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ORIGINAL ARTICLE

Genetic identification as a tool in research of irregular adoptions and abduction of newborns in Spain: National Institute of Toxicology and Forensic Science (Department of Barcelona) experience[☆]



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Abstract

Introduction: Cases of irregular adoptions and abduction of newborns in Spain have had a great social impact which has led to regulatory reforms and the implementation of scientific guidelines and recommendations to help their investigation. The objective of the study was the genetic identification of the samples submitted by kinship studies.

Materials and methods: In the period between the year 2011 and 2014, the Barcelona Department of the National Institute of Toxicology and Forensic Sciences, received a total of 58 cases on which it was judicially required to carry out a study of genetic identification. Exhumed bones or clinical samples were analysed. After previous study (anthropological, anatomic-pathological), the extraction of genetic material and its subsequent analysis were carried out.

Results: The quality and the quantity of recovered DNA allowed carrying out genetic analysis in 67.2% of cases, compared to 32.8%, where it was not possible. The data show that for the majority of the cases studied (87.2%) was established a positive relationship, being minority the cases of exclusion (12.8%). The likelihood ratio (LR) obtained, ranged from 1.75×10^4 – 5.95×10^{17} , and the STR markers studied from 8 to 21.

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PALABRAS CLAVE

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Conclusions: Experience and data show the importance of gathering information, the history related to the case and the importance of the anthropological study prior to genetic analysis. This information allows orienting the genetic studies and, in some cases, it helps to interpret the results. Also, the characteristics of this type of samples require establishing a quality protocol that ensures the authenticity of the final results.

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La identificación genética como herramienta en la investigación de adopciones irregulares y sustracción de recién nacidos en España: experiencia del Instituto Nacional de Toxicología y Ciencias Forenses (Departamento de Barcelona)

Resumen

Introducción: Los casos de adopciones irregulares y sustracción de recién nacidos en España han tenido una gran repercusión social, lo que ha conllevado la elaboración de reformas normativas y el desarrollo de guías y recomendaciones científicas que ayuden a su investigación. El objetivo del presente estudio fue la identificación genética de las muestras remitidas mediante estudios de parentesco.

Material y métodos: Entre 2011-2014 se recibieron en el Departamento de Barcelona del Instituto Nacional de Toxicología y Ciencias Forenses 58 casos sobre los que se requirió judicialmente un estudio de identificación genética. Se analizaron restos óseos exhumados o muestras clínicas. Tras el estudio previo (antropológico y anatomopatológico) se procedió a la extracción del material genético y su posterior análisis.

Resultados: La calidad y cantidad del ADN recuperado permitió el análisis genético en el 67,2% de los casos, frente al 32,8% donde no fue posible. Los datos muestran que mayoritariamente (87,2%) se estableció una relación de compatibilidad paterno-filial, siendo minoritarias las exclusiones (12,8%). El índice de verosimilitud osciló entre $1,75 \times 10^4$ - $5,95 \times 10^{17}$ y los STR estudiados de 8-21.

Conclusiones: Los datos y experiencia adquirida muestran la importancia de la recopilación de la información, de los antecedentes del caso y del estudio antropológico previamente al análisis genético. Esta información permite orientar los estudios genéticos y, ocasionalmente, ayuda a interpretar los resultados. Asimismo, las características de este tipo de muestras obligan a establecer un protocolo de calidad que garantice la autenticidad de los resultados finales.

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Introduction

In late 2010, victims associations lodged a claim with the General State Prosecutor's Office regarding 400 cases of abductions of newborns and irregular adoptions that took place in Spain from the 60s to the 90s. In recent years, these types of cases have been of great social interest. Resolving these types of cases involves two possible scenarios. In one, people who are alive (progenitor[s] and/or descendants) request to be reunited with other people who are also alive. In the other, relatives (usually progenitor[s], and to a lesser degree siblings) request a kinship investigation with human bone remains or clinical samples (biopsies, histological preparations) from a *post-mortem* analysis.

Several public and private Spanish laboratories are participating (at the request of individuals or on court orders) in the family reunification studies. The lack of coordination between the various laboratories and the fact that

there is not a single shared database for the matching studies has resulted in part of the genetic information generated in the various laboratories not being submitted for comparison, with the subsequent loss of information. The Ministry of Justice has made a commitment to the various victims associations to implement certain measures to respond to the victims' demands and needs. Thus Ministerial Order JUS/2146/2012 of 1 October set forth a procedure whereby all of the genetic profiles that had thus far been generated by the various state laboratories (both public and private) could be recorded in a centralised manner in File 120 "DNA profiles of persons affected by kidnappings of newborns"¹ in order to help make comparisons and detect any genetic compatibilities in the people in the file, and with the National Toxicology and Forensic Sciences Institute (INTCF) being responsible for managing the database.

