Molecular Biology Reports (2019) 46:17–25
https://doi.org/10.1007/s11033-018-4375-5

ORIGINAL ARTICLE

Genetic polymorphism analysis of mitochondrial DNA from Chinese Xinjiang Kazak ethnic group by a novel mitochondrial DNA genotyping panel

Tong Xie[1] · Li Hu[2] · Yu-Xin Guo[3,4,5] · Yu-Chun Li[6,7,8,9] · Feng Chen[2] · Bo-Feng Zhu[1,3,4,5]

Received: 17 April 2018 / Accepted: 10 September 2018 / Published online: 17 November 2018

Abstract
Genetic polymorphism analysis of 60 mitochondrial DNA (mtDNA) loci in Chinese Xinjiang Kazak group was conducted in this study. Blood samples from 141 unrelated healthy volunteers were randomly collected from Chinese Kazak ethnic group in Ili, Xinjiang Uygur Autonomous region. Among these mtDNA loci, single nucleotide transition was the most commonly observed variant (87.93%). A total of 25 haplogroups and 79 haplotypes were found in Kazak group, and Haplogroup D4 was the most common haplogroup (21.28%). Among the entire 79 haplotypes, 53 of them were observed for only once, 14 for twice. The haplotype diversity was 0.978 ± 0.005, and the nucleotide diversity was 0.17449. The detection of $(CA)_n$ and 9-bp deletion polymorphisms could improve the discrimination power of the mtDNA genetic marker. Moreover, Xinjiang Kazak group was compared with other previously reported groups to infer its genetic background. The present results revealed that Xinjiang Kazak ethnic group was genetically closer related to Xinjiang Uygur, Xinjiang Uzbek and Xinjiang Han populations. Meanwhile, our results also indicated the potential closer genetic relationships among Xinjiang Kazak group with Altaian Kazak as well as Xinjiang Xibe group. In conclusion, this novel mtDNA panel could be effectively utilized for forensic applications. Additionally, to further reveal the genetic background of Chinese Kazak group, more relevant populations and genetic markers should be incorporated in our future study.

Keywords mtDNA · SNP · Haplogroup · Kazak

Introduction

Mitochondrial DNA (mtDNA) located outside of the nucleus is a crucial part of human genome. Human mtDNA is a 16,569 bp closed circular double-stranded DNA molecule that encodes essential genes for proper cellular function [1]. While applied to forensic studies, mtDNA exhibits many distinct characteristics when compared to nuclear DNA, like maternal inheritance, lack of recombination, rapid mutation rate, and high polymorphisms [2–7]. Furthermore, many reports have confirmed that mtDNA sequence variations are strongly correlated with human genetic evolution and migration [6, 8]. Meanwhile, mtDNA polymorphism analysis is suitable for highly degraded bio-materials [9, 10]. Hence, the genetic analysis of mtDNA in different populations from disparate regions can be utilized for maternal lineage study. What's more, mtDNA is also a helpful genetic marker for geographic ancestry inference and anthropology researches.

The Kazak group is one of the 56 ethnic groups in China, which has its own language, culture and religion. According to the 6th population census in China (2010), Kazak ethnic group has a population of approximately 1.5 million and most Kazak individuals reside in Xinjiang Uygur Autonomous Region and Gansu Aksai Kazakh Autonomous County. For a long time, the genetic structure of Kazak group had changed greatly due to gene interactions happened between Kazak and the neighboring populations. Besides, more and more studies focusing on Kazak group

Tong Xie and Li Hu have contributed equally to this work as first author.

Feng Chen and Bo-Feng Zhu have contributed equally to this work as the corresponding author.

✉ Feng Chen
fchen@nimu.edu.cn

✉ Bo-Feng Zhu
zhubofeng@i.smu.edu.cn

Extended author information available on the last page of the article

have been conducted in recent years to infer its migratory route and origin [11–13]. In this study, we analyzed the mtDNA genetic polymorphisms of Chinese Xinjiang Kazak group using a novel mtDNA panel, and revealed the genetic relationships between Kazak group and the reference populations as well as provided useful information for the maternal lineage study and migration history of Chinese Kazak group.

Materials and methods

Ethical statement

This study was approved by the Ethical Committee of Southern Medical University and Xi'an Jiaotong University, China. And all the volunteers have given their written informed consents before inclusion. The samples collection and subsequent analysis were conducted under the human and ethical research principles of Southern Medical University and Xi'an Jiaotong University, China.

Samples

The blood samples were collected from 141 unrelated healthy volunteers of Kazak ethnic group in Ili, Xinjiang Uygur Autonomous region. The written informed consent was acquired from each of them and migration events did not exist in their family history of the participants for at least three generations. Blood samples were collected respectively in terms of the standard procedure.

