

Research article

# Contribution of forensic genetics to the recovery of historic memory of the Spanish Civil War

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## Abstract

Forensic geneticists are becoming of great importance referring to the Recovery of Historic Memory of the Spanish Civil War. Spanish geography certainly still hides a lot of graves in which many non-identified civil victims were buried approximately 70 years ago. Amelogenin locus of nuclear DNA analysis may help to confirm whether sex composition coincided with the information gathered in some historic reports, and also with anthropological classification. Occasionally, when there is availability of DNA samples of alleged relatives, the chance exists of analyzing mtDNA to check matching between the maternal lineage of the remains and the alleged relatives. Both, nDNA and mtDNA analyses may provide invaluable information to reconstruct historical episodes and to identify victims of the Spanish Civil War.

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## 1. Introduction

During the Spanish Civil War (1936–1939), many people were killed and buried into graves all over Spain. Currently, various projects addressed to the “Recovering of Historic Memory” [1], in some cases with participation of forensic geneticists, are helping to clarify this kind of events. The slaughter of Valdedios town (Asturias, Northern Spain) is among the most remembered of these episodes. In October 1937, 17 people, most of them workers of a psychiatric hospital, were forced to excavate their own tomb and once inside they were assassinated [2]. Spanish geography certainly still hides a lot of graves with their particular stories. Occasionally, when there is availability of DNA samples of alleged relatives, the chance exists of recovering and identifying the skeletal remains of missing persons.

## 2. Objective

As for the bone remains recovered from the grave of Valdedios (Asturias), the main objective was to check whether sex composition coincided with the information gathered in some historic reports, and also with anthropological classification. On the other hand, we analyzed skeletal remains from other four different graves located elsewhere in Spain (and also related with Civil War events), provided that alleged maternally related relatives were on disposal. In this case, hypervariable segments HVSI and HVSII from the mtDNA control region were analyzed to corroborate whether the recovered bone remains do share female genetic lineage with a group of alleged relatives.

## 3. Methodology

With respect to Valdedios, conservation status was, for the most part, rather bad. Nonetheless, the degradation varied depending on the position of each remain within the grave (Fig. 1). For the other four graves, femur bones were sent to

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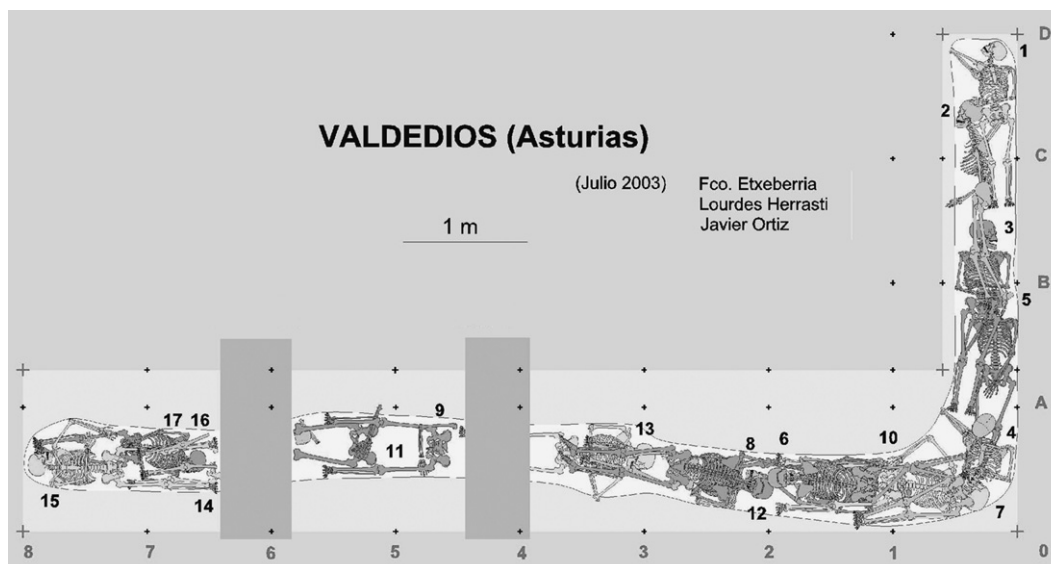


Fig. 1. Scheme of the grave of Valdedios, located in northern Spain, which was excavated in Summer 2003.

our laboratory jointly with a saliva swab of an alleged maternally related relative for each remain. Bone samples were analyzed as described in Ref. [3]. PCRs were performed for microsatellites and mitochondrial DNA, depending on the case, and the product of amplification tested by agarose gel.