Conducting genetic studies of bone remains from exhumations or clinical samples is a crucial tool for being

able to provide an answer in these types of cases.² Yet these types of situations also present major analytical challenges. First, the high degree of deterioration and affectation in a large part of the exhumed remains or clinical samples makes it necessary to use analytical tools and strategies that will offer the best results.^{3–5} Second, there is a limitation due to the type and degree of kinship of the relatives who are available to undergo the genetic identification (total or partial absence of progenitors). And lastly, there is a limitation related to the scant amount of exhumed remains available in some cases.

On the other hand, in these types of cases, the quantity and quality of the genetic material that is recovered is often poor. This entails the appearance of analytical artefacts (allele loss, false alleles, etc.), and a higher propensity towards the appearance of contaminants. These circumstances make it necessary for laboratories to use validated methods and to be certified for conducting these types of analyses, in accordance with standard UNE-EN ISO/IEC 17025, as recommended by the National Commission for the Forensic Use of DNA (CNUFADN) in the document *“Recommendations on genetic identification studies in cases of irregular adoptions and abductions of newborns”*.⁶ The reproducibility of the results that have been issued as far as possible, the use of maximum results strategies, and measures aimed at isolating the contamination of samples are the measures that are necessary in these types of forensic cases for ensuring the reliability and validity of the results and final conclusions.

It is also crucial before the genetic study to have the participation of specialists from other fields, such as forensic or criminal anthropology. These studies provide valuable information on whether there are bone remains, their condition, the number of individuals and their estimated age. The data from these types of studies will make it possible to orient the genetic study, while also helping interpret the final results.

Table 1 Types of samples analysed in the Barcelona Department of the National Toxicology and Forensic Sciences Institute for resolving cases of irregular adoptions. This also includes the type of relatives who were available for cases where a genetic analysis could be performed.

	n (%)
Inconclusive	
Bone remains	49 (84.5)
Clinical sample	9 (15.5)
Paraffin blocks	5 (8.6)
Preparations on slides	4 (6.9)
Total	58
Conclusive	
Both progenitors	25 (64.1%)
One progenitor	11 (28.2%)
Mother	10 (25.6%)
Father	1 (2.6%)
Other relatives (siblings) ^a	3 (7.7%)
Total	39

^a The number of siblings available was 1 (twin sister), 2 (sisters) and 3 (2 brothers and 1 sister).

Lastly, achieving the utmost scientific rigour when exhuming the bone remains is a key aspect for the entire process in order to prevent contaminations and ensure the quality and certainty of the final results. To this end, the Ministry of Justice has published a standard operating procedure for conducting exhumations in these types of cases, the *“Guide of recommendations for performing exhumations in cases of possible abductions of newborns”*.⁷ This guide includes aspects related to preliminary steps focusing on studying the documents related to the case (documents from hospitals, civil registries, interviews with relatives, etc.); the participation of the forensic physician on the scene, distinguishing between confined spaces and spaces underground; procedures in pathology and anthropology laboratories; and how to issue a final report.

The purpose of this study is to explain the experience of the Barcelona Department of the INTCF in genetic identification of samples submitted from kinship studies, from alleged cases of irregular adoptions and abductions of newborns.

Materials and methods

Samples

From 2011 to 2014, the Barcelona Department of the INTCF received a total of 58 investigation requests, from court orders (19%) or from investigation proceedings initiated by prosecutors' offices (81%), from the autonomous communities of Catalonia (51.7%), Valencia (39.7%), Aragon (5.2%), Navarre (1.7%) and the Balearic Islands (1.7%).

In most cases, bone remains that were exhumed in the course of the investigation were submitted as inconclusive evidence (84.5%). On the other hand, to a lesser degree clinical samples were also submitted (biopsies and histological preparations) (15.5%) that came from pathological studies performed on newborns born in various hospitals.

Also, in order to establish kinship relationships, conclusive evidence (oral mucosa) was submitted from relatives with various degrees of kinship. 64.1% of these cases had samples from both progenitors, while in 28.2% there was just one progenitor. In some cases, due to the absence of both progenitors, it was necessary to request conclusive samples from other types of relatives (siblings) (7.7%) (Table 1).

Methods

Before conducting the genetic analysis of the bone remains, they were sent to the Criminology Service of the Madrid Department of the INTCF for an anthropology study. Based on this study, the remains that were most suitable were selected taking into account their state of preservation and the number of pieces retained. In cases where there were clinical samples, a pathological study was conducted before the genetic study to confirm the real attribution of the samples.

For the extraction of the genetic material from the bone remains (long bones, cranial vault), a quantity of bone dust was taken that ranged from 0.2 to 2 g, depending on the affectation and preservation of the samples. DNA was extracted from the bone remains and clinical samples by performing an organic extraction, followed by a

Table 2 Scope of the genetic type results from cases of irregular adoptions studied in the Barcelona Department of the National Toxicology and Forensic Sciences Institute.

