ELSEVIER

Legal Medicine 7 (2005) 314–318

LEGAL MEDICINE

www.elsevier.com/locate/legalmed

Brief communication

DNA typing from skeletal remains following an explosion in a military fort—first experience in Ecuador (South-America)

Fabricio González-Andrade*, Dora Sánchez

Laboratory of Molecular Genetics, Metropolitan Hospital, Edificio Meditrópoli, Subsuelo 2, Av. Mariana de Jesús Oe8 y Occidental, Quito, Ecuador

Received 12 July 2004; received in revised form 5 January 2005; accepted 12 May 2005
Available online 16 August 2005

Abstract

We present individual body identification efforts, to identify skeletal remains and relatives of missing persons of an explosion took place inside one of the munitions recesses of the Armoured Brigade of the Galapagos Armoured Cavalry, in the city of Riobamba, Ecuador, on Wednesday, November 20, 2002. Nineteen samples of bone remains and two tissue samples (a blood stain on a piece of fabric) from the zero zone were analysed. DNA extraction was made by Isoamilic Phenol–Chloroform–Alcohol, and proteinase K. We increased PCR cycles to identify DNA from bones to 35 cycles in some cases. An ABI 310 sequencer was used. Determination of the fragment size and the allelic designation of the different loci was carried out by comparison with the allelic ladders of the PowerPlex©16 kit and Gene Scan Analysis Software® programme. Five possible family groups were established and were compared with the profiles found. Classical Bayesian methods were used to calculate the Likelihood Ratio and it was possible to identify five different genetic profiles in our country. This paper is important because is a novel experience for our forensic services, because this was the first time DNA had been used as an identification method in disasters, and it was validated by Ecuadorian justice like a very effective method.
© 2005 Elsevier Ireland Ltd. All rights reserved.

Keywords: Ecuador; STR; Human remains; Skeletal remains; DNA typing; Explosion; Forensic science

1. Introduction

On Wednesday, November 20, 2002 at approximately 4.45 pm an explosion took place inside one of the munitions recesses of the Armoured Brigade of the Galapagos Armoured Cavalry, in the city of Riobamba, province of Chimborazo, leaving seven dead, over 100 injured and five individuals missing. The explosion took place inside a recess containing military arms and ammunition and it is believed that it was caused by accidental detonation. Analyses were carried out by the Molecular Genetics team of DIAGEN (Diagnostics and Human Identification Co.) using the relevant internal protocols [1]. Various forensic techniques are used today to identify a human corpse, depending on the circumstances and the states of remains. The four most common methods, anatomical and biological, are identification of the remains by a living person who knew the deceased by direct facial recognition of special features, such as scars or marks (tattoos); matching of fingerprints (if pre-mortem linked prints are available); dentition (if pre-mortem dental records are available); and DNA analysis [2].

Relevant efforts have been continuously made to identify cadavers and human remains after wars, socio-political disturbances, and mass disasters. In many cases, the use of DNA typing techniques offers a definitive answer for identification of victims and thus a direct social benefit is realized [3]. Although DNA analysis is a highly discriminatory method, it is not self-sufficient and could not replace an anthropological evaluation [4]. Amplification and typing of DNA extracted from compact bone of human remains could be useful in establishing the identity of a person, as well as in excluding possible false identifications [5]. Body identification is made by using the results from relatives blood samples and information gathered from family trees, to predict the genotype of the deceased family member, in a paternity style analysis [6].

* Corresponding author. Tel.: +593 2 2269354.
E-mail address: fabriciogonzalez@usa.net (F. González-Andrade).

1344-6223/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.legalmed.2005.05.002

Use of personal effects. The true power of DNA testing lies in polymorphism at the individual loci and the number of loci tested. To estimate multi-locus genotype frequencies in this manner, the alleles at each locus must be inherited independently (Hardy–Weinberg equilibrium) and the alleles among loci must be inherited independently (linkage equilibrium) [7]. We used CODIS loci, currently validated for forensic and paternity analysis, and overview of worldwide data on the extent polymorphism at 13 STR[1] loci indicates that together have adequate power to resolve most forensic cases [8].

