ARCHAEOGENETIC RESEARCH OF MEDIEVAL POPULATION FROM WEST SLOVAKIA

Archeogenetický výskum stredovekej populácie pochádzajúcej zo západného Slovenska

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Abstract

Mitochondrial DNA (mtDNA) is often used in archaeogenetic research by migration of populations in the past due to its smaller size than nuclear DNA, higher resistance to physical damage and the maternally inheritance hereby we can monitor the mutations cumulated over the centuries. Most of these mutations are located on so-called hypervariable region, hence is necessary to study this region and find the mutations points. Our aim was the haplotype identification and haplogroup detection in ancient population from modern-day Slovakia. Based on locations of mutations the haplotypes and haplogroups of medieval population from10th-11th century were determined using ten ancient skeletal remains from the cemetery Nitra-Šindolka located in the West Slovakia. After the successful isolation of ancient DNA (aDNA) and amplification of specific sequences of mtDNA we described four different haplogroups of investigated samples: H, J, T and U, which have common European origin.

Keywords: *mitochondrial DNA, haplotypes, haplogroups, medieval population*

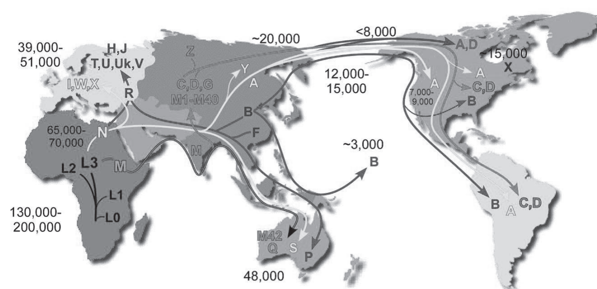
Introduction

The most of archaeogenetic research and population genetics analysis use mtDNA because of its non-recombining property. Based on this marker we can determine origin of populations, assess the relative genetic distance of populations and allocate familial relationships between the findings. Mitochondrial genomes are located in the energy-generating organelles (mitochondria), that are organelles in each eukaryotic cell and their amount per cell (about 8000 copies) depend on type of organism and type of cells (Brown & Brown, 2011). Human mtDNA is a double-stranded closed circular molecule of 16.6 kb in-length and consists of coding and non-coding region (Chinnery & Hudson, 2013). Non-coding region (control region) of 1,1 kb in length contains two hypervariable regions (HVR I and HVR II) which are characterized by mutation rates ten times higher than coding region (Howell et al., 2007). Sequence variation of mtDNA has been generated by the sequential accumulation of new mutations along maternal lineages. Accordingly the human mtDNA contains a molecular recording of the genealogical history and of the migrations of women who transmitted mitochondria through the generations (Torroni et al., 2006). Based on these mutations we can determine haplogroups, which place of origin and extension path are already known (Figure 1). Accordingly, there are Sub-Saharan (African) haplogroups: L0, L1, L2, L3, L4, L5, L6, L7, Western Eurasian haplogroups: H, T, U, V, X, K, I, J, W, Eastern Eurasian haplogroups: A, B, C, D, E, F, G, haplogroups of Native Americans: A, B, C, D, X, and the haplogroups of the South Pacific: P, Q, S (van Oven & Kayser, 2009).

Aim

The aim of our research was the detecting of the mutations points of HVRI region, also of the certain SNP in the coding region of mitochondrial DNA for the purpose of the characterization of haplotype and haplogroup of each sample.

