Int J Legal Med (2002) 116:187–190

SHORT COMMUNICATION

Jose A. Lorente · Carmen Entrala · J. Carlos Alvarez
Miguel Lorente · Blanca Arce · Beatriz Heinrich
Félix Carrasco · Bruce Budowle · Enrique Villanueva

Social benefits of non-criminal genetic databases: missing persons and human remains identification

Received: 21 November 2000 / Accepted: 19 July 2001

Abstract A Missing Persons Genetic Identification Program (Phoenix Program) was implemented in Spain in order to try to identify cadavers and human remains that could not be identified using traditional forensic approaches; to our knowledge, this is the first database ever implemented and in function in the world. Two separate mitochondrial DNA (mtDNA) databases have been generated and comparisons can be made automatically to match identical or similar sequences contained in both databases. One database is called the Reference Database (RD), which contains mtDNA sequences from maternal relatives of missing persons that provide the samples voluntarily after informed consent. The other database is called the Questioned Database (QD) and is comprised of mtDNA data on unknown remains and cadavers that could not be unequivocally identified. The combined database is a civil database designed solely for human identification and because of the informed consent and voluntary donation of reference samples is different from other databases now used to solve criminal cases. It is timely and incumbent on other willing countries to begin an international collaboration so compatibility and full utility can be enjoyed with this kind of non-criminal database.

Keywords Missing persons · Human remains · mtDNA · STRs · Forensic genetics

Introduction

In a recent "Newsletter" of the International Academy of Legal Medicine, Cattaeno et al. [1] pointed out that there are probably more than 1,500 cadavers and human remains still unidentified in the European Union. According to these authors, the identification of human remains represents a growing problem in countries of the EU (European Union). This is a fact, but is also an increasing problem in other countries in Latin America according to surveys (data not published) conducted by the Ibero-American Working Group on DNA Analysis (GITAD) and the Ibero-American Academy of Criminalistics and Forensic Studies (AICEF), and this is probably a fact all over the world.

Simultaneously, large and relevant efforts have been continuously made to attempt to identify cadavers and human remains after wars, socio-political problems [2, 3] and after mass disasters [4, 5, 6]. In many cases, the use of DNA typing techniques assumes a social benefit since these typing techniques offer a definitive answer for identification of victims, perpetrators, and missing persons. Simultaneously, advances and changes in forensic sciences, basically the generation of large databases, are perceived by some as "dangerous" for society [7, 8, 9, 10].

There is a genetic database in the forensic arena that generally should not compromise social or individual rights, because it is based on the voluntary donation of biological reference samples to be analyzed: a missing persons database. Samples are voluntarily donated to a forensic "civil" database after signing an informed consent form, as opposed to forensic "criminal" databases, where samples can be obtained using force. Spain is the first country to implement a forensic civil database (in fact

---

More information on the Phoenix program can be found at www.gitad.org (the web-site for the Ibero-American Academy of Criminalistics and Forensic Studies and its DNA working group, named GITAD)

J. A. Lorente (✉) · C. Entrala · J. C Alvarez · M. Lorente
E. Villanueva
Department of Legal Medicine, University of Granada,
Av. Madrid 11, 18012 Granada, Spain
e-mail: jlorente@ugr.es,
Fax: +34-958-246107

B. Arce · B. Heinrich · F. Carrasco
Servicio de Policía Judicial, C.I.C,
Dirección General de la Guardia Civil. Guzmán el Bueno 110,
28003 Madrid, Spain

B. Budowle
FBI Laboratory Division,
935 Pennsylvania Avenue, N.W. Washington, D.C, 20535 USA

there is no legislation for criminal DNA database in Spain yet).

