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Luka Papac, Bastien Llamas, Alan Cooper, Varsha Pilbrow and Wolfgang Haak
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CONTEXT AND CONNECTION

Studies on the Archaeology of the Ancient Near East in Honour of Antonio Sagona

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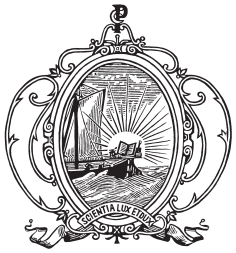

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PRELIMINARY ANCIENT DNA SCREENING RESULTS FROM FIRST TO EIGHTH CENTURY AD SITES IN SAMTAVRO AND TCHKANTISKEDI, GEORGIA

Luka PAPAC, Bastien LLAMAS, Alan COOPER,
Varsha PILBROW and Wolfgang HAAK

ABSTRACT

Ancient DNA allows the analysis of the genetic makeup of past populations. However, due to the highly variable nature of DNA preservation it is often useful to screen samples in order to assess the quality of DNA preservation before deciding how to invest resources into further sequencing efforts. Here we present screening results of 34 human bone and teeth samples showing a high variation in DNA preservation not only across the whole cemetery but also within tombs of the same cemetery. Preliminary analyses show that preservation levels are satisfactory for genetic characterisation of this ancient population.

* * *

INTRODUCTION

A major hurdle in ancient DNA studies is the quality of biochemical preservation. Shortly after death, repair mechanisms that uphold the integrity of the DNA within each cell cease to function and DNA begins to degrade.[1] In addition, microorganisms such as bacteria and fungi then colonise the organism and often the majority of DNA extracted from archaeological organic remains, for example, from human or animal bones or teeth, comes from such environmental contaminants.[2]

The quality of DNA preservation can be estimated by assessing the percentage of DNA fragments of human origin in each DNA extract. This can be done by sequencing a random assortment of fragments from a DNA extract (a so-called DNA library) and determining the proportion of fragments which match DNA sequences found in the human reference genome.[3] Here we report on the screening of 34 samples dated from the first to eighth centuries AD from Samtavro and Tchkantiskedi, sampled as part of Georgian-Australian Investigations in Archaeology, and funded by an Australian Research Council Discovery Project awarded to Professor Antonio Sagona and Professor David Lordkipanidze.

[1] Pääbo *et al*. 2004.
[2] Carpenter *et al*. 2013.
[3] Carpenter *et al*. 2013.

METHODS

We collected 38 bone and tooth samples, representing 33 individuals, currently held in the Mtskheta excavation house near the Samtavro cemetery, Mtskheta. Protective gear and clothing including body suits, gloves and facemasks were worn throughout sampling to minimise risk of modern contamination. Tough, rigid and fresh-looking bone was preferentially sampled and sent to the facility at the Australian Centre for Ancient DNA, Adelaide, Australia. There, all samples were wiped with bleach and UV-irradiated for 20 minutes on each side before having their outer surface mechanically removed using a Dremel drill and disposable cutting disc. 4 of the initial 38 samples were deemed unsuitable for further processing. 34 samples were pulverised in a bone mill and 200 mg of the resulting bone powder was used for DNA extraction.[4] We performed a test polymerase chain reaction (PCR) followed by gel electrophoresis in order to amplify, visualise and determine whether DNA from each sample has been successfully extracted. The gel showed that 28 of the 34 extracts contained human DNA. From these samples we then prepared barcoded genomic DNA libraries following established protocols.[5] DNA libraries were quantified using a Nanodrop and quantitative PCR, and subsequently indexed and pooled (final concentration 2 nanoMolar) for a DNA sequencing run performed on an Illumina MiSeq machine.

Since DNA libraries were pooled together to increase sequencing efficiency, raw sequence data was first sorted into respective samples (demultiplexed). Adapters were removed and the resulting fragments were aligned to the human genome.[6] The proportion of reads (sequenced fragments of DNA) from each DNA library that could be aligned to the human reference genome allowed us to calculate an estimate of endogenous DNA within each library.

