ISSN 0026-8933, Molecular Biology, 2015, Vol. 49, No. 2, pp. 267–274. © Pleiades Publishing, Inc., 2015. Original Russian Text © K.V. Vagaitseva, V.N. Kharkov, K.V. Cherpinskaya, I.Yu. Khitrinskaya, V.A. Stepanov, 2015, published in Molekulyarnaya Biologiya, 2015, Vol. 49, No. 2, pp. 305–312.

> GENOMICS. TRANSCRIPTOMICS

UDC 575.174.015.3

Genetic Variability of X-Linked STR Markers in Siberian Populations

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Abstract—X-chromosome microsatellite markers are a convenient tool for studying genetic variability in human populations and DNA identification, especially during the decreased informativeness of autosomal markers. The results of genetic analysis of Siberian populations by ten X-linked microsatellite markers (DXS8378, GATA172D05, DXS7132, DXS9898, DXS7423, DXS8377, DXS101, DXS6809, DXS6789, and HPRTB) are presented. The allele frequencies, criminalistics parameters, and genetic relations between populations were calculated. The average level of expected heterozygosity (He) was 0.73 in populations. The total level of genetic differentiation in ten populations was relatively low (Fst = 0.031) compared to the level determined by autosomal and Y chromosome markers. The high probability of the establishment of differences (PD) between two unrelated individuals using a ten X-STR marker system was demonstrated. The average PD value in the panel of ten X chromosome microsatellite markers was 0.9999999997 in women and 0.999998 in men. The total level of genetic differentiation in a pool of ten populations is 0.03186.

DOI: 10.1134/S0026893315020132

Keywords: X chromosome, microsatellites (STR), population data, Siberia

INTRODUCTION

The study of the gene pool of human populations using X chromosome markers is one of intensively developing directions, since the X chromosome has a number of advantages in solving problems of population genetics due to peculiarities of inheritance and recombination. In many respects, the X chromosome is similar to autosomes; however, it is more stable at least in placental mammals, since it only recombines in females. The peculiarities of inheritance make X chromosome a convenient tool for population genetic analysis, since male hemizygosity for this chromosome allows to easily determine haplotypes. Due to the low efficient size, this chromosome is largely exposed to the effects of genetic drift, which results in higher indices of interpopulation diversity [1].

DNA identification in criminalistics and forensic medicine is based on the analysis of the frequency of different marker DNA loci (located in autosomes, sex chromosomes (Y and X), and mitochondrial DNA) in biological samples. Each of these systems has a number of both advantages and disadvantages that decrease their informativeness in some complicated cases. The use of a test system based on X-chromosomal markers will allow one to solve this problem. X chromosome markers are especially informative in complicated cases of kinship determination, when only the material from distant relatives is available for the analysis. X chromosome markers are capable to resolve the issue of paternity, when DNA of the supposed father is not available [2], or the issue of paternity is between close relatives [3]. Thus, when determining kinship between father and daughter (and paternal grandmother and granddaughter), X chromosome markers are more informative than autosomal markers 2 and 4 times, respectively. In this case, chromosome and mtDNA markers are useless. Probabilistic calculations of the coincidence of genotypes based on the reference allele frequencies in population (from which the studied DNA profile originates from) are key in DNA identification. The accuracy of the result estimation depends on the reference group; incorrectly the selected reference population decreases a discriminating capacity of the test system. Russian population is extremely heterogeneous ethnically and genetically; therefore, it is necessary to create special databases for reference allele frequencies of the used markers [4-7]. In addition, more intensive genetic drift in small and isolated Siberian populations results in a decrease in heterozygosity, which also influence the identification potential of test systems [8, 9]. In all three systems of markers that are used in DNA identification, systematic knowledge about the frequencies of their alleles in Russian populations, which can be used as reference, were lacking until recently. This problem began to be successfully solved for autosomal STR standard used

| Ethnos | thnos Population | | Localization | Race type | |
|-----------------------------|-------------------------------|-----|------------------------------|--------------------|--|
| Russians | Tomsk (RUS) | 68 | Tomsk oblast | Caucasian | |
| Tuvinians | Kyzyl (TUV) | 127 | Republic of Tuva | Mongoloid | |
| Dumoto | Агинское@ (BUA) | 43 | Chita oblast | Mongoloid | |
| Buryats | Kurumkan district (KUR) | 25 | Republic of Buryatia | Mongoloid | |
| A 14 - i | Beshpel'tir (ALB) | 80 | Republic of Mountain Altai | Mongoloid | |
| Altaians | Kulada (ALK) | 46 | Republic of Mountain Altai | Mongoloid | |
| 171 | Russkinsk (HAR) | 46 | Khanty-Mansi Autonomous Area | Ural | |
| Khanty | Kazym (HAR) | 50 | Khanty-Mansi Autonomous Area | Ural | |
| Siberian Tatars Tomsk (TAT) | | 40 | Tomsk oblast | Ural, Mongoloid | |
| Khakasses | hakasses Askiz district (KHA) | | Republic of Khakassia | Ural | |

