

Short communication

Variability of the human mitochondrial DNA control region sequences in the Lithuanian population

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Abstract. The Lithuanians and Latvians are the only two Baltic cultures that survived until today. Since the Neolithic period the native inhabitants of the present-day Lithuanian territory have not been replaced by any other ethnic group. Therefore the genetic characterization of the present-day Lithuanians may shed some light on the early history of the Balts. We have analysed 120 DNA samples from two Lithuanian ethnolinguistic groups (Aukštaičiai and žemaičiai) by direct sequencing of the first hypervariable segment (HVI) of the control region of mitochondrial DNA (mtDNA) and restriction enzyme digestion for polymorphic site 00073. On the basis of specific nucleotide substitutions the obtained sequences were classified to mtDNA haplogroups. This revealed the presence of almost all European haplogroups (except X) in the Lithuanian sample, including those that expanded through Europe in the Palaeolithic and those whose expansion occurred during the Neolithic. Molecular diversity indices (gene diversity 0.97, nucleotide diversity 0.012 and mean number of pairwise differences 4.5) were within the range usually reported in European populations. No significant differences between Aukštaičiai and žemaičiai subgroups were found, but some slight differences need further investigation.

Key words: haplogroup, hypervariable region I, Lithuanian, mitochondrial DNA, population.

The territory which Lithuania encompasses today was settled relatively late. The land became inhabitable only about 12,000 BP, after the last glaciation. The first people to settle were hunter-gatherers, belonging to late Palaeolithic cultures. Anthropological findings regarding Mesolithic and Neolithic inhabitants

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of present day Lithuania are quite sparse and there is a degree of uncertainty concerning the processes of neolithization, Indo-European dispersal and formation of Baltic tribes who gave rise to present-day Lithuanians. During the period from 2000 till 1000 BP the Baltic tribes, probably inhabited the forested expanses between the Wistula and the Volga and Oka riverheads, the Daugava and the central section of the Dnieper. This large Baltic territory was at that time covered by virtually impenetrable forests and for a long time remained isolated from major migration and trade routes (ZINKEVIČIUS 1998). Since the Neolithic period the native inhabitants of the Lithuanian territory have not been replaced by any other



Figure 1. Lithuanian ethnolinguistic subgroups

ethnic group, so there is a high probability that the inhabitants of present-day Lithuania have preserved the genetic composition of their forebears relatively undisturbed by the major demographic movements (ČESNYS 1991).

Mitochondrial DNA (mtDNA) is a useful tool for investigation of human population history (CANN et al. 1987, RICHARDS et al. 1998, WALLACE et al. 1999, SIMONI et al. 2000 among others) due to such characteristics as maternal mode of

inheritance, high mutation rate, apparent lack of recombination and a high copy number. Recent studies of mtDNA, based on restriction fragment length polymorphism and on sequences of the mtDNA control region hypervariable segments I and II (HVI and HVII) have revealed sequence motifs that define the most common clusters of sequences (TORRONI et al. 1996, RICHARDS et al. 1998, MACAULAY et al. 1999). Some of them have their origin well established and, therefore, can be used to reconstruct the genetic history and composition of extant carrier populations.

In total 120 peripheral blood samples were collected from unrelated individuals representing two main ethno-linguistic groups of Lithuanians: Aukštaičiai and emaičiai (60 samples from each group) (Figure 1). Informed consent and information about birthplace, parents and grandparents were obtained from all donors. Genomic DNA was extracted using the standard salting-out procedure. Primer pairs L15996⁺ H16401 and L29⁺ H408 (VIGILANT et al. 1989) were used for amplifying HVI and HVII, respectively. The nucleotide sequence of HVI was determined by direct sequencing of the products of polymerase chain reaction with primers L15996 and H16401 and DNA sequencing kit on an ABI 310 automated DNA sequencer (Applied Biosystems, Forster City, CA, USA) following the protocol recommended by the supplier. The status of position 00073 was determined by restriction enzyme digestion with *A/w44I*. The basic parameters of molecular diversity were calculated by the computer program Arlequin 2.0 (SCHNEIDER et al. 2000). On the basis of specific nucleotide substitutions in HVI and the status of position 00073, the obtained sequences were classified to specific European haplogroups.

Sequences of the mtDNA HVI region comprising nucleotide positions 16024–16388 (ANDERSON et al. 1981) were determined for 120 individuals. We detected 68 polymorphic sites and 74 distinct haplotypes among the 120 Lithuanian individuals. The proportion of transitions was 88.7%. The nucleotide diversity was the same in Aukštaičiai and emaičiai population samples, and amounted to 0.012 ± 0.007 , while the gene diversity was 0.977 ± 0.011 in Aukštaičiai and 0.971 ± 0.015 in emaičiai. On average, the nucleotide and the gene diversities were similar to those found in other European populations (NASIDZE, STONEKING 2000). The mean number of pairwise nucleotide differences was 4.532 ± 2.261 in Aukštaičiai and 4.448 ± 2.224 in emaičiai. These estimates are within the range of mean pairwise differences found in European populations (3.15–5.03) (COMAS et al. 1997, NASIDZE, STONEKING 2000, HELGASON et al. 2000).

