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Distribution of "Mongoloid" Haplogroups of Mitochondrial DNA Among Indigenous Population of the Tuva Republic

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Abstract—Variation of Mongoloid-specific restriction sites of mitochondrial genome was analyzed in three territorial groups of Tuvinians. Distribution of mitochondrial DNA haplogroups A, B, C, and D on the territory of the Tuva Republic was estimated. The populations studied did not display distinct differentiation in respect to the mtDNA polymorphism. The specific feature of Tuvinian mitochondrial gene pool was the prevalence of only one haplogroup C (over 40%), mainly represented by two mitotypes. The high frequency of this haplogroup makes Tuvinians similar to more northern Siberian populations. On the other hand, the presence of haplogroup B indicates that Tuvinians have affinity to ethnic groups of Central Asia.

INTRODUCTION

Indigenous populations inhabiting the large territory of Siberia are characterized by diverse ethnic composition. Specificity of the ethnogeny of certain Siberian populations, their genetic relatedness, and features of their population structure are among significant problems of modern genetics. One of molecular genetic marker systems routinely used in such studies is the polymorphism of mitochondrial DNA (mtDNA), which was comprehensively analyzed in many ethnic groups. In the early 1990s a number of research groups performed global screening of the main human racial groups with respect to restriction polymorphism of mitochondrial genome. Ancestral mutations, which were the keys for determination of racial-specific clusters, or haplogroups, were described [1]. It was established that in populations of Siberian ethnic groups, haplogroups A, C, and D predominantly occurred; in the Far East, haplogroup F was also found [2–5].

In recent years, studies of racial-specific polymorphism of mtDNA have become even more topical, since they provide information on the origin of the populations and their demographic history, which is very important in the light of the modern concept on the genetics of multifactorial diseases [6]. In addition, mtDNA mutations leading to human diseases arise in the background of particular haplogroups. For instance, the mtDNA 4336C mutation associated with Alzheimer's disease was attributed to the European haplogroup H [7]. Since a large part of mtDNA variation is population-specific, it was suggested that at solution of the question on pathogenic significance of a certain mutation, control mtDNA sample belonging to the same haplogroup as the patient's mtDNA should be used [7]. This approach attaches practical importance to population genetic studies of mtDNA.

The present study describes distribution of the "Mongoloid" mtDNA haplogroups A, B, C and D in three indigenous populations of the Tuva Republic.

MATERIALS AND METHODS

The Tuvinian populations examined inhabited three regions of the Tuva Republic: the settlement of Kungurtug (Shinaan raion, the southeast); the settlement of Teeli (Bai-Taiga raion, the west); and the settlement of Toora-Hem (Todja raion, the northeast). The samples of the indigenous Tuvinian population were represented by the individuals who were maternaly unrelated at least in the last two generations.

Racial-specific restriction polymorphism was determined by means of PRC amplification of total genomic DNA samples, followed by restriction endonuclease digestion and the separation of the reaction products in 2% agarose gels. The *Alu*I, *Bst*DEI, and *Hae*III restriction endonucleases (Sibenzim, Novosibirsk, Russia), and the *Hinc*II endonuclease (ICN, Promega, United States) were used. The PCR reaction was carried out using *Tag* DNA polymerase and the dNTPs purchased from Sibenzim and Laboratoria Medigen, Russia. Oligonucleotide primers analogous to that described in [3] were synthesized in the Institute of Medical Genetics, Tomsk Research Center, Russian Academy of Medical Sciences. The data on deletion in the mtDNA intergenic region V, and on the D-loop region polymorphism were presented earlier [8, 9].

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Interpopulation frequency comparisons were performed using the χ^2 test with Yates's correction and the STATISTICA 5.0 software package. Gene diversity (analogous to heterozygosity) was calculated on an assumption that mtDNA was a single locus with many alleles: $h = = \sum_{i=1}^{k} x_i \frac{n}{n-1}$ [10]. The proportion of rare alleles in the population was calculated using the index suggested by L. A. Zhivotovsky: $h_\mu = 1 - \frac{(\sum_i^k \sqrt{x_i})^2}{k}$ [11]. In the formulas described n is a sample size; k is the number of alleles, and x_i is the frequency of the allele i. The matrices of Nei's genetic distances and the dendrograms reflecting the between-population distances were calculated using the PHYLIP 3.5 software package [12].

