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A maternity case with human remains from a XIII–XIV century burial at Uceda, Guadalajara, Central Spain

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ABSTRACT

In a High Medieval age cemetery, dated from the XIII-XIV century (Uceda, Guadalajara, Central Spain), two bodies were found, buried in a curious position. One of the bodies, an adult, had close to its abdominal area a small number of little bones. It was not clear if it could have been a pregnant woman or, otherwise, two separated burials, at different times.

Anthropological experts confirmed that the second individual should be a fetus, being absolutely impossible to determine the sex. Furthermore, the adult was appointed as a woman.

Concerning the condition of the samples, the adult one was preserved, obeying to the authenticity criteria to select evidences for a critical DNA analysis. But the samples belonging to the second individual were very delicate and fragile, complicating the sampling work.

A genetic study will be carried out to find if there is any biological bond between the individuals, as well as, their biological sex. The analysis procedure had to be somewhat modified due to the sensitivity of the second individual samples.

So far, our preliminary results reveal that, if both individuals are not linked by maternal kinship, they must be, at least, relatives by maternal side, since they share the same maternal lineage.

Conclusions reached in the present study can help in mass disasters cases. In such situations, it is crucial to determine kinships between samples, despite their advanced state of degradation, which makes the improvement of this procedure a crucial point in forensic genetics.

1. Introduction

A maternity test, namely a genetic profile determination, can be the only possibility to disclose the identity of human remains due to its external degradation or inconclusive identity, such as an organ. Effectively, this kind of analysis is mandatory in complex forensic cases, such as terrorist attacks, postwar territories, natural disaster situations, missing people, and cases of refugees or other immigration circumstances.

In this work we analyzed two individuals that have been found in the same grave in a medieval cemetery of Uceda, (Guadalajara, Central Spain), in an uncommon position. Indeed, one of the bodies, an adult, had close to its abdominal area a small number of little bones. The anthropologists identified the adult body as a woman, and due to the proximity with the small bones there is the possibility that she had been buried pregnant. However, it always exists the possibility of them being unrelated, with the same or different sex, since it was not possible to determine the sex of the fetus by anthropological methods.

Regarding the samples condition, the adult skeleton seems to be just somewhat debilitated. Nonetheless, the fetus samples were very fragile, complicating the sampling process.

Accordingly to such evidences, it was our purpose to verify the maternity hypothesis, employing specific genetic markers

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indicated for critical samples, which includes the determination of belonging to the same maternal lineage.

2. Materials and methods

2.1. Sampling

Two teeth were collected from the adult, according to the authenticity criteria, and two different samples were collected from the fetus: a vertebra and a (possible) tibia.

2.2. Extraction

The external layer of the teeth samples was excised with a Sand-blaster, and irradiated for 30 min on each side of the sample. The fetus samples were carefully cleaned with the Sand-blaster and also irradiated, but during a small period (15 min each side) since these samples seemed to be extremely damaged. Then, the samples were pulverized in a freezer mill filled with liquid Nitrogen. Then, the extraction protocol was followed according to the described by Rohland et al. [1]. However, the fetus samples were sent in contact with the buffer extraction 24 h, instead of the usual 12 h, in order to increment the possibility to recover the maximum genetic material possible. DNA extracts were stored at −20 °C until the amplification took place.

2.3. PCR amplification and sequencing

In order to discard/admit a possible maternal relation, the two hypervariable regions (HV1—a 295 base pairs (bp) fragment, and HV2—a 360 bp fragment) of the mitochondrial DNA were analysed, by at least two independent amplifications [2].

On the other hand, to stablish the molecular sex, as well as a maternity kinship, the AmpFLSTR® NGM SElect™ PCR Amplification Kit was used to perform the amplifications of the nuclear genetic material, according to the user's manual [3]. Nonetheless, since we were dealing with critical samples, the number of PCR cycles was extended to 40 cycles.

2.4. Authenticity criteria

In order to obtain replicated and reliable results, sampling, cleaning, extraction and amplification processes had into account international recognized authenticity criteria procedures. Such measures includes, among others [4], exclusive critical DNA laboratory, being the pre-PCR and pos-PCR spaces physically separated; use of negative controls during all the analysis; from the beginning until the end, one single operator, as well as, the achievement of at least two replicated sequences from the same sample, and from different samples of the same individual.

