



Digging up the recent Spanish memory: genetic identification of human remains from mass graves of the Spanish Civil War and posterior dictatorship



Miriam Baeta^a, Carolina Núñez^a, Sergio Cardoso^a, Leire Palencia-Madrid^a, Lourdes Herrasti^b, Francisco Etxeberria^{b,c}, Marian M. de Pancorbo^{a,*}

^a BIOMiCs Research Group, Centro de Investigación “Lascaray” Ikerkunea, Universidad del País Vasco UPV/EHU, Vitoria-Gasteiz 01006, Basque Country, Spain

^b Department of Physical Anthropology, Society of Sciences Aranzadi, Donostia 20014, Basque Country, Spain

^c Department of Legal and Forensic Medicine, Faculty of Medicine, Universidad del País Vasco UPV/EHU, Donostia 20014, Basque Country, Spain

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ABSTRACT

The Spanish Civil War (1936–1939) and posterior dictatorship (until 1970s) stands as one of the major conflicts in the recent history of Spain. It led to nearly two hundred thousand men and women executed or murdered extra-judicially or after dubious legal procedures. Nowadays, most of them remain unidentified or even buried in irretraceable mass graves across Spain. Here, we present the genetic identification of human remains found in 26 mass graves located in Northern Spain. A total of 252 post-mortem remains were analyzed and compared to 186 relatives, allowing the identification of 87 victims. Overall, a significant success of DNA profiling was reached, since informative profiles (≥ 12 STRs and/or mitochondrial DNA profile) were obtained in 85.71% of the remains. This high performance in DNA profiling from challenging samples demonstrated the efficacy of DNA extraction and amplification methods used herein, given that only around 14.29% of the samples did not provide an informative genetic profile for the analysis performed, probably due to the presence of degraded and/or limited DNA in these remains. However, this study shows a partial identification success rate, which is clearly a consequence of the lack of both appropriate family members for genetic comparisons and accurate information about the victims' location. Hence, further perseverance in the exhumation of other intact graves as well as in the search of more alleged relatives is crucial in order to facilitate and increase the number of genetic identifications.

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1. Introduction

DNA analysis has become a key tool for identifying victims from massacres, particularly in cases in which conventional forensic methods (i.e. radiology, anthropology, or odontology) are not enough discriminative.

DNA isolated from skeletal samples offers the possibility of determining the identity of unknown post-mortem remains by comparative genetic analyses with their alleged biological relatives. In the past, mitochondrial DNA (mtDNA) analysis was the first choice -and sometimes the only alternative- for successful

DNA profiling from skeletal remains, due to its high copy number per cell, resistance to degradation, and high mutation rate [1,2]. Because of its maternal inheritance pattern, this analysis was restricted to cases where maternal relatives were available. Fortunately, nowadays the development of more efficient DNA extraction methods, as well as more sensitive marker panels, enables to obtain nuclear DNA profiles from degraded remains [3,4]. Indeed, the study of autosomal short tandem repeats (STRs) is currently the preferred technology for identification purposes because of its simplicity, sensitivity, and high discrimination power [5]. The implementation of STRs of the Y-chromosome (Y-STRs), which reveals the paternal lineage, becomes particularly important for cases in which only male relatives are available for genetic comparisons [6]. Additionally, the study of X-chromosome STRs (X-STRs) has turned into a very useful complement of autosomal STRs in complex kinship analyses, due to its particular inheritance, which differs in male and female descendants [7]. On the other

* Corresponding author at: BIOMiCs Research Group, Lascaray Research Center, University of the Basque Country UPV/EHU, Avda. Miguel de Unamuno, 3, 01006 Vitoria-Gasteiz, Spain. Fax: +34 945 01 4458.

E-mail address: marian.mdepancorbo@ehu.es (M.M. de Pancorbo).

hand, the application of the analysis of reduced size amplicons, known as mini-STRs, has increased the success in typing challenging samples [8].

