

Announcement of Population Data

Y-chromosomal STR haplotypes in Kalmyk population samples

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Abstract

Seventeen Y-chromosomal short tandem repeats (STRs), DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385ab, DYS437, DYS438, DYS439, GATA-H4, DYS448, DYS456, DYS458, DYS635 were typed in DNA samples from the Kalmyk population ($n = 99$). The population is characterized by a high proportion of duplicated DYS19 alleles and deletions of the locus DYS448 on the background of the Central Asian haplogroup C*. AMOVA analysis reveals a close vicinity to Mongolian and Kazakh populations and large genetic distance to geographical neighbours from Russia, Ukraine and the Caucasus.

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1. Population

Ninety-nine random unrelated male DNA samples from Elista (Republic of Kalmykia, Russian Federation) previously typed for Y-SNP and mt-DNA markers [1] were typed for 17 Y-STR markers using the AmpF/STR Yfiler PCR amplification kit [2]. The geographical location of the sampled population is shown in Fig. 1. Historically the Kalmyks belong to the large tribal unions of the Oyrats in Jungaria (Western Mongolia) [3]. Due to rivalry with other tribes in the early 17th century 50.000 families of the Torgouts, Derbets and Khoschuts left their homeland and migrated to the steppes of the Western banks of the Volga river in Russia and were since then named Kalmyk or “xal’mag” (from “qali” = “to spread out” in Mongolian language, or “qal” = “to stay behind” in Turkish language). In

the second half of the 17th century they formed the Kalmyk Khanate, but only a hundred years later they lost independency and about 150.000 Kalmyks tried to return to Jungaria. More than 80.000 people lost their life during this exodus. The Kalmyks remaining in Russia kept their nomadic lifestyle until the end of the 19th century, when they started to adopt agriculture. Today the Kalmyks live in an autonomous republic within the Russian federation, speak a Mongolian language and practise the Buddhist religion. They have now been isolated for some 300 years from their presumed parental population. Genetic studies of the Kalmyks can thus provide insights into a recent historical process (migration and bottleneck) shaping the extant Y chromosome pool of a typical Central Asian nomad population [1].

2. Extraction

Genomic DNA was isolated from cheek-cell samples using a salting-out procedure [4]. Informed consent and information

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Fig. 1. Map of Russia showing the location of Kalmyks and the sampled population of Elista. The figure was modified from [1].

about birthplace, parents and grandparents were obtained from all donors.

3. PCR

A multiplex PCR reaction was carried out for each DNA sample using the AmpF/STR Yfiler PCR amplification kit (Applied Biosystems, Foster City, USA) according to the supplier's protocols. DYS448 deletions were verified by an Octaplex PCR (including this locus) done by the Biotype AG, Dresden [5].

Table 1

AMOVA pairwise distance based on Φ_{st} values between Kalmyks ($n = 99$) and nine other populations ($n = 1375$); p values are shown above, Φ_{st} values below the diagonal

	Elista, Russia [Kalmykian]	Kiev, Ukraine	Egyin Gol, Mongolia	Mongolia [Buryat]	Mongolia [Khalkh]	Moscow, Russia	Siberia [Buryat]	Taraz, Kazakhstan	North Caucasus	South Caucasus
Elista, Russia [Kalmykian]	–	0	0	0.0068	0.2154	0.0025	0	0.0165	0	0
Kiev, Ukraine	0.0815094	–	0	0	0.0011	0.1954	0	0	0	0
Egyin Gol, Mongolia	0.193698	0.343967	–	0	0	0	0	0	0	0
Mongolia [Buryat]	0.0575446	0.165579	0.203368	–	0.2723	0	0	0.0019	0	0
Mongolia [Khalkh]	0.00687419	0.0598084	0.193225	0.00466406	–	0.0634	0	0.6426	0	0
Moscow, Russia	0.0415025	0.00329923	0.296479	0.112866	0.0219137	–	0	0.0093	0	0
Siberia [Buryat]	0.184817	0.261169	0.467863	0.185459	0.185926	0.220993	–	0	0	0
Taraz, Kazakhstan	0.0200029	0.0512212	0.235355	0.0608286	–0.00654626	0.0238254	0.245765	–	0	0
North Caucasus	0.129998	0.177563	0.41475	0.256525	0.164124	0.134347	0.222149	0.184334	–	0.0003
South Caucasus	0.0961689	0.143312	0.376374	0.183242	0.105429	0.0906691	0.19077	0.127244	0.0229455	–

$p > 0.05$.