MtDNA extraction, amplification and genotyping

MtDNA was extracted according to the previous protocol [14]. Multiplex PCR amplification of 60 mtDNA loci (nt10398, 10873, 3010, 709, 7196, 12705, 3970, 13104, 10310, 5178, 13928, 6446, 8414, 8793, 8794, 15043, 16311, 16126, 16129, 8701, 8697, 4883, 10400, CA, 9-bp, 1719, 14668, 12811, 9824, 9123, 7028, 11719, 8584, 11251, 8020, 5460, 2706, 11215, 4216, 12372, 16362, 9698, 1541, 8684, 9477, 4491, 1811, 16316, 16319, 9545, 152, 14569, 8964, 10397, 3348, 4833, 7600, 5417, 5442 and 15784) was conducted using the Expressmarker mtDNA-SNP 60 reagent (AGCU ScienTech Incorporation, Jiangsu, Wuxi). Briefly, the total reaction volume of the PCR amplification system (25 µl) contained 1 µl genomic DNA, 10 µl reaction mix, 5 µl primer set, 5 µl taq DNA polymerase and 4 µl sdH_2O. The cycling parameters were set up according to the manufacturer's instruction, respectively. The PCR production of 1 µl was combined with 0.5 µl Marker SIZ-500 and 12 µl Hi-Di formamide. Capillary electrophoresis was performed by the ABI Prism 3130XL Genetic Analyzer and sample profiling was analyzed by GeneMapper ID software v3.2.1 (Applied Biosystems, USA). Male 9948 and Female 9947A DNA samples were used as the positive control in our experiment.

Data analysis

The genotyping results of Kazak group were aligned with the revised Cambridge Reference Sequence (rCRS) [15] for subsequent statistical analysis. Haplogroups were obtained according to van Oven M, Kayser M (http://www.phylotree.org; [16]). Forensic statistical parameters (haplotype diversity, nucleotide diversity, polymorphic loci and so on) for Kazak group were calculated by DnaSP software version 5.0. The random match probability (RMP) of two individuals from a population having the same haplotype was calculated as $RMP = \sum X_i^2$, X_i is the frequency of the i-th mtDNA haplotype. Besides, the discrimination power (DP) was utilized for evaluating the probability of two unrelated random samples having different haplotypes from a certain population. Haplotype diversity could measure the uniqueness of a particular haplotype in a certain population. Nucleotide diversity (π) is the average number of nucleotide differences per locus between two DNA sequences selected randomly from a given population [17, 18].

Furthermore, Arlequin software version 3.0 was employed to estimate pairwise *Fst* values between Xinjiang Kazak and other neighboring groups [12, 13, 19–24]. Besides, a phylogenetic tree was reconstructed by MEGA software version 4.0 based on genetic distance values of pairwise populations to infer the genetic background of Kazak ethnic group as well as to evaluate the genetic relationships between Kazak and other referenced groups.

Results

Forensic parameter analysis

Allele frequencies of the 60 mtDNA loci detected in Chinese Kazak ethnic group were listed in Table 1. As for the 58 selected mtDNA SNP loci of Kazak ethnic group, the most common polymorphism was single nucleotide transition (87.93%), followed by single nucleotide transversion (nt5178, nt7196, nt13928 variants, 5.17%). At nt9824 locus, single nucleotide transition and transversion were simultaneously observed (A/T/C). While at loci nt3348, nt8697, nt8793, no polymorphisms were detected.

Compared with the previous data of Xinjiang Xibe ethnic group [19], transition and transversion were both observed at the nt9824 lcous. In addition, single nucleotide transversions occurring at nt5178, nt7196 and nt13928 loci were also identically detected in these two groups, while no polymorphisms were found at nt8697, nt8793 loci in Kazak

Table 1 Allele frequencies of 60 mtDNA loci in Chinese Xinjiang Kazak ethnic group

Loci (nt)	Alleles	Frequencies
152	T/C	0.7234/0.2766
709	G/A	0.8794/0.1206
1541	T/C	0.9929/0.0071
1719	G/A	0.9645/0.0355
1811	A/G	0.9716/0.0284
2706	A/G	0.1206/0.8794
3010	G/A	0.6667/0.3333
3348	A	1.0000
3970	C/T	0.9078/0.0922
4216	T/C	0.9787/0.0213
4491	G/A	0.9858/0.0142
4833	A/G	0.9149/0.0851
4883	C/T	0.7092/0.2908
5178	C/A	0.7092/0.2908
5417	G/A	0.9858/0.0142
5442	T/C	0.9929/0.0071
5460	G/A	0.9787/0.0213
6446	G/A	0.9929/0.0071
7028	C/T	0.1206/0.8794
7196	C/A	0.9007/0.0993
7600	G/A	0.9362/0.0638
8020	G/A	0.9645/0.0355
8414	C/T	0.7234/0.2766
8584	G/A	0.9007/0.0993
8684	C/T	0.9787/0.0213
8697	G	1.0000
8701	A/G	0.4823/0.5177
8793	T	1.0000
8794	C/T	0.9149/0.0851
8964	C/T	0.9716/0.0284
9123	G/A	0.9787/0.0213
9477	G/A	0.9645/0.0355
9545	A/G	0.9362/0.0638
9698	T/C	0.9929/0.0071
9824	T/C/A	0.9574/0.0142/0.0284
10310	G/A	0.9078/0.0922
10397	A/G	0.9858/0.0142
10398	A/G	0.4681/0.5319
10400	C/T	0.4894/0.5106
10873	T/C	0.4894/0.5106
11215	C/T	0.9716/0.0284
11251	A/G	0.9929/0.0071
11719	G/A	0.1560/0.8440
12372	G/A	0.9220/0.0780
12705	C/T	0.3688/0.6312
12811	T/C	0.9858/0.0142
13104	A/G	0.9858/0.0142
13928	G/C	0.9078/0.0922
14569	G/A	0.9078/0.0922
14668	C/T	0.7234/0.2766
15043	G/A	0.4894/0.5106
15784	T/C	0.9716/0.0284
16126	T/C	0.9787/0.0213
16129	G/A	0.8794/0.1206
16311	T/C	0.9433/0.0567
16316	A/G	0.9858/0.0142
16319	G/A	0.8723/0.1277
16362	T/C	0.5248/0.4752
CA	4/5/6	0.3050/0.6525/0.0425
9-bp	NORM/DEL	0.9362/0.0638