The number of family members. Several authors [9] describe two types of situations for DNA testing, called close and open studies. Close studies are those in which the remains, where a family member has recognized a personal item they believe belonged to the individual the family member claims is missing, or where some form of identification has been found on or near the body, and there is a general agreement on physical characteristics between ante-mortem and post-mortem data. In other words, when we know personal identity a priori. An open study is when the identities of all the victims are not known a priori. Open cases involve remains, where there is a little or no information as to identify individuals. Our case was a closed study, we knew four missing persons. The fifth identification it was based in the analysis of a corporal fragment of a body previously buried. Given the fragmentary nature of many remains the most likely explanation is that these samples represent part of the same fragmented body. Most of the cases, the quality of the DNA extracted from long bones is higher than extracted from skulls or ribs protocols, and it is possible to see the presence of minimal amounts of degraded human DNA mixed with high amounts of microbial DNA.

2. Material and methods

2.1. Laboratory organization

We used different laminar flow cabinets. Ultraviolet (UV) irradiation and treatment with 10% bleach were used to eliminate possible DNA contaminants from cabinets and laboratory surfaces.

2.2. Selection of samples

Nineteen samples of bone remains and two tissue samples (a blood stain on a piece of fabric) from the zero zone of the explosion were analysed. Samples were taken from the family members of those feared missing.

[1] STRs (micro satellites) analysed: D3S1358, HUMTH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, HUMCSF1PO, Penta D, HUMvWA, D8S1179, HUMTPOX, HUMFGA, Amelogenine.

2.3. DNA extraction

Isoamyl Phenol–Chloroform–Alcohol, and proteinase K were used for the bone remains [10,11]. First, we incubated selected fragment in SDS 10% solution, at 37 °C, overnight with agitation. After, we cleaned the surface and we took deep layer. We weighed 4 g of the bone, and washed it with EDTA 0.5 M, pH 8.0 by three times, for 4 h each time. Then we digest the sample with 8 mL from EDTA, proteinase K (100 mg/mL), 500 mL and Tween 20, 80 μL. Product was incubated overnight at 37 °C. We used organic extraction with Phenol–Chloroform–Alcohol Isoamilic. We washed two times with Phenol equilibrated, and after we used Phenol/chloroform (1:1), twice. Finally, we concentrated liquid phase with Centricon© 100.

2.4. PCR

Amplification was carried out in Genius© model thermocyclers, in accordance with the manufacturer's recommendations. The lower limits of sensitivity recommended by manufacturers of STR multiplex systems [12] are 250 pg. Multiplexes usually work at their optimum efficiency when 1 ng of DNA is analysed and not more than 28–30 cycles of amplification are carried out [13]. We re-analysed 11, 17 and 20 samples because we obtained only a partial profile. We increased PCR cycles to identify DNA from bones to 35 cycles.

2.5. Typing

An ABI310 sequencer was used. We used the PowerPlex 16 system from Promega and Gene Analysis Software® programme [14]. We followed the recommendations of the DNA Commission of the International Society of Forensic Genetics for STR analysis [15,16].

2.6. Procedure control

For internal quality control purposes, target controls were performed in the extraction and amplification of each reaction. A positive human DNA control of cell line 9947A was used following typing by all the systems. For external quality control, the laboratory takes an annual proficiency test with the International Society for Forensic Genetics (GEP-ISFG) [17].

2.7. Data analysis

Having found the genetic profiles of those missing, we proceeded to compare them with those of their family members. Five possible family groups were established and they were compared with the profiles found. Classical Bayesian methods were used for the calculation of the Likelihood Ratios (LR). The system we use includes 13 micro satellites that are part of the CODIS Combined DNA