About 93% of the obtained sequences were classified to haplogroups. Table 1 shows the frequency of specific European haplogroups in Lithuanian samples. The most frequent haplogroup is H, comprising 42–45% of the sequences in the Lithuanian population. TORRONI et al. (1998) calculated the age of haplogroup H and dated its expansion in Europe to 20,000–25,000 BP, i.e. the time intermediate between the appearance of modern *Homo sapiens* in Europe (>40,000 BP) and Neolithic expansion (starting ~10,000 BP) (CAVALLI-SFORZA

Table 1. Haplogroup and subhaplogroup frequencies in the Lithuanian population

Haplogroup, subhaplogroup	Aukštaičiai (sample size N=60)		emaičiai (sample size N=60)		Total Lithuanian sample (sample size N=120)	
	Number of se- quences	Frequency (%)	Number of se- quences	Frequency (%)	Number of se- quences	Frequency (%)
H	27	45.00	25	41.67	52	43.33
V	3	5.00	3	5.00	6	5.00
U	1	1.67	5	8.33	6	5.00
K	3	5.00	1	1.67	4	3.33
U3	2	3.33	1	1.67	3	2.50
U4	3	5.00	4	6.67	7	5.83
U5a1	3	5.00	3	5.00	6	5.00
U5b	2	3.33	3	5.00	5	4.17
U5b1	1	1.67	0	0	1	0.83
U5	0	0	1	1.67	1	0.83
J	3	5.00	4	6.67	7	5.87
J1b1	0	0	2	3.33	2	1.67
T	2	3.33	1	1.67	3	2.50
T1	4	6.67	0	0	4	3.33
I	2	3.33	1	1.67	3	2.50
W	1	1.67	1	1.67	2	1.67
Others	3	5.00	5	8.33	8	6.67

et al. 1994), and suggested that haplogroup H could represent a second Palaeolithic wave of expansion in Europe. The highest frequencies of this haplogroup (40-60%) are in western and northern Europe.

Haplogroup V, comprising 5% of the mtDNA sequences in Lithuanians, has a limited geographical distribution and is observed only in northwestern Europe and North Africa. The most likely homeland of this haplogroup is the Iberian Peninsula and the most likely age is 10,000-15,000 years. This haplogroup has expanded into central-northern Europe after the end of the second Pleniglacial, thus it is a marker of the major late Palaeolithic population expansion from southwestern to northeastern Europe (TORRONI et al. 1998).

The sequences of haplogroup U are characterised by the 00073G nucleotide, a distinction that seems to resolve a more ancient demographic expansion from a fairly recent one (RICHARDS et al. 1998). Haplogroup U appears to be much older than the other haplogroups. It probably originated in Africa ~50,000 BP and subsequently expanded into the Middle East and Europe (TORRONI et al. 1996). Thus it could represent the oldest sequences in Europe. The frequencies

of subhaplogroups K, U3, U4 and U5 in Lithuanians are similar to those found in other European populations, while the frequency of cluster U seems to be slightly higher than that usually reported for European populations (~5% compared with ~1%, HELGASON et al. 2000). This may indicate that the Lithuanian population has preserved a more archaic genetic structure and a more ancient mtDNA pool. It should be pointed out that this difference occurs because of the high frequency of haplogroup U in emaičiai sample.

The clusters J and T originated in the Near East and have been brought into Europe within the last 10,000 years (RICHARDS et al. 1998). Thus they represent the Neolithic component of the early farmers in the modern European population. The frequencies of these haplogroups are the next observable differences between the two major Lithuanian ethnolinguistic groups, Aukštaičiai and emaičiai. An increased sample size is needed to check whether these differences are significant. The frequencies of haplogroups J and T imply that Neolithic component comprises about 15% of the total Lithuanian mtDNA pool.

Haplogroups I and W comprise only 4% of the total data set. These clusters are characterised by the ancestral state at position 16223 (which was probably the major 'out-of-Africa' sequence (CALAFELL et al. 1996, WATSON et al. 1997)), but now are very rare in Europe, comprising 1-2% of sequences each (RICHARDS et al. 1998).

Overall, the molecular diversity in the Lithuanian population is within the range observed throughout Europe. The classification of different sequences into haplogroups revealed the presence of all major European haplogroups in the Lithuanian population, including those that expanded through Europe in the Palaeolithic and those whose expansion occurred during the Neolithic. Only slight differences observed between Aukštaičiai and emaičiai in haplogroup frequencies suggest the need for further studies of the Lithuanian population.

