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To cite this article: Yequan Wang, Ao Gao, Xiudi Hou, Qi Liu, Dan Wang & Zhen Dang (2023) Forensic characterisation and polymorphism analysis of 16 X-chromosomal STRs in the Jining Han population in Eastern China, *Annals of Human Biology*, 50:1, 351-355, DOI: [10.1080/03014460.2023.2213478](https://doi.org/10.1080/03014460.2023.2213478)

To link to this article: <https://doi.org/10.1080/03014460.2023.2213478>



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Published online: 05 Sep 2023.



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RESEARCH PAPER

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Forensic characterisation and polymorphism analysis of 16 X-chromosomal STRs in the Jining Han population in Eastern China

Yequan Wang^{a,b,*}, Ao Gao^{a*}, Xiudi Hou^{b*}, Qi Liu^{a,b}, Dan Wang^{a,b} and Zhen Dang^{a#}

^aInstitute of Forensic Medicine and Laboratory Medicine, Jining Medical University, Jining, PR China; ^bForensic Science Center of Jining Medical University, Jining, PR China

ABSTRACT

Background: X-chromosomal short tandem repeats (X-STRs) are a useful supplementary approach to analysing autosomal markers in forensics and kinship studies; such markers are not well-characterised in many populations.

Aim: To investigate population genetic polymorphism and forensic characterisation of 16 X-STRs in the Jining Han population, and analyse genetic relationships with other Chinese populations.

Subjects and methods: Allele frequencies for 16 X-STR loci were obtained from a sample set of 527 unrelated individuals from the Jining Han population. Population genetic analyses of Jining Han and another 10 reference populations were conducted using phylogenetic tree, principal component analysis and multidimensional scaling.

Results: We detected 149 alleles, with frequencies ranging from 0.0013 to 0.8242. The combined powers of discrimination in males and females were 0.999999997194774 and 0.99999999999995, respectively. The combined mean exclusion change (MEC)_{Krüger}, MEC_{Kishida}, MEC_{Desmarais} and MEC_{Desmarais} values were 0.999974632649096, 0.99999976997582, 0.99999977013201, and 0.99993755768423, respectively. We detected relatively high genetic homogeneity in populations with similar ethnic or geographic origins, and a close relationship between the Jining Han and Beijing Han populations.

Conclusions: The present findings indicate that the 16 X-STR loci examined are highly polymorphic in the Han population of Jining, providing useful information for forensic science and population genetics studies.

ARTICLE HISTORY

Received 7 October 2022

Revised 31 January 2023

Accepted 9 May 2023

KEYWORDS

Forensic genetics; X-STR; Han population; polymorphic marker

1. Introduction

Short tandem repeats (STR) are the main genetic markers used in forensic DNA typing. Autosomal and Y-chromosome STR genetic markers are widely used in individual identification and kinship identification, and many commercial kits have been developed. Relatively little research has focused on X chromosome STR (X-STR) genetic markers in forensic science. However, X-STR markers are of interest to forensic scientists owing to their unique pattern of inheritance. The genetic characteristics of X chromosome genetic markers differ from those of the autosomal and Y chromosomes. The alleles of the two X chromosomes of the mother can be randomly passed to their children; fathers can only pass X chromosome genes to daughters, and the X chromosomes of daughters must come from grandmothers (Diegoli 2015). Owing to the specificity of their inheritance mode, X-STR loci are useful for personal identification, especially the identification of complex genetic relationships involving X chromosome inheritance.

Previous studies have shown that the Goldeneye 17X Kit (Peoplespot, Beijing, China) can be used for applications in forensic genetics, particularly for investigating paternity and genealogy (Sun et al. 2013). However, to date, the STR loci included in the Goldeneye 17X Kit have not been evaluated in the Jining Han population. In this study, we determined the forensic parameters and allele frequencies of the 16 X-STR loci included in the Goldeneye 17X Kit (DX6795, DXS9902, DXS8378, HPRTB, GATA165B12, DXS7132, DXS7424, DXS6807, DXS6803, GATA172D05, DXS6800, DXS10134, GATA31E08, DXS10159, DXS6789, and DXS6810) in the Jining Han population. This investigation of the applicability of the Goldeneye 17X Kit is expected to provide a basis for expanding population genetics data and analysing genetic relationships between the Jining Han and other Chinese populations (Sun et al. 2019; Yang et al. 2019; Chen et al. 2020; Tao et al. 2020; Zhang et al. 2021; Liu, Yao et al. 2022; Liu, Yuan et al. 2022; Qing et al. 2022).

CONTACT Yequan Wang ✉ wangyequan1103@mail.jnmc.edu.cn Institute of Forensic Medicine and Laboratory Medicine, Jining Medical University, Forensic Science Center of Jining Medical University, Jining, Shandong, PR China; Zhen Dang ✉ dangzhen0721@163.com Institute of forensic medicine and laboratory medicine, Jining Medical University, Jining, Shandong, PR China

*These authors contributed equally to this work.

