Archeogenetic analysis of the origin and genetic relations of the Hungarian conquerors

Summary of Ph.D. thesis

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Szeged

2017.

Introduction and background

In the last few decades genetic analysis is playing an increasing role in tracing the origin and relation of human populations. Initially these studies were limited to modern populations, but these studies have limited resolution, as most modern populations are the results of recent admixtures. In contrast archeogenetic data from well dated remains can provide direct information on ancient populations which were sources of their modern descendants.

The Hungarian archeogenetic research began in 2000, in István Raskó's research group in the Biological Research Centre. They isolated the first ancient DNA from the Hungarian conquerors, determined mtDNA haplotypes from a few samples, and tried to elucidate their genetic relation with modern Hungarians. From these first studies it turned out that some of the conquerors had typical Asian haplotypes, while most of them were probably of European origin.

In our research we successfully optimized ancient DNA extraction methods and adapted the latest haplotyping methods. We complemented the traditional PCR based HVR sequencing method with the SNaPshot assay, which is used to determine 22 haplotype defining SNP-s in the mtDNA coding region. In case of bone samples with best DNA preservation the same method could also be used to determine the paternal (Y chromosome) haplogroup.

In the last few years the Next Generation Sequencing method (NGS) has revolutionized the aDNA field, by providing reliable high quality sequence reads, and enabling to sequence even whole ancient genomes. Recently we have adapted the NGS method in our lab, and combined it with hybridization enrichment to sequence whole mtDNA genomes from a large number of Hungarian conqueror samples. Our goal was to examine the genetic composition of three entire cemeteries, which could later be used as references for comparing samples from other conqueror cemeteries and samples from other periods. These data are also suitable to detect possible maternal relationships within and between cemeteries.

Material and methods

- 1. Bone pulverization:
 - Pulverization with Dremel hand tool
 - Pulverization with mill

2. DNA extraction from archeological remains (compact bone, tooth root and pars petrosa)

- 3. PCR amplification
 - mitochondrial DNA amplification
 - Y chromosome DNA amplification
 - multiplex PCR
- 4. SNaPshot assay (GenoCore22 and GenoY25)
- 5. Capillary electrophoresis
- 6. STR analysis
- Next-generation sequencing (Illumina, MiSeq):

 -double-indexing library preparation
 -enrichment of whole mitochondrial genome
 -assembly of genomics capture kit
 -evaluation of sequencing
- 8. Statistical analysis:

-population genetic analysis -phylogenetic analysis

Results and discussion

In the first stage of our research we have successfully amplified mtDNA from 17 samples, of the small Karos-III graveyard, and determined their maternal haplotypes. Our results showed that a significant part of the Karos population probably originated from Central Asia, but most of them were of European origin. Therefore the low-resolution genetic results from the Karos-III cemetery did not differ significantly from previously published results about the Hungarian conquerors.

In the second half of our research, we obtained 98 high resolution mtDNA genome data from multiple conqueror cemeteries with next generation sequencing (11 from Karos-I, 44 from Karos-II, 18 from Karos-III, 6 from Sárrétudvari-Hízóföld, 4 from Kenézlő-Fazekaszug-II, 2 from Szegvár- Oromdülő, and 1-1 from Magyarhomoróg, Orosháza Görbicstanya, Szabadkígyós-Pálliget, Harta-Freifelt). Next we performed phylogenetic analysis with each samples independently, by comparing their mtDNA genomes to all available genomes available from databases. We found that some 40% of the conquerors had East Asian origin, where the geographic origin of the best matching samples nicely corresponded to the region of the ancient Xiongnu empire. Other 40% of the samples had best matches with modern people from Scandinavia,

Germany, or other regions in Western Europe. A smaller third group of the samples (6.7%) matched modern samples from the Caucasus region and the Near East. For a small proportion of samples, the exact origin could not be determined, but these samples were also of East or West European origin. Our data implies that Hungarian conquerors assembled from three major sources before the conquest, corresponding to 3 major distinct populations.

The following individuals had identical mtDNA genomes, so they are possibly direct relatives from the mother's side: Karos-I/1-Karos-I/2, the Karos-I/3-Karos-I/5, the Karos-I/10-Karos-I/38, the Karos-II/9-Karos-II/60-Kenézlő-Fazekaszug-II/1027-Kenézlő-Fazekaszug-II/1045, the Karos-II/16-Karos-II/54, the Karos-II/21-Karos-II/31-Szabadkígyós-Karos-II/22. the Pálliget/7/anc4, the Karos-II/52-Karos-III/11 (chief), the the Karos-III/7-Karos-III/8-Karos-III/5-Karos-III/6. Karos-III/9, the Karos-III/18-Karos-III/19. It can be seen, that within cemeteries there are possible relatives, while between the three neighboring Karos cemeteries there are non, except the leader. Besides, some of the Karos individuals have potential relatives in other cemeteries. This implies that individual tribes might have been split, and their fractions settled together.

Summary

According to our data half of the conqueror population had Xiongnu origin, corroborating the statement of medieval Hungarian chronicles, which all declare Hunnic origin of the Hungarians. The conquerors with Scandinavian-German genetic affinity had most probably Ostrogothic origin, as this group was reported to have been integrated into the European Hun Empire hundreds of years before the conquest. Interestingly this European component also support the Hun affinity of the Hungarian conquerors. We did not find Finno-Ugric genetic connection, so our data do not support the Finno-Ugric origin of the conquerors. Linguistic analyses report a massive Turkic linguistic layer in Hungarian, which can now be directly linked to our Hun ancestors. . Our results are in agreement with conclusions drawn from genomic admixture analysis, historical, anthropological, archaeological data and compel reconsideration much of the implications in Hungarian history and linguistics.

Our results restore the credibility of the medieval Hungarian chronicles, e.g. make credible the Hun tradition of the Hungarian speaking Seklers, and their presence in the pre-conquest Carpathian Basin. Nevertheless our results do not answer the origin of modern Hungarians, and leave open quite a few questions. As the conquerors provided just one major component in the Hungarian ethnogenesis, the other components must be looked for in the numerous preconquest populations of the Carpathian basin. Our work can be considered only the first important step in clarifying Hungarian ethnogenesis.

List of Publications:

MTMT number: 10030445

Genetic data imply Xiongnu origin of the Hungarian Conquerors, who were considerably admixed with germans of Scandinavian origin

<u>Neparáczki E</u>, Maróti Z, Kalmár T, Bihari P, Nagy I, Pálfi G, Fóthi E, Marácz L, Raskó I, Török T

Manuscript in preparation

Revising mtDNA haplotypes of the ancient Hungarian conquerors with next generation sequencing. <u>Neparáczki E</u>, Kocsy K, Tóth GE, Maróti Z, Kalmár T, Bihari P, Nagy I, Pálfi G, Molnár E, Raskó I, Török T. *PLoS One.* 2017 PMID: , IF: 4.411

Genetic structure of the early Hungarian conquerors inferred from mtDNA haplotypes and Y-chromosome haplogroups in a small cemetery. <u>Neparáczki E</u>, Juhász Z, Pamjav H, Fehér T, Csányi B, Zink A, Maixner F, Pálfi G, Molnár E, Pap I, Kustár Á, Révész L, Raskó I, Török T. *Molecular Genetics and Genomics*. 1: 14 p. Paper 10.1007/s00438-016-1267-z. 14 p. 2016 PMID: 27803981, IF: 2.622