$p > 0.01$.

$p < 0.01$.

4. Typing

Individual Y-STR alleles were typed using the ABI310 sequencer/software platform. Allele nomenclature is according to the Y Chromosome Haplotype Reference Database, YHRD [6], the updated recommendations of the ISFG [7] and the manufacturers of the Yfiler kit. The Y chromosomal SNP markers were typed as described in [1].

5. Quality control

GEDNAP (German DNA Profiling Group) blind trial system [8].

6. Analysis of data

Haplotype diversity value was calculated as $D = N/N - 1 (1 - \sum p_i^2)$, where N is the population size and p_i is the frequency of the i th haplotype [9]. Pairwise values of Φ_{st} , an analogue of Wrights F_{st} that takes the evolutionary distance between individual haplotypes into account [10,11], were calculated to measure genetic distances between minimal haplotypes of 10 populations ($n = 1474$) with the statistical significance determined by a permutation test (10,000 replicates) (Table 1). We used our own implementation of AMOVA (available at <http://rprojekt.org/amova/>). To allow the inclusion of 33 haplotypes with duplicated DYS19 alleles in this calculation we had to determine the ancestral (non-duplicated) state for these peculiar mutations at the locus DYS19. We fixed the allele (see Table 2) by minimizing the intra-population variance of all 9-locus haplotypes in the sample (10,000 permutations). The subsequent duplication was included as an additional binary marker, with “1” depicting a second allele, and “0” for the monoallelic state. The biallelic DYS385ab marker was separated into two loci [12]. The DYS389I allele length was obtained by subtracting the shorter allele from the longer allele at DYS389I/II. To illustrate the relationship between populations based on pairwise Φ_{st} values we constructed a Neighbour Joining tree by using the program

