



Research article

Analysis of complex kinship cases for human identification of civil war victims in Guatemala using M-FISys software

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ABSTRACT

Twelve years after the end of Guatemala's 36-year internal conflict, the depth and breadth of loss of human life during this time is now being calculated, and the number is staggering. FAFG has been working for several years trying to reconstruct the historic memory. Now with DNA technology running at FAFG laboratory, our ability to identify victims is much greater, but the data behind these identifications has become much more complicated. To help manage the data FAFG has employed M-FISys software, which contains tools and algorithms for use in elucidating very complex kinship relationships that cross familial as well as generational lines, giving alternative approaches to complex kinship relationships and management of population genetic information. In FAFG's first comparison of 67 victims to 451 family references numerous genetic leads were made and here we describe a family tree compound of 3 family groups that are biologically related to one another and in total consist of 42 individuals spread over 5 generations, including: 14 missing persons; 12 living family members who donated biological samples for DNA analysis and 16 individuals who were either deceased or unavailable for sample collection. This case exemplifies the depth and breadth of the losses as well as the nature of complex kinship between victims and relatives. Through the use of integrated software to combine ante-mortem and post-mortem data into one system, large-scale human identification can be made easier and simpler for those tasked with large-scale or complex human identifications.

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1. Introduction

In 1996 a peace treaty was signed between the Government of Guatemala (GoG) and a group of guerillas known as the *Union Revolucionaria Nacional Guatemalteca* (URNG). The fighting between GoG military forces and the URNG wreaked havoc in Guatemala's peaceful indigenous populations. The 36 years of internal conflict and war left Guatemala with 150,000 dead, 200,000 orphaned children and 50,000 forced disappearances [1].

Following the cessation of hostilities in 1996 and in response to the need for forensic investigative services, the *Fundación de Antropología Forense de Guatemala* (FAFG) began the process exhuming and identifying human remains from mass graves. Currently, FAFG is actively working in 22 regions throughout Guatemala and has conducted 946 investigations of mass graves and reports of forced disappearances [2].

To enhance the reach of these investigations and offer a state of the art tool to victims, FAFG implemented Central America's first fully functional forensic DNA laboratory in November of 2008. The Human ID effort that FAFG is undertaking in Guatemala is an answer

to the demand of justice and closure of the thousands of victims and relatives who suffered from the civil war. The DNA laboratory, though specifically designed to resolve the fate of missing persons from the war, is capable of processing any biological sample for human DNA. The laboratory is currently preparing for ISO 17025 accreditation, which is expected later this year.

2. Materials and methods

Prior to FAFG's laboratory becoming operational, cuttings from femur bones and tooth of 106 bodies found in mass graves, and 451 family and FAFG employee reference buccal swabs were outsourced to The Bode Technology Group (Lorton, VA, USA) to obtain STR profiles using Identifiler[®] (Applied Biosystems). This outsourcing project began FAFG's DNA-based identification process and allowed FAFG to obtain reference material for validation and training. FAFG re-analyzed DNA profiles using GeneMapper ID v3.2 (Applied Biosystems). DNA STR profiles were imported into M-FISys software (Gene Codes, Ann Arbor, MI, USA) to manage data, make statistical calculations and to elucidate cases involving complex kinship using the US Hispanic population database.

For FAFG's human identification efforts it created a STR population allele frequency database from the STR profiles of the 451 outsourced reference samples. The 451 samples were comprised

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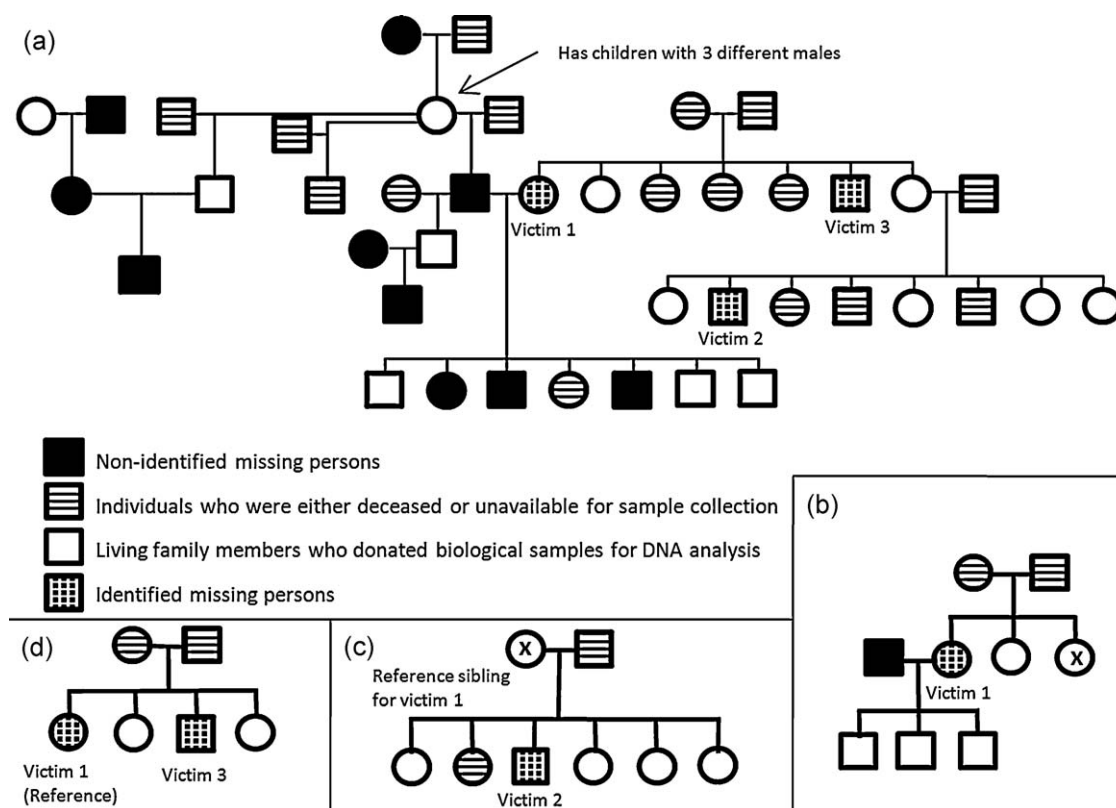


