Genetic variability of the Lithuanian human population according to Y chromosome microsatellite markers

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The human Y chromosome is uniparentally inherited and nonrecombining along most of its length. Polymorphic markers specific for Y chromosome have been already recognised to be highly valuable in human evolutionary studies and population genetics.

We present results of the investigation of genetic features of the Lithuanian population by using seven highly variable Y chromosomal microsatellites (short tandem repeat, STR) systems containing nine loci: DYS19, DYS389 I–II, DYS390, DYS391, DYS392, DYS393, DYS385 I–II. PCR amplified marker fragments were detected using capillary electrophoresis and GenScan Software on the ABI PRISM 310 Genetic Analyzer (ABI/PE, USA). The allele and haplotype frequencies were examined by the Y chromosome-specific STRs in 57 males from two main Lithuanian ethnolinguistic groups, Aukštaičiai (A) and Žemaičiai (Ž). Our results were compared with the data of other European populations.

Key words: population genetics, Y chromosome, microsatellite marker, Lithuanians

INTRODUCTION

Migrations and admixture tend to obliterate the unique and peculiar genetic features of original historical populations. In order to understand the history and evolution of populations it is usually necessary to study a large number of markers. Until recent developments in molecular genetics, analysis of the genotype has usually been indirect and limited to a small number of markers. The study markers at the DNA level are more useful and informative in human evolutionary studies, population genetics, forensic analysis and paternity testing. Human Y chromosome is a good marker for this type of investigations. The nonpseudoautosomal region of the paternal inherited Y-chromosome is male-specific, haploid and free of genetic recombination. The Y chromosome could preserve a unique record of the mutational events that occurred in ancestral generations. The major part of the human Y chromosome consists of polymorphic sequences, which are organised into large interspersed tandemly repeated arrays widespread throughout the Y chromosome and showing a rather high variability among individuals in a population. They have become important in several fields including genetic mapping, linkage analysis, and human identity testing. These tandemly repeated regions of DNA are typically classified into several groups depending on the size of the repeat region. Minisatellites (variable number of tandem repeats, VNTRs) have core repeats with 9–80 bp, while microsatellites (short tandem repeats, STRs) contain 2–5 bp repeats. STRs are presently of particular use in the field of forensic science, evolution and anthropology. Y-chromosomal STR systems are widely used for population genetics, population history, and for male identification in forensic cases. At least 17 highly variable Y chromosomal STR systems have already been described (Short Tandem…, 1997).

A set of seven informative Y-chromosomal STR systems, including nine loci, DYS19, DYS389 I–II, DYS390, DYS391, DYS392, DYS393 and DYS385 I–II, has been recommended to use in Europe (Kayser et al., 1997; Roewer et al., 2000). Six of the marker systems are characterised by a tetrameric repeat unit, with two markers (DYS389 I–II, DYS385 I–II) representing two loci with different numbers of repeat units. One marker system (DYS392) is a trimeric repeat. These markers (Fig. 1) are easy to use and sensitive for genetic study.

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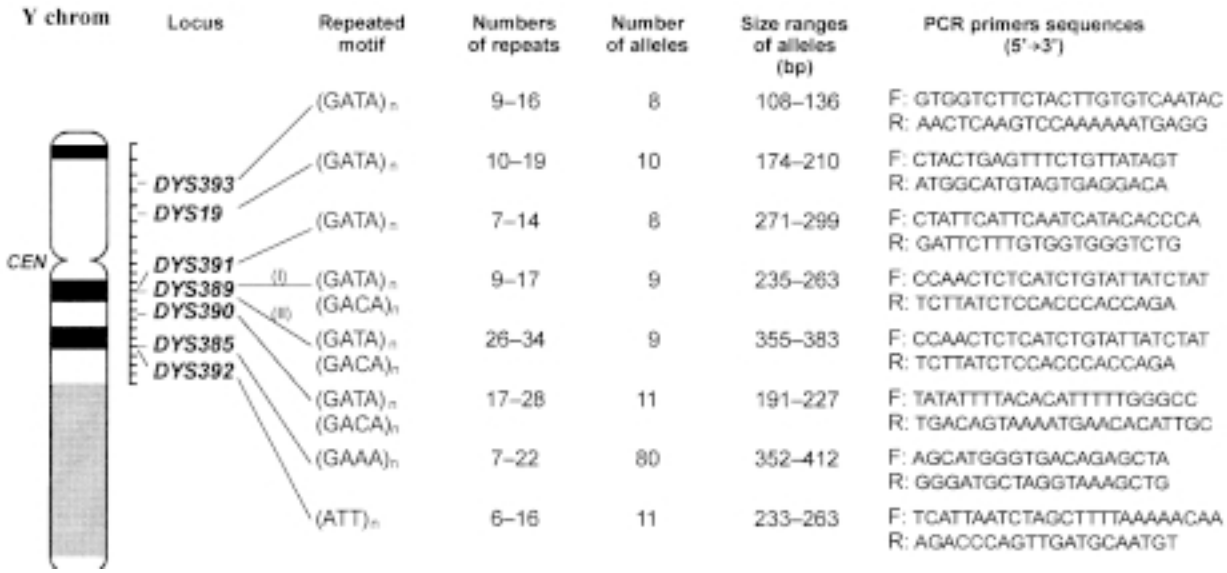