Figure 1. *MtDNA haplogroups migration patterns (Mitomap, 2013)*



Material and Methods

The samples were provided by the Institute of Archaeology of the Slovak Academy of Science in Nitra and Slovak National Museum in Bratislava. The bones and teeth come from the cemetery Nitra-Šindolka and were selected according to sex, age, objects in graves, bone preservation and the particular horizons. The burial Nitra-Šindolka was excavated in years 1985–1986 during the construction of the highway around the town of Nitra. This burial consists of two sections: F and E. The section F contains 204 graves, the section E contains 99 graves and they are 30 meter far from each other. The cemetery is from 10th–11th century, and can be divided into three horizons based on the orientations of graves and objects in graves (jewellery, coins, etc.) (Fusek, 2012). We selected ten samples from different horizons (Table 1) from the cemetery Nitra-Šindolka for the purpose of determine their haplogroups. Ten bones and teeth samples from ancient remains were included in the analysis. We were taken two samples (A and B) of each sample. Sample A constituted tooth and sample B part of femur diaphysis. If the grave did not have any teeth, sample A was the part of femur diaphysis and sample B was the part of tibia diaphysis. The burial sites and bones were archaeologically and osteologically well defined before the analysis.

In order to prevent possible contamination, all stages of the work were carried out under sterile conditions. All appliances, containers and work areas were cleaned and irradiated with UV light. In order to detect possible contamination by exogenous DNA, extraction and amplification blanks (with no bone powder) were used as negative controls. Haplotypes of all persons involved in processing the samples were determined and compared with the results obtained from the ancient bone samples. The samples were prepared in accordance with the protocols described by authors (Kalmár et al., 2000) and (Shapiro & Hofreiter, 2012). The bone and teeth samples were irradiated with UV light. A 3 × 3 cm portion was cut from each femur diaphysis. After removing the surface (at least 2–3 mm) of the sample with a fresh drilling bit at slow speed the clean bone and teeth

Table 1. *The location, sex, age, type of investigated samples and the detected haplogroups*

Number of samples	Number of graves	Horizons of burial	Sex	Age	Sample A	Sample B	Haplogroup
S1	44a	F2	Female?	20–29	Tooth (37)	Femur	T1a
S2	174	E2	?	7–14	Tooth (75)	Femur	H
S3	5	F1	Male	35–45	Tooth (37)	Femur	J2
S4	16	F2	Female	55–65	Femur	Tibia	T1a
S5	64	F1	Male	40–59	Tooth (46)	Femur	T2
S6	76	F1	Female	20–29	Tooth (17)	Femur	J1
S7	104	F2	Male	60+	Tooth (46)	Femur	H2a2
S8	239	F1	Female	20–29	Tooth (36)	Femur	T2b
S9	246	F1	Male	20–29	Tooth (48)	Femur	T2f
S10	220	F3	?	20–29	Tooth (28)	Femur	U5a1

Table 2. *Primers of the HVR I and control region of mtDNA (Tömöry et al., 2007)*

	Nucleotide position	Substitution	Primers	Detection of mutation
HVR I	16020–16259	Whole sequence	5´-TCTGTTCTTTCATGGGGAAG-3´ 5´-GTGGCTTTGGAGTTGCAGTT-3´	Sequencing
	16182–16420	Whole sequence	5´-AACCCCCTCCCCATGCTTAC-3´ 5´-TGATTTCACGGAGGATGGTG-3´	Sequencing
Coding region	7028	C→T	L6962 5`-TTTTCACCGTAGGTGGCCTG-3` H7126 5`-TGAAATGGATTTTGGCGTAGG-3`	Restriction 17025*AluI*
	12308	A→G	L12214 5`-CCCCTTATTTACCGAGAAAGC-3` H12398 5`-TTGTTAGGGTTAACGAGGGTGG-3`	Restriction *EcoRI*
	12705	C→T	L12622 5`-CATCCCTGTAGCATTGTTCG-3`, H12764 5`-AATTCCTACGCCCTCTCAGC-3	Sequencing

fragments were treated with UV light (1.0 J/cm^2, 20 min) and mechanically ground into fine powder in a sterile mixer mill.

