

**Comparison of maternal lineage and biogeographic analysis
of ancient population of the 10th-11th century Carpathian
Basin and recent Hungarian-speaking populations**

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Introduction

Several disciplines try to approach from different aspects the extremely complex question of the Hungarian prehistory. Numerous results and theories came to light about the ancestry and prehistory of Hungarians, which often conflict with each other.

The earlier stage of their history must have been far further east, on the forest steppes of western Siberia (Fodor, 1982). They migrated for hundreds of years until they arrived to the Carpathian Basin. On their way they were neighbors of numerous peoples like the Turkic-speaking Khazars, Onogur-Bulgars, Savirs and Kabars, and Iranian-speaking Alans from whom the Caucasian Ossetes are descended (Berta Róna-Tas 2002). The contact between these people and the ancient Hungarians is reflected in both Hungarian language and culture. In 895 seven major Magyar and Kabar hordes forced the Verecke, Uzsok and Dukla passes across the mountains. This migration took the Magyars into the great plain drained by the Danube and Tisza rivers, a region that corresponds roughly to present-day Hungary (Fodor 1996), and into the more fertile parts of Transylvania among the mountains. These regions had been settled for thousands of years before the Magyars' arrival, by Dacians, Romans, Sarmatians, Goths, Huns, Avars, Slavs, and others: it is probable on the eve of the Hungarian Conquest the overwhelming majority of the indigenous population was Slavic.

However Hungarians, have been living in the Carpathian Basin for more than 1000 years, surrounded by speakers of unrelated Indo-European languages, preserved their Uralic language.

Several Hungarian-speaking ethnic groups nowadays live isolated outside the homeland (e. g. Seklers and Csangós). Seklers composes an isolated minority in the south-eastern part of Transylvania. The Csangós of Gyimes live outside of the Carpathian Mountains in the valley of river Tatros, while the Csangós of Moldva are Hungarians living between the Eastern-Carpathian and the river Prut.

In the last years we had the opportunity to analyze the mitochondrial lineages of human bone samples originating from archaeologically characterized graves from the 10th-11th century, in the Carpathian Basin. So we could study the Hungarian conquerors' gene pool directly in the concerned population. In contrast to the recent Hungarian-speaking populations this is free of the genetic effect of the latest yearhundreds.

Mitochondrial DNA (mtDNA) was used, because its high copy number in cells (usually 1000-10000) which is especially advantageous in case of archaeological remains. Furthermore its mutation rate is appropriate to analyse phylogenetic processes, it is transmitted as a non-recombining unit only through the mother, so its sequence variation has been generated solely by the sequential accumulation of new mutations along radiating maternal lineages. Numerous stable mutations were detected along the, which define related groups, the so called haplogroups. Its evolution happen parallel with the colonisation of the world, so a part of the mtDNA variations can be correlated to geographical sites or populations. Thus it enables to follow the migration history of populations in maternal lineages.

In archaeological remains DNA survives most of all in long bones and teeth for several thousands of years. As an effect of the environmental circumstances however, the quality of the preserved ancient DNA (aDNA) is very poor and its quantity is limited, it bears numerous oxidative and hidrolitic modifications of bases and crosslinks. This fact raises difficulties in the aDNA work. The greatest challenge is the possibility of contamination by exogenous DNA. Thus, in the interest of the authenticity, all work phases should be carried out according to the required serious standards.

The aim of the work was to examine the homogeneity of the ancient population and to determine the frequency of west Eurasian and the occurring Asian genetic elements derived from the original homeland population. Recent Hungarian-speaking populations were involved in the study (Hungarians from the homeland, Seklers and Csángós from Gyimes and Moldva) in order to examine the genetic continuity between ancient Hungarian and modern Hungarian-speaking populations in maternal lineages, to study the rate of isolation from the Indo-European people living in the neighbourhood for more then 1000 years, to determine the rate of the isolation effect in the gene pool of Seklers and Csangós, and to detect the occurring archaic elements in the populations.

Several publications were written about the ancestry and genetic relationships of Hungarian peoples, but this is the first one which tries to approach the problem by the help of archaeological findings originated from the age of the Hungarian conquest.

Materials and methods

Bone samples from ancient remains from the age of the Hungarian Conquest were included in the analysis. Samples were excavated in cemeteries from the 10th - 11th centuries from different regions of the Carpathian Basin. Burial sites and bones were archeologically and anthropomorphologically well defined before the analysis. Hair samples were collected from maternally unrelated recent Hungarian individuals from all regions of Hungary, from Sekler individuals living in Romanian Transylvania and from Csangós living in Gyimes and Moldva region of Transylvania.

aDNA was isolated from femoral bones. Standard isolation methods were used as described by Kalmar et. al, (2000) and alternatively when needed, a modified method incorporating the DNeasy or Qiaamp DNA isolation kits (Qiagen) were used . Thereinafter the informative DNA regions were amplified.

DNA isolation from the recent hair samples was performed by using Chelex, according to the published protocol (Walsh et al., 1991).

mtDNA analysis was performed on the hypervariable region I (HVSI) of the mtDNA control region. When needed, analysis of the diagnostic polymorphic sites in the HVSI region and mtDNA coding region was also performed. In cases of ancient samples, two possibly four partially overlapping sub-regions were amplified. Polymorphic sites in the HVSI and mtDNA protein coding regions were analyzed either by restriction enzyme cleavage or by sequencing.

mtDNA haplogroups were assigned to each sample relative to the revised Cambridge Reference Sequence (rCRS) (Andrews et al., 1999). Ancient and modern Hungarian-speaking populations were compared on the basis of haplotypes and haplogroups as well as several statistical parameters. Population genetic analysis was performed by the use of ARLEQUIN version 2.000 (Schneider et al., 2000). Finally, the sequences were compared with the ones originated from European and Asian populations, including some Finno-Ugric speaking ethnic groups. The resulting matrix of interpopulation F_{st} values (genetic distances) was summarized in two-dimensional scaling (MDS) performed by SPSS package version 5.0 and XLSTAT version 2006.3.