Table 2
17-locus Y-STR haplotypes and haplogroups in 99 Kalmykian males

Ht	Pop (YHRD format)	DYS 19	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385ab	DYS 438	DYS 439	DYS 437	DY S448	DYS 456	DYS 458	DYS 635	GATA-H4	Hg ¹	n
1	Elista, Russia [Kalmykian]	14	12	28	23	10	10	14	12,19	11	10	16	19	15	17	24	12		10
2	Elista, Russia [Kalmykian]	16,17	14	31	24	9	11	13	12,12	10	11	14	20	15	18	23	10	K*	6
3	Elista, Russia [Kalmykian]	16,17	14	31	24	9	11	13	12,13	10	11	14	20	15	18	23	10	C*	3
4	Elista, Russia [Kalmykian]	16,17	14	31	24	9	11	13	12,12	10	11	14	20	15	18	22	10	C*	2
5	Elista, Russia [Kalmykian]	16,17	14	31	25	9	11	13	12,12	10	11	14	20	15	18	23	10	C*	2
6	Elista, Russia [Kalmykian]	16,17	15	32	24	9	11	13	12,13	10	11	14	20	15	18	23	10	C*	2
7	Elista, Russia [Kalmykian]	15	13	28	23	10	11	14	11,18	10	12	14	21	15	17	21	11		2
8	Elista, Russia [Kalmykian]	15	13	28	23	10	11	14	11,18	10	13	14	21	15	19	21	11		2
9	Elista, Russia [Kalmykian]	15	13	28	23	10	11	14	11,11	10	11	14	21	15	18	22	11		2
10	Elista, Russia [Kalmykian]	15	13	28	23	10	11	14	11,11	10	11	14	21	15	17	22	11		2
11	Elista, Russia [Kalmykian]	17	14	31	24	9	11	13	12,12	10	11	14	20	15	19	23	10		2
12	Elista, Russia [Kalmykian]	16	13	29	25	10	11	13	12,13	10	10	14	22	15	18	21	11		1
13	Elista, Russia [Kalmykian]	16	13	29	25	10	11	13	12,13	10	10	14	22	14	18	21	11		1
14	Elista, Russia [Kalmykian]	15,17	14	32	24	9	11	13	12,12	10	11	14	20	15	18	22	10	C*	1
15	Elista, Russia [Kalmykian]	15,17	14	31	24	9	11	13	12,12	10	11	14	20	15	18	23	10	C*	1
16	Elista, Russia [Kalmykian]	16,17	14	31	24	9	11	13	12,12	10	12	14	20	16	18	23	10	C*	1
17	Elista, Russia [Kalmykian]	16,17	14	30	23	9	11	13	12,12	10	11	14	20	15	18	23	10	K*	1
18	Elista, Russia [Kalmykian]	16,17	14	33	24	9	11	13	12,12	10	11	14	20	15	18	23	10	C*	1
19	Elista, Russia [Kalmykian]	16,17	14	31	24	9	11	13	12,12	10	12	14	20	15	18	23	10	C*	1
20	Elista, Russia [Kalmykian]	16,17	14	30	24	9	11	13	12,12	10	11	14	20	15	18	23	10	C*	1
21	Elista, Russia [Kalmykian]	16,17	14	31	24	9	11	13	12,12	10	11	14	20	15	18	24	10	C*	1
22	Elista, Russia [Kalmykian]	16,17	14	31	24	9	11	13	11,12	10	11	14	20	15	19	23	10	C*	1
23	Elista, Russia [Kalmykian]	16,17	14	31	24	9	11	13	12,12	10	12	14	20	15	18	24	10	C*	1
24	Elista, Russia [Kalmykian]	16,17	14	32	24	9	11	13	12,12	10	11	14	20	15	18	24	10	C*	1
25	Elista, Russia [Kalmykian]	16,17	15	32	24	9	11	13	12,12	10	11	14	20	15	18	23	10	C*	1
26	Elista, Russia [Kalmykian]	16,17	15	31	23	9	11	13	12,12	10	12	14	20	15	18	22	10	C*	1
27	Elista, Russia [Kalmykian]	16,17	14	31	24	9	11	13	12,12	10	11	14	20	14	18	23	10	C*	1
28	Elista, Russia [Kalmykian]	16,17	14	31	25	9	11	12	12,12	10	11	14	20	15	18	23	10	C*	1
29	Elista, Russia [Kalmykian]	17,18	14	31	24	9	11	13	12,12	10	11	14	20	15	17	23	10	C*	1
30	Elista, Russia [Kalmykian]	17,18	14	31	24	9	11	13	12,12	10	11	14	20	16	18	24	10	C*	1
31	Elista, Russia [Kalmykian]	15,16	14	31	24	9	11	13	12,12	10	11	14	del	15	18	23	10	C*	1

Table 2 (Continued)

