B180 Ashes to Ashes: An Analysis of Enhanced Methods for Genetic Identification of Human Cremated Remains

Kelly Grisedale, PhD, Western Carolina University, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723; and Maureen Hickman, MS, Western Carolina University, 111 Memorial Drive, NSB 231, Cullowhee, NC 28723*

After attending this presentation, attendees will better understand the potential and limitations of genetic investigations of cremated human remains.

This presentation will impact the forensic science community by providing a way to gain maximum information from cremated human remains, which have historically been very difficult to identify.

Human cremation is a common funeral practice in many cultures throughout the world. In the United States, the preference for cremation continues to increase, with the rate of cremation soon expected to surpass that of burials. After cremation, there may be a need to confirm the identity of the remains for reasons including civil or criminal cases, paternity or kinship analysis, or identification of missing or deceased individuals. This has traditionally fallen to anthropologists, who can employ various metric analyses to assess whether the contents of an urn are in fact human cremated remains and can assist in determining a biological profile including sex, stature and weight.

Historically, genetic examination of cremated remains has been limited due to lack of remaining DNA and concerns regarding contamination. The cremation process involves burning at temperatures up to 1,000°C, reducing the corpse to skeletal remains or bone fragments, which are then often further ground to a sand-like consistency; however, depending on the method used to grind the remains, sizeable bone fragments may still be found in the urn returned to family members. Previous investigations into DNA analysis from cremated remains have shown limited success. Studies testing the sand-like portion of the remains found that small amounts of nuclear and mitochondrial DNA were recoverable, but the potential for contamination was too great to ensure reliable results, while examination of remaining bones or teeth resulted in little to no DNA recovery; however, advances in DNA extraction and detection methodologies may present an opportunity to re-examine the process of genetic identification from the remaining bone fragments.[1,2]

This work examines adjustments or enhancements along the entire DNA analysis workflow to improve identification methods from human cremated remains. DNA was extracted from bone fragments using either a commercial silica-based method or an enhanced extraction method that modifies the commercial kit to determine which process resulted in maximum DNA recovery. Multiple extracts from a single bone fragment were pooled, then concentrated using an Eppendorf Vacufuge™. All extracts were quantified using real-time Polymerase Chain Reaction (PCR) to assess nuclear or mitochondrial DNA (mtDNA) recovery. Concentrated extracts were then amplified using either the GlobalFiler® PCR Amplification Kit with an increased PCR cycle protocol and analyzed using capillary electrophoresis or an in-house-developed whole mtDNA genome multiplex for sequencing on the Illumina® MiSeq®. Multiple samples from individual sets of remains were examined to assess consistency across results.

Results indicate that low levels of nuclear DNA can be recovered from cremated bone, with partial Short Tandem Repeat (STR) profiles obtained from pooled and concentrated extracts; however, exaggerated stochastic effects such as increased stutter, allele drop-out, and peak-height imbalance were observed in some profiles due to the low amount of starting template and use of an increased-cycle PCR, thereby complicating the profile interpretation.

Results also revealed that sufficient mtDNA can be recovered from cremated remains for whole mtDNA genome sequencing using a multiplex amplification approach. Massively parallel sequencing of amplified mtDNA yielded an average coverage of 10,500 reads across the genome, with 97% of the genome covered by at least 100 reads. Preliminary variant analysis suggests the remains are likely to be from a single source.

Reference(s):

1. von Wurmb-Schwark N., Simeoni E., Ringleb A., Oehmichen M. Genetic investigation of modern burnt corpses. *Int Congr Ser.* 2004:1261:50-52.
2. Tsuchimochi T., Iwasa M., Maneno Y., Koyama H., Inoue H., Isobe I., Matoba R., Yokoi M., Nagao M. Chelating resin-based extracting of DNA from dental pulp and sex determination from incinerated teeth with Y-chromosomal alphoid repeat and short tandem repeats. *Am J Forensic Med Pathol.* 2002:23(3):268-271.

Cremated Remains, STR Profiling, MtDNA Sequencing