Table 1. Characteristics of populations

in Russia, including in our previous works. However, it remains very urgent for X chromosome markers [10, 11].

In the present work, the genetic diversity of ten Siberian populations for X-chromosome STR markers was analyzed in order to estimate identification potential of the test system based on ten X-STR markers and to determine the allele frequencies for these markers in populations.

EXPERIMENTAL

DNA samples of 604 men (unrelated, without cross-breeding in three generations) belonging to ten Siberian population samples were analyzed. These populations represent three racial types, i.e., Caucasian, Mongoloid, and Ural (Table 1).

The best-studied markers in world populations are included in the multiplex (DXS8378, GATA172D05, DXS7132, DXS9898, DXS7423, DXS8377, DXS101, DXS6809, DXS6789, and HPRTB) and were selected for population genetic analysis [12].

Total DNA was isolated from venous blood samples by phenol-chloroform extraction. Genotyping was carried out using the PCR of each locus with subsequent multiplex analysis of fragments based on the method of capillary gel electrophoresis on genetic analyzer (AbiPrism3130xl, AbiPrism3730xl). PCR was conducted in 20 μ L of mixture, which included 1– 1.5 ng DNA, 10× PCR buffer, 2.5 mM MgCl₂, 0.2 mM each dNTP, 0.75 units of activity Taq-DNA-polymerase, and 0.01–0.02 optical units of each primer. The number of repeats was determined by sequencing of several samples with different allele variants for each locus on AbiPrism3130xl genetic analyzer. The structure of repeats is presented in Table 2.

The allele frequencies and expected heterozygosity were determined by standard biostatistical methods. The estimation of linkage disequilibrium (LD), analysis of genetic diversity and differentiation (AMOVA) were conducted by means of Arlequin v. 2000 program package [13]. Since the gametic phase of men's DNA samples is known, haplotype frequencies were determined without using additional statistical algorithms. In order to determine the association of the average expected heterozygosity with geographical indices, the Spearman correlation coefficient was calculated. Analysis was carried out based on the principal components method using STATISTICA 6.0 program. The identification potential of the system based on X-chromosome markers was estimated using standard population statistical indices. These indices include the probability of determining the differences between two unrelated individuals (PD), exclusive capacity (PE), and the probability of random genotype coincidence (MP) [14].

RESULTS AND DISCUSSION

Analysis of Genetic Diversity

An analysis of the allele frequency distribution of STR markers demonstrated that modal allele mainly coincide in populations of different ethnicity; however, there are loci, for which the distribution of frequencies differs in Caucasians and Mongoloids (Fig. 1). The allele frequencies are given in electronic supplementary to the article (http://www.medgenetics.ru/ UserFile/File/Doc/Evolution%20Doc/Simonova% 202014.pdf). No identical haplotypes were found in individuals from ten population groups.

The average level of expected heterozygosity (He) was 0.73 (Table 3). Out of ten X-STR markers, the