Standard isolation methods were used as described by collective of authors (Kalmár et al., 2000; Tömöry et al., 2007) with a little modification. Before the isolation the samples (250 mg bone powder) were washed with 8 ml EDTA (0.5 M, pH = 7.5) overnight at laboratory temperature with continuous vertical rotation. The supernatant was discarded and the samples were suspended in 1.6 ml extraction puffer (0.1 M EDTA, 20% Sarcosyl and 20 mg/ml proteinase K) and incubated overnight at 37 °C with continuous vertical rotation. After phase separation by centrifugation at laboratory temperature at 13000 rpm for 10 min, 350 µl supernatant was transferred to a 1.5 ml Eppendorf tube and 350 µl NH4-acetate (4M) and 700 µl absolute ethanol were added and incubated overnight at –20 °C. For the isolation was used the DNeasy Tissue Kit (Qiagen): the mixture was transferred into DNeasy Mini spin column and centrifuged at 6500 rpm for 1 min. The column was washed twice and DNA was eluted in a final volume of 70 µl.

The amplification reaction (PCR) of HVR I and coding region of mtDNA contained 6 µl template DNA, 20.5 µl H_2O, 1x PCR puffer, 0.8 M dNTP mix, 0.18 M $MgCl_2$, 0.16 mg/ml BSA, primers 0.625 µM, 1.5 U AmpliTaq Gold DNA polymerase in 40 µl total volume. The primers are described in Table 2. Amplicons were checked on 8% native polyacrylamide gel and visualized after ethidium bromide staining with UV transilluminator (Syngene GeneGenius Bio Imaging System). Sequencing reactions were performed using the ABI PRISM BigDyeTM Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and the sequences were determined on ABI Prism 310 (PE Applied Biosystems). The restriction enzyme cleavage reaction mix contained 35 µl PCR product, 9.5 µl H_2O, 5 µl puffer and 0.5 µl 3 U enzyme in 50 µl final volume. Reaction was performed for 1 hour at 37 °C. The sizes of the resulted fragments were checked on 8% native polyacrylamide gel and visualized with an UV transilluminator after ethidium bromide staining.

Results and discussion

Ten sequences derived from ancient bones of the cemetery Nitra-Šindolka were analysed for mtDNA polymorphisms. All sequences could be assigned to definitive haplogroups. Eight different haplotypes (mutations points on HVR I) were distinguished, which could be assigned to four different haplogroups (Table 1). The complete HVR I was sequenced of each samples and the SNPs in coding region were analysed by RFLP or sequencing depending on which haplogroups belong the samples. The haplogroups J and T (S1, S3, S4, S5, S6, S8, S9) containing position 12 705 C in coding region was analysed by sequencing. The haplogroup H (S2 and S7, position 7028 in coding region) was detected by RFLP analysis using the restriction enzyme AluI and the haplogroup U (S10, position 12308) with EcoRI. We isolated the DNA from sample A and sample B to detect of possible contamination. Each of ancient samples has the same polymorphism in the sample A and sample B. The mutations points had to be detected three times from two isolated DNA and all sequence had to read each nucleotide positions. The positions of mutations (polymorphisms) were detected based on comparison with rCRS (Andrews et al., 1999) in the program GeneDoc.

The mtDNA profile of two samples (S1 and S4) belonging to haplogroup T1a is completely identical, they share a common maternal ancestor, but this ancestor might have lived a generation or thousands of years ago. This result supports the matrilineal family relationship between those samples.

The origins and spread of haplogroups can be associated with certain geographic regions. The detected haplogroups of investigated population (H, J, T, and U) belong to most frequently European haplogroups.

Haplogroup H is the most common (40–50%) haplogroup in all European population (Torroni et al., 2006), and reaches its highest frequencies in Western Europe yet was less common (19%) among Early Neolithic farmers and virtually absent in Mesolithic hunter-gatherers (Brotherton et al., 2013). This haplogroup is also common in the Caucasoid populations of the

Near East and North Africa and is also observed in Northern India. Even though haplogroup H is more common in Europe than in the Near East, analysis of sequence divergence appears to indicate that haplogroup H has a much higher diversity in the Near East than in Europe. These divergence values suggest that haplogroup H originated in the Near East 25,000–30,000 years ago and expanded into Europe before the Second Pleniglacial (Torroni et al., 1998).

