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Genetic polymorphism of 27 Y-STR loci in Kazakh populations from Eastern Kazakhstan

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ABSTRACT

Background: The establishment of a national haplotype database is important for forensic and genetic applications and requires studying genetic polymorphisms at Y-STR sites. However, the genetic structure of the Eastern Kazakhstan population is poorly characterised.

Aim: To investigate the genetic polymorphisms of 27 Y-STR loci in the Kazakh population from Eastern Kazakhstan and analyse the population genetic relationships of the Eastern Kazakhs with other populations.

Subjects and methods: The Yfiler Plus kit was utilised to genotype 246 healthy, unrelated males from Eastern Kazakhstan. Based on the raw data, haplotype and allele frequencies along with forensic parameters were calculated, and an MDS plot was constructed.

Results: A total of 207 haplotypes were detected, of which 186 were unique. The haplotype diversity and discrimination capacity were 0.997 and 0.841, respectively. Population comparisons showed that Eastern Kazakhs have close genetic relationships with Kazakhs from Xinjiang, China. At the same time, a difference was found between the studied population and the previous one in the same part of Kazakhstan.

Conclusions: The obtained haplotypes will help to expand the Kazakhstan Y-chromosome reference database and will be useful for future genetic research and forensic applications.

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Y-STR; Yfiler Plus; Haplotype; YHRD; Kazakh population

Background

The analysis of Y-chromosomal short tandem repeats (Y-STRs) is extensively utilised in forensics (Kayser 2017), genealogical and anthropological studies, as well as population genetics (Jobling and Tyler-Smith 2003; Purps et al. 2014; Xu et al. 2015). Y-STRs are crucial and stand out, in particular, when it comes to forensic investigations since they allow the detection of male individuals in mixed male and female stains (Roewer 2009). As a result, several commercial Y-STR kits became available and were utilised to expand public Y-STR reference databases (Oostdik et al. 2014; Gopinath et al. 2016). The National Database "Kazakhstan" of the Y-Chromosome STR Haplotype Reference Database contains 2294 haplotypes, of which only 723 are of Kazakhs that were characterised using the 27 Y-STR system; there are 382 samples of Kazakhs from Northern Kazakhstan (Ashirbekov et al. 2022) and 341 samples of the general Kazakh population (Zhabagin et al. 2019; YA004185), in which Kazakhs from Eastern Kazakhstan are represented by only 17 samples. Most of the haplotypes presented in YRHD were obtained using the 17 Y-STR system (Khussainova et al. 2021). Population studies of the Central (Balanovsky et al. 2015), Western (Zhabagin et al. 2021), Southern (Zhabagin et al. 2020), and Eastern (Tarlykov et al. 2013) regions were also limited to only 17 Y-STR markers. The population sample size of previously studied Eastern Kazakhs was only 67, the smallest among all other regions. Furthermore, a low degree of genetic diversity was observed for 17 Y-STR data from Eastern Kazakhs (Tarlykov et al. 2013).

Eastern Kazakhstan occupies a large area (401.8 thousand km^2) in the east of the country and is a border area with China and Russia. Along the border stretches the Altai mountain range, where the steppes on both sides are a historical corridor of bilateral latitudinal migrations and the mixing of Asians and Europeans (González-Ruiz et al. 2012). Recent studies of the ancient population of this region, the Scythians, demonstrate their high genetic diversity (Gnecchi-Ruscone et al. 2021). Several caravans on their way to the Dzungarian Gates went through the area. The Dzungarian Gates are a natural passageway between the Dzungarian Alatau and the Barlyk Range. The Great Silk Road also went through this area. With these connections, it is not surprising to expect a large genetic diversity in the population of this

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region. Most of the people in the area are Kazakhs, especially from the Naiman and Kerei tribes (Zhabagin et al. 2018).

Thermo Fisher's Yfiler Plus, which was used in the current study, allowed for the characterisation of 27 Y-STR loci, including nine rapidly mutating ones, increasing the discrimination power of the analysis (Ballantyne et al. 2010; Ballantyne et al. 2012).

This is especially of current interest to the Kazakh population, which consists of tribes and clans. Members of such patrilineal groups are closely related to each other. Consequently, it is important for forensic genetics to distinguish between them. Therefore, this study will contribute to population genetics research in East Kazakhstan and analyse the genetic links between the Eastern Kazakh population and other Kazakh groups.

Sample

To conduct research, 246 saliva samples were collected from healthy Kazakh male volunteers, with direct family history of being residents of the Eastern Kazakhstan region for at least three generations. In addition, they had not been connected in at least three generations. All participants in this study provided their written informed consent prior to sample collection. The study was approved by the Ethics Committee of the National Centre for Biotechnology (No. 2 of 1 August 2019) and the Ethics Committee of the Asfendiyarov Kazakh National Medical University for the M. Aitkhozhin Institute of Molecular Biology and Biochemistry (#6 of 29 October 2012). All experimental procedures were performed following the standards of the Declaration of Helsinki 1964.