3. Results

Concerning mtDNA, the results obtained seems to indicate a good quality of the genetic material, which required only two amplifications per sample. The haplotypes obtained for the adult and fetus are exactly the same (Table 1), and replicated results were always obtained, both with the forward and reverse sequence.

On the other hand, contrary to what was observed for the mitochondrial DNA, the nuclear information seems to have suffered extensive damage. In fact, the autosomal results are very limited, since it was not possible to obtain any information from the first three amplifications, and from 16 markers, only from 5 it was possible to get information (Table 1). According to our preliminary information, the genotype obtained for the fetus was XY, meaning a male biological sex.

All results were compared with the investigators' profiles and in both cases there are no sign of contamination (Table 1).

4. Discussion

From the mitochondrial point of view, all of the obtained sequences were positively replicated, indicating always the same haplotype, which seems to point out a possible maternal relation. However, from the genetic point of view, all of the maternal relationships such as mother – son, maternal grandmother-grandchild, maternal aunt – nephew or siblings are impossible to exclude, due to the way of mitochondrial transmission.

Regarding autosomal information, such results indicated the degraded and the damaged degree of the samples. It was quite difficult to obtain an autosomal profile, and only with the fourth amplification attempt it was possible to achieve some alleles from the second sample from both individuals. From the first sample it was not possible to amplify any kind of nuclear information. On the other hand, although only 5 from 16 markers were amplified, in 3 from these 5 there is a possible coincidence between 1 of the 2 alleles between the fetus and adults alleles. This information seems to support the maternity theory. However, such results must be taken into account with precaution, for two main reasons: it was not possible until now to replicate autosomal results and, for both individuals, the dropout rate is very high. Effectively, only for three markers (Amelogenin, D21S11 and D22S1045) it was viable to amplify two alleles in the fetus, and in the adult only in one marker (D21S11).

Finally, although it was not possible to amplify all of the genetic information from the 4 samples, it was an important achievement the recovery of some information from the fetus, since the samples were in a serious damaged condition. In fact, it is quite important a future replication of such results, mainly for the Amelogenin marker, since it seems to be the only way to determine the sex of a

Table 1
Mitochondrial and STRs NGM® kit results for four samples, belonging to two individuals (31-I-UC, an adult and 31-II-UC, a fetus). The mtDNA results seem to indicate that both individuals belong to the same maternal lineage, since they share the same mitochondrial haplotype. Concerning to STRs results, both individuals share one allele for each amplified marker, except for vWA marker, where there is no result for the adult individual.

Individual	Sample	Mitochondrial DNA results	Autosomal STRs				
		Haplotype	vWA	Amel.	D8S1179	D21S11	D22S1045
31-I-UC	31-I-UC-1	73G 152C 263G 285T 295T 16126C 16145A 16172C 16222T 16261T	–	–	–	–	–
	31-I-UC-2	73G 152C 263G 285T 295T 16126C 16145A 16172C 16222T 16261T		X	19	34.2 / 35	11
31-II-UC	31-II-UC-1	73G 152C 263G 285T 295T 16126C 16145A 16172C 16222T 16261T	–	–	–	–	–
	31-II-UC-2	73G 152C 263G 285T 295T 16126C 16145A 16172C 16222T 16261T	13	XY	19	33.2/34/ 34.2	11/17
Investigator 1		152C 236C 263G 16304C	18/19	XX	10/13	29/30	19/19
Investigator 2		263G 309.1C 315.C 16257T	14/15	XX	9/11	30/30	17/18

Amel.: Amelogenin marker.

fetus, as well as, the only way to obtain reliable information for such degraded biological samples.

Conflict of interest

None.

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References

[1] N. Rohland, H. Siedel, M. Hofreiter, A rapid column—based ancient DNA extraction method for increased sample throughput, Mol. Ecol. Resour. 10 (2009) 677–683.
[2] C. Gamba, E. Fernández, A. Oliver, et al., Population genetics and DNA preservation in ancient human remains from Eastern Spain, Forensic Sci. Int. Genet. Suppl. Ser. 1 (2008) 462–464.
[3] Applied Biosystems Life Tecnologies, AmpFLSTR® NGM SElect™ PCR Amplification Kit. User Guide, Thermo Fisher Scientific Inc. (2015).
[4] S. Pääbo, H. Poinar, D. Serre, et al., Genetic analyses from ancient DNA, Annu. Rev. Genet. 38 (2004) 645–679.