

## Identification of Missing Persons: The Spanish "Phoenix" Program

Jose A. Lorente, Carmen Entrala, J. Carlos Alvarez, Blanca Arce<sup>1</sup>, Beatriz Heinrichs<sup>1</sup>, Miguel Lorente, Felix Carrasco<sup>1</sup>, Bruce Budowle<sup>2</sup>, Enrique Villanueva

*Department of Legal Medicine, University of Granada, Granada; <sup>1</sup>Servicio de Policía Judicial, C.I.C. Dirección General de la Guardia Civil, Madrid, Spain; and <sup>2</sup>Laboratory Division, Federal Bureau of Investigation, Washington, D.C., USA*

In 1999, Spain was the first country to officially start a National Program to try to identify cadavers and human remains which could not be identified by the use of traditional forensic approaches. This attempt is called "Phoenix Program". Two independent mitochondrial DNA (mtDNA) databases were generated, which can automatically compare and match identical or similar sequences. One is the Reference Database, with mtDNA sequences from maternal relatives of missing persons, who provide the samples (buccal swabs) voluntarily; the other is the Questioned Database, comprised of mtDNA data of unknown remains and cadavers. Although the first phase of the program (typing of all unidentified human remains) will probably not be completed until December 2003, positive identifications are being made in the interim. To date, more than 1,200 families have contacted Phoenix, and at least 280 reference samples and 48 questioned evidences have been analyzed. When mtDNA matches are found, another independent analysis is performed as a part of the quality control mechanism. Once a match is confirmed (so far in 6 cases), an attempt is made to analyze short tandem repeat (STR) loci. We call for international collaboration to make this effort valuable worldwide.

**Key words:** DNA, mitochondrial; fluorescent probes; forensic medicine; polymerase chain reaction; polymorphism; polymorphism, restriction fragment length; Spain; United States

Advances in the molecular tools in genetics which have been applied to the forensic field have had a beneficial impact, such as exonerating the innocent, offender identification, and generation of criminal databases (1-3). Regardless of the anonymous nature of the DNA profile data and security measures in place, the use of the data and the mishandling of samples are sometimes raised as threats to social and individual rights and freedom (1,4-6). However, some genetic identity applications may not compromise social or personal rights, such as the identification of missing persons and human remains (7).

Large and relevant efforts have been continuously made to identify cadavers and human remains after wars, socio-political disturbances (2,3), and mass disasters (8,9). 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.

A database on missing persons is a forensic genetic database that should not compromise social or individual rights, because it is based on the voluntary donation of biological samples. Since samples are donated to a forensic "civil" database voluntarily, DNA profile data are processed only after an informed consent form has been signed by the donor, as opposed to forensic "criminal" databases, where samples can be obtained by force. Spain is the first country to im-

plement a forensic civil database (actually, there is no legislation for a criminal DNA database in Spain yet).

There are probably more than 1,500 cadavers/remains unidentified in the European Union (7). According to the Spanish Guardia Civil statistics (data not shown), this number could be much higher and therefore it is difficult to determine the magnitude of the task at hand. Because of the social dynamics of modern societies, there are always missing persons reported and unidentified cadavers and human remains found. This is a universal problem, a common situation not related only to wars or natural catastrophes. It is obvious that some of the unidentified remains and some of the cadavers belong to reported missing people.

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

The Phoenix program contains and generates two independent databases, which can automatically compare DNA sequences to identify matching or related profiles, so that identifications of unknown remains may be possible. One of the databases is known as the Reference Database. It contains mitochondrial DNA (mtDNA) sequences from maternally related relatives of missing persons. Since the Reference Database is not a criminal database, only individuals who agree to voluntarily provide samples are DNA typed and their data are placed in the Reference Database. In all cases, these donors are apprised of the informed consent protocol and asked to sign appropriate documentation. All samples are bar coded. Data are treated as confidential and will be deleted at anytime if so requested by the donor(s). The second database is known as the Questioned Database. The Questioned Database 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 a permission from a judge, as mandated by the Spanish law. To allow and facilitate mtDNA profile comparisons between the Reference Database and Questioned Database, the MitoSearch software program (kindly provided by Dr Budowle, FBI, Washington, D.C., USA) is used.

## Methods

### Informed Consent and Contacting Procedure

General procedure in the Phoenix Program is shown in Figure 1. Only persons signing a valid informed consent protocol are allowed to participate in the program. People who 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 file with full information about Phoenix Program, what should they

expect, and what should they not expect, is mailed to the volunteers along with a form containing a formal request for collaboration. Once this form is received and processed, trained police officers visit the family home of the donors and collect the samples. There is no financial cost whatsoever for participating families.