Data collection

DNA extraction and Y-STR fragment analysis

Genomic DNA was isolated from the saliva samples using the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA). Amplification of 27 STR loci of the Y chromosome was performed using the Yfiler® Plus PCR Kit (Thermo Fisher Scientific, Waltham, MA, USA) on a SimpliAmp Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA). Amplicon fragmentary analysis was performed on an 8-capillary Applied Biosystems 3500 genetic analyser (Thermo Fisher Scientific, Waltham, MA, USA). Further, the results were analysed using the GeneMapper IDx v.1.5 software. (Thermo Fisher Scientific, Waltham, MA, USA) according to the reference allelic ladder.

Data management and statistical analysis

The haplotype data were submitted to the YHRD (http://www.yhrd.org) with the accession number YA006011. To contribute to the haplotype data, the laboratories passed the Quality Control Test of the YHRD (YC000343).

The direct counting approach was utilised to determine the allele and haplotype frequencies in the Eastern Kazakh population. The number of analysed samples (n) and the frequency of the i-*th* allele or haplotype were used to calculate the gene diversity (GD) and haplotype diversity (HD) using the formula (Nei and Tajima 1981): $HD = n^*(1-\sum p_i^2)/(n-1)$. The formula used to compute the haplotype match probability (HMP) is $HMP = \sum p_i^2$ where p_i is the haplotype frequency. Discrimination capacity (DC) was equal to the ratio between the number of distinct haplotypes and the total number of haplotypes. The STRAF 1.0.5 program (http://cmpg.unibe.ch/shiny/STRAF/) was used to calculate forensic metrics for each locus, including frequency for each locus, Typical Paternity Index (TPI), Power of Exclusion (PE), Gene Diversity (GD), and Polymorphism Information Content (PIC) (Gouy and Zieger 2017). Pair-wise genetic distances (Rst) and multidimensional scaling were computed using the "AMOVA and MDS" tools from the YHRD website (https://yhrd.org/). Multidimensional scaling (MDS) was visualised using the Statistica 13.5 software (https://docs.tibco.com/products/tibco-statistica-13-5-0). The population data for Eastern Kazakhstan was compared to the population data for Kazakhstan and its neighbouring countries that are available in the YHRD (Tarlykov et al. 2013; Shan et al. 2014; Gao et al. 2016; Wang et al. 2019; Zhabagin et al. 2019; Liu et al. 2020; Li et al. 2020; Adnan et al. 2021; Khussainova et al. 2021; Semikhodskii et al. 2021; Ashirbekov et al. 2022).

Results

Haplotype/allele frequencies and forensic parameters

Supplementary Table S1 shows the haplotype distribution of Kazakhs from Eastern Kazakhstan. 246 samples yielded 207 different haplotypes by 27 Y-STR, the frequencies of which are presented in Table S2. The number of unique haplotypes is 186 (90%), while the other 21 (10%) were observed at least twice. The most prevalent haplotype appeared in 10 individuals, followed by another that was detected in 5 individuals; 7 haplotypes were shared among 3 people; and 12 haplotypes appeared twice. Haplotype diversity (HD), discrimination capacity (DC), and haplotype match probability (HMP) were equal to 0.997, 0.841, and 0.007, respectively (Table 1). Previously, low values of haplotype diversity (HD = 0.629) and discrimination capacity (DC = 0.338) on 17 Y-STR were reported for Eastern Kazakhs (Tarlykov et al. 2013). Furthermore, Tables S3 and S4 contain information about the 27 Y-STR allele frequency and corresponding gene diversity (GD). Overall, at single-copy loci (Table S3), 153 alleles were detected with their frequencies varying from 0.041 to 0.821. DYS391 was the least polymorphic locus with only three allele combinations observed and GD equal to 0.31, while the most polymorphic among single-copy loci was DYS449, with 13 allele variants and GD = 0.83. On the other hand, 37 and 29 allele combinations were detected at two multi-copy loci DYF38FS1 and DYS385, respectively, with gene diversity of 0.92 and 0.84, correspondingly (Table S4). Abnormal alleles are presented in Table S5. Overall, three intermediate alleles, 15 samples with double alleles, and 5 samples with triallelic patterns were observed at loci DYS458, DYS19, and DYF387S1, respectively. Null alleles were present at loci DYS448 and DYS481.

Table 1. Comparison of genetic polymorphism of 27 Y-STR haplotypes in the Kazakh populations.

Population	Number of samples	Number of distinct haplotypes	Frequency of unique haplotypes	Discrimination capacity	Haplotype match probability	Haplotype diversity
General Kazakh (Zhabagin et al. 2019)	300	270	82%	90%	0.0042	0.9991
Northern Kazakh (Ashirbekov et al. 2022)	382	326	78%	85.34%	0.0044	0.9982
Eastern Kazakh	246	207	75.61%	84.15%	0.0069	0.9971