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| Locus | Repeat structure | Allele | | | | |
|------------|---|--------|--|--|--|--|
| DXS8378 | PF-N18-(CTAT)n-N20-PR | | | | | |
| | PF-N18-(CTAT)10-N20-PR | | | | | |
| GATA172D05 | PF-N5-(TAGA)n-N39-PR | | | | | |
| | PF-N5-(TAGA)10-N39-PR | 10 | | | | |
| DXS7132 | PF-(CTTA)n-N38-PR | | | | | |
| | PF-(CTTA)11-N38-PR | 11 | | | | |
| DXS9898 | PF-N15-(TATC)2-(ATC)-(TATC)n-N58-PR | | | | | |
| | PF-N15-(TATC)2-(ATC)-(TATC)10-N58-PR | 10 | | | | |
| DXS7423 | PF-N52-(TCCA)n-N47-PR | | | | | |
| | PF-N52-(TCCA)10-N47-PR | 10 | | | | |
| DXS8377 | PF-N31-(AGA)n-(GGA-AGA)m-(AGA)2-GGA-(AGA)6-N25 | | | | | |
| | PF-N31-(AGA)19-(GGA-AGA)5-(AGA)2-GGA-(AGA)6-N25 | 38 | | | | |
| DXS101 | PF-N80-(CTT)n-(ATT)m-N14-PR | | | | | |
| | PF-N80-(CTT)6-(ATT)9-N14-PR | 15 | | | | |
| DXS6809 | PF-N47-(CTAT)n-(ATCT)3-N9-(TATC)3-(ATCT)5-N10-(ATCT)m-N14-PR | | | | | |
| | PF-N47-(CTAT)8-(ATCT)3-N9-(TATC)3-(ATCT)5-N10-(ATCT)11-N14-PR | 30 | | | | |
| DXS6789 | PF-N130-(GATA)n-(CATA)m-N23-PR | | | | | |
| | PF-N130-(GATA)7-(CATA)7-N23-PR | 14 | | | | |
| HPRTB | PF-N107-(TCTA)n-N76-PR | | | | | |
| | PF-N107-(TCTA)14-N76-PR | 14 | | | | |

Table 2. Structure of repeats and allele nomenclature

DXS8377 (He = 0.886), DXS101 (He = 0.814), DXS6809 (He = 0.764), DXS6789 (He = 0.776) loci demonstrated the largest variability, and DXS7423 (He = 0.613), DXS8378 (He = 0.667) had the lowest variability. The highest level of average expected heterozygosity for ten loci was detected in Siberian Tatars (He = 0.781) and Russians (He = 0.774), which is apparently due to the development of these peoples based on ancestral groups that are heterogeneous in origin. The maximal level of heterozygosity in the other eight populations belongs to Buryats (BUA) and is 0.750.

No significant association between He value (for all system of markers) and geographical parameters was found (He/latitude 0.214 p = 0.644; He/longitude -0.143, p = 0.760).

Genetic Differentiation of Populations

The total level of genetic differentiation in ten populations was relatively small (Fst = 0.031), which is slightly higher than values obtained using autosomal

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microsatellites (Fst = 0.025), but significantly less than those determined by Y-STR (Fst = 0.186) [4].

The GATA172D05 (Fst = 0.062, p = 0.000), DXS101 (Fst = 0.045, p = 0.000), HPRTB (Fst = 0.034, p = 0.000), and DXS7132 (Fst = 0.034, p =0.000) loci mostly contribute to interpopulation diversity. Fst values for other loci are lower than average, and minimal values belong to DXS8378 (Fst = 0.007, p = 0.125), DXS8377 (Fst = 0.007, p = 0.011) loci. The genetic distances matrix (Rst) between ten populations is presented in Table 4.

Genetic Relationships between Populations

Integral characteristics (that cause variability in allele frequencies of the genes in populations) were detected using principal components method (Fig. 2). Data on allele frequencies in 15 world populations were involved for analysis [12, 15–28].

The first two principal components in total explain 25% of the variability of allele frequency in the studied populations. Two clusters (African and Mongoloid) are clearly marked out in the space of two principal

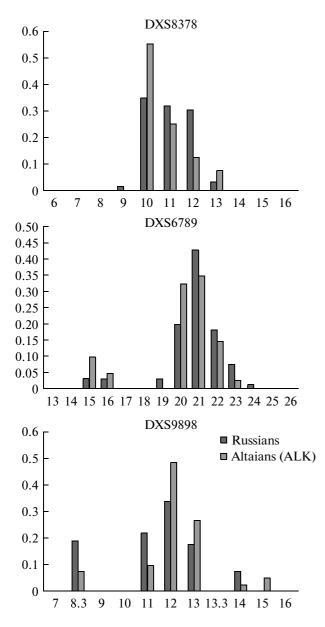


Fig. 1. Distribution of allele frequencies of DXS8378, DXS6789, DXS9898 loci in populations of Russians and Altaians. Allelic variants are presented on the abscissa axis, and allele frequencies are presented on the ordinate axis.