group and at nt4491, nt6446, nt8684, nt13104 loci in Xinjiang Xibe group. Results demonstrated that allele frequencies of some mtDNA loci differed among different populations. Therefore, more population data should be collected to verify the efficiency of this novel mtDNA panel in forensic field.

With the exclusion of $(CA)_n$ locus, mtDNA haplogroups of the overall 141 individuals in Xinjiang Kazak group were presented in Fig. 1. While Table 2 and Fig. 2 showed haplogroups and haplotypes based on polymorphisms of the 60 mtDNA loci in Xinjiang Kazak ethnic group. Fifty-seven polymorphic loci (excluding nt3348, nt8697, and nt8793 loci) defined 25 haplogroups and 79 haplotypes. Moreover, Haplogroup D4 was the most common haplogroup (21.28%) in Xinjiang Kazak group, followed by the H haplogroup (14.18%). Among the total 79 haplotypes, 53 of them were observed for only once, 14 for twice, and 12 for three times or more, with the detailed information shown in Table 3.

Based on the total 60 mtDNA loci, the values of RMP and DP were 0.0270 and 0.9730 in Xinjiang Kazak group, respectively. In addition, more forensic statistical parameters of the 58 mtDNA loci excluding $(CA)_n$ and 9-bp deletion were presented in Table 4. The haplotype diversity was 0.978 ± 0.005, and the nucleotide diversity was 0.17449. As presented in Table 4, the values of RMP and DP were 0.0291 and 0.9709, respectively. In Chinese Xinjiang Kazak group, the DP value of 58 mtDNA SNP loci was lower, which was consistent with the previous report regarding other Chinese groups (Han population and Uyghur group) [25].

Interpopulation differentiation and phylogenetic analysis

As shown in Table 5, the pairwise *Fst* and *p* values between Chinese Xinjiang Kazak group and other previously reported groups [12, 13, 19–24] were calculated. Values below the

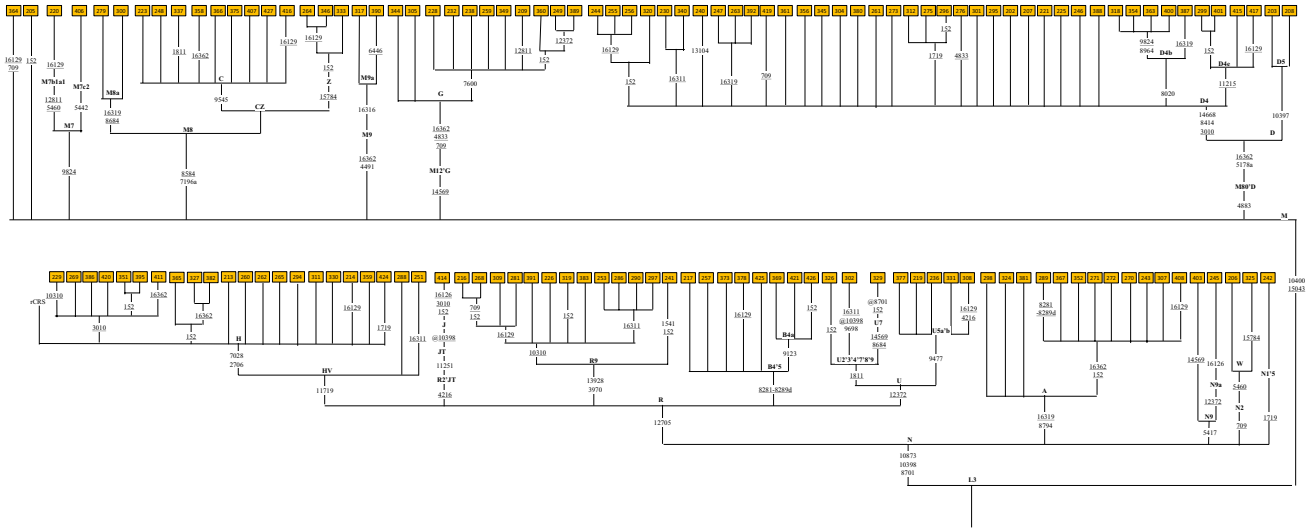

Fig. 1 MtDNA haplogroups of 141 individuals recruited from Chinese Xinjiang Kazak ethnic group using online software phylotree

diagonal were *Fst* values, while above the diagonal were *p* values. Data with statistical significances were labelled in bold. It was obvious that the studied Xinjiang Kazak ethnic group had the smallest genetic differentiation with Xinjiang Uzbek group ($Fst = 0.00808$, $p > 0.05$), followed by Xinjiang Han population ($Fst = 0.00828$, $p > 0.05$), and Xinjiang Uygur ethnic group ($Fst = 0.00935$, $p > 0.05$). Oppositely, Italian population was observed to have the largest genetic differentiation with Kazak ethnic group ($Fst = 0.20159$, $p < 0.05$).