According to the Spanish Guardia Civil statistics (personal communication) there are over 2,500 reported missing persons and approximately 1,000 unidentified human remains, but these numbers could be much higher and therefore a problem in itself is to determine the magnitude of the task at hand, as already pointed out for the EU [1]. Because of the social dynamics of modern societies, there are always missing persons being reported and unidentified cadavers and human remains are always being found. This is a universal problem and a common situation not related to wars or natural catastrophes. It is obvious that some of the unidentified remains and some of the cadavers belong to reported missing persons.

In November 1998, the Spanish Ministry of the Interior decided to support an initiative from the University of Granada presented to the Guardia Civil (the largest national law enforcement agency in Spain) to implement a National Program to attempt to identify cadavers and bones from missing persons. The program was named the "Phoenix program" (Programa Fénix, in Spanish) based on its purpose and classical Greek mythology.

The Phoenix program contains and generates two independent databases that can automatically compare DNA sequences to identify matching or related profiles, such that identification of unknown remains may be possible. One of the databases is known as the Reference Database (RD) which contains mtDNA sequences from maternally related relatives of missing persons. Since the RD is not a criminal database, only individuals that voluntarily agree to provide samples will be DNA typed and their data placed in the RD. In all cases, these donors will be apprised of the informed consent protocol and sign appropriate documentation. All samples are bar-coded, data are treated as confidential and can be deleted at anytime if requested by the donor(s). The second database is known as the Questioned Database (QD) which is comprised of mtDNA sequences obtained from bones or cadavers that cannot be identified or that were not identified by routine and standard procedures, such as fingerprints, anthropology, odontology, X-rays, etc. In all cases, the analysis and storage of mtDNA profiles from unidentified remains requires permission from a judge, as mandated by Spanish law. To enable and facilitate mtDNA profile comparisons between the RD and QD, the MitoSearch software program (now included in the USA CODIS system) is used. The program allows searching for exact matches and can be configured for identifying sequences that differ by as much as three nucleotides (or greater differences if required).

Materials and methods

Informed consent and contacting procedure

Only persons signing a valid informed consent protocol are allowed to participate in the program (general procedure outlined in Fig. 1). People that have reported missing relatives are requested to voluntarily contact the Phoenix Program by calling a toll-free telephone number (in Spain +900-150-759) to communicate that they are willing to participate. A full file with information regarding what Phoenix is, what they should expect and what they should not expect is mailed along with a form to formally ask for collaboration. Once this form is received and processed, samples are obtained by trained police officers who come to the family home. Thus, there is no financial cost for participating families.

Reference database samples

Two buccal cotton swabs (Clue Profile Collector kit, Swisforensix, Bern, Switzerland) are obtained from a minimum of two and a maximum of four maternal relatives (when available); also, buccal swabs are taken from relatives whose nuclear DNA could help to identify the missing person using STRs (e.g. parents, offspring, siblings, etc.). Swabs are allowed to dry at room temperature, placed in the appropriate box included in the kit and sent to the laboratory for further analysis. Once obtained, all samples are barcoded and data dissociated to avoid manipulation and to maintain confidentiality.

Questioned database samples

Two to four fragments of at least 25 g of compact bone, and/or teeth (preferable molars, maximum of six), and/or blood stains (when available, spotted on cotton-swabs or, preferably, FTA paper; Whatman, Maidstone, UK) are obtained from non-identified cadavers and human remains by Guardia Civil's trained specialists, appropriately packaged and sent to the laboratory for analysis. Questioned samples are not immediately analyzed; rather, mtDNA analysis only starts once routine techniques (fingerprints, odontology, etc.) and law enforcement investigations yield no identification results.

