Byggðasafn Skagfirðinga - Rannsóknaskýrslur

Who were the people of Keldudalur?

A status report on aDNA studies on skeletons from Keldudalur

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Introduction

Researches especially over the last 10 years have shown that DNA can be isolated from archaeological material. The Research laboratory at the Institute of Forensic Medicine at the University in Copenhagen has been working at ancient DNA studies from human material for some years and has obtained good results on Viking material and skeletons from the Iron Age.

The most reliable results are obtained when ancient DNA (aDNA) from the mitochondria (mtDNA) is isolated from the teeth of the skeleton. That is because there are up to 1000 copies of mtDNA per cell compared to two copies of nuclear DNA (nDNA). This gives a greater possibility of retrieving mtDNA from the material than nDNA. Another important quality that mtDNA have is that it is only inherited from mother to daughter. That means that mtDNA gives information about maternal family relations because they have identical mtDNA. Only when mutations happen it diverges. The mutations are rare but because of them the human race can be divided into 25, so called, haplotypes and many subtypes. The human evolution and origin can be traced trough these haplotypes.

Working with aDNA is very difficult because of various reasons; i) there is little intact DNA in the archaeological remains, if any ii) the DNA that still remains is degraded and usually damaged. These damages can lead to misinterpretations and wrong results iii) there are big risks of contamination with modern DNA. Those risks exist because the modern human DNA is everywhere in high numbers and it has not been damaged. Because of these contamination risks it is very important that the skeletons are treated right after excavation and that the DNA extractions are done in a specialty aDNA laboratory.

It has been shown that skeletons from cold, dry regions are more likely to contain useful DNA than skeletons from warmer and more humid regions. This is because the break down of the DNA is slowest in the former condition. Therefore, material from Iceland should be very good for aDNA analysis.

In august 2002 an old burial place was found in Keldudalur in Iceland. Studies have shown that it is a cemetery from the 11th century. 54 skeletons were found at the location. Most of the bones were well preserved. It was therefore decided to try to make DNA analyses of some of these skeletons at the Research Laboratory at the Institute of Forensic Medicine in the University of Copenhagen.

Kristnihátíðarsjóður donated 800.000 ísl. kr and Byggðasafn Skagfirðinga 400.000 ísl. kr to the project. The purpose of the project was to see:

-If the haplotype diversity was different from today's population in Iceland

-If there was maternal kinship between the individuals

The skeletons had been extensively washed after their excavation. That gives concern about contamination with external DNA.

Materials and methods

Human remains

The material consisted of samples from 7 individuals from the cemetery in Keldudalur. Only teeth were used as DNA source. Only teeth that showed good physical preservation were selected. Three teeth were taken from each individual and they had to be in place in the jaw. The latter was to secure that the teeth taken for DNA extraction were indeed from the skeleton in question and that the root tip had been protected, at least to some degree, during the previous archaeological handling of the remains.

Precautions regarding work with ancient DNA

To ensure the highest possible reliability of the work all pre- PCR work was performed as DNA sterile as possible. For this purpose all chemical and reagents were of analytical grade or highest purity available. PCR tubes and micro centrifuge tubes for extracts and primers were free of human DNA as guaranteed by the manufacturer ("PCR-Clean" and "Biopure" tubes, Eppendorf, Hamburg, Germany). Also to ensure the purity of the extracts all pre-PCR work was performed in so called Clean-Laboratory.

Clean-laboratory (clean-lab)

The clean-lab is dedicated solely to aDNA work. It is situated in a building, which is separated from the other DNA analysis laboratories. Staffs entering the laboratory were equipped with full body suits, hairnets, filter-containing facemasks and gloves; staff only entered the laboratory when coming directly from home. The laboratory was fitted out with positive, filtered airflow, hanging UV-lights for sterilization of surfaces and a Millipore set-up for the production of de-ionized lab-grade, filtered (12 kDa cut-off) water. The Millipore de-ionization set-up was fed with autoclaved, de-

ionized water produced outside the laboratory. Cutting and drilling of teeth, filling of dialysis tubes with the drilled powder and mixing of PCR reactions (see below) were performed in a glove box (glass/plastic, 60 cm (w) x 40 cm (d) x 50 cm (h)) within the Clean-Laboratory. After usage the glove box was cleaned with 5% commercial bleach. Likewise, after each cutting and drilling all surfaces of the Clean-laboratory were sprayed with the 5% bleach using an electric air gun. All equipment to be used in the lab was sterilized, metal tools and glassware by baking at 250 °C for 30 min, other equipment by wiping the surfaces with a cloth soaked with 5% bleach.