As shown in Fig. 3, a phylogenetic tree was constructed based on *DA* distances between Xinjiang Kazak and other groups to further demonstrate the genetic relationships among those popualtions. Similarly, Italian, African Americans, Estonian and Caucasian groups were in the same cluster, while the rest groups were in another. Xinjiang Kazak group had closer genetic relationships with Xinjiang Uygur and Uzbek groups, and they shared a sub-branch of the phylogenetic tree collectively. Furthermore, Xinjiang Kazak group had relatively closer genetic distances with Xinjiang Han, Xinjiang Xibe and Altaian Kazak groups, which meant these groups might have closer genetic relationships in a way.

Discussion

The genetic polymorphism analysis of mtDNA plays an essential and irreplaceable role in population genetic studies. The analysis of hypervariable regions in human mtDNA is widely used in forensic applications in recently years. Due to the inheritance traits, high polymorphism, small amplicon size, it can be utilized for highly degraded bio-materials analysis, maternal ancestry inference and anthropology study. Hence, mtDNA could be a powerful genetic marker in forensic applications.

The highly polymorphic mtDNA loci can reveal some important genetic features of the studied population. Single nucleotide transition and transversion are the common polymorphisms. $(CA)_n$ is a kind of length polymorphism of which the n represents the number of CA dinucleotide repeats (from nt00514 to nt00524 in the rCRS). Besides, $(CA)_n$ has a strong correlation with geographic origin and could be applied to individual identification because of relatively higher DP [25]. 9-bp deletion is the deletion of CCC CCTCTA sequence, and the occurence of 9-bp deletion or not in different geographic distribution is related with human migration [26–28]. A combination of $(CA)_n$ and 9-bp deletion polymorphisms could improve the efficiency of mtDNA genetic marker in forensic applications.

In our present study, the results of pairwise *Fst* values and phylogenetic tree simultaneously demonstrated that Xinjiang Kazak ethnic group might be closely related to Xinjiang Uygur, Xinjiang Uzbek and Xinjiang Han populations. Meanwhile, Altaian Kazak and Xinjiang Xibe groups also had relative closer genetic distances with Xinjiang Kazak group which indicated closer genetic relationships among these groups, besides, it could be supported by historical records as well. The origin of Kazak group in Chinese history could trace back to Western Han dynasty, the inhabitants who lived in ill River valley and Issyk Kul were regarded as the forefather of Kazaks. [29–32]. In addition, 'Silk Road' accelerated the interaction of culture and gene between Kazak group and other populations [12, 33]. Furthermore, during 1932 to 1933, a large

Table 2 The numbers of mtDNA haplogroups and haplotypes based on 60 mtDNA loci in the 141 Xinjiang Kazak individuals

Haplogroups	$(CA)_n$	Number	Haplotypes
A	4	12	4
B4′5	6	2	1
	5	1	1
	4	2	2
B4a	4	3	2
C	5	9	4
D4	4	1	1
	5	28	10
	6	1	1
D4b	4	5	2
D4e	5	4	3
D5	4	2	1
G	4	2	2
	5	9	5
H	4	2	2
	5	18	9
HV	5	2	2
J	5	1	1
M	5	2	2
M7b1a1	5	1	1
M7c2	4	1	1
M8a	5	2	1
M9a	5	1	1
	6	1	1
N1′5	5	1	1
N9	5	2	2
R9	4	12	6
	5	1	1
U2′3′4′7′8′9	5	1	1
	6	1	1
U5a'b	5	5	2
U7	4	1	1
W	5	1	1
	6	1	1
Z	5	3	2

amount of foreign Kazaks migrated to China for severely famine [34]. Therefore, gene interaction between different populations inevitably happened in consideration of above mentioned historical events. Modern records indicated that after long-term residing with the Uygurs and other ethnic groups in Xinjiang, Kazaks broadly assimilated their culture, language and custom in Northwest China [35, 36]. Hence, according to the genetic analysis results, the Xinjiang Kazak ethnic group had colser genetic relationships with Xinjiang Uygur, Xinjiang Uzbek and Xinjiang Han populations.