Table 1
Samples analysed

Sample No.	Tissue type	Initial description given by the forensic physician	Description given by DNA laboratory
1	Bone	Long, unidentified bone fragment	Distal epiphysis of right fibula
2	Bone	Head of undetermined joint	Head of right humerus
3	Bone	Rib fragment	Rib fragment
4	Bone	Knee joint	Distal epiphysis of right femur
5	Bone	Patella	Patella
6	Bone	Skull fragment	Skull, parietal bones (sagittal suture)
7	Bone	Skull fragment	Skull, temporal fragment
8	Bone	Head of fibula	Charred, not identified
9	Bone	Femur fragment (exhumation)	Femur fragment (exhumation)
10	Tissue	Muscle fragment (exhumation)	Muscle fragment (exhumation)
11	Bone	Knee joint	Distal epiphysis of left femur
12	Bone	Talus fragment	Left calcaneum
13	Tissue	Piece of uniform—blood stains	Piece of uniform with blood stains
14	Bone	Not identified	Not identified
15	Bone	Wrist fragment	Fragment of left wrist
16	Bone	Flat unidentified bone	Flat bone, fragments of right iliac
17	Bone	Humerus fragment	Fragments of talus and left calcaneum
18	Bone	Fragment of left radius	Fragments of distal epiphysis of left ulna
19	Bone	Not identified	Not identified
20	Bone	Not identified	Not identified
21	Bone	Upper jaw	Left upper jaw with six teeth

Index System (FBI-USA). We used our own databases published previously [18,19].

3. Results

Table 1 shows samples analysed. We selected several skeletal remains and were reclassified by our team. Table 2 shows genetic profiles found in all the samples, and in Table 3, we can observe correlation with presumptive relatives. We established five familiars groups, with different situations each one. It was difficult to locate relatives because everybody had their home far from disaster zone.

4. Discussion

The utility of STRs to analyse highly degraded DNA samples in real casework was dramatically demonstrated by the identification of human remains from disasters such as Waco, Texas (14,15,77); the Spit Bergen disaster (53); TWA flight 800 (6); Swiss-air flight 111 (29); the 1998 Philippines air-crash (40). However, Olaisen was able to use minisatellites in the Spit Bergen disaster with a 100% success rate because prior to collection, the bodies were at an ambient temperature of ∼0 °C and were well preserved. Conversely, with the Waco disaster bodies were badly burned and subsequently highly decomposed after several days at an ambient temperature of ∼30 °C., hence

Table 2
Genetic profiles found

Genetic system analysed	Profile 1	Profile 2	Profile 3	Profile 4	Profile 5
	Samples 2–14–16–18	Sample 17	Samples 3–5–10–12–15–21	Samples 1–4	Samples 11–20
D3S1358	14–15	15–15	15–16	15–16	16–17
HUMTH01	6–7	7–9.3	7–9.3	7–8	6–9
D21S11	29–33.2	29–30	31.2–32.2	28–31.2	29–33.2
D18S51	15–17	14–15	14–15	13–14	12–14
Penta E	15–20	–	12–19	10–15	12–21
D5S818	10–11	11–12	11–12	11–12	11–14
D13S317	9–11	9–9	9–12	8–11	11–13
D7S820	10–11	11–11	10–11	11–12	8–12
D16S539	9–14	10–12	9–12	9–12	10–11
HUMCSF1PO	11–11	11–12	10–10	11–12	10–12
Penta D	10–10	9–13	10–10	10–11	11–12
HUMvWA	14–18	16–17	15–16	17–18	16–17
D8S1179	12–13	8–13	15–15	10–16	10–17
HUMTPOX	8–11	8–11	8–12	8–8	11–11
HUMFGA	23–26	19–25	25–25	21–24	22–25
Amelogenine	XY	XY	XY	XY	XY

Table 3
Correlation with presumptive family members

Family	Missing	STR amelogenine	Family members analysed	Relevant samples	Likelihood ratio (LR)
Family 1	Individual 1	XY	Presumptive mother	2–14–16–18	39,794.122
Family 2	Individual 2	XY	Presumptive father	17	38.494
Family 3	Individual 3	XY	Presumptive mother	3– 5–10–12–15–21	396.497
Family 4	Individual 4	XY	Presumptive mother	1–4	1537.512
Family 5	Individual 5	XY	Presumptive child	11–20	1236.270

mini-satellite analysis was not possible with these samples. The success rate (with STRs) was 66–83%. A similar success rate was achieved with the TWA-800 disaster, where bodies were decomposed and often skeletonised.