components. The Mongoloid cluster is located in the space of negative values of principal components, it included the following population, including Altaians, Buryats, Tuvinians, Japanese, Taiwanese, Han, Kazakhs (living in China territory), Uighurs, Americans of Asian origin, Khakasses, and Colombians. The location of the Khakasses population (belonging to Ural race type) in Mongoloid cluster indicates larger Mongoloid component in this population. The entrance of the Colombian population in this cluster is rather logical, since the colonization of America occurred from the territory of Siberia, and populations of indigenous ethnic groups in Southern and Central Siberia have the largest genetic similarity to modern American Indians of South America [29]. The African cluster is located aside from Eurasian populations, and it contains four population groups (African Americans and residents of Angola, Mozambique, and Uganda. Two first principal components explain the 36% of allele frequency variability among Siberian populations (Fig. 3). The placement of populations in the space of principal components allows one to isolate three clusters. The majority of Siberian populations are located in the cluster of Southern and Eastern Siberia, two populations of the Western Siberian Khanty generate a separate group, while Siberian Tatars cluster with Russian population of Siberia, which probably reflects a considerable degree of crossbreeding of Siberian Tatars with newly arrived Slavic population [30, 31].

Linkage Disequilibrium

It is necessary to take into account the linkage of loci when calculating the test system identification potential. An analysis of linkage disequilibrium of all pairs of loci in ten populations using Bonferroni correction (p < 0.001) detected linkage in six populations, including DXS7423 and DXS8377 in Khakasses and Tuvinians; GATA172D05 and DXS8377 in Buryats (BUA); GATA172D05 and DXS7423, DXS8378 and DXS101 in Siberian Tatars; and DXS6809 and DXS6789 in Buryats (KUR) and Altaians (ALK). However, the rather large distance between GATA172D05–DXS8377, GATA172D05–DXS7423, and DXS8378–DXS101 loci and the absence of linkage between intermediate loci allow one to attribute these results to the effect of sample.

Criminalistics Indices

In order to estimate the identification potential of the test system based on ten X-STR, the following indices, including the probability of discrimination of unrelated individuals (PD), exclusive capacity (PE), and probability of random genotype coincidence (MP), were calculated.

The average PE value for ten loci in ten populations was 0.999097. A maximal PE value (0.9999) was in population of Siberian Tatars.

The average value of the probability of establishing the existence of differences between two unrelated individuals (PD) was 0.999999997 on women and 0.999998 in men. In the Russian population, MP value is 0.839×10^{-12} in two randomly selected unrelated women and 0.235×10^{-7} in men. Average MP values for ten populations are 0.326×10^{-10} and 0.186×10^{-6} , respectively.

Obvious differences between population groups indicate the need to select maximally accurate reference group for DNA identification. The panel out of ten microsatellite X chromosome markers containing DXS8378, GATA172D05, DXS7132, DXS9898,

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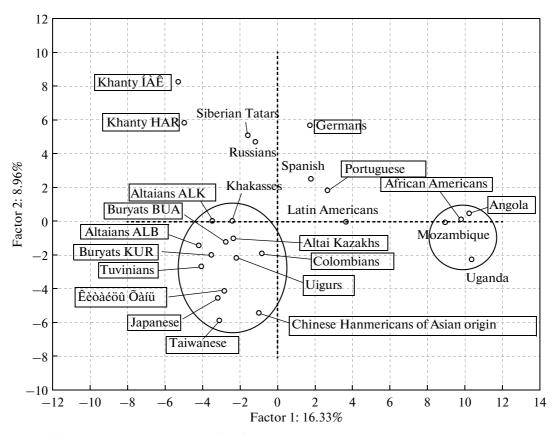


Fig. 2. Location of world populations in the space of two first principle components. Population designations are given in Table 1.

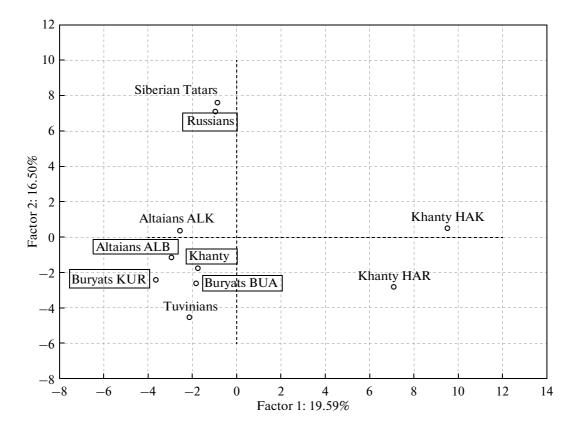


Fig. 3. Location of Siberian populations in the space of two first principle components. Population designations are given in Table 1. MOLECULAR BIOLOGY Vol. 49 No. 2 2015