Conclusion

Genetic polymorphisms of the 60 mtDNA loci were investigated to evaluate the efficiency of the overall 60 mtDNA loci for being a supplementary tool for individual identification and matrilineal parentage testing in Chinese Xinjiang Kazak group. Among these loci, single nucleotide transition was the most common polymorphism (87.93%), followed by single nucleotide transversion (5.17%). Single nucleotide transition and transversion were observed simultaneously at nt9824 locus, while there were three loci (nt3348, nt8697 and nt8793) that had no polymorphisms. There were 25 haplogroups and 79 haplotypes in the studied Kazak groups. Haplogroup D4 was the most common haplogroup (21.28%) in Chinese Xinjiang Kazak group. Among the total 79 haplotypes, 53 of them were observed for only once, 14 for twice, and 12 for three times or more. The haplotype diversity was 0.978 ± 0.005, and the nucleotide diversity was 0.17449. $(CA)_n$ and 9-bp deletion polymorphisms could improve DP of the mtDNA haplotypes. Finally, the genetic background of Chinese Xinjiang Kazak group and its genetic relationships with other referenced groups were also exploited through phylogenetic analysis. It was indicated that Xinjiang Kazak ethnic group had closer genetic relationships with Xinjiang Uygur, Xinjiang Uzbek and Xinjiang Han populations. However, in order to further reveal the genetic background of Chinese Kazak group, more referenced populations and genetic markers would be collected and studied in our future study.

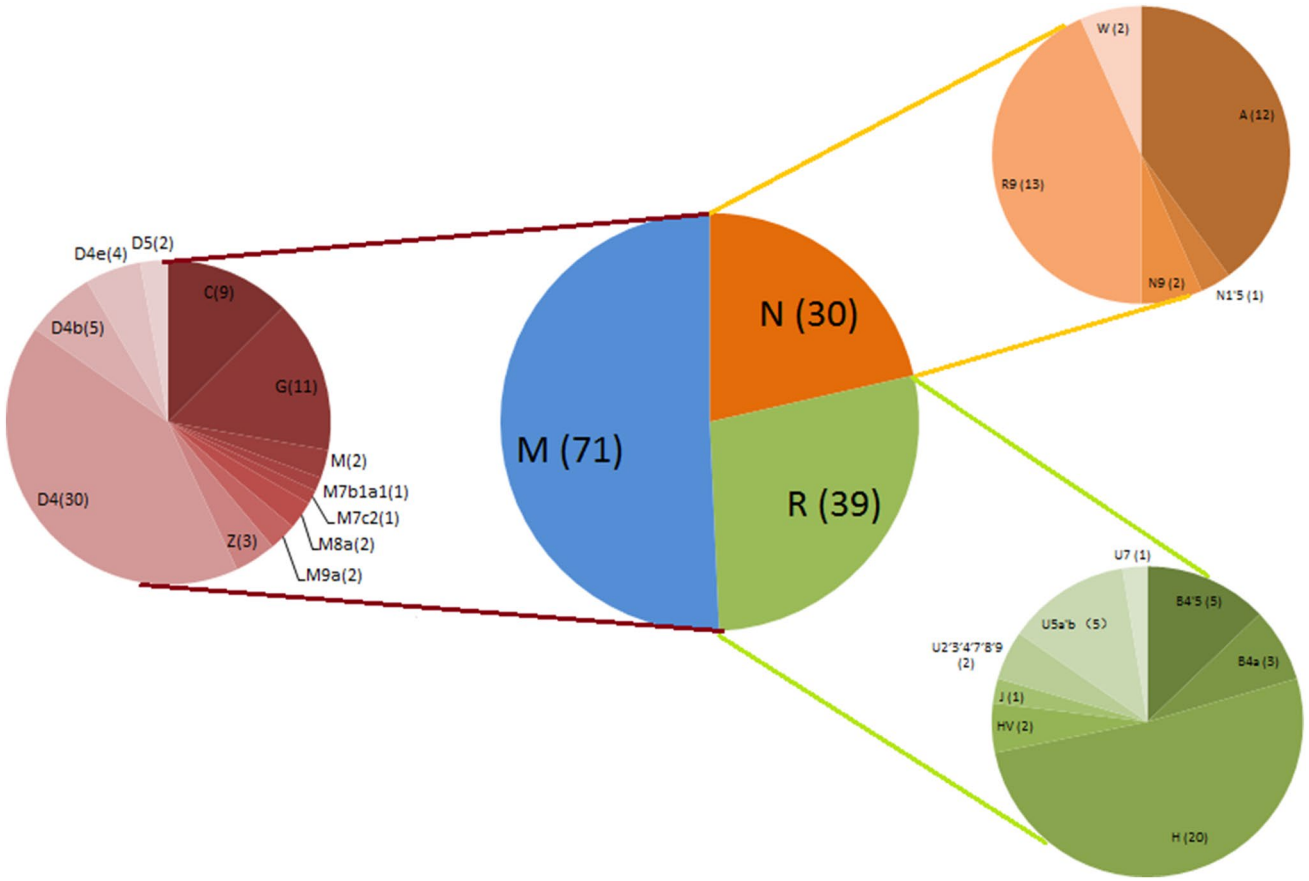

Fig. 2 The pie chart showing the mtDNA haplogroup and haplotype frequencies based on the 60 mtDNA loci in Chinese Xinjiang Kazak group

Table 3 Haplotypes observed times and the corresponding quantities of the 60 mtDNA loci in Chinese Xinjiang Kazak ethnic group

