Russian Journal of Genetics, Vol. 37, No. 10, 2001, pp. 1185–1189. Translated from Genetika, Vol. 37, No. 10, 2001, pp. 1411–1416.

HUMAN GENETICS

Mitochondrial DNA Variation in Russian Populations of Krasnodar Krai, Belgorod, and Nizhni Novgorod Oblast

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Received March 27, 2001

Abstract—Mitochondrial DNA (mtDNA) polymorphism was examined in three Russian populations from the European part of Russia (Krasnodar Krai, Belgorod, and Nizhnii Novgorod oblast). This analysis revealed that mitochondrial gene pool of Russians was represented by the mtDNA types belonging to groups H, V, pre-V, HV*, J, T, U, K, I, W, and X. The major groups (average frequency over 5%) were H, V, J, T, and U. Mongoloid admixture in Russians, constituting only 1%, was revealed in the form of mtDNA types of groups C and D. Analysis of the frequency distribution of the mtDNA type groups indicated the absence of genetic differences between the Russian populations studied.

INTRODUCTION

An interest to the studies of the origin of eastern Slavs (Russians, Ukrainians, and Byelorussians) is caused by their extremely complex ethnic history and high anthropological diversity of territorial ethnic groups [1, 2]. Eastern Slavs were formed in the forest and forest-steppe zones of Eastern Europe. This process was accompanied by assimilation of ancient populations of the region. Because of this, the gene pool of contemporary eastern Slavs is expected to contain genetic components corresponding to Slavs proper as well as to Finno-Ugric, Skyto-Sarmatian, and Letto-Lithuanian populations [3]. However, the origin of the main anthropological component of eastern Slavs, introduced by Slavonic colonization, remains obscure. According to anthropological data, Slavonic community was formed as a result of long-term contacts between the northern and southern Caucasoids [1]. For this reason, at the beginning of Slavonic expansion to the Eastern Europe the ratio between the basic components in these groups could be different. Colonization of East European territories in different directions was accompanied by the development of polymorphism caused by interactions with local populations.

Based on the development of genetic methodology, different approaches have been applied for studying ethnic territorial groups of Eastern Europe [4]. The key approach in these studies is the gene geographic method, since it implies obtaining information on genetically determined characters at the population level [1, 5]. Analysis of variability of the genetic systems lacking recombination and having uniparental mode of inheritance seems to be a most promising tool for studying ethnic processes. The examples of such systems are maternally inherited mitochondrial DNA (mtDNA) and paternally inherited nonrecombining part of the Y chromosome [6, 7].

MtDNA variability in populations of eastern Slavs has been examined in numerous works [8–14]. However, most studies on mtDNA variation were conducted using substantially differing approaches (restriction analysis of mtDNA D-region, restriction mapping of the entire mtDNA molecules, and sequencing of hypervariable segment of the main noncoding region of mtDNA). Moreover, analysis of mtDNA polymorphism in populations of Russians from the European Russia was carried out only in one of the works cited [13]. Thus, the currently existing data has only laid the foundation for further systematic studies on the origin and ethnic history of eastern Slavs.

In the present study, we examined the structure of mitochondrial gene pools of the three Russian populations of the European part of Russia using restriction analysis of mtDNA. This approach allowed us to determine polymorphic variants characteristic of certain groups of phylogenetically relative mtDNA types [15]. It has been already established that mitochondrial gene pools of Caucasoids are mainly composed of 11 mtDNA groups, H, V, HV*, J, T, U, K, I, W, X, and N [16, 17]. Screening of group-specific sites is a necessary stage for subsequent analysis of variation of the nucleotide sequences of the hypervariable segments in the main noncoding region of mtDNA, because the heterogeneity of the rates of mutation accumulation at different nucleotide positions of these genomic regions