Genetic type studies	n (%)	Marker(s) studied	LR range
1. With genetic profile results	39 (67.2)		
1.1. Kinship compatibility	34 (87.2)		
Both progenitors/child kinship	21 (61.8)	STR	$45,270-5.9 \times 10^{17}$
Mother/child kinship	10 (29.5)	STR	$17,586-2.1 \times 10^8$
Father/child kinship	1 (2.9)	STR	2.4×10^7
Sibling kinship	2 (5.8)	STR	$84,481-6.7 \times 10^{10a}$
1.2. Exclusion of kinship	5 (12.8)	STR/mtDNA	0
2. No profile results	19 (32.8)		

^a Value obtained after comparison with the genetic profile of a twin sister.

final supplemental purification phase in *QIAquick™* (QIAGEN Inc.) columns. The DNA extracts were quantified using *Polymerase Chain Reaction* (PCR) in real time with the *Quantifiler® Duo kit* (Life Technologies Corporation).

The genetic marker type that was used (nuclear DNA or mitochondrial DNA [mtDNA]) depended on how well the submitted samples were preserved and on the type of relative that was available. Various commercial kits were used to study the autosomal *short tandem repeat* (STR) markers (*MiniFiler®*, *Identifiler®* and *NGM®-Life Technologies Corporation*), following the usage and amplification conditions recommended by the manufacturer. In some cases, changes were made to the amplification reaction (increasing the final amplification volume, adding *bovine serum albumin*-BSA) and/or subsequently treating the amplified products (purification with ultrafilter columns). When an mtDNA study was necessary, the HVSI II and III regions were studied jointly, individually or even in fractions, depending on the foreseeable degradation of the DNA (down to a minimum amplicon fragment of 167 base pairs) following the protocols and primers described by Wilson et al.⁸ and Lutz et al.⁹ The fragments were detected using an ABI PRISM® 310 sequencer (*Applied Biosystems Inc.*) and for the subsequent editing, the *GeneMapper®* ID v3.2 programme was used to study the STR markers, and *SeqScape®* v2.1.1 for the mtDNA study.

Results

Quality control of results

The results were ensured by means of the reproducibility of the analyses using independent studies of extractions of two or more specimens from the cadaver, or in some cases, from various blocks or clinical samples from the same cadaver. Secondly, the negative controls and extraction buffers used in the various analysis phases were checked and confirmed. Thirdly, the results were mapped independently by two specialists, and in some cases the results were reproduced and confirmed by an external laboratory. Cases of parent/child incompatibility were confirmed independently for each progenitor, where they were both available. Lastly, all of the results were contrasted against a discard database, comprised by the personnel from the laboratory itself or others involved in the sample processing phases.

Paternity studies

In 39 cases (67.2%) it was possible to obtain a valid genetic profile that could be contrasted. In 34 of the cases (87.2%) a parent/child kinship relationship could be defined between the remains that were analysed and the relatives who had requested the kinship study. On the other hand, in 5 cases (12.8%) the results were incompatible for the requested kinship study. Lastly, in 19 cases (32.8%) the quality of the results was not sufficient for issuing a conclusion, mainly due to the presence of various factors (sample scarcity, affectation of the sample, or unsuitability of the available relatives) (Table 2).

In the cases where compatibility was established between the bone remains (or *post mortem* clinical samples) and the relatives who requested the study, in view of the scientific recommendations,^{6,10,11} the compatibility was assessed by using the *likelihood ratio* (LR) statistical parameter, which ranged from 1.75×10^4 to 5.95×10^{17} . The LR value that was obtained depended on the number of available progenitors and the number of STR markers with valid results (from 8 to 21). In 25 of the cases of parent/child compatibility (64.1%), it was possible to use both progenitors, while in 11 cases (28.2%) only one could be used. In another 3 cases (7.7%) another type of relative (sibling) was used (Table 1).

In all cases where the analysis of the autosomal polymorphism markers was negative, an mtDNA study was performed. Yet using this tool was not successful, in spite of making changes to the analysis in order to improve the results (double volume amplification reactions, double purifications of the DNA extract, increasing the *Taq* polymerase quantity). In each case, even where the DNA extracts were submitted to a double purification process, the internal positive control (IPC) included in the quantification kit (*Quantifiler® Duo*) yielded *threshold cycle* (Ct) values over 31, thus showing the presence of PCR inhibitors in the quantified extract.

Conflictive cases

In addition to these case studies in the study, two other cases were also submitted that incorrectly had remains from more than one individual in them; in another case bone fragments were submitted from an adult. Conducting the

anthropometric and criminological study prior to the genetic study of the bone remains turned out to be absolutely crucial for revealing errors in the exhumation phase.