Haplotypes observed times	Haplotypes quantities
1	53
2	14
3	5
4	3
5	1
6	1
7	1
15	1

Table 4 Forensic statistical parameters of 58 mtDNA SNP loci excluding $(CA)_n$ and 9 bp deletion polymorphisms in Chinese Xinjiang Kazak ethnic group

Forensic statistical parameters	Values
Number of haplotypes	71
Number of polymorphic loci	55
Haplotype diversity	0.978 ± 0.005
Nucleotide diversity	0.17449
RMP	0.0291
DP	0.9709

Table 5 *Fst* and *p* values between Kazak and other previously reported groups based on 60 mtDNA loci (the data below the diagonal were *Fst* values and above the diagonal were *p* values)

Populations	Xinjiang_ Kazak	Italian	Xinjiang_ Xibe	Guangdong_ Han	Xinjiang_ Han	Yunnan_ Han	Estonian	US_African Americans	US_ Caucasians	Southern China_ Han	Altaian_ Kazak	Xinjiang_ Uygur	Xinjiang_ Uzbek
Xinjiang_ Kazak	*	0.00000	0.01802	0.07207	0.79279	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.21622	0.09009
Italian	**0.20159**	*	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Xinjiang_ Xibe	**0.01388**	**0.15331**	*	0.91892	0.67568	0.01802	0.00000	0.00000	0.00000	0.07207	0.20721	0.52252	0.09009
Guangdong_ Han	0.01692	**0.25350**	−0.01584	*	0.45946	0.19820	0.00000	0.00000	0.00000	0.31530	0.31532	0.44144	0.06306
Xinjiang_ Han	−0.00828	**0.19978**	−0.00435	−0.00560	*	0.02703	0.00000	0.00000	0.00000	0.01802	0.18018	0.66667	0.27027
Yunnan_ Han	**0.09642**	**0.22158**	**0.02715**	0.00955	**0.04806**	*	0.00000	0.00000	0.00000	0.74775	0.00000	0.00901	0.00000
Estonian	**0.18089**	**0.05950**	**0.12411**	**0.15518**	**0.15560**	**0.16072**	*	0.00000	0.54054	0.00000	0.00000	0.00000	0.00000
US_African Americans	**0.16607**	**0.14887**	**0.09087**	**0.08142**	**0.12067**	**0.07974**	**0.05147**	*	0.00000	0.00000	0.00000	0.00000	0.00000
US_ Caucasians	**0.14945**	**0.07514**	**0.09197**	**0.10993**	**0.11627**	**0.11048**	−0.00204	**0.04086**	*	0.00000	0.00000	0.00000	0.00000
SouthernChina_ Han	**0.08777**	**0.17057**	0.02081	0.00195	**0.04597**	−0.01097	**0.12277**	**0.05208**	**0.08747**	*	0.00901	0.05405	0.00000
Altaian_ Kazak	**0.02864**	**0.11168**	0.00364	0.00217	0.00819	**0.04422**	**0.06768**	**0.05516**	**0.04443**	**0.02961**	*	0.85586	0.15315
Xinjiang_ Uygur	0.00935	**0.14296**	−0.00459	−0.00201	−0.00830	**0.04104**	**0.08986**	**0.07821**	**0.05616**	0.03250	−0.00928	*	0.55856
Xinjiang_ Uzbek	0.00808	**0.18087**	0.01585	0.02921	0.00629	**0.10164**	**0.12124**	**0.13294**	**0.08723**	**0.08775**	0.00807	−0.00516	*

Data with statistical significances were labelled in bold

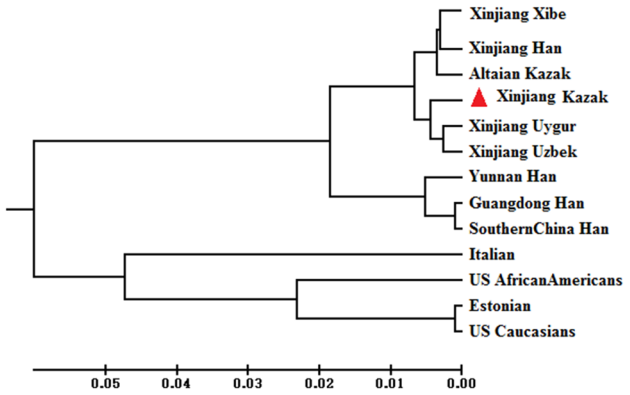

Fig. 3 A phylogenetic tree reconstructed by MEGA software version 4.0 based on 58 mtDNA loci excluding $(CA)_n$ and 9-bp deletion polyporphisms revealing the genetic relationships between Chinese Xinjiang Kazak group labeled in red and other previously reported groups

Acknowledgements This project was supported by the National Natural Science Foundation of China (NSFC, No. 81772031), GDUPS (2017). The authors sincerely thank all the volunteers for providing blood samples and Southern Medical University for providing the research platform.

Compliance with ethical standards

Conflict of interest The authors have declared no conflicts of interest.

Ethical approval Blood samples were obtained respectively according to standard procedures. The study was conducted in accordance with the human and ethical research principles of Southern Medical University and Xi'an Jiaotong University, China.

Informed consent All volunteers gave their written informed consents before inclusion.

