STR analysis of skeletal remains exhumed from a dry "water well" used as a massive clandestine burial in Argentina

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ABSTRACT

The military dictatorship that took power in Argentina from 1976 to 1983, brought enforced disappearance and death to thousands of people in the country. "Pozo de Vargas" is a 3.5 m wide and 60 m deep dry water well used as a massive clandestine burial site in Tucumán, Argentina. The site contained commingled remains as well as partially articulated body parts. Since 2011, EAAF Forensic Genetics Laboratory (LGF-EAAF) has been processing post mortem (PM) samples exhumed from the well to achieve the intra-skeletal re-association and the identification of the bodies or body parts. 981 skeletal remains, with different states of preservation, were analyzed for STR typing using commercial available kits. Different bone elements were exhumed for intra-skeletal re-associations (PM-PM genetic comparisons): teeth, femur and tibia represented more than 65% of sampling and almost all of them were successfully re-associated; spongy bones as scapula, vertebrae and clavicles yielded worse results, although almost 80% of them were also re-associated. 871 skeletal remains (89%) were successfully typed for autosomal STR markers and re-associated into 147 unique STR profiles meaning that at least 147 different individuals were thrown into the water well. After re-association, unique STR profiles obtained from the skeletal remains were compared to ante mortem (AM) family reference database in order to identify victims (AM-PM genetic comparisons), being possible to restore 113 victims to their families. Another challenge of this identification project was the presence of biologically related victims inside the well.

1. Introduction

The Argentinian military dictatorship resulted in the disappearance and death of thousands of people in the country. Bodies of a large number of victims were usually buried in the ground in primary (individual or mass) graves, but also in secondary burial sites, which usually resulted in remains intermingling. Since 1984, the Argentine Forensic Anthropology Team (EAAF) [1] is working to carry out the identification of the victims and to restore the remains to their families by searching and exhuming the victims' bodies. Exhumation process of skeletal remains in the "Pozo de Vargas" began in 2004 and was carried out in several stages, being the first remains found at 20 m deep. Bone sampling for genetic studies consisted mainly of diaphysis of long bones and teeth taken from skulls, although other bone remains such as vertebrae, ribs and clavicles, were also exhumed from the depths of the well. Since 2011, LGF-EAAF has been continuously processing post mortem (PM) samples exhumed from the well; at present it is working on more than 60 skeletal remains of this case (results not shown in this article). After the re-association of the skeletal elements exhumed from the well, it is necessary to continue working on the identification of these remains, therefore, unique STR profiles obtained are compared to ante mortem (AM) family reference database.

2. Materials and methods

981 skeletal remains that have been buried in the dry water well with 30 years post-mortem interval were analyzed. 65% of them were compact long bones and teeth, while the remaining 35% were spongy or less compact bones. Since the LGF-EAAF has been working in this case for almost 10 years, laboratory procedures used in different stages of the analysis have been updated. DNA extraction protocols have changed, from 2 g of bone manually processed using silica-based DNA extraction method with QIAGEN maxi columns [2,3] to an automated platform silica-based DNA extraction, using Automate Express DNA extraction system and PrepFiler™ BTA Forensic DNA Extraction Kit (Applied Biosystems), processing 1 g of bone with full demineralization [4,5]. Quantifiler Human and Quantifiler HP were used for DNA quantitation. Identifiler and Power Plex Fusion were the commercial kits used for STR typing. DNAview software (unmixed stain and kinship analysis tools) was used for statistical calculations [6]. Prior odd values of 1/866 and 1/160 were considered for male and female samples respectively, according to the non-DNA data. STR profiles were considered as reportable when the RMP were 1.2e-7 (males) and 6.3e-7 (females) according to the mentioned prior odds. A posterior odd value of 10,000 was the threshold to report intra-skeletal re-association and identification results.

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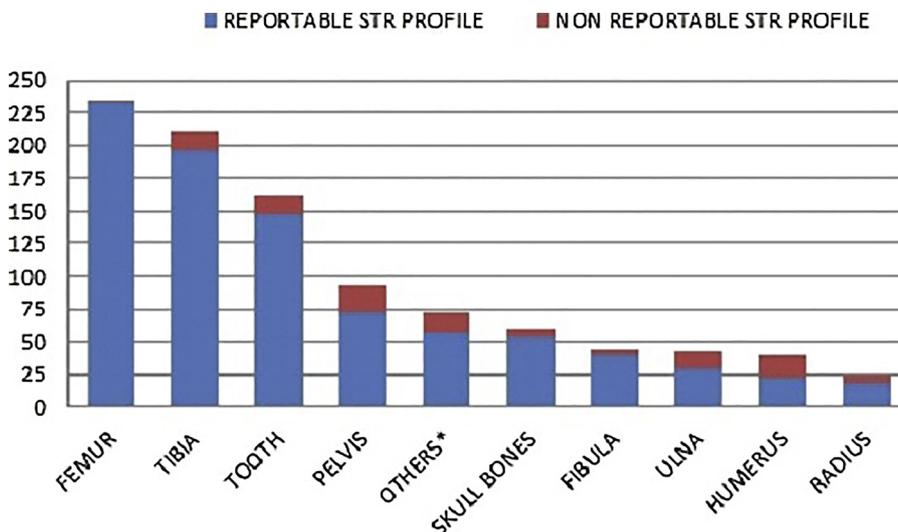 *E-mail address:* marialauracatelli@gmail.com (C. Vullo).