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Table 1. Polymorphic restriction variants determining groups of mtDNA types in the population of Eurasia

mtDNA Group	Group-specific restriction variants
H	–14766 *Mse*I, –7025 *Alu*I
V	–14766 *Mse*I, +15904 *Mse*I, –16297 *Mse*I, –4577 *Nla*III
pre-V	–14766 *Mse*I, +15904 *Mse*I, –16297 *Mse*I
HV*	–14766 *Mse*I
U	+12308 *Hin*fI
K	+12308 *Hin*fI, –9052 *Hae*II, +10394 *Dde*I
J:	–13704 *Bst*NI, +10394 *Dde*I
J1	–13704 *Bst*NI, +10394 *Dde*I, +16143 *Mse*I
T:	+13366 *Bam*HI, +15606 *Alu*I
T1	+13366 *Bam*HI, +15606 *Alu*I, –12629 *Ava*II
I	–4529 *Hae*II, +8249 *Ava*II, +16389 *Bam*HI, +10032 *Alu*I
W	+8249 *Ava*II, –8994 *Hae*III
X	–14465 *Acc*I
M:	+10394 *Dde*I, +10397 *Alu*I
C	+10394 *Dde*I, +10397 *Alu*I, –13259 *Hinc*II/ +13262 *Alu*I
D	+10394 *Dde*I, +10397 *Alu*I, –5176 *Alu*I
E	+10394 *Dde*I, +10397 *Alu*I, –7598 *Hha*I
G	+10394 *Dde*I, +10397 *Alu*I, +4830*Hae*II/ +4831 *Hha*I
A	+663 *Hae*III
B	9-bp deletion
F	–12406 *Hpa*I/*Hin*cII

Note: Groups of mtDNA types were designated according to classification proposed in [16–18]. Positions of polymorphic sites are indicated with respect to the Cambridge Reference Sequence of human mtDNA [20].

often leads to formation of identical but unrelated mtDNA nucleotide sequences [16–19].

Here, the data on the structure of the gene pools of Russian populations of Krasnodar krai, as well as of Belgorod and Nizhnii Novgorod oblast are presented. The populations examined reside at the borders of ethnic neighborhoods of Russians, the territories where the processes of interethnic relationships were most intense. According to anthropological data [1], Russian population inhabiting the south of the European part of Russia (Krasnodar krai) was formed under the influence of the ancient population of the steppe zone. In turn, Russian population of the Volga region (Nizhnii Novgorod oblast), which by origin is thought to be associated with the mentioned in the chronicles Krivichi tribes of Vladimir-Ryazan and Novgorod groups, was formed under the influence of Finno-Ugric and Turkic ethnic populations. The territory of Belgorod oblast lies in the contact zone between the areas previously occupied by the mentioned in the chronicles tribes of Severyans and Polyans. For these reasons, the formation of Russian population of this region was influenced by the Ukrainians from the central and eastern parts of the Ukraine.

MATERIALS AND METHODS

Experimental material (whole blood and blood serum samples) were obtained from oblast and raion clinical hospitals and blood transfusion centers. Subjects for the study were chosen based on preliminary surveys and analysis of case records. The samples were taken from Russian individuals who were unrelated at least for two generations. Population samples from Krasnodar krai ($n = 49$), Nizhnii Novgorod oblast ($n = 78$), and Belgorod oblast ($n = 69$) were examined.

Total DNA was extracted from biological materials using standard techniques.

Screening for polymorphic sites determining the main groups of mtDNA types distributed in the populations of Eurasia (Table 1) was conducted through the analysis of mtDNA fragments amplified in polymerase chain reaction with the primers proposed in [15, 16, 21]. Restriction fragments were separated by electrophoresis in 8% polyacrylamide gel. Gels were stained with ethidium bromide and DNA fragments were visualized in the UV light.

Polymorphism was scored by the presence (+) or absence (–) of restriction endonuclease recognition sites. MtDNA types were identified based on classification of the groups of mtDNA types in the populations of Eurasia [16, 19]. According to this classification, the groups of mtDNA types are denoted by single Roman letters (excluding group HV), and subgroups within the groups, by digits added to the letter code of the group (Table 1). The mtDNA types that belong to a particular group but cannot be attributed to any of known subgroups, were designated by asterisks (e.g., J = J* + J1 in the present work).