| PopulationDXS8378Altaians ALB0.619594Altaians ALB0.663200Khanty HAR0.663200Khanty HAK0.682775 | GATA172D05 0.682957 0.693600 | DXS7132 | DXS9898 | DXS7423 | | | | DXS6789 | | |
|---|------------------------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| | 0.693600 | | | | 11000000 | DXS101 | DX56809 | | нрктв | Average |
| | 0.693600 | 0.639782 | 0.672122 | 0.638690 | 0.876798 | 0.772062 | 0.738178 | 0.736250 | 0.567918 | 0.694435 |
| | | 0.699048 | 0.702557 | 0.454618 | 0.887500 | 0.835470 | 0.684282 | 0.769194 | 0.584590 | 0.697406 |
| | 0.692748 | 0.610848 | 0.700056 | 0.509722 | 0.899778 | 0.804129 | 0.728316 | 0.723450 | 0.673958 | 0.702578 |
| Tuvinians 0.683520 | 0.740649 | 0.771886 | 0.595460 | 0.539392 | 0.888344 | 0.733284 | 0.767294 | 0.774124 | 0.664038 | 0.715799 |
| Buryats KUR 0.631054 | 0.645666 | 0.718332 | 0.695138 | 0.652722 | 0.819840 | 0.850970 | 0.739082 | 0.809388 | 0.711944 | 0.727414 |
| Altaians ALK 0.688746 | 0.773238 | 0.624366 | 0.527152 | 0.675466 | 0.908652 | 0.778606 | 0.760032 | 0.845278 | 0.765752 | 0.734729 |
| Khakasses 0.670250 | 0.715978 | 0.727818 | 0.752332 | 0.599830 | 0.901054 | 0.860228 | 0.785952 | 0.758570 | 0.598062 | 0.737007 |
| Buryats BUA 0.715961 | 0.724318 | 0.766226 | 0.611184 | 0.606720 | 0.882862 | 0.817980 | 0.839936 | 0.838220 | 0.695272 | 0.749868 |
| Russians Tomsk 0.693856 | 0.775640 | 0.753030 | 0.763982 | 0.713958 | 0.899058 | 0.806658 | 0.810133 | 0.731838 | 0.793596 | 0.774175 |
| Siberian Tatars 0.622500 | 0.806815 | 0.766578 | 0.788750 | 0.744470 | 0.898949 | 0.878257 | 0.784382 | 0.773750 | 0.742617 | 0.780707 |
| Average 0.667146 | 0.725161 | 0.707791 | 0.680873 | 0.613559 | 0.886284 | 0.813764 | 0.763759 | 0.776006 | 0.679775 | 0.731412 |

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Values that exceed the average level of heterozygosity are highlighted in gray

| Popula- tion | RUS | TUV | BUA | KUR | ALB | ALK | НАК | HAR | КНА | TAT |
|-----------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|-----|
| RUS | _ | | | | | | | | | |
| TUV | 0.06092 | — | | | | | | | | |
| BUA | 0.02960 | 0.02086 | — | | | | | | | |
| KUR | 0.03622 | 0.03488 | 0.00568 | — | | | | | | |
| ALB | 0.02006 | 0.03357 | 0.02177 | 0.03759 | — | | | | | |
| ALK | 0.03735 | 0.03704 | 0.01419 | 0.02638 | 0.02134 | - | | | | |
| HAK | 0.04290 | 0.07160 | 0.04862 | 0.06193 | 0.05392 | 0.06505 | — | | | |
| HAR | 0.05680 | 0.08112 | 0.06061 | 0.07360 | 0.06701 | 0.06913 | 0.00966 | _ | | |
| KHA | 0.02471 | 0.03413 | 0.03164 | 0.03838 | 0.02790 | 0.05100 | 0.04694 | 0.06398 | _ | |
| TAT | 0.01188 | 0.04532 | 0.02386 | 0.02136 | 0.02247 | 0.03285 | 0.05471 | 0.06296 | 0.01795 | _ |

Table 4. Genetic distances matrix

DXS7423, DXS8377, DXS101, DXS6809, DXS6789, and HPRTB has a high identification potential. The collected data on allele frequencies in ten populations allow one to use this panel of markers for the DNA identification of members of ethnic groups of Siberia.

ACKNOWLEDGMENTS

The work was supported by the Federal Targeted Program for Research and Development in Priority Areas of Development of Russian Scientific and Technological Complex for 2014–2020. Competitive selection of two-year applied scientific studies directed on creation of production and technologies on the priority area "Life Sciences" within the event 1.2 of the Program. Agreement Number 14.604.21.0019.

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Translated by A. Barkhash

SPELL: 1. OK