RESULTS

Diversity of mtDNA haplotypes in Tuvunians. The data on polymorphic restriction sites associations showed that in Tuvinians a total of 13 different haplotypes were present (Table 1). In addition to haplogroups A, B, C, and D, the haplotype X, defined by the association of the *Dde*I 10394/*Alu*I 10397 restriction sites was revealed. This haplotype belongs to the Mongoloid superhaplogroup M, which is determined by this polymorphism. Since haplotype X is a combined one, it can contain haplogroups E and G, as well as certain lineages of superhaplogroup M. It is necessary to clarify that this haplotype corresponds to haplotypes X6 and X7 described in [13], but not to the European haplogroup X [14]. The remaining haplotypes were assigned the numbers from I to VIII. By the site association, haplotype I of this group corresponds to the Cambridge mtDNA sequence [15]. It can include the representatives of the Mongoloid haplogroup F, which was not examined in this study, and the lineages of possible European origin. Haplotype II can be of European origin, since it differs from the Cambridge sequence only by the *Dde*I 10394 site. Furthermore, the association of the *Dde*I 10393+/*Alu*I 10397 sites is not typical to Mongoloids [1, 16]. Haplotypes III and IV could arise as a result of a mutation leading to the loss of the *Dde*I 10394 and *Alu*I 10397 sites: haplotype III, against the background of haplogroup D, and haplotype IV, against the background of haplogroup C. Haplotypes from V to VIII carry either deletion or insertion of the intergenic region V. Haplotype V differs from haplogroup B only by the presence of the *Dde*I 10394 site. Its origin could be associated either with recurrent mutation resulted in the appearance of this site, or with independent emergence of the deletion. The latest variant seems most probable in case of haplotype IV, which differs from haplogroup B as much as by the three sites, and corresponds to haplogroup C. Haplotypes VII and VIII carry the region V insertion along with site association typical to haplogroups C and D respectively.

Distribution of mtDNA haplogroups over the territory of the Tuva Republic. The Bai-Taiga population was characterized by highest number of haplotypes detected (Table 1). In this population the whole haplotype spectrum revealed in Tuvinians was represented. Some specific features of the distribution of the region V insertion–deletion polymorphism on the Tuva territory should be mentioned. In the Kungurtug population, characterized by the lowest deletion frequency of all populations studied, the deletion was associated only with haplogroup B. However, the deletion was also observed in two other Tuvinian populations, which carried haplotypes V and VI. The region V insertion in the Toora-Hem population was associated with one haplotype, and in the Teeli population it was associated with two haplotypes.

The three Tuvinian populations examined were generally characterized by similar frequencies of certain haplotypes. Haplogroup C with the frequency exceeding 40% was most frequent. The frequencies of haplogroups A and B were rather low. In the Bai-Taiga population the frequency of haplogroup B was statistically significantly higher than in the Kungurtug population. In addition, the Kungurtug population was distinguished by the frequency of haplogroup D. In this population this frequency was statistically significantly higher than in two other populations. The frequency of haplotype II (*Dde*I 10394+) in the Bai-Taiga population was statistically significantly higher than in other Tuvinian populations.

Differentiation of haplotypes in respect to the D-loop polymorphism and diversity of mitochondrial gene pool in Tuvinians. To analyze genetic heterogeneity within the haplogroups, we studied the distribution of the lineages isolated in different haplogroups by the polymorphic restriction sites in the D-loop region. The structure of the D-loop mitotypes was described earlier [8]. In three Tuvinian populations a total of 48 different mtDNA lineages, distinguished by 14 polymorphic restriction sites were revealed (Table 2). Interestingly, high frequency of haplogroup C was mostly associated with one lineage, represented by mitotype 1. The frequency of this lineage in Tuvinians overall was 31%. The frequency of the second shared lineage, haplogroup C / mitotype 2 (C/2), was 15%. Thus, about 46% of all Tuvinian mtDNAs were represented by two lineages. In addition, three lineages with the frequencies about 5% were found in the Kungurtug population, one lineage was detected in the Toora-Hem population, and another three lineages were detected in the population of Bai-Taiga (Table 2).