Diversity of mtDNA types (h) was calculated according to Nei and Tajima [22]:

$$h = (1 - \Sigma x^2)N/(N - 1),$$

where x is the population frequency of each mtDNA type and N is the sample size.

Statistical significance of interpopulation differences with respect to the frequencies of mtDNA type groups was evaluated using the measure of relatedness, r, and the index of identity, I, [23]. To estimate genetic similarity with respect to the distribution of mtDNA type groups among the populations, cluster analysis (k-means clustering, STATISTICA/w 5.0) was used.

RESULTS AND DISCUSSION

Analysis of the structure of mitochondrial gene pools of Russian populations from the European part of Russia showed that Russians were characterized by the

same Groups of mtDNA types (Table 2) which are distributed in the European and Middle Eastern gene pools [15–18]. Groups H, U, J, and T were most prevalent in Russian gene pools. Group T, represented in European gene pools by the subgroups T* and T1 [16, 24], was found in the Russian populations examined with the frequencies ranging from 5 to 12%. Moreover, in all samples tested mtDNA types belonging to subgroup T* were most prevalent. The frequency of group J in Russians varied from 9 to 18%. Note that in all previously examined Russian samples [10, 13, 25] group J was represented only by subgroup J*. However, the results of the present study show that some Russian populations can be characterized by prevalence of mtDNA types belonging to subgroup J1. For instance, in Russians from Nizhnii Novgorod oblast the frequency of subgroup J1 (5.1%) was higher than that of subgroup J* (3.9%).

Group V was found in all samples examined with the frequency varying from 2.6 to 7.3%. Similarly, mtDNA types belonging to group HV* occurred, albeit at low (not exceeding 5%) frequencies, in all samples studied. The types of mtDNA belonging to groups K, I, W, and X were detected at very low frequencies and not in all samples. Low frequencies of groups I, W, and X is characteristic of European population as a whole [18], while the frequency of group K in the majority of European populations studied is substantially (about an order of magnitude) higher than in Russians.

Mongoloid populations are characterized by the presence of mtDNA types from group A, B, and F, along with groups C, D, E, and G, belonging to macrogroup M (Table 1). In Russian populations only a few cases of mtDNA types from groups C and D were observed. Their total frequency constituted only 1%. Interestingly, mtDNA type of group D observed in Russians was characterized by the presence of the 4-bp insertion in mtDNA region V. This D-type variant of mtDNA is known to be distributed among Mongoloid populations of Southern Siberia [26].

The scheme of restriction analysis of mtDNA polymorphism used in the present study permitted rather detailed characterization of Russian gene pools. Only about 5.6% of the samples remained uncharacterized. Taking into consideration our previous experience of the analysis of mtDNA hypervariable segment I sequence variation in eastern Slavs [10, 13, 14], it can be hypothesized that "other" mtDNA types in Russians mainly belong to groups pre-HV and N. However, the presence of isolated mtDNA types from group Z cannot be excluded.

As shown in Table 2, mtDNA types belonging to group pre-V are found in Russian gene pools with low frequency (at most 2%). It is suggested that mtDNA types belonging to this rare group served as a basis for group V, distributed among the population of Europe with the frequency of 2 to 5% [27, 28]. Types of mtDNA belonging to groups pre-V and V are characterized by the +15904*Mse*I/–16297*Mse*I marker combination along with the presence of variant 00072C in hypervariable segment 2. Group V is characterized by the presence of an additional variant, –4577*Nla*III. In Russian populations tested mtDNA types belonging to group HV (–14766*Mse*I; see Table 1) and characterized by the presence of variant –16297*Mse*I and the lack of variant +15904*Mse*I were found. These mtDNA types revealed in Russians from Belgorod and Nizhnii Novgorod oblast with the frequencies of 1.5% and 1.3%, respectively, were attributed to group HV* (Table 2). However, it cannot be excluded that these mtDNA types are the predecessors of group pre-V. A solution of this problem, which is very important for the understanding of the origin of group V in Europe, requires further investigation.

