim was so powerful, public opinion doubted his identity. For this reason all effort was made to obtain maximum identificatory data. In addition to all the conventional procedures employed, during the autopsy abundant tissue samples were selected from diverse organs for molecular analysis. This strategy was twofold, on the one hand to ensure that no person would be able to survive without these fragments, and on the other hand to provide enough material for additional molecular tests.

The combined use of different DNA based typing approaches including 27 systems allowed certifying the paternity of the man whose tissues were investigated in comparison with his putative sons.

In a short span of time the forensic genetic trend was towards the development of an increasing number of more sensitive and reproducible genetic markers to be employed in human identification. However, this exponential growth has not been accompanied by public opinion accepting such overwhelming objective information. Should we therefore increase further the number of such markers?

CONCLUSIONS

1. All tissue samples belonged to a single person. The match probability was in order 1 $x10^{27}$

2. These remains and two putative sons belonged to the same patrilineage

3. The Paternity Index obtained, with respect to each son was $3x10^{13}$ and $1x10^{11}$, respectively. Accordingly the Probability of Paternity was over 99.99%.

EPILOGUE

After the publication of the DNA report the photographer's family requested the judge to order another DNA study. Due to the robustness of the identification techniques applied, the judge rejected the request. However, even today some people still doubt that the businessman is dead.

ACKNOWLEDGMENT

This work was supported in part by FA019 UBACYT grant to D. Corach.

REFERENCES

1. Budowle B., Chakraborty R., Giusti A.M., Eisemberg A.J., Allen R.C. (1991) Analysis of the VNTR locus D1S80 by the PCR followed by high resolution PAGE. *Am.J.Hum.Genet.* **48**: 137-144.
2. Budowle B., Baechtel F.S., Comey C.T., Giusti A.M., and Klevan L. (1995) Simple Protocols for Typing Forensic Evidence: Chemiluminescent Detection for Human DNA Quantitation and Restriction Fragment Length Polymorphism (RFLP) Analyses and Manual Typing of Polymerase Chain Reaction (PCR) Amplified Polymorphisms. *Electrophoresis* Sep **16(9**): 1559-1567.
3. National Research Council (1996) The Evaluation of Forensic DNA Evidence. USA.
4. Sala A., Penacino G., and Corach D. (1997) VNTR Polymorphism in the Buenos Aires-Argentina Metropolitan Population. *Hum. Biol.* Dec. **69(6)**:777-783.
5. Sala A., Penacino G. and Corach D. (1998) Comparison of Allele Frequencies of Eight STR Loci from Argentinian Amerindian and European Populations. *Hum. Biol*. Oct.**70(5)**:937-947.
6. Kayser M., Caglia A., Corach D., Fretwell N., Gehrig C., Graziosi G., Heidorn F., Herrmann S., Herzog B., Hidding M., Honda K., Jobling M., Krawczak M., Leim K., Meuser S., Meyer E., Oesterreich W., Pandya A., Parson W., Penacino G., Perez-Lezaun A., Piccinini A., Prinz M., Schmitt C., Roewer L., et al (1997) Evaluation of Y-chromosomal STRs: a Multicenter Study. *Int. J. Legal Med*. 110(3):125-133
7. Krawczak M., and Bockel B. (1993) The Formal Analysis of Multilocus DNA Fingerprints. EXS **67**: 249-255.
8. Hagelberg E., Gray I.C., Jeffreys A.J. (1991) Identification of the Skeletal Remains of a Murder Victim by DNA Analysis. *Nature* Aug 1, **352(6334)**: 427-429
9. Holland M.M., Fischer D.L., Mitchell L.G., Rodriguez W.C., Canik J.J., Merril C.R., and Weedn V.W. (1993) Mitochondrial DNA Sequence Analysis of Human Skeletal Remains: Identification of Remains from the Vietnam War. *J. Forensic Sci.* **38**: 542-553
10. Corach D., Sala A., Penacino G., and Sotelo A. (1995) Mass Disasters: Rapid Molecular Screening of Human Remains by Means of STRs Typing. *Electrophoresis* **16 (9)**:1617-1623.
11. Olaisen B., Stenersen M., Mevag B. (1997) Identification by DNA Analysis of the Victims of the August 1996 Spitsbergen Civil Aircraft Disaster. *Nat. Genet.* Apr, **15(4)**: 402-405.
12. Corach D., Penacino G., Sala A., Iannucci N., Bernardi P., Doretti M., Fondebrider L., Ginarte A., Inchaurregui A., Somigliana C., Turner S., and Hagelberg E. (1997) Additional Approaches to DNA Typing of Skeletal Remains: The Search for Missing Persons Killed During the Last Dictatorship in Argentina. *Electrophoresis* **18(9)**: 1608-1612.

Table 1: Matching probabilities across loci

Microsatellites

	CSF1PO	TH01	TPOX	FES	F13A01	vWA	D13S317	D7S820	D16S539	D6S366	FABP
MP	1:11	1:19	1:3.8	1:4.8	1:7.8	1:9.8	1:20	1:13	1:6.8	1:21	1:3
MP_T		1:215	1:817	$1:3.9X10^3$	$1:3.0X10^4$	$1:3.0X10^5$	$1:6.1X10^6$	$1:8.2X10^7$	$1:5.7X10^8$	$1:1.2X10^{10}$	$1:3.7X10^{10}$

Minisatellites

	D1S80	YNH24	TBQ7	EFD52	LH1	MS1	PH30	CEB42
MP	1:67	1:53	1:110	1:58	1:558	1:109	1:84	1:82
MP_T	1:67	1:3551	$1:3.9X10^5$	$1:2.2X10^7$	$1:1.2X10^{10}$	$1:1.4X10^{12}$	$1:1.1X10^{14}$	$1:9.5X10^{15}$

MP: Matching Probability
MP_T: Cumulative Matching Probability

Table 2: **Cumulative Matching Probabilities**

	Y-Specific STRs	Autosomal STRs	AmpFLP	SLPs	Total
MP_T	1:33	$1:3.7x10^{10}$	1:67	$1:1.4x10^{14}$	$1:2.7x10^{27}$