DNA analysis for purposes of forensic identification is at the very early stages in Ecuador, even. In fact there is only one laboratory (DIAGEN laboratory) with capacity for DNA analysis on forensic remains. The force of the explosion left four individuals, all of whom were fulfilling their obligatory military service, missing. Following the analysis, we found five different profiles, four of which applied to those missing and the fifth to fragments of an incomplete corpse.

All methods used to analyse low copy number (LCN) DNA suffer from several disadvantages that are primarily derived from stochastic variation. When present in low-copy-number, a molecule that is amplified by chance during the early rounds of the PCR is likely to be preferentially amplified. There are, therefore, several consequences that cannot be avoided: allele drop out may occur because one allele of a heterozygote locus can be preferentially amplified; stutters may be preferentially analysed (these are sometimes known as false alleles); the method is prone to sporadic contamination, amplifying alleles that are unassociated with the sample. This means that different DNA profiles may be observed after replicate PCR analyses.

In samples 13 and 19 it was not possible to extract DNA, which meant a specific profile could not be established due to the high degree of contamination of the samples. The success rate for this study it was of 90% (19 samples identified from 21).

The phenomenon random allelic dropout, which is a consequence of the very low human DNA template input in the PCR reaction, was the most common artefact observed. In some cases, increasing the amount of DNA input helped to overcome this problem. In other cases, we increased PCR cycles to identify DNA from bones to 35 cycles. Anthropologists to identify ancient DNA from bones, to increase the sensitivity, routinely use increased PCR cycles. Gill et al. used 38–43 cycles to analyse STRs from 70 year old bone from the Romanov family.

Due to transfer of soft tissue and other commingling of remains. DNA testing alone would have led to problems if only soft tissue would have been tested. This was one of the reasons that forensic specialists were needed evaluate the consistency between all linked body parts. Careful considerations must be given not only to the environmental inhibitors that often accompany bone samples found in soil or other metal ion-rich environments, such as humic acid, but also to the inhibitors that are naturally inherent in bones, such as collagen derivatives and calcium ions. For that, we recommended to use EDTA to wash and remove calcium ions.

There are two broad categories of evidence types: discrete (e.g. bone, hair) and non discrete (e.g. blood stains). When using LCN, it is generally easier to associate a DNA profile with a discrete evidence type. This is because analysis of bone samples is not attempted without removing the outermost layer by physical methods (e.g. sandpaper) in order to minimise the possible contamination from modern DNA. Similarly, hair shafts can be washed in a detergent solution to remove adhering DNA. This cannot be done with evidence types that are not discrete, e.g. blood stained cloth, hence the chance is increased that a DNA profile may not be directly associated with the evidential body fluid that is 'apparently' analysed.

Although STR DNA typing has become the 'gold standard' of human identification, evidential value of a genetic match can easily be misinterpreted and careful use of statistical methods is essential for proper evaluation of laboratory results. A statistical evaluation, in the form of a Likelihood Ratio (LR), for each of the cases was undertaken. A LR is a numeric expression of the weight of the DNA evidence. It was used to establish how likely it is that remains in question have originated from an individual related to the family in question, compared to LR of observing the genotype at random in the population. In some cases even a relatively high Paternity Index over 10,000 might be sufficiently high for the correct determination of identity. We observed values in the range of 38–39 millions. Higher LR was obtained for those cases in which there were fewer probabilities in the profile predicted for the deceased. Whenever possible, multiple relatives should be analysed, and other evidence based on the information about time, place, and other conditions of disappearance. The interdisciplinary work is very important in these cases, as well as classical forensic data. We analysed samples in a period of 2 months. After that, we have some reflections about identification of remains. Without a body to bury, without assurance of death, real grieving cannot even begin, because it is viewed as disloyal. This is not denial, but reality. Without the certainty of death, the status of family member remains unclear. Principal purpose of identification is to change the status of the missing to death. It is most

important factor for the relatives and legal services in our country. But we have some questions about these situations that is not clear for authorities: what is the best way to collect samples in disasters, in which cases DNA technology must be applied, how much of a body is the minimum to be considered complete, when is necessary to identify bodies and when fragments. These rules are not clear even. We hope that in future these questions will be solved.