Ht	Pop (YHRD format)	DYS 19	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385ab	DYS 438	DYS 439	DYS 437	DY S448	DYS 456	DYS 458	DYS 635	GATA-H4	Hg ¹	n
32	Elista, Russia [Kalmykian]	15	13	28	24	9	11	13	12,15	11	11	15	del	16	16	21	11	C*	1
33	Elista, Russia [Kalmykian]	15	13	30	24	10	11	13	12,14	11	12	14	del	17	17	21	11	C*	1
34	Elista, Russia [Kalmykian]	15	13	28	23	9	11	13	12,15	11	12	15	del	17	16	21	11	C*	1
35	Elista, Russia [Kalmykian]	15	13	29	24	10	11	13	12,13	11	11	14	del	17	17	21	11	C*	1
36	Elista, Russia [Kalmykian]	15	13	28	24	9	11	13	12,15	11	11	15	del	17	16	21	11	C*	1
37	Elista, Russia [Kalmykian]	15	13	28	24	9	11	13	12,15	11	12	15	del	17	16	21	11	C*	1
38	Elista, Russia [Kalmykian]	14	12	27	23	10	14	13	11,13	11	11	14	19	14	17	20	12		1
39	Elista, Russia [Kalmykian]	14	13	28	23	10	14	13	12,13	10	10	14	19	16	16	24	12		1
40	Elista, Russia [Kalmykian]	13	13	28	23	10	16	13	13,18	11	11	14	22	16	16	23	9		1
41	Elista, Russia [Kalmykian]	17	14	32	23	11	11	13	11,14	11	10	14	20	16	16	23	12		1
42	Elista, Russia [Kalmykian]	14	12	28	23	10	10	14	11,19	11	10	16	19	15	18	24	12		1
43	Elista, Russia [Kalmykian]	15	13	28	23	10	13	14	11,11	10	11	14	21	15	17	22	11		1
44	Elista, Russia [Kalmykian]	15	13	28	23	10	11	14	11,11	10	12	14	21	15	19	22	11		1
45	Elista, Russia [Kalmykian]	17	15	32	24	9	11	13	12,12	10	11	15	20	15	18	23	10		1
46	Elista, Russia [Kalmykian]	15	13	30	24	10	13	14	12,17	10	11	14	18	15	15	20	12		1
47	Elista, Russia [Kalmykian]	17	13	30	24	10	13	12	13,18	10	13	14	19	14	20	22	12		1
48	Elista, Russia [Kalmykian]	15	13	31	24	9	11	13	12,13	10	11	14	20	15	18	25	10		1
49	Elista, Russia [Kalmykian]	14	13	30	23	12	13	13	14,16	10	13	15	20	15	16	23	11		1
50	Elista, Russia [Kalmykian]	14	12	28	23	10	10	14	12,19	11	10	16	19	15	16	24	12		1
51	Elista, Russia [Kalmykian]	15	14	31	24	10	13	14	12,17	10	11	14	18	15	15	21	12		1
52	Elista, Russia [Kalmykian]	13	13	31	23	10	14	14	15,16	11	13	13	19	15	16	23	11		1
53	Elista, Russia [Kalmykian]	16	13	28	23	10	11	14	11,11	10	11	14	21	15	19	22	11		1
54	Elista, Russia [Kalmykian]	14	12	28	23	10	10	14	12,19	11	11	16	19	15	17	24	12		1
55	Elista, Russia [Kalmykian]	14	14	30	19	11	13	13	13,13	10	13	15	19	15	17	24	11		1
56	Elista, Russia [Kalmykian]	14	14	30	23	10	14	13	12,13	10	10	14	19	16	17	24	12		1
57	Elista, Russia [Kalmykian]	15	14	30	24	10	13	14	12,17	10	11	14	18	15	15	21	12		1
58	Elista, Russia [Kalmykian]	14	12	28	23	10	10	14	12,19	11	10	16	19	16	17	25	12		1
59	Elista, Russia [Kalmykian]	15	13	29	23	10	11	14	11,11	10	11	14	21	15	18	22	11		1
60	Elista, Russia [Kalmykian]	15	14	30	23	9	15	12	15,15	10	11	16	19	15	17	19	11		1
61	Elista, Russia [Kalmykian]	15	11	28	23	9	10	15	15,17	9	11	16	21	15	17	22	12		1
62	Elista, Russia [Kalmykian]	15	14	31	24	9	11	13	12,13	10	11	14	21	15	18	24	10		1
63	Elista, Russia [Kalmykian]	14	12	28	23	10	10	14	12,19	11	10	16	19	15	16	24	12		1

Table 2 (Continued)