Fig. 1. (a) Family tree representing all 42 individuals from the extended family: victims, reference sample donors and people with sample not available. (b) Victim 1 identification using two siblings and three children as reference samples. (c) Adding mother as a reference (which also was used as a reference for sibling for victim 1, marked with an X) increase statistical power for the identification of victim 2. (d) Once victim 1 is identified its STR profile could be used as a reference to identify victim 3.

of 83 Mestizo, 269 Kaqchikel, and 99 Ixil. FAFG used Arlequin 3.1 [3] to analyze the STR allele frequencies from the three population to (1) determine if the allele frequencies of the three groups were similar enough to be combined into one database, which they were and FAFG did combine; and (2) which samples to use in the combined allele frequency database so that allele frequencies are in HWE. The final database consisted of a total of 205 of the 451 profiles, comprised of 83 Mestizo, 92 Kaqchikel, and 30 Ixil. Previous work by Martínez-Espin et al. [4] genetically described 200 individuals from the Mestizo population of Guatemala.

3. Results

Fig. 1a shows a family tree compound of three family groups that are biologically related to one another and in total consist of 42 individuals spread over 5 generations, including: 14 missing persons; 12 living family members who donated biological samples for DNA analysis and 16 individuals who were either deceased or unavailable for sample collection. From this case three identifications were made. The first identification (Fig. 1b) was made comparing STR profiles from siblings and children from the victim 1 with a profile from a bone sample and a combined kinship index (KI) of 6.5×10^{10} was obtained, thus the sample cannot be excluded as the victim's remains. Based on pedigree information, a reference sample provided to be used as a sibling reference for victim 1 was used also as a mother reference for victim 2 (Fig. 1c). The KI value obtained for victim 2 when using also the mother (6.5×10^{10}) increased 71 times compared to the KI obtained when using just the siblings (9.1×10^8). The identification of victim 1 allowed the use of its profile as a sibling reference to identify victim 3 generating a higher KI (2.0×10^{11} instead of 8.3×10^9), thus opening the possibility of identifying a third victim (Fig. 1d). Of particular interest is one female, as noted in Fig. 1a, that has

children with three different males and coincidentally links the families together.

4. Discussion and conclusions

The magnitude of the number of victims has a direct impact in the complexity of the pedigrees and the amount of data to be managed. As a result mistakes can be committed during the establishment of donor–victim relation. This mistake would be reflected as miscalculations of KI and therefore the lack of identifications. M-FISys is a powerful tool that allows for the proper administration of family relationships and family trees. Using the Kinship Sketch function of M-FISys, FAFG was able to modify the pedigrees to quickly re-asses data for alternative hypotheses in real-time. Similarly, roles of family references can be changed, allowing for correction of misstated familial relationships. This flexibility also permits the user to relate reference samples to different scenarios pertaining to different victims, easily allowing an identified person to serve as a reference for another unidentified person, as is the case for victim 3. As identifications proceed, more reference information is available for the other non-identified victims.

Implementing DNA technology in post-conflict Guatemala presented several challenges: time since the events occurred, lack of understanding about DNA within indigenous populations, lack of direct references, the existence of 23 separate ethnic populations (each of which speak a separate language) that have never been genetically described, the high number of missing persons per family, the highly degraded skeletal remains and the extraordinarily complex kinship relationships within the indigenous populations. As FAFG DNA laboratory became online, the amount of genetic information, both from victims and references related, will increase, and so will the chances of identifying the missing.

Conflict of interest

None.

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