Fig. 1. STR results success rate, according to the type of skeletal element: Femur 99%, Tibia 93%, Tooth 91%, Pelvis 77%, Others* (vertebra, rib, clavicle, scapula, foot bones) 79%, Skull bones 92%, Fibula 91%, Ulna 69%, Humerus 58%, Radius 75%.
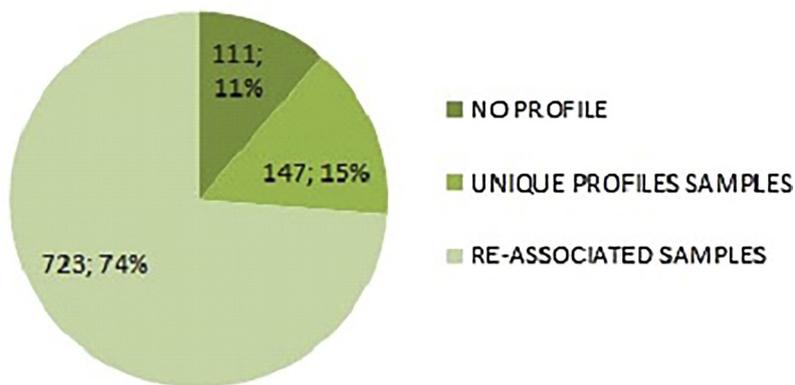

Fig. 2. Global results. Unique profile represents number of different individuals found. Re-associated samples according to profiles found.

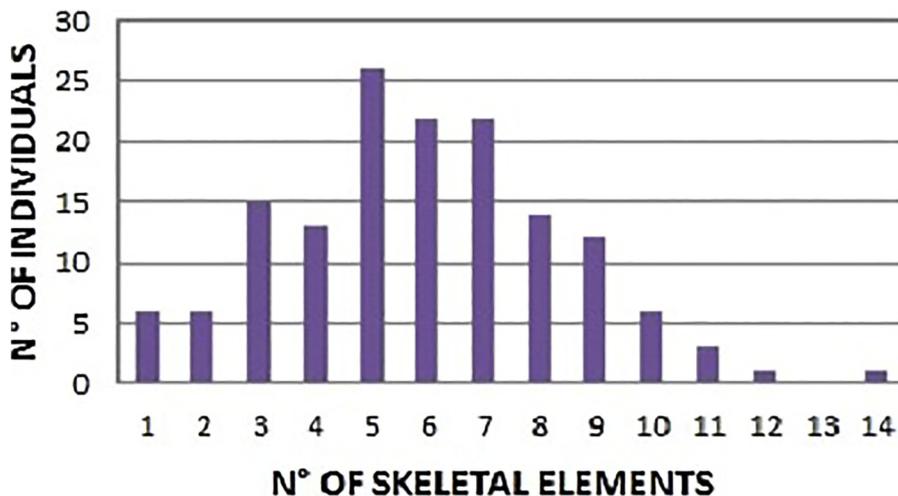**Fig. 3.** Number of individuals re-associated into "n" skeletal elements.

3. Results

Out of 981 samples analyzed, autosomal STR markers typing results allowed the investigation of intra-skeletal re-association in 870 samples (89%). Fig. 1 summarizes the number of samples analyzed of each type of skeletal remain and their results for STR analysis. Long bones of the lower body, teeth and some bones of the skull as mastoid and petrous were the skeletal elements that yielded best results. A lower success rate in STR typing was observed in the extremities of the upper body. According to the information described in Figs. 2 and 3, skeletal remains were re-associated into 147 unique STR profiles; it means at least 147 different individuals were exhumed from the water well. Most of the individuals were re-associated into 5 to 7 elements. After intra-skeletal re-associations, remaining unique profiles were compared by kinship analysis against AM family reference database representing almost 6000 missing persons [7,8]. As a result of this large scale comparison, identification of 116 individuals (79%) was achieved. Biologically related victims could be individualized in 3 families, as the family reference pedigree allowed distinguishing between them; on the other hand, related victims could not be individualized in 4 families because of limited family reference pedigree.

4. Conclusion

DNA analysis by autosomal STR typing allowed the investigation of intra-skeletal re-association of almost 1000 samples, resulting in a minimum number of 147 individuals exhumed from 60 m deep dry water well. It also allowed the identification of 116 victims. Correlation between type of skeletal element and autosomal STR typing success rate was observed, as described in [9]. As bone remains are still being processed and family reference database is frequently updated, LGF-EAAF continues working on PM-PM and large scale AM-PM genetic comparisons periodically.

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