Fig. 1. Characteristics of the analyzed Y-STR loci according to Z. Zinkevičius (1998).
n – number of variable repeats, F – forward primer, R – reverse primer

The Lithuanian population is an interesting object for genetic studies. Genetic differentiation within the Lithuanian population and the relationship between Lithuanians and other European populations was analysed by means of blood groups, serum protein polymorphisms and DNA markers including mt-DNA, Alu sequences and Y chromosome biallelic markers (Kučinskas et al., 2001; Kučinskas et al., 2001; Kučinskas et al., 2001). The aim of this study was to investigate the genetic differentiation within the Lithuanian population and the relationship between Lithuanians and other populations, using the Y chromosome markers (DYS19, DYS389 I–II, DYS390, DYS391, DYS392, DYS393, DYS385 I–II).

MATERIALS AND METHODS

For the population study, 57 blood samples were taken from unrelated Lithuanian males from two main groups of Lithuanians, Žemaičiai and Aukštaičiai (Zinkevičius, 1998).

The genomic DNA was isolated from peripheral blood lymphocytes using a standard procedure (Miller et al., 1988). DNA was amplified by the polymerase chain reaction (PCR) using system-specific primers (Fig. 1), whose forward primers were labelled with fluorescent dyes. PCR fragments were analysed by capillary electrophoresis (ABI PRISM 310 Genetic Analyzer, ABI/PE, USA). Allele assignment was performed by comparison to a self-established allelic ladder. The nomenclature was according to Kayser et al. (1997) and electronic-database information (Forensic…, 2001; Short…, 1997; Y-STR Haplotype…, 2000).

PCR amplification conditions

Amplification of the loci was performed in two non-overlapping multiplex hot start PCR systems (group I – DYS19, DYS389 I, DYS389 II, DYS390, group II – DYS391, DYS392, DYS393) and singleplex reaction for DYS385 I–II.

In groups I and II multiplex PCR reactions were carried out from 5–10 ng DNA template using 0.2 mM dNTP, 1 × AmpliTag Gold Buffer with $MgCl_2$ (PE Applied Biosystems, USA), 0.8 U AmpliTag Gold polymerase (PE Applied Biosystems, USA), 0.85 M Betain (Sigma) (for group II) in a 25 µl reaction volume.

PCR reaction for DYS385 loci I–II was carried out from 5–10 ng DNA template using 0.2 mM dNTP, 1× AmpliTag Gold Buffer without $MgCl_2$ (PE Applied Biosystems, USA), 1 mM $MgCl_2$, 0.75 U AmpliTag Gold polymerase (PE Applied Biosystems) in a 25 µl reaction volume.

The STR forward and reverse primer sequences and concentrations, the forward primer dyes and amplified fragment sizes were used according to recommendations of Kayser et al. (1997) and are shown in Fig. 1.

Amplification was carried out using the GeneAmp PCR system 2400 (PE Applied Biosystems, USA). The following cycling conditions were applied for the hot start multiplex PCR: 95 °C – 11min (initial denaturation), 94 °C – 1 min, 55 °C – 1 min, 72 °C – 2 min, 31 cycles; 60 °C – 45 min (final extension); for DYS385: 95 °C – 11 min (initial denaturation); 94 °C – 30 s, 59 °C – 30 s, 72 °C – 30 s, 2 cycles; 94 °C – 30 s, 58 °C – 30 s, 72 °C – 30 s, 2 cycles; 94 °C – 30 s, 57 °C – 30 s, 72 °C – 30 s, 2 cycles; 94 °C – 30 s, 56 °C – 30 s, 72 °C – 30 s, 29 cycles; 72 °C – 10 min (final extension).