### Reference Database Samples

Two buccal cotton swabs (Clue™ Profile Collector kit, Swisforensix AG, 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 by use of short tandem repeat (STR) strategy (parents, offsprings, 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 bar-coded and data dissociated to avoid manipulation and maintain confidentiality.

### Questioned Database Samples

Fragments of at least 25 g of bone (maximum of six), 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 investigation yield no identification results.

### Mitochondrial DNA Analysis

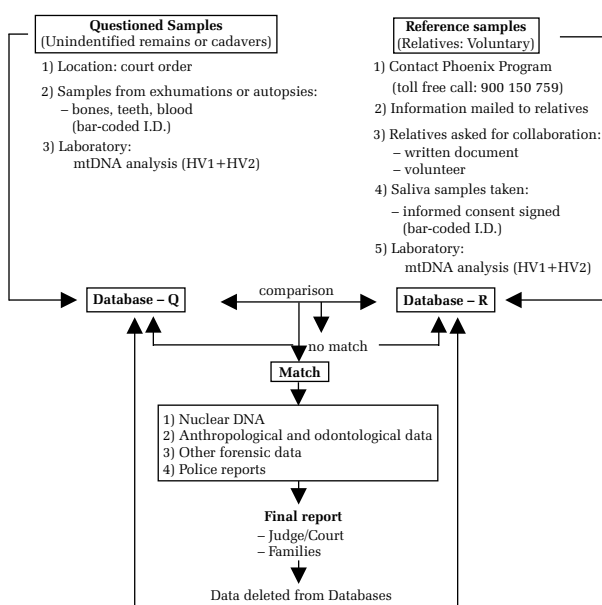
DNA from one buccal swab from two maternally related individuals per case is immediately sequenced for the complete hypervariable region 1 (HV1) and hypervariable region 2 (HV2) of the control region (or d-loop) of the mtDNA genome. DNA extraction from bones is performed by organic extraction and filtration for purification (13); DNA from teeth is also extracted by use of organic reagents (14); saliva (15), and blood (16,17) from reference samples are extracted according to previously published protocols. All samples are quantified by slot-blot and chemiluminescent methods (18). To avoid contamination and DNA carry-over, proper positive and negative controls are used, and work is carried out only in designated and separated areas. Also, Questioned Database samples are analyzed in one of the participating laboratories, and Reference Database samples are analyzed in another laboratory. STRs are not systematically analyzed for matching purposes in the database, but are used to confirm hits when possible. Polymerase chain reaction (PCR) amplification and sequencing follows standard operational procedures for mtDNA analysis and nomenclature is similar to that validated for forensic purposes (12).

### Nuclear DNA Analysis

Nuclear DNA analysis is performed using the PowerPlex 16 kit (Promega Corporation, Madison, WI, USA), 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). Thirteen of the loci in the kit (FGA, vWA, D3S1358, HUMTH01, HUMTPOX, HUMCSF1PO, D5S818, D7S820, D8S1179, D13S317, D18S51, and D21S11) are the core loci in the United States Combined DNA Index System (CODIS). There are two additional highly discriminative pentameric STR 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 280 reference samples and 48 questioned evidences 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



**Figure 1.** Identification of unidentified remains or cadavers in the "Phoenix Program".

far in 6 cases), an attempt is made to analyze STR loci by the use of the PowerPlex 16™ kit.

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

1) Analyses have to be based on standard operating protocols and universally accepted genetic markers. The techniques should be reproducible, widely used, and accepted by the forensic community and the courts around the world. Only techniques fulfilling technical and legal criteria will provide international compatibility.

2) Results must be valid and reliable. It is important to emphasize 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-national and international searches and comparisons. Data have to be easily and unequivocally converted into alphanumeric 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, and court order to handle human remains are among some of the requirements of the Spanish database.

5) To build up a truly operative database, the analysis and data have to be useful in as many cases as possible, particularly so that the 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 fulfill this requirement. The goal of the Phoenix 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 nor advocating that the DNA 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 tool to help determine the identity of remains that had never been identified by non-genetic approaches. Final identification relies not only on DNA results (despite the undoubted value), but also on other police investigation information and forensic data (10). So far, in the first 8 months of the Program, 6 cases have been solved, some of them belonging to human remains at least 8 to 10 years old. It is anticipated that, with the analysis of more cases in the next two years, and the relative increase in the number of Questioned Database sam-

ples compared with that of Reference Database samples, a larger percentage of cases will be solved.