Table 2. Frequency distribution patterns (%) of the groups and a number of subgroups of mtDNA types in three Russian populations

Groups/subgroups of mtDNA	Krasnodar krai (n = 49)	Belgorod oblast (n = 69)	Nizhnii Novgorod oblast (n = 78)	Average (n = 196)
H	38.78	37.68	44.87	40.82
V	6.12	7.25	2.56	5.10
pre-V	2.04	0	1.28	1.02
HV*	4.08	2.90	3.85	3.57
J*	14.29	11.59	3.85	9.18
J1	4.08	0	5.13	3.06
T*	8.16	7.25	3.85	6.12
T1	4.08	1.45	1.28	2.04
U	10.20	18.84	23.08	18.37
K	0	1.45	0	0.51
I	2.04	4.35	0	2.04
W	0	1.45	1.28	1.02
X	0	1.45	0	0.51
C	0	1.45	0	0.51
D	0	0	1.28	0.51
"Others"	6.13	2.89	7.69	5.62

Russian population samples examined were characterized by similar values of genetic diversity. Higher values were observed in populations of Krasnodar krai and Belgorod oblast (h values were equal to 0.797 and 0.804, respectively). In Nizhnii Novgorod population, lower genetic diversity value (h = 0.736) was observed. Estimation of the identity index I showed that by the mtDNA type frequency distribution no statistically significant differences between the populations studied were observed (the values of I varied from 11.35 to 18.95; $P > 0.1$). However, the data in Table 2 indicate the existence of some regularities in the groups J, T and U frequency distribution patterns. For instance, the fre-

Table 3. Frequencies (%) of mtDNA groups J, T and U in Russian populations

Population	J	T	U
Krasnodar krai	18.37	12.24	10.20
Belgorod oblast	11.59	8.70	18.84
Kursk oblast*	11.76	5.88	17.65
Nizhni Novgorod oblast	8.98	5.13	23.08
Kostroma oblast*	5.45	16.36	12.72

* According to [13].

quency of group U increased from 10.2 to 23.1% in the direction from Krasnodar population to Nizhnii Novgorod one. On the contrary, in this direction the frequencies of groups J and T decreased from 18.4 to 12.2%, and from 9.0 to 5.1%, respectively. The literature evidence, however, does not confirm the existence of the frequency gradient of the mtDNA types examined. For the analysis we used the data on the mtDNA HVSI sequence variation in the populations of Kostroma and Kursk oblast [13]. Note that using the data on mtDNA HVSI polymorphism permits obtaining only rough estimates of the distribution of mtDNA groups in the populations. Only for several mtDNA groups (including groups J, T, and a number of subroups of group U), a good agreement between the mtDNA HVSI nucleotide motifs and their phylogenetic status determined by the polymorphisms of the coding regions of mitochondrial genome was observed [15–19]. Analysis of the frequency distribution of groups J, T and U in Russian populations, including expected frequencies of these groups in Kostroma and Kursk populations (Table 3), pointed to the existence of substantially more complex pattern of mtDNA geographic variability. According to the data of cluster analysis, mtDNA group frequency distribution patterns observed in populations of Kursk and Belgorod oblast were closest to each other. The populations of Krasnodar krai, Kostroma, and Nizhnii Novgorod oblast were distant from each other and from the cluster of the populations mentioned above. It should be noted, however, that interpopulation differences in respect to the distribution of mtDNA type groups were very low.

The data on mtDNA variation in Russian populations from the European part of Russia obtained so far are in a good agreement with anthropological data. It was established that Russians are characterized by homogeneity of anthropological traits and by prevalence of local trait variation over the typological one [1, 2]. Moreover, according to the data of craniology and dentistry, variation of anthropological traits was not the function of geographical variation [2]. Our data on the distribution of mtDNA groups in different Russian populations from the European part of Russia confirmed conclusions of the anthropologists. More detailed data on the differentiation of Russian populations could be obtained using gene geographic approach, which requires examination of the larger number of Russian and neighboring Eastern European populations.

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research (grant nos. 00-06-80448 and 00-06-88006) and by the Russian State Program “Frontiers in Genetics” (grant no. 99-04-30).

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