Detection system for PCR products

The PCR products that were fluorescein-labeled by the forward primers were analysed by capillary electrophoresis in the denaturing polymer POP 4 on an ABI PRISM 310 Genetic Analyzer (ABI/PE, USA). One to 3 µl of the PCR product was mixed with 12 µl of deionized formamide and 0.5 µl of internal DNA size standard GS500 TAMRA. Before electrophoresis, samples were denatured for 5 min at 95 °C and subsequently snap-cooled in an ice bath. Capillary electrophoresis was performed using the POP 4 polymer, a 47 cm 50 µM i. d. capillary, run module GS STR POP4 (1 ml) C and standard conditions. Electrophoreses were performed and the data were analysed automatically with Collection 1.0.4. and GeneScan 3.1 (ABI/PE, USA) software using the local Southern sizing and light smoothing algorithms. Genotype classification was carried out in comparison to control DNA samples kindly provided by Dr. Manfred Kayser (Max Planck Institute for Evolutionary Anthropology, Germany) and self-made ladders. The nomenclature was according to Kayser et al. (1997) and **e**lectronic-database information (Forensic..., 2001; Short..., 1997; Y-STR..., 2000).

Statistical analysis

Gene or haplotype diversity was calculated according to the formula: $D = 1 - \sum_{i=1}^{n} x_i^2$, where x is the frequency of the i^{th} allele or haplotype and n is the total number of alleles or haplotypes (Nei, 1987). In Y-linked systems this value is identical to the discrimination index DI and to the power of exclusion.

RESULTS AND DISCUSSION

The presence of four, two/three, five, four, six, three and seven alleles (nine different genotypes) for Y chromosome STRs systems DYS19, DYS389 I–II, DYS390, DYS391, DYS392, DYS393 and DYS385 I–II respectively were detected in 57 DNA samples from unrelated males representing two main Lithuanian ethnolinguistic groups, Aukštaičiai (A) and Žemaičiai (Ž). The frequencies of the single alleles or genotype representative for each polymorphism are reported in Table. Some frequency variations were detected in these groups. The frequencies of DYS19 allele 15 (60%), DYS389II allele 30 (68%) and DYS392 allele 14 (39%) appeared to be slightly higher in Žemaičiai, while DYS390 allele 25 (59%) and DYS392 allele 11 (65%) were higher in Aukštaičiai. Comparison of the distribution of allelic frequencies in two ethnolinguistic groups did not reveal any statistically significant va-

Table. **Allele/genotype frequencies and discrimination power obtained for nine Y-STRs loci studied in the general Lithuanian (L) population and in main ethnolinguistic groups, Aukštaičiai (A) and Žemaičiai (Ž)**

Locus	Allele	Number of individuals L and A/Ž		Frequency (%) among L and A/Ž		Discrimination power
		L (57)	A/Ž (29/28)	L	A/Ž	
DYS19	14	5	2/3	8.8	6.9/10.7	0.579
	15	30	13/17	52.6	44.8/60.7	
	16	21	13/8	36.8	44.8/28.6	
	17	1	1/0	1.8	3.5/0	
DYS389-I	13	41	20/21	72.0	69.0/75	0.404
	14	16	9/7	28.0	31.0/25	
DYS389-II	29	17	10/7	29.8	34.5/25	0.561
	30	33	14/19	57.9	48.3/67.9	
	31	7	5/2	12.3	17.2/7.1	
DYS390	22	1	0/1	1.8	0/3.6	0.645
	23	15	7/8	26.3	24.1/28.6	
	24	12	5/7	21.0	17.2/25	
	25	28	17/11	49.1	58.6/39.3	
	26	1	0/1	1.8	0/3.6	
DYS391	9	1	0/1	1.8	0/3.6	0.526
	10	24	11/13	42.0	37.9/46.4	
	11	31	17/14	54.4	58.6/50	
	12	1	1/0	1.8	3.4/0	
DYS392	10	1	0/1	1.8	0/3.6	0.542
	11	34	19/15	59.6	65.5/53.6	
	13	2	1/1	3.5	3.4/3.6	
	14	18	7/11	31.6	24.1/39.3	
	15	2	2/0	3.5	6.9/0	
DYS393	13	35	18/17	61.4	62.1/60.7	0.487
	14	21	10/11	36.8	34.5/39.3	
	15	1	1/0	1.8	3.4/0	
DYS385I/II	10/13	1	0/1	1.8	0/3.6	0.695
	10/14	3	2/1	5.2	6.9/3.6	
	11/12	2	2/0	3.5	6.9/0	
	11/13	18	8/10	31.6	27.6/35.7	
	11/14	25	11/14	43.9	37.9/50	
	11/15	5	5/0	8.7	17.2/0	
	12/13	1	0/1	1.8	0/3.6	
	13/14	1	0/1	1.8	0/3.6	
	13/15	1	1/0	1.8	3.4/0	