For the last decades, DNA analyses have played a major role in victim identification of natural disasters such as the Asian tsunami disaster in 2004 and Hurricane Katrina in 2005 [9]. Likewise, DNA profiling was fundamental in the identification of missing persons from terrorism attacks including the World Trade Center mass fatality in September 2001 [10] and Madrid in March 2004 [11]; dictatorship victims from Chile [12], Argentina [13] or Guatemala [14] in the 1970s and 1980s; or from war conflicts such as the Balkans in the 1990s [15,16] or more recent conflicts in Iraq or Libya; and even soldiers from the II World War [6].

Spain is the second country in the world, after Cambodia, in number of enforced disappearances whose remains in mass graves have not been recovered or identified [17]. During the Spanish Civil War (1936–1939) and the posterior dictatorship period (until 1970s), nearly 200,000 men and women were executed or murdered extra-judicially or after dubious legal procedures [18]. More than 300 mass graves and 5000 skeletons have been already exhumed across the Spanish territory [19], and some of these victims have been identified by multidisciplinary studies [20–23]. However, this barely represents 15% of the estimated 2000 burial sites; consequently, there is still a vast reservoir of remains and thousands of families who keep searching for the fate or whereabouts of their relative ones who disappeared during that inauspicious period.

Here we present the results of our experience of the last six-years (2010–2015) in genetic identification of human remains recovered from Spanish Civil War mass graves in the north of Spain.

2. Materials and methods

A total of 252 human remains recovered from 26 graves of the Spanish Civil War and posterior dictatorship, located in the north of Spain, were analyzed. More specifically, the remains of the following graves were studied (Fig. 1): La Tejera (Alava) ($n=1$), Intxorta (Gipuzkoa) ($n=3$), Ziardamendi (Gipuzkoa) ($n=4$), Etxaguen (Alava) ($n=15$), and Bóveda (Alava) ($n=1$) in the Basque Country; Ezkaba ($n=3$), Añezcar ($n=1$), Berriosuso ($n=1$), Aibar ($n=3$), Corella ($n=2$), Tudela ($n=1$), Urzante ($n=21$), Sima del Raso de Urbasa ($n=10$), Peralta ($n=1$), and Elia ($n=3$) in Navarre; Santa Eulalia de Gállego ($n=13$), Movera ($n=4$), and Vera de Moncayo ($n=10$) in Aragon; La Pedraja (Burgos) ($n=103$), Loma de Montija (Burgos) ($n=24$), Espinosa de los Monteros (Burgos) ($n=9$), Sorriba (Burgos) ($n=4$), Villamediana (Palencia) ($n=3$), Ágreda (Soria) ($n=3$), and Barcones (Soria) ($n=6$) in Castile and Leon, and Vilarmaior ($n=3$) in Galicia. Prior to genetic analysis, anthropological studies were carried out in order to individualize the skeletal remains and obtained basic biological data on sex, age-at-death, stature, ante mortem fractures, or possible cause of death, among other data.

In order to avoid contamination, recommendations suggested for work with ancient DNA (aDNA) were followed [24,25]. These include physically isolated work area laboratories for skeletal remains and reference samples, quantitation studies, and contamination monitoring in all steps using blank samples and negative controls.

For genetic analysis, long bones (i.e. femur, tibia or humerus) or teeth were preferably sampled. A 5 to 10 cm fragment was taken from each bone, and in the case of teeth, the complete piece was used. Prior to DNA extraction, bone surface was cleaned by physical



Fig. 1. Map of the mass graves under study from Spanish Civil War and posterior dictatorship. Basque Country: (1) La Tejera (Alava); (2) Intxorta (Gipuzkoa); (3) Ziardamendi (Gipuzkoa); (4) Etxaguen (Alava); (5) Bóveda (Alava). Navarre: (6) Ezkaba; (7) Añezcar; (8) Berriosuso; (9) Aibar; (10) Corella; (11) Tudela; (12) Urzante; (13) Sima del Raso de Urbasa; (14) Peralta; (15) Elia. Aragon: (16) Santa Eulalia de Gállego; (17) Movera; (18) Vera de Moncayo. Castile and Leon: (19) La Pedraja (Burgos); (20) Loma de Montija (Burgos); (21) Espinosa de los Monteros (Burgos); (22) Sorriba (Burgos); (23) Villamediana (Palencia); (24) Ágreda (Soria); (25) Barcones (Soria). Galicia: (26) Vilarmaior.

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