**Amplification and analysis of nDNA:** DNA was analysed for sex determination of the Valdedios' bone remains. Two PCR were carried out. Firstly, we amplified the amelogenin locus in a standard PCR for subsequent RFLP analysis by cutting the amplicon with restriction enzyme Tsp509I. This enzyme recognizes the AATT target at the amelogenin locus of the Y chromosome. Secondly, for double check, we carried out a PCR with labelled primers for fragments size analysis on an ABI3130 Genetic Analyzer (Fig. 2).

**Amplification and analysis of mtDNA:** regions HVSI and HVSII were PCR amplified for four remains and their

respective alleged relative. Sequences were edited with ChromasPro 1.22 and aligned against those of the alleged relatives with ClustalX Multiple Sequence Alignment Program (1.81) [4]. rCRS (revised Cambridge Reference Sequence) [5] was used to report polymorphisms.

#### 4. Results and discussion

We have analyzed so far 13 DNA samples from Valdedios' remains for sex determination. Seven of them rendered amplified DNA and, according to results double-checked by RFLP and fragment analysis, they could be classed as female remains. The lack of DNA amplicons for several remains was attributed to their position at the bottom of the grave, directly on the soil, which is known to accelerate the degradation process. In sum, degradation status of bone remains largely determined the obtained results for 13 out of 17 samples examined. Yet,

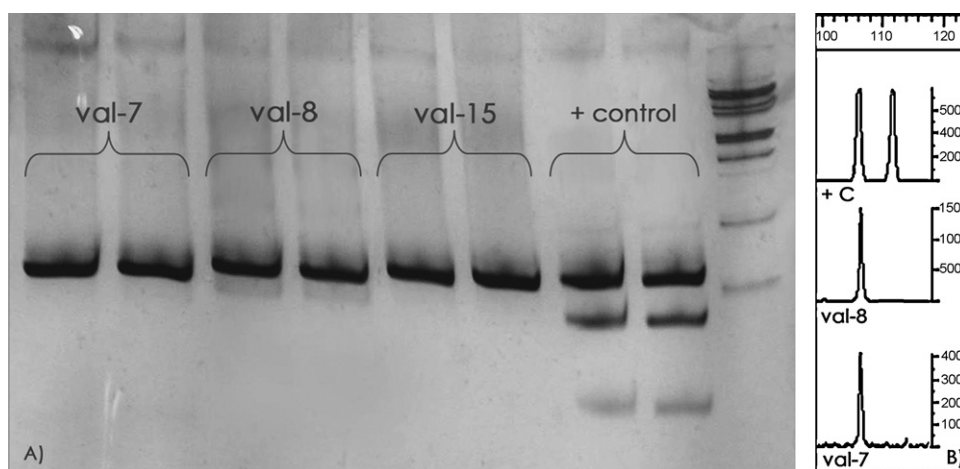


Fig. 2. (A) 15% acrilamide gel was used to check result of incubation with enzyme Tsp509I. Each sample was amplified twice. Cut with Tsp509I restriction enzyme signals presence of Y-chromosome, thus confirming male condition of the questioned sample. The three samples depicted herein proved to be female and (B) previous to RFLP confirmation analysis, samples were explored by fragment analysis of the amelogenin locus on a Genetic Analyzer ABI3130.

amelogenin analysis ratifies itself as a rapid and useful tool in those cases where checking sex composition of bone remains from a specific grave is needed to be contrasted with data recorded by Historic Register, or merely by local people memory.

As for maternal lineages analysis, regions HVSI and HVSII of mtDNA were successfully amplified in the four remains examined. In contrast with the expected, mtDNA data failed to detect close genetic affinity between the remains and the alleged maternally related relatives in all cases. This finding indicates how sometimes written or orally transmitted data are not completely trustworthy.

Summarizing, forensic genetics, in conjunction with other areas such as Archeology, Anthropology and even memories of survivors or their relatives, may provide invaluable information to reconstruct historical episodes and to identify victims of the Spanish Civil War.

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### **Conflict of interest**

None.

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