mtDNA analysis

DNA from one buccal swab from two maternally related individuals per case is immediately sequenced for the complete hypervariable region 1 (HV1) and hypervariable region 2 (HV2) of the control region (or d-loop) of the mtDNA genome. DNA extraction from bones is performed using organic extraction and filtration for purification [11] and DNA from teeth is also extracted using organic reagents [12]; for reference samples, DNA from saliva is extracted using a modified Chelex protocol [13] and DNA from blood is extracted according to previously reported protocols [14, 15]. All samples are quantified using slot-blot and chemiluminescent methods [16]. To avoid contamination and DNA carry-over, appropriate positive and negative controls are used, and work is carried out only in designated and separated areas. Also, questioned database samples (QD) are analyzed in one of the participating laboratories, and reference database samples (RD) are analyzed in the other laboratory. No STRs are systematically analyzed for matching purposes in the database, but when possible they are used to confirm hits. PCR amplification and sequencing follow standard operational procedures for mtDNA analysis and the nomenclature is similar to that validated for forensic purposes [17].

Nuclear DNA analysis

This is performed using the PowerPlex16 kit (Promega Corp. Madison, Wis.), as described in the manufacturer's technical manual included with the kit (Part. DC6530). This kit with primers and PCR reagents for typing includes 15 polymorphic STR loci and the locus amelogenin (a gender marker). Of the loci in the kit 13 (FGA, vWA, D3S1358, HUMTHO1, HUMTPOX, HUMCSF1PO, D5S818, D7S820, D8S1179, D13S317, D18S51, and D21S11) are the core loci in the United Stated Coordinated DNA Index System