Figure 1 shows the clean lab and how the staffs is dressed when working in there. There can be seen dried bleach on the windows. That is rests from when the laboratory have been cleaned with the air gun and bleach.

Extraction of DNA

First of all the tooth was soaked in 50% commercial bleach containing approximately 2% sodium hypochlorite and rinsed in sterile water (5 min each). The tooth surface was then sealed with cellulose lacquer and allowed to dry at room temperature for 24 h. The root was cut horizontally approximately 5 mm from the tip. Dental pulp was collected from the wall of the pulp chamber by drilling, using a dental drill (fig.2).

Figure 2 shows a tooth where the root tip has been cut off and the dental pulp has been drilled out.

For half of the teeth the powder was treated with 2% sodium hypochlorite for 5 minuets and then washed with DNA free water. The powder was then transferred to a dialysis tube where proteinase K (20mg/ml), Invitrogen) were added and the tube closed. The powders that were not treated with sodium hypochlorite were transferred directly to the dialysis tube. The tubes were dialysed against 0.5 M di-sodium EDTA, ph 8.0, at room temperature for 24 hr. The EDTA solution was discarded and the tubing dialyzed against digest buffer (1 % SDS, 10mM DTT, 10mM Tris-HCl, pH 8.0) for 24 hr at room temperature. Finally the the tubing was rinsed and the contents briefly dialyzed by submersion in 200 ml deionized, autoclaved water for 15 min. The extracted DNA was collected by centrifugation on a 30 kDa filter (Microcon, Millipore) and purified using the silica based QIAamp DNA mini kit (Qiagen, Venlo, ***The Netherlands***) and eluted with 2x100µl d-ionized, autoclaved water. The purified DNA was finally concentrated by centrifugation on a 30 kDa filter (Microcon, Millipore) and recovered from the filter with 100 µl autoclaved, de-ionized and filtered water. The extract was stored at –20^{o}C in 25 µl aliquots. Subsequently, the lacquer was removed from the tooth by washing with acetone and the tip of the root was glued into place. Independent extraction(s) of DNA from a second and in some instances also a third tooth from the same individual as well as all subsequent analytical steps were performed by other researchers in our laboratory at intervals of several weeks.

PCR, cloning and sequencing

DNA was amplified and sequenced over the mitochondrial Hyper Variable Region 1 (HVR-1) between nucleotides 16064-16405 (the revised Cambridge Reference Sequence, CRS) using 4 overlapping segments of between 135-141 bp. An 113bp segment containing the H-specific position at nt 7028 was also amplified for all samples. In addition, all non-H samples were amplified over coding region segments containing haplogroup specific substitutions. Table I shows details of the primers used. The 25 µL PCR reactions contained 2 µL extract, 2.5 µL High-Fidelity PCR Buffer (Invitrogen), 2.5 mM $MgSO_4$, 0.1 mM of each dNTP, 0.8 µM of each primer, 0.5 U Platinum Taq High-Fidelity polymerase (Invitrogen). The polymerase was activated by heating at 94 °C for 7 min followed by 40 PCR cycles: 94 °C for 20 s, 50-56 °C (depending on the primers, see Table 1) for 20 s, 72 °C for 30 s. For each sample PCR blanks, PCR with "room controls" (i.e. tubes with water which had been sitting on the bench with an open lid during the work in the laboratory) and PCR with mock extractions were also run.