Results and Discussion

The sequences of 38 ancient bone samples were determined and analysed. The ancient Hungarian population was heterogeneous at the mitochondrial haplogroup and haplotype levels. Nineteen haplogroups and 33 haplotypes were distinguished among the samples studied. However, most of the ancient samples (91%) belong to potentially west Eurasian haplogroups, three sequences were assigned to Asian specific (A, B, M) clades. The difference is conspicuous however, when two groups according to apparent social status as judged by grave goods was analysed: commoners, originated from cemeteries with poor archaeological remains show a clear dominance of haplotypes and haplogroups common in west Eurasia. Only one sequence belongs to Asian specific haplogroup (M). On the other hand, classical Hungarian conquerors excavated from cemeteries with rich grave goods, show a more heterogeneous haplogroup representation. Among the studied Hungarian-speaking populations here is the highest gene diversity. Contrary to the commoners, in this group the rate of Asian-specific haplogroups is higher (11%), moreover all sequences belonging to the N1a haplogroup pertains a mutation (16189C) which characterises the Asian lineage of the given clade. Thus, 28% of the classical conquerors' lineages are of Asian origin.

The highest rate of haplotype identities exist between the Csángós of Gyimes and the Hungarians, followed by the Seklers and the Hungarians. The Hungarian conquerors share most of identities to recent Hungarians then to Seklers, but among classical conquerors only two haplotypes, (the CRS -which is common throughout Europe- and one more) show identities with recent populations. So the linguistic and cultural heritage which traces back 1000 years does not mean genetic continuity and direct genetic relationship between the Hungarian conquerors and the recent Hungarian-speaking populations.

The AMOVA suggests that the genetic groups are formed according to cultural and historical relations rather than geographical localizations; Seklers with Hungarians, while Csángós with each other compose genetical groups. Hungarian conquerors show the lowest diversity to Csángós of Moldva. Classical conquerors show massive difference to all other ethnic groups, while commoners suit into the group of recent Hungarian-speaking populations.

Although the 10th-11th century samples as well as the classical conquerors show significant differences from other Hungarian-speaking populations, neither of them composes a separated phylogenetic cluster, and the origin of each population is polyphyletic.

The Hungarian-speaking groups were compared with sequences from 71 other populations. Genetic distances were estimated between all populations as linearised F_{st} statistics by pair wise comparison using the Tamura-Nei model, and F_{st} values were summarised in multi-dimensional scaling (MDS). The MDS plot reflects the geographical localization of the involved populations. Modern Hungarians and Seklers are localized unambiguously among west Eurasian ethnic groups. Csángós of Gyimes are mapped a bit eastern, but the Csángós of Moldva are far more eastern, together with peoples from Eastern Europe (Ukrainian, Komi) and Asia Minor (Turkish, Kurdish, North-Ossetian), and the Hungarian conquerors were placed here as well. By analyzing the two groups of the ancient samples separately, the difference is significant. The localization of the classical Hungarian conquerors suggests a clear relationship with populations from Central Asia, while commoners are mapped at the edge of the west Eurasians towards to Middle Eastern and ethnic groups of Asia Minor. According to our results commoners show closer relationships with recent Hungarian-speaking populations and other west Eurasian ethnic groups. The explanation could be that the commoners' cemeteries might contain the remains of pre-Hungarian populations in the Carpathian Basin, as it is suggested by some historical assumptions.

Recent Hungarians, Seklers and the Csángós from Gyimes show significant genetic distances from classical conquerors, while only recent Hungarians and Seklers have significant distances from the 10th-11th century samples.

The genetic effect of populations who lived in close contact with the Hungarians during their migration from the Ural region to the Carpathian Basin – Khazars, Petchenegs, Onogur-Bulgars, Savirs and Iranian-speaking Alans – seems to have left imprints in the ancient Hungarian gene pool, as well as in Hungarian language and culture.

Recent Hungarian-speaking populations seem to be specifically European populations with few Asian affinities (1-6%), which is the lowest in the Hungarian population and the highest in Csangós from Gyimes. A shift to east is apparent from recent Hungarians and Seklers towards to Csángós of Gyimes then of Moldva, to the Hungarian conquerors and classicals at the end.

The geographical isolation of Csángós from other Hungarian-speaking populations is remarkable, they may have more archaic elements, but the direct effect of the conquerors can not be detected in their gene pool.

In the analysed Hungarian-speaking ethnic groups compared to the Finno-Ugric populations only the relatively high frequencies of haplogroup U seems to be common, otherwise these populations are genetically rather different.

On the basis of the results it is probable that a relatively small number of Hungarian conquerors arrived in the Carpathian Basin, who mixed with other populations had been living here earlier (Slavs, Avars, Germans etc.).

This study shows that the linguistic isolation of Hungarian-speaking populations in the Carpathian Basin has not lead to significant genetic isolation. Gene flow from neighbours has affected the Hungarian gene pool: maternal lineages in the modern Hungarian gene pool bear the imprints of populations who have been living in the region for centuries. In the recent Hungarians, there is a dominating effect of Slav populations (Slovakian, Czech, Ukrainian, Croatian), with influence from the Balkans and West Eurasia, while in the Seklers and mostly in the Csángós the genetic effect of Eastern and Southern Europeans is more visible.

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