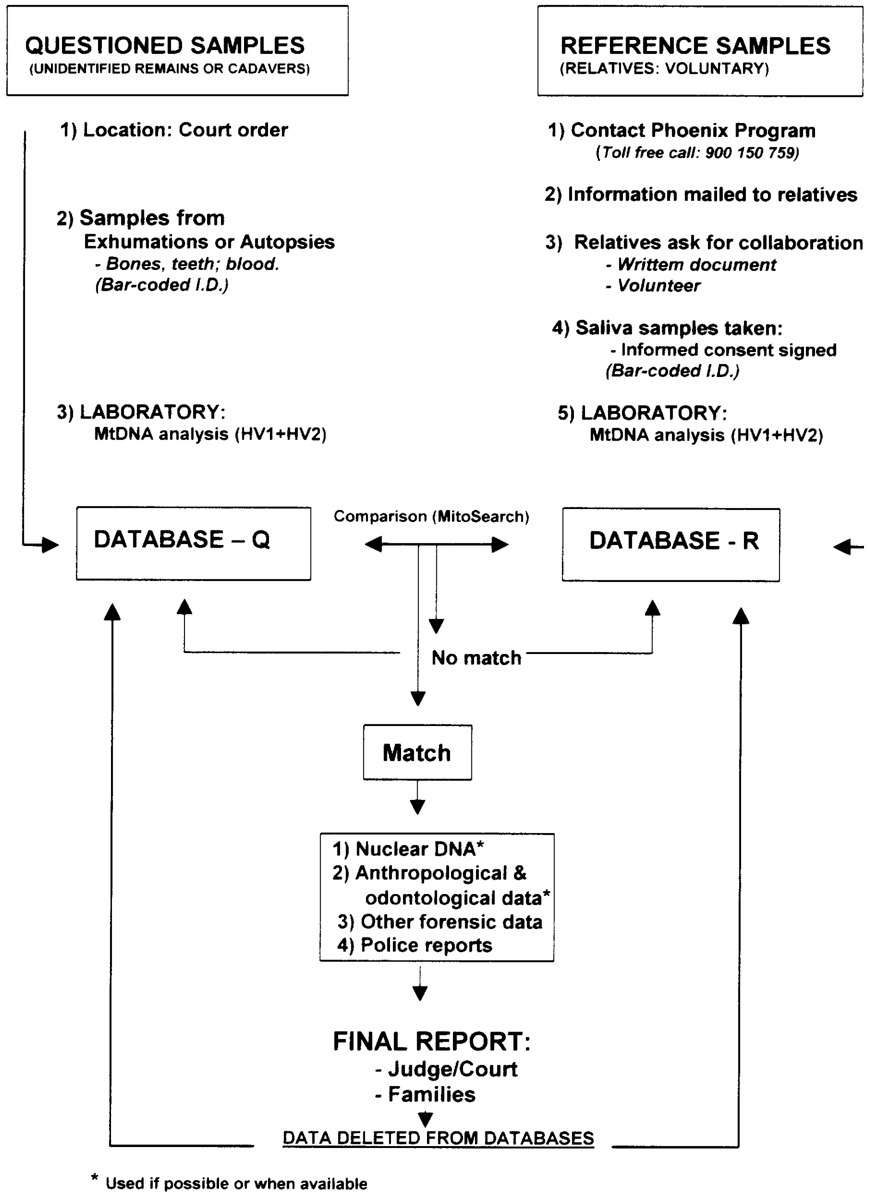

Fig. 1 Spanish missing people identification procedure (Phoenix Program)

(CODIS). There are also two highly discriminative pentameric loci (Penta D and Penta E) included in the multiplex.

Results and discussion

To date more than 1,200 families have contacted Phoenix, and at least 140 reference samples and 48 unidentified remains have been analyzed. When mtDNA matches are found, a second and independent analysis is performed as part of the quality control mechanism. Once a match is confirmed (so far six cases), an attempt is made to analyze short tandem repeat (STR) loci using the PowerPlex 16 kit (Promega, Madison, Wis.).

Nationally and internationally compatible protocols leading to identification of human remains or skeletons ideally will require the use of databases that fulfil five basic requisites, as follows:

1. Analyses have to be based on standard operating protocols and universally accepted genetic markers. The techniques should be widely used, reproducible, and accepted by the forensic community and courts around the world. Only techniques fulfilling technical and legal criteria will enable international compatibility.
2. Results must be valid and reliable. It is important to stress that only laboratories and techniques subjected to strict quality assurance and quality control programs should be used.
3. The technology should be amenable to automation, to facilitate the typing of the anticipated large volume of samples and to allow intra- and international searches and comparisons. Data have to be easily and unequivocally converted into alpha-numeric characters. DNA results already meet this criterion. Also, there must be programs available to compare and find matches. For example, mtDNA data meet this criterion, and the

FBI's MitoSearch program (among others) is used for searching purposes.

4. It is desirable to generate data that provide little or no personal or confidential information about the individual. Proper use of this mtDNA database according to national laws, dissociation of data, restricted access, informed consent from voluntary donors, court orders to handle human remains are among some of the requirements of the Spanish database.
5. In order to build up a truly operative database, the analysis and data have to be useful in as many cases as possible, particularly so that results can be obtained on challenging materials such as a skeleton or partial remains. In our experience, mtDNA is the best genetic marker system to fulfil this requirement. The goal of this database is to be able to generate some information about the identity of the remains where classical techniques did not yield positive identification; all samples should be typed for mtDNA.

We are neither proposing or advocating that DNA should be the only tool for identification. The Phoenix program is using DNA just to find matches between relatives of missing persons and unidentified cadavers or human remains of previously unsolved cases. Therefore, DNA is just another piece in the puzzle to help to determine the identity of remains that had never been identified using non-genetic approaches. Final identification relies not only on the DNA results (despite the undoubted value), but also on other police investigation information and forensic data [6]. So far, in the first 8 months of operation, 6 cases have been solved, some of them belonging to human remains that are at least 8–10 years old. It is anticipated that with the analysis of more cases in the next 2 years, and as the relative proportion of QD samples increases compared with that of RD samples, a larger percentage of cases will be solved.

Beneficial social applications of scientific advances is a commitment that science and the scientist must pursue. There is no doubt that genetic advances have played a major role in the forensic community over the past 15 years, helping to solve difficult criminal cases where biological evidence is found, exonerating the innocent, supporting the creation of criminal databases and resolving identity in mass disaster cases. A structured DNA database program (as implemented through the Spanish Phoenix program) is needed to help to solve many cases. International collaboration has started in the Latin American area through GITAD/AICEF [18], but further international cooperation is necessary to make this a valuable world-wide effort for identification of missing persons.

