Population genetic and diagnostic mitochondrial DNA and autosomal marker analyses of ancient bones excavated in Hungary and modern samples

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INTRODUCTION

Hungary is located in the heart of Europe, in the Carpathian Basin. Since the dawn of civilization this area has been inhabited by human beings, a meeting point where cultures blended together. Hungarians represent an amalgamation of populations, which settled down into the Carpatian basin since the palaeolithic age to present time. Cumanians formed one of settled groups. Cumanians appear in Byzantine history in the 11th century. According to historical data after the ravage of Hungary by Tartar hordes (1241-42) the Cumanians settled down on the area of the early Hungary. The ethnic origin of the Cumanians are uncertain, although their anthropological characteristics suggest that their geographical origin might be in Inner-Asia, South-Siberia (Horváth, 2001).

There are two possibilities to examine the origin of populations. First, to study the genetic structure of the present-day populations and second, to examine the ancient genetic trace. Cumanians completely assimilated, both genetically and culturally into Hungarians. Analysis of ancient DNA from the remains of such a group of early settlers is the only reliable mean of study the genetic affinities and relationships of the Cumanians in Hungary.
In the first part of our study we examined a mediaeval Cumanian group and the modern Hungarian population in order to determine the maternal genetic origin and relationships of these populations.

The mitochondrial genome is maternally inherited (Giles et al., 1980). The high flux of oxygen radicals could cause substantial oxidative damage to mitochondrial DNA (mtDNA) which is not protected by histones (or other proteins). The mutation rate of mtDNA is about 10 times greater than in the nuclear genome (Richards and Macaulay, 2001). Human mtDNA mutations have accumulated sequentially along radiating maternal lineages during and after the process of human colonization of different geographical regions of the world. Hence, groups of mitochondrial DNA types often show geographic specificity (Torroni and Wallace, 1995). Analysis of mtDNA in populations therefore allows reconstruction of their maternal lineages, making it possible to study the genetic traces of migration and admixture of different human communities, and helping to estimate the degree of relationships within and between populations (Vigilant et al., 1991). Mitochondrial DNA is commonly used in archaic DNA (aDNA) studies, on account of its high copy number (up to 5,000–10,000 times that of single-copy nuclear sequences). In the first part of our study we concentrated on the analysis of mtDNA.

The quality and quantity of aDNA in fossils is usually very poor. The most obvious archaic DNA damage types are stand breaks, oxidative and hidrolythic lesions (Paabo et al., 2004).

To prevent contamination from modern DNA drastic laboratory precautions and systematic controls were used. We applied rigorous physical separation of modern and ancient DNA laboratories and pre-and post-PCR laboratories.

Our paper is the first aDNA characterisation of one of the many historically attested Eastern pastoral nomad populations that migrated into Europe; in this case, into the Carpathian basin during the 13th century.

In the second part of our work an autosomal SNP marker detecting method for ancient DNA analyses was established. The studied SNP marker is associated with a trait, which shows characteristic geographic pattern. Using linkage disequilibrium and haplotype analysis of Finnish families a genetic variant located 13,910 bp upstream of the start codon of the human lactase gene was identified which is strongly associated with hereditary lactase persistence/non-persistence (Enattah et al., 2002). This genetic variant (–13910C/T variant) was studied.

The adult-type lactose intolerance is inherited as an autosomal recessive trait. The mutant type –13910T allele is dominant. The adult-type hypolactasia varies widely among populations, both ethnically and geographically, the prevalence in adults is 3-70% among Caucasian population in Europe and it reaches approximately 100% in Asians (Swallow, 2003). Wide ethnic variation in the age of onset has been reported in adult-type hypolactasia.

We tried to execute the –13910C/T genetic variant analyses in case of ancient bone samples and evaluated the prevalence of different –13910 variant genotypes in the recent Hungarian population.

In co-operation with Pediatrics Clinic, University of Szeged we studied the applicability of our molecular genetic screening method for the -13910C/T variant detection as a diagnostic test for adult-type hypolactasia during childhood in Hungarian children.