Despite the fact that the number of haplotypes revealed in the Kungurtug population by use of racial-specific restriction sites was the lowest (Table 1), the total number of mtDNA lineages (33) revealed in this population was the highest (Table 2). Furthermore,

Table 1. The structure and frequencies of mtDNA haplotypes revealed in Tuvinians in comparison with other Asian populations (%)

Haplotype	Polymorphic sites*							Kungurtug, *N* = 156	Toora-Hem, *N* = 130	Teeli, *N* = 172	Tuvinians, *N* = 458	Mongols, *N* = 103 [26]	Altaians, *N* = 28 [3]	Chukchi, *N* = 70	Koryaks, *N* = 107	Nganasans, *N* = 49	Evens, *N* = 43	South-Eastern Asia, *N* = 153 [16]
	1	2	3	4	5	6	7	present study						[2]				
A	+	+	N	–	–	+	–	4.49	2.31	2.32	3.05	2.91	3.57	28.57	3.74	2.04	–	2.61
B	–	+	D	–	–	+	–	1.92^{B}	2.31	6.40^{K}	3.71	7.77	3.57	–	–	–	–	9.80
C	–	+	N	+	+	–	+	51.92	53.85	42.44	48.91	14.56	35.71	8.57	28.04	38.78	58.14	0.65
D	–	–	N	+	+	+	–	16.67^{TB}	7.69^{K}	5.24^{K}	9.82	20.39	14.28	7.14	1.87	36.74	6.98	3.92
X	–	+	N	+	+	+	–	10.26	5.38	8.14	8.08	23.30	7.14	12.86	46.73	6.12	34.88	37.25
I	–	+	N	–	–	+	–	10.25	18.46	13.96	13.97	22.33	35.71	27.14	–	16.33	–	36.64
II	–	+	N	+	–	+	–	1.92^{B}	3.08^{B}	9.88^{KT}	5.24	2.91	–	–	18.69	–	–	1.3
III	–	–	N	–	–	+	–	2.56	0.77	0.58	1.31	3.88	–	4.29	–	–	–	0.65
IV	–	+	N	–	–	–	+	0	0	0.58	0.22	–	–	11.43	–	–	–	–
V	–	+	D	+	–	+	–	0	2.31	6.40	3.06	1.94	–	–	–	–	–	3.92
VI	–	+	D	+	+	–	+	0	0	0.58	0.22	–	–	–	–	–	–	–
VII	–	+	I	+	+	–	+	0	3.85	1.74	1.75	–	–	–	–	–	–	–
VIII	–	–	I	+	+	+	–	0	0	1.74	0.66	–	–	–	–	–	–	–
h								0.6826	0.6682	0.7763	0.7517							

Note: Upper indices show statistically significant frequency differences ($p < 0.05$) with: B, Bai-Taiga population; K, Kungurtug population; T, Toora-Hem population;
* Polymorphic sites: 1, *Hae*III 663; 2, *Alu*I 5176; 3, del/ins 8272–8289; 4, *Dde*I 10394; 5. *Alu*I 10397; 6, *Hinc*II; 7, *Alu*I 13262.

Table 2. Distribution of different mtDNA lineages (>1%) in Tuvinians*

Haplotype	Mitotype number according to [6]	Kungurtug (156)	Toora-Hem (130)	Teeli (172)	Overall (458)
A	1	<1	<1	1.16	<1
	3	3.21	1.54	1.16	1.97
B	1	<1	1.54	2.91	1.75
	2	1.28	–	–	<1
	4	–	<1	3.49	1.53
C	1	32.69	36.92	25.00	31.00
	2	14.74	13.85	16.86	15.28
	6	1.28	3.08	<1	1.53
	7	1.28	–	–	<1
D	1	5.77	1.54	2.33	3.28
	2	1.28	–	–	<1
	3	5.77	5.38	2.91	4.59
	4	3.85	<1	–	1.53
X	1	–	4.62	2.91	2.40
	2	–	–	1.16	<1
	3	1.92	–	<1	<1
	5	5.77	–	<1	2.18
	7	1.28	–	2.33	1.31
I	1	1.92	2.31	6.98	3.93
	2	1.92	<1	<1	1.09
	3	1.92	11.54	6.40	6.55
	4	–	2.31	–	<1
	8	1.92	–	–	<1
	9	1.28	–	–	<1
II	1	1.28	–	2.33	1.31
	3	<1	–	4.65	1.97
	5	–	2.31	2.91	1.75
III	2	1.28	–	–	<1
V	1	–	<1	6.40	2.62
	3	–	1.54	–	<1
VII	1	–	3.85	1.74	1.75
VIII	3	–	–	1.74	<1
Total number of alleles**		33	24	27	48
Diversity *h*		0.8607	0.8270	0.8915	0.8686
Proportion of rare alleles h_μ		0.33	0.34	0.28	0.41