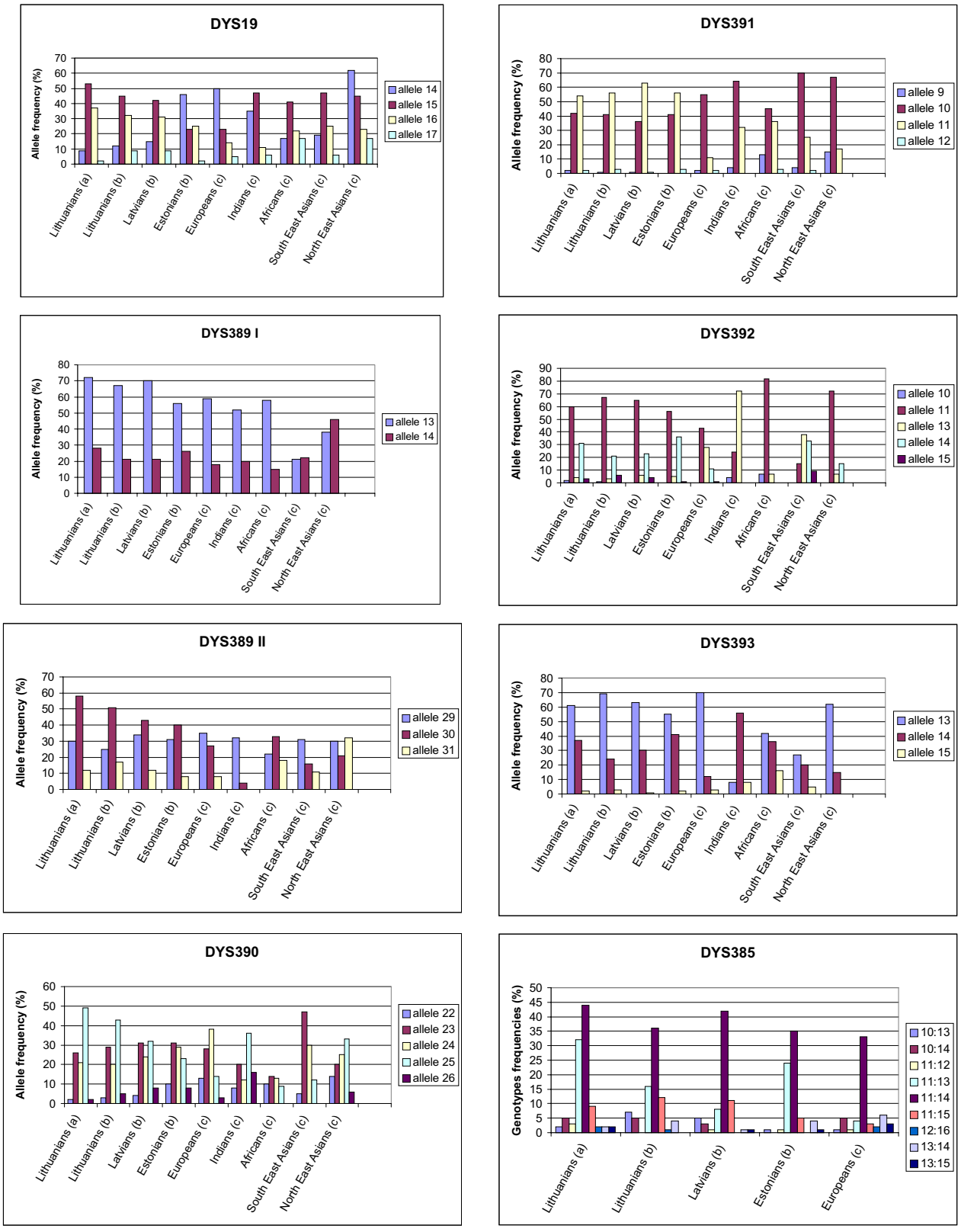

Fig. 2. Y chromosome microsatellite marker alleles and genotypes detected in this study in Lithuanians: comparison of frequencies in different populations.
a – present study, b – *Study 1* (Lessig et al., 2001), c – *Study 2* (Kayser et al., 1997; Short…, 1997)