MATERILAS AND METHODS

I. Mitochondrial DNA analyses

Samples:

- 11 human bone remains derive from two archeologically well-documented Hungarian excavations, dating from the late 14th and early 15th century
- single hairs or blood samples of 74 modern Hungarians

Methods:

- DNA extraction from ancient bone remains (Kalmar et al., 2000), and modern blood or single hair samples (Walsh et al., 1991)
- PCR amplification of mitochondrial control and coding regions
- Polyacrilamid gel electrophoresis
- Post-amplification purification and direct sequencing of PRC products
- Cloning and sequencing of PRC products
- Restriction analyses of PCR products
- Authenticate the results
- Estimate the genetic distances between populations

II. Lactose intolerance examination
(–13910 variant analyses)

Samples:

- 5 human bone remains, derived from the 10th century, cementery of Balatonújlak
- 110 anonymous modern Hungarian adults
- 149 children and adolescent subjects, suffered in abdominal complaints

Methods:

- DNA extraction from ancient bone remains (Qiagen, Dneasy Tissue Kit), and modern single hair or buccal smear samples (Walsh et al., 1991)
- Whole genom preamplification with PEP (Primer Extension Preamplification) method (Zhang et al., 1992)
- dCAPs-PCR based amplification of region contains the -13910 variant (Neff et al., 1998)
- Polyacrilamid gel electrophoresis
- Restriction analyses of PCR products
- -13910 variant genotypes determination with ALF (Automated Laser Fluorescent fragment analyser) system in case of ancient samples

RESULTS AND DISCUSSION

Cumanian samples belong to 6 haplogroups. Out of the eleven remains, 4 samples belonged to H, 2 to U, 2 to V and one each to the JT, U3 and D haplogroups, respectively. Haplogroup D is characteristic for Eastern Asia. All of the others are West Eurasian haplogroups (H, V, U, U3 and JT).

Our results suggest that the Cumanians, as seen in the excavation at Csengele, were very far from genetic homogeneity. We conclude therefore that the mitochondrial motifs of Cumanians from Csengele show the genetic admixtures of the Cumanians with other populations rather than the ultimate genetic origins of the founders of Cumanian culture. So the maternal lineages of a large part of the group would reflect the maternal lineage of those populations that had geographic connection with Cumanians during their migrations. Nevertheless, the Asian mitochondrial haplotype in Cu26 may still reflect the Asian origins of the Cumanians of Csengele. Considering genetic distances Cumanians are nearest to the Finnish, Komi and Turkish populations.

Modern Hungarian samples represent 15 haplogroups. All but one is West Eurasian haplogroup, the remaining one is East Asian (F). Haplogroup F is almost absent in continental Europe. Four haplogroups, present in the ancient samples (H, V, U*, JT), can also be found in the modern Hungarians, but identical haplotypes only in the case of haplogroup H and V were

found. U3 and D clades occur exclusively in ancient group and 11 haplogroups (HV, U4, U5, K, J, J1a, T, T1, T2, W and F) occur only in the modern Hungarian population.

Haplogroup frequency in the modern Hungarian population is very similar to other European populations. Although the presence of F haplogroup in the modern Hungarian population is interesting and can reflect some past contribution. According to haplogroup frequencies of Hungarians we concluded that the ancestors of the majority of modern Hungarians are those paleolithic hanter-gartheners, who lived in Europe before the Last Glacial Maximum of Ice Age. The presence of Asian maternal lineage is 4.1% among the studied modern Hungarians. The neolithic source haplogroup J –associated with the spread of agriculture- occurs with 8.2% frequency.

The –13910 variant genotype determination associated with adult-type lactose intolerance was succesfully executed in cases of two ancient samples.

The prevalence of -13910CC genotype associated with lactose intolerance in the recent Hungarian population proved to be 37%.

Out of the 149 examined children and adolescents, who suffered in abdominal complains, 100 had CC genotype connected to lactose intolerance and 49 had CT or TT genotypes associated with lactose tolerance.

Our results suggest that the adult-type hypolactasia does not manifest under the age of five. The lactose intolerance has been manifested between the age of 6-11 years in 87%, above 12 years in 90% of the examined children with CC genotype. Over the age of 13 years all but one subjects with CC genotype were lactose intolerant.

Ten of the 49 examined young persons with CT or TT genotype had lactose intolerance symptoms. In 8 cases the subjects suffered in other diseases affecting large areas of the small intestinal mucosa, which can cause secondary lactose malabsorption.

In our study the invented examination method is proved to be suitable for the analyses of autosomal SNP markers in case of ancient samples. We established the prevalence of different lactase genotypes in the recent Hungarian population. We confirmed that the genetic screening for the –13,910 polymorphic variant could be a useful tool to diagnose the adult-type hypolactasia, especially over the age of 13 and to distinguish the primery and secondary lactase deficiency in Hungarian population.

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