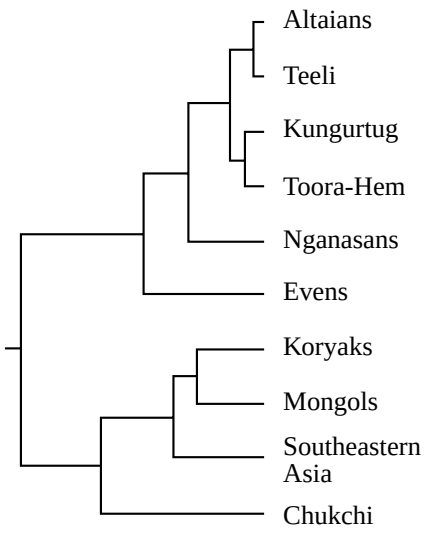
* Alleles with the frequencies less than 1% in all of the populations (sporadic cases) were not included in the Table.
** Taking into consideration the alleles with the frequencies <1%.

most of these lineages were rare. Thus, the Shinaan population from the settlement of Kungurtug differs from other Tuvinian populations by higher diversity within the haplogroups, because in this population such lineages as A, C, D, X, I, and III are represented by the highest number of different D-loop region mitotypes.

The lack of consistent association between most of the mitotypes formed by the polymorphic sites within the D-loop region of mtDNA and a particular haplogroup can be explained by the fact that mutation rate in the D-loop region is higher than that in the coding regions of mitochondrial genome. Moreover, in this

region both direct and reverse mutations leading to recurrent alterations in the restriction sites as a result of independent mutation events have been observed. Specifically, mitotype 1 was found practically in all of the haplogroups analyzed (Table 2).

The value of genetic diversity, calculated based of the haplotype frequencies determined by the coding region polymorphisms, varied from 0.66 in Toora-Hem to 0.77 in Teeli (Table 1). For combined mtDNA lineages, the average value of this parameter in Tuvinians constituted 0.87. It was the highest in the Bai-Taiga population (Table 2). The observed distribution of genetic diversity values was opposite to that observed earlier at the analysis of the mtDNA D-loop region polymorphism. According to that data, the highest degree of genetic diversity was typical to the Kungurtug population, while the lowest degree of genetic diversity was observed in Teeli [8]. The proportion of rare alleles in different populations varied about 0.3, and in the pooled Tuvinian sample it constituted 0.4 (the proportion of rare alleles increases at population pooling [17]).



Dendrogram reflecting the degree of genetic relatedness between the Asian populations (according to Nei's genetic distances).

The effect of contemporary migrations of the Tuvinian gene pool can be estimated based on the data of demographic studies [18, 19]. In the Todja raion migration processes have recently intensified [18]. Analysis of the data on the birthplaces of the individuals examined showed that the Kungurtug and Bai-Taiga population samples were for the most part comprised by the native inhabitants of those settlements, while 38% of the Toora-Hem population sample was represented by the inhabitants of other, mostly western and central, Tuva territories. Correlating the data on individual birthplaces with the polymorphism of their mtDNAs showed that the presence of some mtDNA lineages in the Todja population resulted from migrations. These lineages were A/mitotype 1 (A/1), D/1 and D/4, X/4, I/6, III/3, V/1. After the exclusion of migrants from the sample, the frequency of the most common C/1 lineage in the population of Toora-Hem increased up to 49.38%, while the frequency of C/2 lineage decreased to 9.88%. The frequencies of other lineages did not change considerably, and genetic diversity *h* was 0.7441. Thus, contemporary migration processes resulted in the decrease of the major mitotype frequency and in the increase of genetic diversity in the population from the Todja raion of the Tuva Republic.

Polymorphism of mtDNA in Tuvinians in comparison with other Asian populations. Nei's genetic distances [10] were calculated between Tuvinians and some other Asian populations listed in Table 1, which represented the main linguistic groups of Siberia. To calculate the distances, the frequencies of the main lineages, A–D, II, and I were used. The values of genetic distances varied from 0.02 to 1.09. The lowest values were obtained at comparison of Tuvinian populations with each other and with northern Altaians (Table 3). Of three Tuvinian populations examined the highest distance value was observed between the populations of Kungurtug and Teeli (0.11), and the lowest value, between the populations of Teeli and Toora-Hem (0.03). Generally, Tuvinian populations were closer to each other and to Altaians than to other Asian populations.

