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Rapid Communication

Y-STR analysis of highly degraded DNA from skeletal remains over 70 years old

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Abstract

The goal of the following study is to clarify whether the skeletal remains over 70 years old from missing persons and their alleged relatives shared identical Y-STR loci. Nowadays, advances in ancient DNA extraction techniques and approaches of using multiple different Y-STRs have significantly increased the possibility of obtaining DNA profiles from highly degraded skeletal remains. Given the ages and conditions of the skeletal remains, ancient DNA extraction methods can be used to maximize the probability of DNA recovery. Considering that information about distant relatives is more relevant for long-term missing persons and alleged family members are male, Y-STR loci analysis is considered the most appropriate and informative approach for determining paternal lineage relationship. In this study, Y-STR genotypes obtained from these alleged relatives were identical to each other and to the alleles of missing persons' consensus profiles at more than 22 loci examined, whilst not being found in Y-STR population database from Y-Chromosome STR Haplotype Reference Database. Therefore, Missing Person No.7 and Missing Person No.18 have a patrilineal relationship with reference samples from Family1 and Family2, respectively. In addition, the fact that Y-STR haplotypes obtained from skeletal remains of missing persons and reference samples are not found in the Han Chinese people from East Asian demonstrates its rarity and further supports a paternal lineage relationship amongst them.

Keywords: Y-STR analysis; skeletal remains; highly degraded DNA; forensic genetics

Introduction

There are numbers of forensic case-work scenarios in which skeletal remains may be the only viable sample type for DNA testing, including war conflicts, natural disasters, airline crashes, fires, etc. [1–5]. In addition, skeletal remains are a valuable source of DNA in historical, anthropological, and archaeological investigations [6–9]. Skeletal remains are one of the most challenging sample types for DNA testing because of their long-term exposures to various environment insults, including the effects of soil acidity. Humic and fulvic acids in soil can also inhibit PCR amplification. Since DNA recovered from skeletal remains is often degraded and in low quantities, it is necessary to perform more than one type of marker analysis on such samples in order to compile sufficient data for identification. Autosomal STR typing sometimes fails or results in partial profiles that may not be sufficient for rendering an identification. Nevertheless, lineages markers, such as Y-STR loci and mitochondrial DNA (mtDNA), can lead to complement autosomal STR results and anthropological metadata to increase statistical confidence in identification.

Although the identification accuracy of skeletal remains increases with the number of relatives typed, in some cases the number of reference samples available may be quite limited [10]. One approach to improving the power of identification is to type additional markers [11–14]. The mtDNA testing is useful to trace the matrilineal ancestry of an individual since mtDNA is passed down unchanged by the mother to all her children, both male and female. Consequently, mtDNA tests can be taken by both men and women. Lineage-based Y-chromosome markers can provide additional data to support or refute putative familial relationships. In some cases, lineage markers may be the only informative markers to associate unidentified remains and their living relatives [15, 16]. Currently, there is barely any report on the identification of decade-old skeletal remains on the basis of circumstantial evidence and lineage-based marker analysis. Thus, it is necessary to determine potential kinship between missing persons and their alleged relatives.

In this study, considering the genetic recombination that occurs in autosomal DNA over the generations within a family, Y-STR analysis was determined to be the most appropriate and informative approach for confirmation of potential kinship [17, 18]. Skeletal remains of six missing persons and five reference samples from the supposed biological

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relatives of these missing persons were selected and analyzed by employing an optimized method for efficient ancient DNA (aDNA) extraction. Y-STR results were then generated using three commercial kits (Yfiler® Platinum PCR Amplification kit, Goldeneye™ DNA Y Plus PCR Amplification kit, and FastDirect DNA 44Y PCR Amplification kit) to confirm alleged relationships. All of the three kits contain reagents to simultaneously amplify 38 Y-STRs, 41 Y-STRs, and 44 Y-STRs, respectively, along with the core Y-STR loci advocated by the Scientific Working Group on DNA Analysis Methods and European minimal haplotype (https://www.swgdam.org/publications).