riation. In general, low allelic frequency variation of Y chromosome microsatellite markers points to a significant homogeneity of the Lithuanian population.

Data on the frequencies of Y chromosome microsatellite marker alleles and genotypes in Lithuanian and other populations are summarised in Fig. 2. Results of the present study appeared to be identical to those of Study 1 (Lessig et al., 2001). The data of Y chromosome microsatellite markers in Lithuanian and Latvian populations are in consistence with the common development of these two populations, which belong to the same (Baltic) branch of Indo-Europeans. Results of Y chromosome STR marker analysis lead to the same conclusion. The set of Y chromosome markers identified in Estonians, which belong to the Finno-Ugric language group, is of different origin if compared to Lithuanians and Latvians, despite close geographical neighbourhood. Comparison of the data on these three populations obtained in the present study and Study 2 (Kayser et al., 1997; Short…, 1997) implies an influence of various migration waves carrying different sets of Y chromosome alleles.

Variation at different Y-STR loci was combined to different haplotypes. A total of 44 different haplotypes in 57 unrelated Lithuanians resulted from combining 32 alleles of seven Y-linked systems containing nine loci. Four of them were identified thrice, five haplotypes were observed twice and 35 haplotypes were seen only once. The haplotype diversity/discrimination index is 0.9721.

Twenty different haplotypes were detected in the Aukštaičiai group (of them, two haplotypes were identified twice) and 17 different haplotypes in the Žemaičiai group. Seven haplotypes were detected in both ethnolinguistic groups (in the Žemaičiai group four haplotypes were identified twice). These two ethnolinguistic groups had been developing over a long time as two independent Baltic tribes, Lietuviai (Aukštaičiai) and Žemaičiai, and our data are in consistence with the common historical origin of these groups.

Investigation of the Y chromosome microsatellite markers shows that the Lithuanian population is an old group residing on its territory for a long period and that various migration forces have influenced its formation.

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ŽMOGAUS Y CHROMOSOMOS MIKROSATELITINIŲ ŽYMENŲ PANAUDOJIMAS, TIRIANT LIETUVOS POPULIACIJOS ĮVAIROVĘ

S a n t r a u k a

Tiriant Lietuvos populiacijos genetinę įvairovę, buvo naudojamos žmogaus Y chromosomos septynių mikrosatelitinių žymenų sistemos, apimančios devynias Y chromosomos genetines sritis: DYS19, DYS389 I/II, DYS390, DYS391, DYS392, DYS393, DYS385 I–II. Siekta nustatyti Lietuvos populiacijos genetinio įvairavimo ypatumus ir palyginti juos su kitų Europos populiacijų duomenimis.

Tyrimo metodai. Kiekvienos sistemos DNR fragmentai buvo pagausinti standartinės ar sudėtinės polimerazinės grandininės reakcijos (PGR) metodu. Reakcijai buvo naudojamos specifinės pradmenų poros, kuriose vienas iš pradmenų buvo žymėtas fluorescuojančia žyme. Pasikartojimų skaičius buvo nustatomas vykdant kapiliarinę elektroforezę genetiniu analizatoriumi ABI PRISM 310 Genetic Analyzer (ABI/PE), naudojant kompiuterinę įrangą ir programas.

Išanalizavus lietuvių populiaciją pagal žymenų alelinių variantų ir haplotipų dažnius, galima teigti, kad lietuvių ir latvių populiacijos yra artimos ne tik etnolingvistiškai, bet ir genetiškai. Lietuviai, kaip atskira Europos populiacija, yra sena konservatyvi etninė grupė, bet neizoliuota nuo kitų Europos populiacijų migracinių bangų poveikio.

Raktažodžiai: populiacinė genetika, Y chromosoma, mikrosatelitiniai žymenys, lietuviai