The dendrogram constructed from the matrix of genetic distances (figure) showed that all of the grouped into two distinct clusters. The first cluster included the populations of Southeastern Asia, Mongols, Koryaks, and Chukchi. The second cluster was comprised by the three Tuvinian populations, Altaians, and by slightly distant from these Evens and Nganasans. Thus, Siberian populations clustered separately from the populations of Central and Eastern Asia, and the populations from the Pacific coast.

DISCUSSION

Intraethnic differentiation of Tuvinians by mtDNA polymorphism. According to anthropological studies, the population of the Tuva Republic is heterogeneous in respect to the expression of Mongoloid anthropological features. The highest degree of manifestation of Mongoloid features was typical to the inhabitants of the southern part of the Republic, where the family names of Mongoloid origin occurred more frequently (the settlement of Kungurtug [19, 20]). The population of the western part of the Republic (including the Bai-Taiga raion) is characterized by relatively weak manifestation of Mongoloid features [20], and the inhabitants of the Todja raion (the settlement of Toora-Hem) belong to the Baikal anthropological type [21].

As shown earlier, Tuvinian populations did not display clear-cut differences in respect to the mtDNA D-

Table 3. Nei's genetic distances between Tuvinians and other Asian populations according to the data on mtDNA polymorphism

	Population	1	2	3	4	5	6	7	8	9	10
1	Kungurtug	–	0.0408	0.1098	0.4020	0.1217	0.7663	0.3869	0.0975	0.1171	1.0696
2	Toora-Hem	**0.0408**	–	0.0266	0.4566	0.0563	0.5832	0.4251	0.1886	0.2208	0.8412
3	Teeli	**0.1098**	**0.0266**	–	0.3343	0.0213	0.3839	0.3630	0.2517	0.3236	0.5075
4	Mongols	0.3960	0.3833	0.2479	–	0.1616	0.2497	0.2350	0.2939	0.5741	0.1345
5	Altaians	0.1217	0.0563	0.0213	0.2489	–	0.3034	0.3979	0.1620	0.4023	0.4557
6	Chukchi	0.7663	0.5832	0.3839	0.3404	0.3034	–	0.5594	0.7578	1.3139	0.2443
7	Koryaks	0.3869	0.4251	0.3630	0.1536	0.3979	0.5594	–	0.6121	0.1898	0.2648
8	Nganasans	0.0975	0.1886	0.2517	0.3179	0.1620	0.7578	0.6121	–	0.3409	1.0760
9	Evens	0.1171	0.2208	0.3236	0.4480	0.4023	1.3139	0.1898	0.3409	–	1.0968
10	Southeastern Asia	1.0696	0.8412	0.5075	0.1595	0.4557	0.2443	0.2648	1.0760	1.0968	–

loop region polymorphisms that could be associated with their specific anthropological features [8]. Our analysis of racial-specific polymorphism, however, has revealed a weakly expressed differentiation, which corresponded to the anthropological one. In particular, the frequency of haplogroup D in Tuvinians from Kungurtug was more similar to that in Mongols, than to other Tuvinian populations. Moreover, in Bai-Taiga population an increased frequency of lineage II, presumably including the mtDNAs of European origin, was observed (Table 1). On the dendrogram the Bai-Taiga population is placed in one cluster with Northern Altaians, which are characterized by South Siberian anthropological type, formed with the participation of the European component. Nevertheless, Tuvinian populations tested were characterized by some shared specific features of mtDNA polymorphism, which distinguished them from other Siberian populations. These are the high frequency of haplogroup C (from 42 to 54%) and the presence of haplogroup B. The first feature defines Tuvinians as a Siberian population and brings them close to Tungus and Samoyed populations. The second feature indicates the affinity of Tuvinians to Turkic and Mongoloid populations. The association of these two features is specific to Tuvinians, and is probably a characteristic feature of the population structure of mtDNA polymorphism in this ethnic groups.