PCR reactions were estimated on agarose yield gels. Positive reactions were cloned using the pGEM® Easy Vector system (Promega). An average of 8 clones were sequenced for each PCR reaction using conserved primers M13 and the ABI Prism 310 DNA single capillary DNA analyzer and the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturers instructions. The sequences were aligned against the CRS sequence using ALFWIN 2.10 software (Pharmacia Biotech), analyzed for post-mortem damage induced miscoding lesions and the consensus sequence was determined.

Haplogroup analysis

Haplogroups were assigned using the online mitochondrial haplogroup motif database and the supplementary data of Richards et al. located at http://www.stats.gla.ac.uk/~vincent/founder2000/index.html.

Summary of steps taken to ensure authentication

A wide range of measures to ensure the authenticity of the aDNA results was observed: i) Isolation of work areas, ii) Negative PCR controls and mock extractions, iii) Appropriate molecular behavior, iv) Reproducibility, v) Cloning of PCR products, vi) Independent replication, vii) All results were based on the analysis of at least 2 different samples from the same individual, each analysis being

carried out by a different researcher, viii) It was controlled that each ancient haplotype made phylogenetic sense and that it did not appear to be an odd sequence combination and ix) To exclude that staff that handled the samples being the source of the DNA, all aDNA sequences obtained were compared with the HVR-1 sequences of these individuals.

Results and discussion

Out of the 7 individuals that were analyzed 5 yielded useful results. The teeth from the 2 others didn't show consistent sequences; this is probably due to the presence of modern DNA in the samples, which may have been contaminated during previous handling and washing. The sequences from the 5 individuals are shown in table 1.

Table 1:

Sample ID	**Non-HVR**	**HVR – A2 16064-16160 A!: 16036-16118**	**HVR –B1 16132-16227**	**HVR – C1 16226-16324**	**HVR – D3 16308-16405 D4/D5: 16294-16404**	**Haplogroup**
Keldudalur						
Person 1: KEH-B-10	7028 C 7113 G>A	CRS	CRS	CRS	CRS	**H**
Person 2: KEH-A-29	7028 C>T 13708 G>A	CRS	16193 C>T 16221 C>T	16278 C>T	CRS	**J2**
Person 3: KEH-B-7	7028 C>T 13708 G>A	16069 C>T 16126 C>T	CRS	CRS	CRS	**J**
Person 4: KEH-D-	7028 C>T 12308 A>G	CRS	16224 T>C	16311 T>C 16320 C>T	16311 T>C 16320 C>T	**K1**
Person 5: Commingled	7028 C	16093 T>C	16221 C>T	CRS	CRS	**H5**
Person 6: KEH-A-6	This person didn't yield reliable results.					
Person 7: KEH-C-1	This person didn't yield reliable results.					

The numbers in the table represent the location of a substitution in the mtDNA. CRS means that there was no divergence found from the CRS reference sequence. A letter, an arrow and a letter, f.ex. T>C, means that there is a substitution. For example 16093 T>C means that there is substitution at location 16093 were T has become a C.

None of the 5 individuals that were typed had identical haplotypes (see table 1). That means that none of these persons were maternally related. However, does this not exclude that they were paternally related e.g. sharing a father.

The haplotypes that were found all exist in modern Icelanders so none of them are unexpected or unusual. The frequencies for the respective haplotypes in modern Icelanders can be seen in table 2. Because of the small sample size from Keldudalur the frequencies for the haplotypes can't be calculated.

Table2:

Haplotypes	Frequencies in Iceland (%)	Frequencies in Ireland (%)	Frequencies in Scandinavia (%)
H	28,27	41,41	39,69
J2	1,28	0,78	0
J	6,85	11,72	6,82
K1	2,36	0,78	0,47
H5	0,43	0	0,62

Table 2 shows the frequencies of the observed haplotypes in Modern Icelandic, Scandinavian and Irish people.

Conclusion

Despite the skeletons had been extensively washed it was possible to obtain reliable results on 5 out of 7 individuals.

Interestingly none of the 5 skeletons that were DNA typed had identical haplotype and they can therefore not be maternally related. All the haplotypes that were found are present amongst modern Icelanders. Interestingly all these haplotypes are found in either modern Scandinavian people or modern Irish people.