Ht	Pop (YHRD format)	DYS 19	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385ab	DYS 438	DYS 439	DYS 437	DY S448	DYS 456	DYS 458	DYS 635	GATA-H4	Hg ¹	n
64	Elista, Russia [Kalmykian]	13	13	29	24	10	11	13	17,18	10	13	14	20	17	16	23	12		1
65	Elista, Russia [Kalmykian]	13	13	31	23	11	15	13	15,16	11	14	13	19	15	17	23	11		1
66	Elista, Russia [Kalmykian]	17	13	29	24	10	13	13	14,20	10	11	16	20	15	17	20	11		1
67	Elista, Russia [Kalmykian]	17	14	31	24	9	11	13	12,12	10	11	14	20	15	18	23	10		1
68	Elista, Russia [Kalmykian]	15	12	29	23	10	12	12	11,17	10	13	14	19	16	18	19	11		1
69	Elista, Russia [Kalmykian]	16	14	33	25	11	11	13	11,14	11	10	14	21	15	15	23	12		1
70	Elista, Russia [Kalmykian]	17	13	29	25	10	12	13	12,12	10	10	14	22	15	18	21	11		1
71	Elista, Russia [Kalmykian]	14	12	27	23	10	15	12	12,18	11	13	15	20	15	17	20	12		1
72	Elista, Russia [Kalmykian]	15	13	28	23	10	11	14	11,18	10	13	14	21	15	18	21	11		1
73	Elista, Russia [Kalmykian]	14	13	30	22	11	13	13	13,17	10	12	15	20	15	15	23	11		1
74	Elista, Russia [Kalmykian]	15	13	28	23	10	11	14	10,11	10	11	14	21	15	18	22	11		1
75	Elista, Russia [Kalmykian]	15	13	28	23	10	11	14	11,18	10	11	14	21	15	18	21	11		1

1 The SNP-defined haplogroup has been determined in all samples with **DYS19** duplication or **DYS448** deletion.

2 The **DYS19** duplication alleles chosen for AMOVA calculations are written in bold (see text for method).

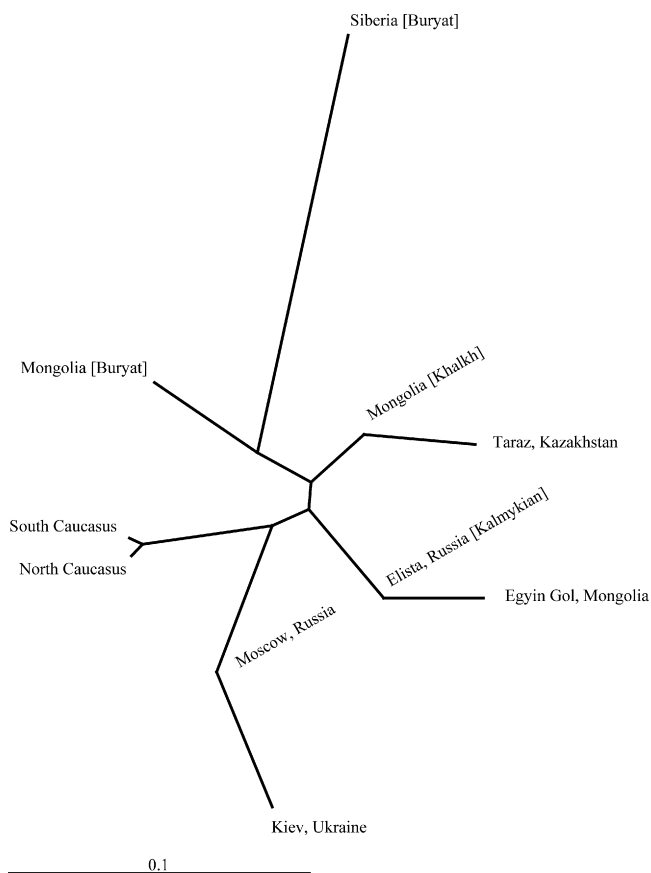


Fig. 2. NJ tree for Kalmyks and 9 reference populations.