As mentioned above, in respect to the frequencies of the main mtDNA lineages, there were no considerable differences between contemporary population of the Todja raion, which included migrants, and the population sample comprised only by native inhabitants of this territory. Hence, the uniformity of Tuvinian populations in respect to the mtDNA polymorphism is most likely an ancestral character not associated with contemporary migrations. Since mitochondrial gene pool of Tuvinians is generally characterized only by slight territorial differentiation, it can be considered as a uniform system, which also includes Toja Tuvinians. Our findings that migrations northwestward from the west of Tuva led to an increase of the number of rare alleles in the Toora-Hem population confirm the existence of a current gene flow on the territory of the Republic, which was first described by Yu. G. Rychkov [22]. This gene flow may be the reason for the increased degree of genetic diversity observed in Todja Tuvinian populations.

Genetic diversity, calculated on the basis of the mtDNA haplogroup frequencies, reflects the ancestral structure of mitochondrial genome, because haplogroups correspond to ancestral lineages that remain unchanged during many generations. At the same time, genetic diversity calculated based on the frequencies of all revealed mtDNA lineages can reflect the current state of the gene pool, because preservation or elimination of the new lineages, arising as a result of mutations, depends on migration and stochastic factors. The h value calculated by use of haplogroup frequencies (Table 1) was the highest in Teeli (0.78). In the Kungurtug and Toora-Hem populations, the h values were lower (0.67–0.68). The genetic diversity values calculated using the second approach were nearly equal in all of the three Tuvinian populations (Table 2), favoring the proposal on the homogeneous action of population dynamics factors on the whole Tuva territory. The most probable explanation for higher mtDNA haplogroup diversity revealed in Bai-Taiga population is the historically formed heterogeneity of the western part of the Tuva Republic, inhabited by different ethnic groups [23–25].

Interestingly, in Tuvinian gene pool two main mtDNA lineages, C/1 and C/2, with summarized frequency of about 50% prevail. This fact can reflect some specific features of the founder population demographic history, specifically, dramatic decrease of its population number with subsequent increase, which resulted in the decrease of genetic diversity and the alteration of the gene frequencies (the bottleneck effect). Note that while the total frequency of haplogroup C in Mongols is lower that in Tuvinians, it is

mainly represented by mitotypes 1 and 2 [26]. It can be suggested that appearance of haplogroup C in the Altai and Sayan upland and in the Central Asia dates back to the same geological period and has the common source. In any case, this component of mitochondrial gene pool is suggested to be one of the oldest. From the other hand, the population contains numerous rare mitotypes (Table 3). As mentioned above, this situation can be the reflection of intense migrations from other territories. The confirmation of this suggestion can be found in the history of Tuva. Over the last several thousand years the territory of Sayans and the Minusinsk depression were invaded by the tribes of different ethnic origin, and even of different races (see [18] for review).

Genetic relationships between Tuvinians and other Asian populations. Contemporary Tuvinians along with Mongols and Buryats belong to Central Asian anthropological type of Mongoloid race with some variations in different region of Tuva [19, 21]. However, Tuvinians are characterized by complex ethnogeny, and different heterogeneous ancient tribes, which replacing one another inhabited the territory of ancient Tuva, could leave their traces in the gene pool of contemporary population.

Taking into consideration the fact that in ancient times (the Bronze Age) the territory of Altai and Sayans was inhabited by Caucasoids [23], it seems interesting to reveal the presence of presumptive Caucasoid admixture in the Tuvinian gene pool. In spite of the fact that in this study the "Mongoloid-specific" set of polymorphic sites was used, haplotypes of presumptive Caucasoid origin can be isolated by the method of exclusion. As mentioned above in the Results, these were haplotypes I and II. Their summarized frequency was the highest in the Bai-Taiga population (about 24%), in Toora-Hem it was 21.5%, and in Kungurtug, about 12%. The average haplotype frequency for Tuva as a whole was 19%. These findings indicate that maximum contribution of Caucasoid component to the Tuvinian gene pool does not exceed 19%. However, haplotypes I and II can also include the lineages of the Mongoloid origin. Since the frequency of haplotypes I and II in other Mongoloid populations, and in Mongols in particular, is also high (Table 1), it can be suggested that at least some of the lineages included in haplotypes I and II in Tuvinians can be of Mongoloid origin. Thus, it is likely that Caucasoid admixture in the Tuvinian gene pool is low. The contribution of the Caucasoid component seems to be higher in the populations inhabiting western raions of the republic, where the frequency of haplotypes I and II is the highest, and the features of Mongoloid race, according to anthropological data, are less expressed [19]. Interestingly, the data on Y chromosome polymorphism showed that about 24% of Y chromosomes in Tuvinians were of ancient Caucasoid origin [27]. This situation can reflect ethnically differentiated contribution of males and females to the formation of contemporary gene pool.