Acknowledgments The authors are grateful to the nine Spanish Foundations supporting this program (BBV, Caja Madrid, Endesa, Juan March, Marcelino Botín, Pedro Barrié de la Maza, Ramón Areces, Tabacalera, and Telefónica), and the Spanish Ministry of Education and Science for supporting research on human identification through project PM97-0175.

References

1. Cattaneo C, Ritz-Timme S, Schutz HW, Collins M, Waite E, Boorman H, Grandi M, Kaatsch HJ (2000) Unidentified cadavers and human remains in the EU: an unknown issue. The Newsletter of the International Academy of Legal Medicine 1-2000. Int J Legal Med 113:N1
2. Primorac D, Andelinovic S, Definis-Gojanovic M, Drmic I, Rezic B, Baden MM, Kennedy MA, Schanfield MS, Skakel SB, Lee HC (1996) Identification of war victims from mass graves in Croatia, Bosnia and Herzegovina by use of standard forensic methods and DNA typing. J Forensic Sci 41:891–894
3. Watson A (2000) A new breed of high-tech detectives. Science 289:850–854
4. Olaissen B, Stenersen M, Mevag B (1997) Identification by DNA analysis of the victims of the August 1996 Spitsbergen civil aircraft disaster. Nat Genet 15:402–405
5. Martin-de las Heras S, Valenzuela A, Villanueva E, Marques T, Exposito N, Bohoyo JM (1999) Methods for identification of 28 burn victims following a 1996 bus accident in Spain. J Forensic Sci 44:428–431
6. Ballantyne J (1997) Mass disaster genetics. Nat Genet 15: 329–331
7. Editorial (1999) DNA, not d.o.a. Nat Genet 26:243–244
8. TOUCHINGbase (1999) Wanted: TCACCGTT... Nat Genet 23:261
9. Dwyer J, Neufeld P, Scheck B (2000) Actual innocence. Five days to execution and other dispatches from the wrongly convicted. Doubleday, New York
10. Erzinclioglu Z (1998) British forensic science in the dock. Nature 392:859–860
11. Holland MM, Fisher DL, Mitchell LG, Rodriguez WC, Canik JJ, Merril CR, Weedn VW (1993) Mitochondrial DNA analysis of human skeletal remains: identification of remains from the Vietnam War. J Forensic Sci 38:542–553
12. Pfeiffer H, Hühne J, Seitz B, Brinkmann B (1999) Influence of soil storage and exposure period on DNA recovered from teeth. Int J Legal Med 112:142–144
13. Sweet DJ, Lorente M, Valenzuela A, Lorente JA, Alvarez JC (1996) Increasing DNA extraction yield from saliva stains with a modified chelex method. Forensic Sci Int 83:167–177
14. Jung JM, Comey CT, Baer DB, Budowle B (1991) Extraction strategy for obtaining DNA from bloodstains for PCR amplification and typing of the HLA-DQ alpha gene. Int J Legal Med 104:145–148
15. Del Rio S, Marino MA, Belgrader P (1996) Reusing the same blood-stained punch for sequential DNA amplification and typing. Biotechniques 20:970–974
16. Budowle B, Baechtel FS, Comey CT, Giusti AM, Klevan L (1995) Simple protocols for typing forensic biological evidence: chemiluminescent detection for human DNA quantitation and RFLP analysis and manual typing for PCR amplified polymorphism. Electrophoresis 16:1559–1567
17. Wilson MR, Dizzino JA, Polanskey D, Replogle J, Budowle B (1995) Validation of mitochondrial DNA sequencing for forensic casework analysis. Int J Legal Med 108:68–74
18. Lorente JA (1999) Human identification and DNA typing in Latin America: the "GITAD" project. Profiles in DNA 3:8–9