RESULTS

The quality of DNA preservation not only varies considerably across the cemetery, resulting in quantities of endogenous human DNA ranging from as low as 0.08 per cent (Samtavro T15 SM3) to 37 per cent (Samtavro T27 SM3) but also between samples coming from the same tomb (**Table 1**).[7] In Tomb 27 the quality of preservation varies between 0.27 per cent and 37 per cent; likewise, in Tomb 15, it varies between 0.08 per cent and 27 per cent.

CONCLUSION

The high variation in endogenous DNA preservation across the cemetery makes the investment of screening prior to further sequencing worthwhile.

With this information, resources can be invested according to each sample's quality of preservation. Sequencing of better-preserved samples is more efficient, as a greater proportion of the sequencing effort is invested in the target of interest, in this case human DNA. Samples with preservation levels of greater than 25 per cent (n = 3) can be targeted for

[4] Brotherton *et al*. 2013.
[5] Meyer and Kircher 2010.
[6] Laziridis *et al*. 2014; Llamas *et al*. 2014.
[7] Michalewicz 2014.

Table 1. Summary of sample information and corresponding quality estimates of DNA preservation, *i.e*. proportion of endogenous human DNA.

Specimen	Burial Type	Year Excavated	Skeletal Element	% Endogenous DNA
Samtavro T27 SM3	Stone cist	2009	Bone	37.02
Samtavro T1 SM2	Stone cist	2008	Molar	32.64
Samtavro T15 SM1	Stone cist	2010	Bone	27.07
Samtavro T20 SM1	Stone cist	2009	Canine	18.95
Samtavro T20 SM3	Stone cist	2009	Canine	11.87
Samtavro T21 SM1	Stone cist	2009	Bone	8.29
Samtavro T740 2167	Stone cist	1984	Bone	7.28
Samtavro T608	Stone cist	1982	Bone	7.07
Samtavro T308 SM1	Stone cist	1977	Bone	6.65
Samtavro T36 SM2	Clay sarcophagus	2009	Bone	3.43
Samtavro T33 SM1	Earthen pit	2009	Bone	3.40
Samtavro T576 SM1	Tile lined	1980	Bone	2.36
Samtavro T15 SM1	Stone cist	2010	Canine	2.27
Samtavro T206	Stone cist	1977	Bone	1.88
Tchkantiskedi I11N SM1	Extended pit	2011	Bone	1.80
Samtavro T15 SM2	Stone cist	2010	Bone	1.69
Samtavro T292 1872 SM1	Stone cist	1978	Bone	1.36
Samtavro T290 1889 SM1	Stone cist	1978	Bone	0.70
Tchkantiskedi Q6/R6 4002	Clay sarcophagus	2011	Bone	0.64
Samtavro T21 SM3	Stone cist	2009	Molar	0.64
Samtavro T46 SM1	Composite	2010	Bone	0.63
Samtavro T23 SM2	Stone cist	2009	Bone	0.49
Samtavro T308 SM3	Stone cist	1977	Bone	0.40
Samtavro T478 SM1	Earthen pit	2010	Bone	0.31
Samtavro T6 SM1	Tile lined	2008	Bone	0.27
Samtavro T27 SM1	Stone cist	2009	Bone	0.27
Samtavro T543 SM1	Earthen pit	1979	Bone	0.19
Samtavro T15 SM3	Stone cist	2010	Molar	0.08

cost-effective whole genome sequencing, samples with preservation levels between 5 and 25 per cent (n = 6) are candidates for genomic single nucleotide polymorphism (SNP) analyses, while samples with preservation levels between one and five per cent (n = 8) can still have their mitochondrial genomes sequenced after targeted capture.

Sequencing a greater portion of the genome can aid in better understanding of population history, affiliations and demographic events. It is clear from these results that the remains at Samtavro and Tchkantiskedi have levels of DNA preservation conducive to population genetic studies of these ancient human populations.

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