Power Plex 16 system proved to be accurate reliable and robust. Consequently, a high success rate was achieved. These data reinforce the potential of DNA identification for use in routine casework. This paper is important because is a novel experience for our forensic services, because this was the first time DNA had been used as an identification method in disasters, and it was validated by Ecuadorian justice like a very effective method.

References

[1] González-Andrade F, Sánchez D, Martínez-Jarreta B. Evaluation of 1495 cases of Disputed Paternity in Ecuador (South America) resolved with STR-PCR polymorphisms. Proceedings of International Association of Forensic Sciences; Italy. IAFS; 2002, p. 225–30.

[2] Alonso A, Andenilovic S, Martín P, Sutlovic D, Ercef I, Huffine E, et al. DNA typing from skeletal remains: evaluation of multiplex and megaplex STR system on DNA isolated from bone and teeth samples. Croat Med J 2001;42(3):260–6.

[3] Lorente J, Entrala C, Alvarez C, Arce B, Heinrichs B, Lorente M, et al. Identification of missing persons: Spanish 'Phoenix' program. Croat Med J 2001;42(3):267–70.

[4] Budimlija Z, Prinz M, Zelson-Mundorff A, Wieserma J, Bartelink E, MacKinnon G, et al. World Trade Center human identification project: experiences with individual body identification cases. Croat Med J 2003;44(3):259–63.

[5] Hochmeister M, Budowle B, Borer U, Eggmann U, Comey C, Dirnhofer R. Typing of DNA extracted from compact bone from human remains. J Forensic Sci 1991;36(6):1649–61.

[6] Clayton TM, Whitaker JP, Maguire CN. Identification of bodies from the scene of a mass disaster using DNA amplification of STR loci. Forensic Sci Int 1995;76:7–15.

[7] Tracey M. STR identification of individuals and parents. Croat Med J 2001;42(3):233–8.

[8] Chakraborty R, Stivers D, Su B, Zhong Y, Budowle B. The utility of STR loci beyond human identification: implications for development of new DNA typing systems. Electrophoresis 1999;20:1682–96.

[9] Huffine E, Crews J, Kennedy B, Bomberger K, Zinbo A. Mass identification of persons missing from the break-up of the former Yugoslavia: structure, function, and role of the international commission on missing persons. Croat Med J 2001;2(3):271–5.

[10] González F, Martínez B. Técnicas Instrumentales en Genética Forense. 1st ed. Zaragoza:España; 2001.

[11] Valverde E, Cabrero E, Cao R, Rodríguez Calvo MS, Díaz A, Barros F, Alemany J, Carracedo A. Population genetics of three VNTR polymorphisms in two different Spanish populations. Int J Legal Med 1993;105:251–6.

[12] Promega Corporation. PowerPlex ® 16 System. Technical Manual (TMD. No.012), 2002, USA.

[13] Gill P. Application of low copy number DNA profiling. Croat Med J 2001;42(3):229–32.

[14] Gene Scan 3.1.2 Analysis Software® Applied Biosystems, 2000.

[15] International Society for Forensic Haemogenetics. Recommendations of the DNA commission of the international society for forensic haemogenetics relating to the use of PCR-based polymorphisms. Forensic Sci Int 1992;55(1):1–3.

[16] Bär W, Brinkmann B, Budowle B, Carracedo A, Gill P, Lincoln P, et al. DNA recommendations Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. Forensic Sci Int 1997;87(3):181–4.

[17] Gómez J, Alonso A, García O, Carracedo A. The proficiency testing program on DNA typing of the Spanish and Portuguese working group pf the ISFG international congress series 1239. Prog Forensic Genet 2003;(9):837–40.

[18] Sánchez D, González-Andrade F, Martínez Jarreta B. Population genetics of 12 STR loci in a sample of Mestizos from Ecuador (South America). J Forensic Sci 2003;48(2):453–4.

[19] González-Andrade F, Sánchez D, Martínez-Jarreta B. Genetic profile of the Ecuadorian Mestizo population by using the PowerPlex 16 System kit. Forensic Sci Int 2003;135:64–6.