Furthermore, in one of the cases under analysis there was a false exclusion because the clinical remains that were analysed (paraffin blocks) did not match those which the hospital attributed to a newborn that needed to be identified. Subsequent studies on the blocks in the Pathological Anatomy Service corroborated that they came from an adult, and that the tissues and pathologies that were detected had no correlation with those that might be attributed to a newborn. This study made it possible to authenticate the *ante mortem* samples and avoid a false exclusion.

Discussion

The importance of gathering preliminary data prior to exhumation

The data show a very high percentage of cases in which a parent/child relation was established (87.2%) compared to those where one was not (12.8%). In the latter cases, an analysis of the documents provided by the prosecutor's office, courts and forensic physicians showed a certain correlation between the incompatibility of paternity that was obtained and problems ascribable to doubts as to the exact location of the exhumation, the cadaver being identified as being buried together with other relatives, or the remains having been moved from the place where the cadaver was initially buried. One of the steps prior to exhumation outlined by the "*Guide of recommendations for performing exhumations in cases of possible abductions of newborns*",⁷ published by the Ministry of Justice, is conducting a detailed study of the documents related to the facts under investigation (clinical history, registry documents, information from relatives, etc.). Information such as the gender of the newborn being investigated, the exact location of the exhumation, whether the burial was in an individual or mass grave, and information on the subject's clothes or belongings at the time of burial are all aspects that help minimise errors attributable to false exclusions.

The information that was collected or provided in the five cases where paternity was deemed incompatible showed the existence of circumstances that might explain the incompatibilities (doubts regarding the exact burial location or mass burials).

Multi-disciplinary study

As indicated in the "*Recommendations on genetic identification studies in cases of irregular adoptions and abductions of newborns*",⁶ issued by the National Commission for the Forensic Use of DNA, the anthropological and criminological study prior to the genetic study of the bone remains is crucially important. Information such as the number of individuals exhumed, their estimated age, and the state of preservation of the various recovered bone pieces, made it possible to select the fragments that would be most suitable for the study, and to explain certain genetic results that were obtained.

In a similar vein, in cases where clinical samples can be used, an pathological study is conducted before the genetic study to confirm the real attribution of the samples.

Quality of the results

The experience of the cases studies that were analysed confirms that the special characteristics of these types of samples makes it necessary to have laboratories certified with standard UNE-EN ISO/IEC 17025 in order to ensure the authenticity of the final results by establishing and complying with the necessary quality requirements, including: conducting the analyses in specially dedicated work areas, with specific equipment and materials; reviewing negative controls and extraction buffers; independent duplication of results; and contrasting results with a discard base. The high degree of affectation of the inconclusive samples is often times an obstacle to obtaining a sufficiently high number of STR markers. It needs to be possible to establish a minimum paternity index (or LR) value in the laboratory from which a conclusion can be drawn on whether the paternity under investigation is compatible. In the cases presented in this study, there was not always a direct match between the number of markers and the LR value that was obtained. The number of progenitors available was the most important factor for obtaining a high LR value.

Limitations derived from the preservation of the genetic material

In 32.8% of the cases the quantity or the quality of the retrieved DNA made it impossible to conduct the genetic analysis, and therefore issue conclusions on the investigations. Yet since there was a limited number of cases (19 in total), and in some of these cases there was no information available on the case, it was impossible to draw a direct correlation between the impossibility of conducting a genetic analysis and the taphonomical conditions of the burial (weather and environmental conditions of the exhumation area [temperature, humidity, pH, soil composition], burial age)⁵ or, if applicable, data on the preparation and preservation of the clinical samples.

Use of haplotypic markers

In spite of the limited resolution offered by analysis of haplotypic markers, they do provide a response to certain situations where studying STR markers fails due to the quality and/or quantity of the DNA that is retrieved, or because the available relatives are not suitable for an autosomal STR study. Yet conclusive results were not obtained in any of the cases under study where an mtDNA study was used. This circumstance might be explained by the fact that certain inhibitors could not be eliminated which may have affected the reaction of the PCR.^{12,13} The buffer that was used in this reaction is less optimised for acting with a certain inhibitor type than the one used in commercial kits for amplifying autosomal markers. Moreover, the DNA being highly degraded cannot be discarded as a probable cause of the absence of results.

On the other hand, the use of specific Y-chromosome markers was not a strategy that was used in the laboratory because the relatives who were available for the test were not suitable for doing so. In any case, studying haplotypic markers is yet one more strategy that should not be discarded when dealing with future cases of irregular adoptions and abductions of newborns, especially where the direct progenitors are not available. Even so, the conclusions derived from the exclusive study of these types of markers should be considered with caution.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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