Materials and methods

Sample preparation

Upon exhumation, it was discovered that skeletal remains of martyrs had been buried in soil with plenty of vegetation on the surface. Their teeth and bones (tibia, femur, and mandible) with adequate structural integrity were retrieved from the burial site and sent to the Key Laboratory of Evidence Identification in Universities of Shandong Province, Shandong University of Political Science and Law, China for analysis. According to other studies and our previous experiences with ancient samples [19–22], DNA extracted from teeth is often of higher quality than DNA extracted from bones, thus teeth were chosen here as samples for DNA extraction. When preceding DNA extraction, in order to remove surface contaminants, six samples of human teeth over 70 years old (postmortem) were bathed in 5% Na-hypochlorite (Sinopharm Chemical Reagent, Shanghai, China) for 5 min and rinsed once with distilled water. Next, the tooth samples were soaked in absolute ethanol (Sinopharm Chemical Reagent) for 5 min, then transferred to a clean disposable urinalysis cup and left to air-dry overnight. Before drilling the teeth into powder, all samples were exposed to UV-light for 30 min on each side. In this study, protocols for minimizing contamination during the handling of skeletal remains were performed according to the archaeological recommendations [23]. In this study, blood spot of reference samples from alleged family members were collected using FTA cards. Alleged relatives from two different families claimed to share the same paternal line with missing persons.

DNA extraction and purification

DNA extraction of teeth samples were performed at the clean DNA laboratory at Shandong University of Political Science and Law, China, for analysis following a published DNA extraction method [24, 25]. Prior to DNA extraction, laboratory areas and equipment were cleaned with DNA-off and 75% ethanol, then irradiated by UV-light overnight. To maximize DNA yield, a large-scale silica-based extraction method combined with complete demineralization was employed [26]. Approximately 50 mg of powder was drilled and incubated for 24 h at 37°C in 1 mL of extraction buffer containing 0.45 mol/L EDTA (pH 8.0) and proteinase K (0.25 mg/mL) with 0.05% Tween 20. Subsequently, the supernatant was transferred into 13 mL of binding buffer containing quinidine hydrochloride and purified with silica-based MinElute spin columns (Qiagen, Hilden, Germany), then the DNA was eluted in 50 μL of elution buffer.

DNA quantification, amplification, and DNA profiling

DNA quantification of the extracts using Qubit™ 1X dsDNA High Sensitivity assay kits was done for the purpose of evaluating the final concentration of DNA. In order to increase the number of amplified Y-STR loci, we used three different kits for the amplification of Y-STRs: Yfiler® Platinum PCR Amplification kit (Thermo Fisher Scientific, Waltham, MA, USA) including 38 Y-STR loci and 3 Y-InDel loci, Goldeneye™ DNA Y Plus PCR Amplification kit (Peoplespot, Beijing, China) including 41 Y-STR loci and 3 Y-InDel loci, and FastDirect DNA 44Y PCR Amplification kit (Biotech Original, Beijing, China) including 44 Y-STRs and 3 Y-InDels, as per the manufacturers' recommendations. Amplifications were performed in ABI GeneAmp® 9700 PCR System, and all teeth extracts had been processed prior to the amplification of reference sample DNA. To avoid contamination and cross-contamination, PCR preparation was done in the laboratory dedicated particularly to PCR amplification.

DNA separation, detection, and data analysis

Amplified DNA products were separated by size and detected on an ABI 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) with pop-4 polymer and standard (default) injection parameters. Raw data retrieved were analyzed using the GeneMapper® ID-X software v1.5 (Thermo Fisher Scientific). An allelic ladder was included at least once per injection on the 96-well plate. During the process, the DNA typing, quality control, and assignment of nomenclature all went by the recommendations of the International Society of Forensic Genetics [27].

Calculation of likelihood ratios

To confirm the alleged relationship, the likelihood ratio (LR) of patrilineal relationship versus non-relationship of male pairs given their Y-STR profiles was calculated [28]. LR was calculated as LR = P(E/H0)/P(E/H1), where H0 is hypothesis that ancestor and offspring are related and H1: hypothesis that ancestor and offspring are unrelated. For more detailed calculation formula can see the document (https://yhrd.org/downloads/YHRD_kinship_formula.pdf). LRs based on Y-STR were obtained using the inverse of frequency of Y-STR observed from Y-Chromosome STR Haplotype Reference Database (YHRD; www.yhrd.com) [29], and were estimated by accounting for the uncertainty because of sampling errors [30]. To calculate the cumulative LRs including Y-STR data, the Y-STR haplotype was taken into account only once. In consideration of allelic drop-out, a partial match was allowed for the calculation of Y-STR haplotype frequencies.

Results and discussion

In general, autosomal STR markers provide results highly accurate for the analysis of kinship, but not of distant relatives. Uniparental makers are much more informative for this type of analysis. Considering that information about distant relatives is more relevant for long-term missing persons, the analysis of uniparental markers, such as Y and mitochondrial markers, is routinely used to study not only individual origin and migrations, but also identifications of missing and unknown persons [9, 22, 31]. In this study, alleged family members claimed to be from the same paternal line, so Y-STR

analysis was considered the most appropriate and informative approach for determining potential kinship. According to other studies [19–22] and our previous experiences with ancient samples, DNA extracted from teeth is often of higher quality than DNA extracted from bones, thus we used tooth samples for genetic analysis.