Haplogroup U is the most ancient haplogroup in Europe, embracing phylogeographically different subclades (Peričić et al., 2005). Ancient mtDNA sequences recovered from three Upper Palaeolithic and 14 Mesolithic and Neolithic hunter-gatherers all belong to the haplogroup U (Bramanti et al., 2009), but now currently found at frequencies 1–7% in most modern European populations, but at up to 20% in Baltic populations and around 40% in Saami (an indigenous minority in the Scandinavian countries) (Pinhasi et al., 2012). Haplogroup U comprise subhaplogroups U1–U6, U8, and K (Peričić et al., 2005). The oldest branch is U5, the oldest European haplogroup whose age was estimated at 53000 to 40000 years ago (Richards et al., 2000). Phylogenetically more refined studies of U5 suggest that it underwent a postglacial expansion phase (Tambets et al., 2004).

Haplogroups J and T are sister clades presented by European population at similar frequencies (Peričić et al., 2005). Haplogroup T comprises 8% of the maternal European gene pool variation, with the highest frequency among populations of the middle Mediterranean (12%) (Richards et al., 1998). In all other hunter-gatherer samples, the now common mtDNA lineages as J and T are absent, suggesting that these mtDNA lineages were introduced during the Neolithic period (Pinhasi et al., 2012). The small immigration in the Neolithic (and later) seems to have brought some younger sub-haplogroups, such as J1b1, J2a and T1a (Torroni et al., 2006). The origin of these Neolithic founder clusters was dated about 9000 years BP (Malyarchuk et al., 2008).

Conclusion

All of determined haplogroups are typically European haplogroups, which suggest, that the people from Nitra-Šindolka belong probably to characteristically European population. It is interesting, that the most common and widespread haplogroups H was found only by two samples, and the haplogroups T and J – which are nowadays in frequency 9–10% – were detected by five and two samples, respectively. We can concluded, that the medieval population had the same haplogroup-representation as the modern-day Europeans, but they are in different frequency. We need more ancient results to identify of genetic composition of medieval era by using the statistical analysis too.

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Súhrn

Pri archeogenetických výskumoch najviac zaužívaným markerom je mitochondriálna DNA (mtDNA), pretože sa vyznačuje vysokou početnosťou na bunku, v porovnaní s jadrovou DNA je relatívne odolnejšia voči degradácii a fyzikálnych vplyvoch pôsobiacich na ňu počas stároči. Okrem toho mitochondrie oplodnenej zygoty pochádzajú takmer výlučne z vajíčka, čo umožňuje sledovanie materskej línie dedičnosti. Na základe analýz súboru konkrétnych mutácií v mtDNA je možné určiť tzv. haplotypy – bodové mutácie, ktoré sú významným zdrojom variability genetického materiálu. Na základe kombinácií viacerých bodových mutácií v mitochondriálnom genóme jedinca sú definované haploskupiny, ktoré predstavujú súbor podobných haploptypov odvodených od spoločného predka. Fylogenéza mitochondriálnych haploskupín sa medzi jednotlivými kontinentmi a populáciami líši, a preto je možné ich využitie na mapovanie migrácií a pôvodu prehistorických populácií. Hlavným cieľom nášho výskumu bola identifikácia haplotypov a haploskupín vzoriek z 10. storočia, v rámci ktorého sme izolovali historickú DNA (aDNA) z desiatich jedincov pochádzajúcich zo stredovekého pohrebiska Nitra-Šindolka zo západného Slovenska. Pomocou PCR sme amplifikovali hypervariabilný región I (HVRI) a určité úseky kódujúceho regiónu mtDNA. Na základe detekcie SNP polymorfizmov mtDNA sme uručili haplotypy jedincov, ktoré sme úspešne zatriedili do štyroch haploskupín. Detekované haploskupiny z 10.–11. storočia (H, J, T a U) sú európskeho pôvodu, avšak ich frekvencia výskytu sa medzi stredovekou a dnešnou populáciou líši. Najviac zastúpenú haploskupinu H (40–60% populácie) sme zistili v 2 prípadoch, kým haploskupiny T a J (s frekvenciou 9–10%) sme určili pri 5, resp. 2 jedincoch.

Kľúčové slová: *mitochondrial DNA, haplotypes, haplogroups, medieval population*

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