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REFERENCES

- ANDERSON S., BANKIER A.T., BARRELL B.G., DE BRUIJN M.H., COULSON A.R., DROUIN J., EPERON I.C., NIERLICH D.P., ROE B.A., SANGER F., SCHREIER P.H., SMITH A.J., STADEN R., YOUNG I.G. (1981). Sequence and organization of the human mitochondrial genome. *Nature* 290: 457-465.
- CALAFELL F., UNDERHILL P., TOLUN A., ANGELICHEVA D., KALAYDJIEVA L. (1996). From Asia to Europe: mitochondrial DNA sequence variability in Bulgarians and Turks. *Ann. Hum. Genet.* 60: 35-49.
- CANN R.L., STONEKING M., WILSON A.M. (1987). Mitochondrial DNA and human evolution. *Nature* 325: 31-36.

- CAVALLI-SFORZA L.L., MENOZZI P., PIAZZA A. (1994). The history and geography of human genes. Princeton, NJ: Princeton University Press.
- COMAS D., CALAFELL F., MATEU E., PEREZ-LEZAUN A., BOSCH E., BERTRANPETIT J. (1997). Mitochondrial DNA variation and the origin of the Europeans. *Hum. Genet.* 99: 443-449.
- ČESNYS G. (1991). Anthropological roots of Lithuanians. *Science, Arts and Lithuania* 1: 4-10.
- HELGASON A., SIGURETHARDOTTIR S., GULCHER J.R., WARD R., STEFANSSON K. (2000). mtDNA and the origin of the Icelanders: deciphering signals of recent population history. *Am. J. Hum. Genet.* 66: 999-1016.
- MACAULAY V., RICHARDS M., HICKEY E., VEGA E., CRUCIANI F., GUIDA V., SCOZZARI R., BONNÉ-TAMIR B., SYKES B., TORRONI A. (1999). The emerging tree of west Eurasian mtDNAs: a synthesis of control-region sequences and RFLPs. *Am. J. Hum. Genet.* 64: 232-249.
- NASIDZE I., STONEKING M. (2001). Mitochondrial DNA variation and language replacements in the Caucasus. *Proc. R. Soc. Lond. B. Biol. Sci.* 268: 1197-1206.
- RICHARDS M.B., MACAULAY V.A., BANDELT H.-J., SYKES B.C. (1998). Phylogeography of mitochondrial DNA in western Europe. *Ann. Hum. Genet.* 62: 241-260.
- RICHARDS M., MACAULAY V., HICKEY E., VEGA E., SYKES B., GUIDA V., RENGÓ C., SELITTO D., CRUCIANI F., KIVISILD T., VILLEMS R., THOMAS M., RYCHKOV S., RYCHKOV O., RYCHKOV Y., GOLGE M., DIMITROV D., HILL E., BRADLEY D., ROMANO V., CALI F., VONA G., DEMAINE A., PAPIHA S., TRIANTAPHYLIDIS C., STEFANESCU G. (2000). Tracing European founder lineages in the Near Eastern mtDNA pool. *Am. J. Hum. Genet.* 67: 1251-1276.
- SIMONI L., CALAFELL F., PETTENER D., BERTRANPETIT J., BARBUJANI G. (2000). Geographic patterns of mtDNA diversity in Europe. *Am. J. Hum. Genet.* 66: 262-278.
- SCHNEIDER S., ROESSLI D., EXCOFFIER L. (2000). Arlequin v. 2.000: a software for population genetics data analysis. Geneva, Switzerland: Genetics and Biometry Laboratory, University of Geneva.
- TORRONI A., BANDELT H.J., D'URBANO L., LAHERMO P., MORAL P., SELITTO D., RENGÓ C., FORSTER P., SAVONTAUS M.L., BONNE-TAMIR B., SCOZZARI R. (1998). mtDNA analysis reveals a major late Paleolithic population expansion from southwestern to northeastern Europe. *Am. J. Hum. Genet.* 62: 1137-1152.
- TORRONI A., HUOPONEN K., FRANCALACCI P., PETROZZI P., MORELLI L., SCOZZARI R., OBINU D., SAVONTAUS M.-L., WALLACE D.C. (1996). Classification of European mtDNAs from an analysis of three European populations. *Genetics* 144: 1835-1850.
- ZINKEVIČIUS Z. (1998). The history of the Lithuanian language, 1st edn. Vilnius: Mokslo ir enciklopedijų leidykla p. 333.
- VIGILANT L., PENNINGTON R., HARPENDING H., KOCHER T.D., WILSON A.C. (1989). Mitochondrial DNA sequences in single hairs from a southern African population. *Proc. Natl. Acad. Sci. USA* 86: 9350-9354.
- WALLACE D. C., BROWN M. D., LOTT M. T. (1999). Mitochondrial DNA variation in human evolution and disease. *Gene* 238: 211-230.
- WATSON E., FORSTER P., RICHARDS M., BANDELT H.J. (1997). Mitochondrial footprints of human expansions in Africa. *Am. J. Hum. Genet.* 61: 691-704.