Forty-four STR loci on the Y-chromosome of extracts from reference samples and tooth samples were examined, and complete Y-STR profiles of the reference samples were obtained. Tooth samples were buried underground for more than 70 years so the DNA was highly degraded and present at low copy number as expected. Notwithstanding, as shown in Supplementary Table S1, nearly complete Y-STR profiles could be generated. For the sample Missing Person No.17, only 21 Y-STR loci in total amplified, which could be attributed to poor preservation of analyzed tooth sample. The alleles observed in the partial profiles from each sample were compared for concordance, and a consensus Y-STR haplotype was generated each with three difference kits. Alleles observed in the partial profiles from one of the three kits were to identify the final and effective Y-STR loci genotype. Allele calls generated for the same loci amongst the three kits were consistent, except DYF387S1, DYS444, DYS518, and DYS527 from sample Missing Person No.7.

The principal goal of this study was to determine whether familial relationships existed amongst missing persons and their alleged relatives. The Y-STR haplotypes obtained from Family1-B2 and Family1-B3 were identical to each other and to the alleles in consensus profile of Missing Person No.7 at 27 Y-STR loci and 28 Y-STR loci examined, respectively (Table 1). In general, comparative analysis of Y-STR profiles revealed that all individuals shared the same Y-haplotype, indicating patrilineal kinship [17]. However, an exception occurred at DYS437 locus, where allelic variant 15 was detected in the case of individual Family1-B2, indicating the possibility of single-step mutation [32]. In the cases when only one mismatch was detected and kinship was not rejected, a one-step mutation was considered to have occurred. The Y-STR haplotypes obtained from Family2-B1 to B3 were completely consistent and identical to Missing Person No.18 at 22 Y-STR loci (Table 1). The Y-STR haplotypes obtained from Missing Person No.6, Missing Person No.11, Missing Person No.17, and Missing Person No.19 were not consistent with reference samples form Family1 and Family2. Since the missing persons were known to be of East Asian (Han Chinese) descent, LRs were calculated using similar affine populations. As shown in Tables 1 and Supplementary Table S2, the LRs based on Y-STR of YHRD database were 13 and 19 491 for Missing Person No.7 with two reference samples (Family1-B2 and Family1-B3), respectively. Missing Person No.18 and the alleged relatives shared a common allele at 22 Y-STR loci, and the LRs were mostly over 19 425 (Table 1). In other words, it was 19 425 times more likely to observe the Y-STR results above when Missing Person No.18 and the alleged relatives were paternally related as opposed to unrelated. Therefore, Missing Person No.7 and Missing Person No.18 have a patrilineal relationship with reference samples from Family1 and Family2, respectively. In addition, the fact that the Y-STR haplotype obtained from skeletal remains of missing persons and reference samples was not found in either YHRD population database demonstrated the rarity of its occurrence and further strengthened this conclusion of kinship.

Conclusion

DNA genotyping can be successfully performed for old skeletal remains with a highly effective DNA extraction method and the application of low copy number DNA interpretation rule. As might be expected with old skeletal remains, incidences of allele drop-in were observed in a few samples, whereas incidences of allele drop-out were observed in all ancient samples tested. Cumulative LRs obtained from Y-STR results confirmed the alleged relationships between missing persons and their relatives with great probabilities. Overall, this study emphasizes the efficiency and usefulness of the DNA extraction method in old skeletal remains as well as the enlightenment of employing different Y-STR loci in archaeogenetics studies.

Authors' contributions

Xuebo Li and Suhua Zhang conceptualized the research study, designed the methodology, and supervised the project. Jiashuo Zhang and Liangliang Li collected the data, conducted the analysis, and wrote the original draft. Jiashuo Zhang, Anqi Chen and Suhua Zhang provided critical revisions and edited the final manuscript. All authors reviewed and approved the final manuscript.

Compliance with ethical standards

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Shandong University of Political Science and Law.

Disclosure statement

None declared.

Table 1. Summary of statistical analyses from between skeletal remains over 70 years old and reference samples from alleged family members.

Skeletal sample	Reference sample	Number of consistent Y-STR loci	The LR of patrilineal relationship *versus* non-relationship
Missing Person No.7	Family1-B2	27	13
Missing Person No.7	Family1-B3	28	19 491
Missing Person No.18	Family2-B1	22	19 425
Missing Person No.18	Family2-B2	22	19 425
Missing Person No.18	Family2-B3	22	19 425

LR: likelihood ratio.

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