Population analysis of the Kazakh population

Based on the 27 Y-STR haplotype data, the Eastern Kazakh population haplotypes were compared to 16 neighbouring populations: "Afghanistan [Hazara]" (260 haplotypes), "Hohhot, China [Mongolian]" (240 haplotypes), "Hulun Buir, China [Mongolian]" (508 haplotypes), "Ordos, China [Mongolian]" (213 haplotypes), "Xinjiang, China [Mongolian]" (182 haplotypes), "Aksu, China [Uighur]" (150 haplotypes), "Karamay, China [Uighur]" (129 haplotypes), "Kashi, China [Uighur]" (77 haplotypes), "Korla, China [Uighur]" (141 haplotypes), "Urumqi, China [Uighur]" (49 haplotypes), "Kazakhstan [Kazakh]" (341 haplotypes), "North Kazakhstan [Kazakh]" (382 haplotypes), "Balochistan, Pakistan [Hazara]", "Russian Federation [Russian]" (895 haplotypes), "Ural, Russian Federation [Russian]" (91 haplotypes), "Russian Federation [Yakut]" (34 haplotypes) using the analysis of molecular variance (AMOVA) at YHRD database (https://yrhd.org/amova). Resulting pairwise genetic distances (Rst) along with corresponding p values ($p < 0.05/136 = 0.0004$ after Bonferroni correction) are presented in Table S6. The highest Rst was for people from the eastern part of Kazakhstan. The next highest was for Yakuts from the Russian Federation (Rst = 0.4487). On the other hand, the Mongolian populations from Hohhot, China (Rst = 0.0634) and Ordos, China (Rst = 0.0657), and the Uighur population from Urumqi, China (Rst = 0.0746), showed the lowest genetic distances with Eastern Kazakhs. At the same time, the general Kazakhs sample (Rst = 0.0841) is also close to the Eastern Kazakhs, unlike the Northern Kazakh population (Rst = 0.165). The multidimensional scaling (MDS) plot is given in Figure S1. Overall, it can be seen that there are four clusters of populations. The first cluster is composed of Kazakhs from Altai, Xinjiang and Gansu, China; the second cluster is composed of Kazakhs from Xinjiang, China, along with Eastern and Southern Kazakhs; the third cluster and the fourth cluster were formed by the general Kazakh population of Kazakhstan and Northern Kazakhs, respectively. The results showed that Eastern Kazakhs from this study are closely related to Kazakhs from neighbouring Xinjiang, China, which can also be observed from the MDS plot. At the same time, Eastern Kazakhs are genetically distinct from Yakuts. Furthermore, the Kazakh population comparison was performed on 17 Y-STR haplotype data, and 7 Kazakh populations were chosen as reference populations: "Altai, Xinjiang, China [Kazakh]" (428 haplotypes), "Gansu, China [Kazakh]" (93 haplotypes), "Xinjiang, China [Kazakh]" (114 haplotypes), "East Kazakhstan, Kazakhstan [Kazakh]" (67 haplotypes), "Kazakhstan [Kazakh]" (1476 haplotypes), "North Kazakhstan, Kazakhstan [Kazakh]" (337 haplotypes), "South Kazakhstan [Kazakh]" (99 haplotypes). The pairwise genetic distances (Rst) and corresponding p values ($p < 0.05/18 = 0.001786$ after Bonferroni correction) are listed in Table S7. The maximal distance was detected between Eastern Kazakhs and Kazakhs from Altai, Xinjiang, China (Rst = 0.2726) and Gansu, China (Rst = 0.2724), while there was a minimal distance between Eastern Kazakhs and Kazakhs from Xinjiang, China (Rst = 0.0079).

It should be noted that the previously studied sample of Eastern Kazakhs (Tarlykov et al. 2013) was not close (Rst = 0.1713) to the Eastern Kazakhs of this study. This indicates the existence in East Kazakhstan of population groups of different paternal origins, which corresponds to the settlement of at least two large clans in East Kazakhstan: Naiman and Kerey. Much closer to the Eastern Kazakhs of this study are the Kazakhs of the North (Rst = 0.1163) and the South (Rst = 0.0612).

Conclusion

This is the first study characterising the genetic polymorphism of the Eastern Kazakh population using a comprehensive 27 Y-STR loci system. This allowed for the addition of 17 Y-STR haplotypes from Eastern Kazakhs to the Kazakhstan National Y-chromosome National Haplotype Database. As a result, the generated population haplotype data have a higher genetic diversity and better discrimination capacity in comparison with the previous study, allowing the usage of the data for forensic purposes. Furthermore, population comparisons indicated the genetic relatedness of Eastern Kazakhs and Chinese Kazakhs from Xinjiang, China. This study made it possible to characterise a new and different sample of Kazakhs in East Kazakhstan from the previous sample (Tarlykov et al. 2013), which was shown using Rst and MDS. However, more research is needed to elucidate the complex population structure of Eastern Kazakhs.

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Author contributions

Conceived and designed the experiments: MZ; Performed the experiments: YA, AA; Analysed the data: AN, AZ; Contributed reagents/materials/analysis tools: YA, AA, AZ, ZS; AN, YA contributed to writing the paper; MZ edited the manuscript. Read and approved the final version of the paper: all co-authors.

Ethical statement

The study was approved by the Ethics Committee of the National Centre for Biotechnology (No. 2 of 1 August 2019) and the Ethics Committee of the Asfendiyarov Kazakh National Medical University for the M. Aitkhozhin Institute of Molecular Biology and Biochemistry (№6 of 29 October 2012).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary material.

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