Beneficial social application of scientific advances is the objective that science and scientists 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 was 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, may assist in solving many cases. International collaboration has started in the Latin American area through Grupo Iberoamericano de Trabajo en el Análisis del DNA (GITAD) and Academia Iberoamericana de Criminalística y Estudios Forenses (AICEF) (11). We call for further international cooperation to make this a valuable worldwide effort for identification of missing persons.

#### Acknowledgment

We acknowledge 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). Spanish Ministry of Education and Science supports the research on minimal amounts of DNA through the project PM97-0175.

More information on the Phoenix program can be found at [www.gitad.org](http://www.gitad.org) and [www.aicef.org](http://www.aicef.org).

#### References

- 1 DNA, not d.o.a. [editorial]. *Nat Genet* 1999;26:243-4.
- 2 Primorac D, Andelinović S, Definis-Gojanović M, Drmić I, Režić B, Baden MM, et al. Identification of war victims from mass graves in Croatia, Bosnia and Herzegovina by use of standard forensic methods and DNA typing. *J Forensic Sci* 1996;41:891-4.
- 3 Corach D, Sala A, Penacino G, Iannucci N, Bernardi P, Doretti M, et al. Additional approaches to DNA typing of skeletal remains: the search for "missing" persons killed during the last dictatorship in Argentina. *Electrophoresis* 1997;18:1608-12.
- 4 TOUCHINGbase. Wanted: TCACCGTT... *Nat Genet* 1999;23:261.
- 5 Dwyer J, Neufeld P, Scheck B. Actual innocence. Five days to execution and other dispatches from the wrongly convicted. New York (NY): Doubleday; 2000.
- 6 Erzincliglu Z. British forensic science in the dock. *Nature* 1998;392:859-60.
- 7 Cattaneo C, Ritz-Timme S, Schutz HW, Collins M, Waite E, Boorman H, et al. 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* 2000;113:N1.
- 8 Olaissen B, Stenersen M, Mevag B. Identification by DNA analysis of the victims of the August 1996 Spitsbergen civil aircraft disaster. *Nat Genet* 1997; 15:402-5.
- 9 Martin-de las Heras S, Valenzuela A, Villanueva E, Marques T, Exposito N, Bohoyo JM. Methods for identification of 28 burn victims following a 1996 bus accident in Spain. *J Forensic Sci* 1999;44: 428-31.
- 10 Ballantyne J. Mass disaster genetics. *Nat Genet* 1997; 15:329-31.

- 11 Lorente JA. Human identification and DNA typing in Latin America: the "GITAD" project. Profiles in DNA 1999;3:8-9.
- 12 Wilson MR, Dizzino JA, Polansky D, Reploge J, Budowle B. Validation of mitochondrial DNA sequencing for forensic casework analysis. Int J Legal Med 1995;108:68-74.
- 13 Holland MM, Fisher DL, Mitchell LG, Rodriguez WC, Canik JJ, Merrill CR, et al. Mitochondrial DNA analysis of human skeletal remains: identification of remains from the Vietnam war. J Forensic Sci 1993; 38:542-53.
- 14 Pfeiffer H, Hühne J, Seitz B, Brinkmann B. Influence of soil storage and exposure period on DNA recovered from teeth. Int J Legal Med 1999;112:142-4.
- 15 Sweet D, Lorente M, Valenzuela A, Lorente JA, Alvarez JC. Increasing DNA extraction yield from saliva stains with a modified chelex method. Forensic Sci Int 1996; 83:167-77.
- 16 Comey CT, Koons BW, Presley KW, Smerick JB, Sobieralski C, Stanley DM, et al. DNA extraction strategies for amplified fragment length polymorphism analysis. J Forensic Sci 1994;39:1254-69.
- 17 Del Rio S, Marino MA, Belgrader P. Reusing the same blood-stained punch for sequential DNA amplification and typing. BioTechniques 1996;20:970-4.
- 18 Budowle B, Baechtel FS, Comey CT, Giusti AM, Klevan L. Simple protocols for typing forensic biological evidence: chemiluminescent detection for human DNA quantitation and RFLP analysis and manual typing for PCR amplified polymorphism. Electrophoresis 1995; 16:1559-67.

Received: April 11, 2001

Accepted: May 2, 2001

**Correspondence to:**

Jose A. Lorente

Department of Legal Medicine

University of Granada

18012 Granada, Spain

*jlorente@ugr.es*