PHYLIP [13]. The tree was visualized by using the software TREEVIEW [14] (Fig. 2).

7. Comparative population data

Minimal 9-locus haplotypes of the Kalmyks were compared via AMOVA with 241 Northern and 263 Southern Caucasians [15], 85 Russians from Moscow, 243 Ukrainians from Kiev, 181 Kazakhs from Taraz, 215 Buryats from Siberia [16], 66 Mongolians from Egin Gol [17], 39 Mongolian Khalks [18] and 42 Mongolian Buryats [19]. All reference samples are included in the Y Chromosome Haplotype Reference Database, YHRD (www.yhrd.org) [6].

8. Discussion

A total of 99 samples were investigated in this study and only 75 different 17-locus haplotypes were detected leading to a comparably reduced haplotype diversity value of 0.986 (Table 2). The Kalmyks have the highest number of **DYS19** duplications observed so far in any single population group. One-third (33 out of 99) of the Y chromosomes show a biallelic pattern; among these the allelic pair 16,17 has the highest frequency ($n = 28$), followed by 15,17 (2), 17,18 (2) and 15,16 (1). All but 2 chromosomes with **DYS19** duplications (hg K*) belong to haplogroup C* which is predominant in Central Asia. The haplotypes/haplogroup background is very similar for all chromosomes showing **DYS19** duplications (see Table 2). Moreover a high number of deletions at locus **DYS448** has been observed (7 out of 99), all of these chromosomes belong to the

haplogroup C* as well (Table 2). It is very likely that the DYS448 deletion is caused by a reciprocal deletion of the b2/b3 segment within the ampliconic AZFc region of Yq [20]. Many other complete deletions of the locus DYS448 have been found on the background of haplogroup C* (Jobling et al., manuscript in preparation). Most probably both the DYS19 duplication and the DYS448 deletion pattern already originated in Central Asia. We suggest bottlenecks and/or founder effects due to the recent emigration and subsequent isolation in the Russian steppe as responsible for the high frequency of Kalmyk-specific Y chromosomal lineages.

The Kalmyks showed the lowest genetic distance to the Mongolian (except the outlier Egyin Gol and Siberian Buryat groups) and Kazakh populations confirming their historical ancestry. The *p* values for the Taraz Kasakh and the Mongolian Khalk comparison turned out to be non-significant at the 99% and 95% CI level, respectively. The DYS19 duplications are also frequent in these reference populations. Among the 175 Kazakhs the typical Kalmykian minimal haplotype 16,17-14-31-24-9-11-13-12,12 occurs 3 times; altogether 6/175 Kazakh chromosomes are duplicated at DYS19. In the combined Mongolian samples (see www.yhrd.org → Mutations → Duplications) a similar (reduced) 7-locus haplotype 16,17-14-31-24-9/10-11-13 (without DYS385) occurs 6 times among 147 samples. The close geographical neighbours of the Kalmyks, the Slavic-speaking Russians and Ukrainians as well as the Northern Caucasian populations, show much higher and significant Φ_{st} values ($p = 0$). Based on the AMOVA results the population sample “Elista, Russia [Kalmyk]” uploaded to release 19 of the YHRD has been assigned to the “1.Eurasian, 1.1 Altaic” metapopulation defined as a reference population in the YHRD for calculating match probabilities [21].

The occurrence of two recent, rare and independent mutations in Kalmykians on Yp (DYS19) and Yq (DYS448), in combination with a uniform haplotype/haplogroup C* mutational background and well-sampled ancestral populations, opens the possibility to precisely synchronize the genetic and historical chronology (manuscript in preparation).

Forensic analysts using the popular YFiler kit in casework should be aware of unusual mutations which may rise to high frequencies in certain populations. Misinterpretation of Y-STR results could be avoided by consulting the public databases depicting the variety and the population genetic background of the typical mutations affecting the Y chromosome (segmental deletions/insertions, duplications etc).

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