Research involving human and animals participants This study involved the 141 healthy volunteers of Kazak ethnic group in Ili, Xinjiang Uygur Autonomous region.

References

1. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F (1981) Sequence and organization of the human mitochondrial genome. Nature 290(5806):457–465
2. Ginther C, Issel-Tarver L, King MC (1992) Identifying individuals by sequencing mitochondrial DNA from teeth. Nat Genet 2(2):135–138. https://doi.org/10.1038/ng1092-135
3. Holland MM, Parsons TJ (1999) Mitochondrial DNA sequence analysis—validation and use for forensic casework. Forensic Sci Rev 11(1):21–50
4. Wittig H, Augustin C, Baasner A, Bulnheim U, Dimo-Simonin N, Edelmann J, Hering S, Jung S, Lutz S, Michael M, Parson W, Poetsch M, Schneider PM, Weichhold G, Krause D (2000) Mitochondrial DNA in the Central European population. Human identification with the help of the forensic mt-DNA D-loop-base database. Forensic Sci Int 113(1–3):113–118
5. Holland MM, Cave CA, Holland CA, Bille TW (2003) Development of a quality, high throughput DNA analysis procedure for skeletal samples to assist with the identification of victims from the World Trade Center attacks. Croatian Med J 44(3):264–272
6. Macaulay V, Hill C, Achilli A, Rengo C, Clarke D, Meehan W, Blackburn J, Semino O, Scozzari R, Cruciani F, Taha A, Shaari NK, Raja JM, Ismail P, Zainuddin Z, Goodwin W, Bulbeck D, Bandelt HJ, Oppenheimer S, Torroni A, Richards M (2005) Single, rapid coastal settlement of Asia revealed by analysis of complete mitochondrial genomes. Science 308(5724):1034–1036. https://doi.org/10.1126/science.1109792
7. Gilbert MT, Djurhuus D, Melchior L, Lynnerup N, Worobey M, Wilson AS, Andreasen C, Dissing J (2007) mtDNA from hair and nail clarifies the genetic relationship of the 15th century Qilakitsoq Inuit mummies. Am J Phys Anthropol 133(2):847–853. https://doi.org/10.1002/ajpa.20602
8. Boattini A, Castri L, Sarno S, Useli A, Cioffi M, Sazzini M, Garagnani P, De Fanti S, Pettener D, Luiselli D (2013) mtDNA variation in East Africa unravels the history of Afro-Asiatic groups. Am J Phys Anthropol 150(3):375–385. https://doi.org/10.1002/ajpa.22212
9. Kohnemann S, Pfeiffer H (2011) Application of mtDNA SNP analysis in forensic casework. Forensic Sci Int Genet 5(3):216–221. https://doi.org/10.1016/j.fsigen.2010.01.015
10. Kohnemann S, Pennekamp P, Schmidt PF, Pfeiffer H (2010) qPCR and mtDNA SNP analysis of experimentally degraded hair samples and its application in forensic casework. Int J Legal Med 124(4):337–342. https://doi.org/10.1007/s00414-010-0466-0
11. Zerjal T, Wells RS, Yuldasheva N, Ruzibakiev R, Tyler-Smith C (2002) A genetic landscape reshaped by recent events: Y-chromosomal insights into Central Asia. Am J Hum Genet 71(3):466–482. https://doi.org/10.1086/342096
12. Yao YG, Kong QP, Wang CY, Zhu CL, Zhang YP (2004) Different matrilineal contributions to genetic structure of ethnic groups in the silk road region in china. Mol Biol Evol 21(12):2265–2280. https://doi.org/10.1093/molbev/msh238msh238
13. Gokcumen O, Dulik MC, Pai AA, Zhadanov SI, Rubinstein S, Osipova LP, Andreenkov OV, Tabikhanova LE, Gubina MA, Labuda D, Schurr TG (2008) Genetic variation in the enigmatic Altaian Kazakhs of south-central russia: insights into turkic population history. Am J Phys Anthropol 136(3):278–293. https://doi.org/10.1002/ajpa.20802
14. Walsh PS, Metzger DA, Higuchi R (2013) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 54(3):134–139
15. Andrews R, Kubacka I, Chinnery P, Lightowlers R, Turnbull D, Howell N (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23:147
16. van Oven M, Kayser M (2009) Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat 30:E386–E394
17. Nei M, Tajima F (1981) DNA polymorphism detectable by restriction endonucleases. Genetics 97(1):145–163
18. Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76(10):5269–5273
19. Shen CM, Hu L, Yang CH, Yin CY, Li ZD, Meng HT, Guo YX, Mei T, Chen F, Zhu BF (2017) Genetic polymorphisms of 54 mitochondrial DNA SNP loci in Chinese Xibe ethnic minority group. Sci Rep 7:44407. https://doi.org/10.1038/srep44407
20. Kivisild T, Tolk HV, Parik J, Wang YM, Papiha SS, Bandelt HJ, Villems R (2002) The emerging limbs and twigs of the East Asian mtDNA tree. Mol Biol Evol 19(10):1737–1751
21. Yao YG, Kong QP, Bandelt HJ, Kivisild T, Zhang YP (2002) Phylogeographic differentiation of mitochondrial DNA in Han Chinese. Am J Hum Genet 70(3):635–651. https://doi.org/10.1086/338999
22. King JL, Larue BL, Novroski NM, Stoljarova M, Seo SB, Zeng XP, Warshauer DH, Davis CP, Parson W, Sajantila A, Budowle B (2014)