It was established that the territory of Tuva was inhabited by the ancient Samoyed and Keto-speaking tribes, whose influence is most pronounced in the northeast of Tuva [24]. Earlier, it was shown that in respect to mtDNA D-loop polymorphism Tuvinians are close to the Samoyed-speaking Sel'kups [8]. The genetic distances between Tuvinian populations and Nganasans and Evens are low, which is reflected in the dendrogram topology (figure),and confirms the participation of the "northern" component in the Tuvinian ethnogeny. The studies carried out in some other populations showed that high frequency of haplogroup C is typical to the populations inhabiting the territories of Siberia north of Tuva [2, 3].

The next stage of the Tuva history was associated with the invasion of Huns and Turkic tribes. In this respect note that judging by genetic distances, Tuvinians are very close to Altaians, which belong to Turkic group (Table 3), and cluster together with them on the dendrogram (figure). Thus, historical data along with the results of anthropological studies point to the relatedness between Altaians and Tuvinians [19, 23]. These suggestions are confirmed by the data on mtDNA polymorphism. Earlier studies of length polymorphism of mtDNA intergenic region V led to a suggestion that this polymorphism in Tuvinians is associated with the Turkic influence [9]. Further studies of this problem would permit elucidation of the degree of genetic differentiation of the Turkic-speaking people. It should be also noted that insertion in this region in Tuvinians is associated with haplogroups C and D (see also [28]). This finding suggests recurrent independent emergence of the interegenic region V insertion in the history of humankind.

Numerous data suggest that during the last millennium, nomad Mongolian tribes migrated to the territory of Tuva. In view of this, it is surprising that Mongolians and Tuvinians do not cluster together on the dendrogram (figure). Indeed, Tuvinians are remarkably different from Mongols with regard to the frequencies of the main haplogroups. In Tuvinians the frequency of haplogroup C is higher, while the frequencies of haplogroups D and X are lower than in Mongols (Table 1). These findings indicate that although Tuvinian and Mongolian people are similar in their culture and anthropological types, there are the differences in the formation of these populations.

Central Asian ancestry of Native Americans. It was established that mitochondrial genome of American Indians can be considered as a "subsample" of Mongoloid mtDNA. Its diversity was considerably lower than that of Asian populations, and furthermore, it was influenced by the founder effect. For these reasons, the search of the Asian ancestry of American Indians, who are supposed to move to the New World through the land bridge, which was located in the place currently occupied by the Bering Strait, has become the subject of many studies [1–4, 13, 26, 28–30]. However, only a

few Asian populations possess all of the four main haplogroups, A, B, C, and D, found in American Indians. According to Derenko, *et al.* [28], confirmed in the present study, the summarized frequencies of the four New World-founder haplogroups in Tuvinians was the highest (over 70%). Taking into consideration ancient migrations of Mongolian and Turkic tribes to the south of Siberia (Altai, Tuva, and Buryatia), it can be suggested that the presence of haplogroup B, very scarce in other Siberian populations, in this territory is rather the result of migrations, than the initial characteristics of prehistoric populations. Nevertheless, the data on the ubiquitous presence of haplogroups A, B, C, and D in Tuvinians support the hypothesis that the ancestors of American Indians may have originated in Central Asia. Mongolia along with Altai and Sayan upland could be the potential locations for the origin of these populations [13, 26, 28].

Analysis of mtDNA restriction polymorphism in the three Tuvinian populations showed that, compared to other Asian populations, this ethnic group is characterized by unique diversity and frequencies of the main mtDNA lineages. The gene pool of Tuvinian mtDNA contains haplogroups A, B, C, and D, which is typical to the populations of Central Asia, and supports the suggestion that American Indians may have originated in Central Asia. Substantial prevalence of haplogroup C brings Tuvinians closer to more northern Siberian populations. Despite of intense ancient migrations from the surrounding territories and mutual isolation of Tuvinian tribes at the late 19th–early 20th beginning centuries [24], mitochondrial gene pool of Tuvinians represents a unified structure with weak territorial differentiation.

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