High-quality and high-throughput massively parallel sequencing of the human mitochondrial genome using the Illumina MiSEq. Forensic Sci Int 12:128–135. https://doi.org/10.1016/j.fsigen.2014.06.001
23. Bodner M, Iuvaro A, Strobl C, Nagl S, Huber G, Pelotti S, Pettener D, Luiselli D, Parson W (2015) Helena, the hidden beauty: resolving the most common West Eurasian mtDNA control region haplotype by massively parallel sequencing an Italian population sample. Forensic Sci Int 15:21–26. https://doi.org/10.1016/j.fsigen.2014.09.012
24. Stoljarova M, King JL, Takahashi M, Aaspollu A, Budowle B (2016) Whole mitochondrial genome genetic diversity in an Estonian population sample. Int J Legal Med 130(1):67–71. https://doi.org/10.1007/s00414-015-1249-4
25. Nie Y, Zhang C, Jiao H, Zhao Z, Zhou H (2014) Development of a multiplex PCR system of 59 mitochondrial SNPs and genetic analysis in Chinese population. Electrophoresis 35(12–13):1903–1911. https://doi.org/10.1002/elps.201400047
26. Lum JK, Cann RL (1998) mtDNA and language support a common origin of Micronesians and Polynesians in Island Southeast Asia. Am J Phys Anthropol 105 (2):109–119
27. Merriwether DA, Friedlaender JS, Mediavilla J, Mgone C, Gentz F, Ferrell RE (1999) Mitochondrial DNA variation is an indicator of austronesian influence in Island Melanesia. Am J Phys Anthropol 110 (3):243–270
28. Alves-Silva J, Santos MS, Pena SD, Prado VF (1999) Multiple geographic sources of region V 9-bp deletion haplotypes in Brazilians. Hum Biol 71(2):245–259
29. Barthold VV (1962) Four studies on the history of Central Asia, vol 3. Brill Publishers, Leiden
30. Millward JA (2007) Eurasian crossroads: a history of Xinjiang. Columbia University Press, New York
31. Perdue PC (2005) China marches west: the qing conquest of Central Eurasia. Harvard University Press, Cambridge
32. Lattimore O (1950) Pivot of Asia; Sinkiang and the inner Asian frontiers of China and Russia. Little, Brown and Company, Boston
33. Comas (1999) Trading genes along the silk road: mtDNA sequences and the origin of central Asian populations (vol 63, pg 1824, 1998). Am J Hum Genet 65(1):277–277
34. Conquest R (1987) The harvest of sorrow: soviet collectivization and the terror-famine. Oxford University Press, Oxford
35. The Annals of the American Academy of Political and Social Science, vol 277 (2010). vol 276–278. American Academy of Political and Social Science
36. The Annals of the American Academy of Political and Social Science, Volume 277 (2012) A group of Kazakhs, originally numbering over 20000 people when expelled from Sinkiang by Sheng Shih-ts'ai in 1936, was reduced, after repeated massacres by their Chinese coreligionists under Ma Pu-fang, to a scattered 135 people. American Academy of Political and Social Science

Affiliations

Tong Xie[1] · Li Hu[2] · Yu-Xin Guo[3,4,5] · Yu-Chun Li[6,7,8,9] · Feng Chen[2] · Bo-Feng Zhu[1,3,4,5]

Tong Xie
xietong123@i.smu.edu.cn

Li Hu
huijieh@nimu.edu.cn

Yu-Xin Guo
guoyuxin_004@stu.xjtu.edu.cn

Yu-Chun Li
liyuchun@mail.kiz.ac.cn

[1] School of Forensic Medicine, Southern Medical University, Guangzhou 510515, People's Republic of China

[2] Department of Forensic Medicine, Nanjing Medical University, Nanjing 210029, Jiangsu, China

[3] Key Laboratory of Shaanxi Province for Craniofacial Precision Medicine Research, College of Stomatology, Xi'an Jiaotong University, Xi'an, Shaanxi, People's Republic of China

[4] Clinical Research Center of Shaanxi Province for Dental and Maxillofacial Diseases, College of Stomatology, Xi'an Jiaotong University, Xi'an, Shaanxi, People's Republic of China

[5] College of Medicine & Forensics, Xi'an Jiaotong University Health Science Center, Xi'an 710061, Shaanxi, People's Republic of China

[6] State Key Laboratory of Genetic Resources and Evolution, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming 650223, People's Republic of China

[7] Center for Excellence in Animal Evolution and Genetics, Chinese Academy of Sciences, Kunming 650223, China

[8] Kunming Key Laboratory of Healthy Aging Molecular Mechanism Study, Kunming 650223, China

[9] KIZ/CUHK Joint Laboratory of Bioresources and Molecular Research in Common Diseases, Kunming 650223, China