#Corresponding author.

Supplemental data for this article can be accessed online at <https://doi.org/10.1080/03014460.2023.2213478>

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2. Subjects and methods

2.1. DNA extraction and PCR

Peripheral blood samples of 527 unrelated, healthy individuals randomly chosen from the Jining Han population were collected with informed consent. Samples were obtained and used after obtaining the approval of the Medical Ethics Committee of Jining Medical University (Approval No. JNMC-2021-YX-008). FTA cards were used to save as samples. DNA was extracted from blood samples according to the Chelex-100 protocol, as described previously (Walsh et al. 2013). Multiplex PCR was performed using the Goldeneye 17X Kit (Peoplespot, China) and the ProFlex™ PCR System (Thermo Fisher Scientific, Waltham, MA, USA).

2.2. Genotyping and quality control

Amplification products were separated by capillary electrophoresis on an Applied Biosystems® 3500 Genetic Analyser (Thermo Fisher Scientific, Waltham, MA, USA). Allele designations were determined by the comparison of sample PCR fragments with allelic ladders provided with the Goldeneye 17X kit. All steps were conducted using laboratory internal control standards and kit controls. Raw data were analysed using GeneMapper ID-X v.1.6 (Thermo Fisher Scientific, Waltham, MA, USA) for genotype assignment. All experiments were conducted at the Forensic Science Centre of Jining Medical University, an accredited laboratory (ISO 17025), in accordance with quality control standards (Dang et al. 2020).

2.3. Data analysis

A modified Powerstats spreadsheet was used to calculate forensic parameters, including allele frequencies for the 16 X-STR loci. Exact tests of Hardy–Weinberg equilibrium (HWE) for each locus were performed using Genepop version 4.2 (<http://genepop.curtin.edu.au/>). The polymorphism information content (PIC), gene diversity (GD), power of discrimination in males (PDM), power of discrimination in females (PDF), and mean exclusion chance (MEC) in trios and duos ($MEC_{Krüger}$, $MEC_{Kishida}$, $MEC_{Desmarais}$, and $MEC_{Desmarais\ Duo}$) were estimated using StatsX (Statistics for X-STR) version 2.0 (Lang et al. 2019). A principal component analysis (PCA) was implemented in SPSS version 21.0. Phylip 3.695 was used to analyse the genetic distances among the Jining Han and other populations. F_{ST} and p -values were estimated for 16 X-STR loci to evaluate differentiation between Jining Han populations and other groups using Arlequin 3.5. A phylogenetic tree was reconstructed using Mega 7.0 with the neighbor-joining method. Furthermore, a two multidimensional scaling (MDS) plot was built using SPSS version 21.0. Values of $p < 0.05$ were considered significant.

3. Results and discussion

We used the Goldeneye 17X Kit to genotype 527 volunteers (292 males and 235 females) residing in Jining city, Shandong

province, based on 16 X-STRs. Allele frequencies for males and females are presented in Table S1. We detected 137 alleles with frequencies ranging from 0.0021 to 0.8085 in females, and 128 alleles with frequencies ranging from 0.0034 to 0.8493 in males. Among these, 21 alleles were found exclusively in females and 12 were found exclusively in males (Table S1). As determined by chi-squared tests, there were no significant differences in allele frequencies between males and females for the 16 loci (Table S2). Therefore, samples were pooled and the allele frequency distribution and forensic parameters for the 16 X-STR loci in the Jining Han population are shown in Table S3 and Figure 1. After Bonferroni correction ($p = 0.05/16 = 0.003$), no loci showed deviations from Hardy–Weinberg equilibrium ($p > 0.003$). We detected 149 unique alleles, with allele frequencies ranging from 0.0013 to 0.8242. DXS10134 was the most polymorphic (PIC = 0.8440) with 20 unique alleles, and DXS6800 was the least polymorphic (PIC = 0.2888) with six alleles. Excluding DXS6800, the PIC values for the other 15 loci exceeded 0.5, indicating that the loci had high genetic diversity and can be used for forensic paternity identification. The PDF and PDM values were 0.5016–0.9649 and 0.3073–0.8592, respectively. The combined PDM and CPDF were 0.99999997194774 and 0.99999999999995, respectively. The combined high values of $MEC_{Krüger}$, $MEC_{Kishida}$, $MEC_{Desmarais}$, and $MEC_{Desmarais\ Duo}$ were 0.999974632649096, 0.999999976997582, 0.999999977013201, and 0.999993755768423, respectively.

We did not detect significant linkage disequilibrium (LD) between any pair of X-STR loci after Bonferroni correction ($p = 0.05/120$) (Table S4, Table S5). These results indicated that all X-STR loci in the Goldeneye 17X Kit are independent, which is beneficial for maximising the combined polymorphism and diversity indexes, power of individual discrimination, and MEC.

We further explored the genetic differences between the Jining Han population and Han and minority groups in other regions based on previously reported allele frequency data for the 16 loci, including data for the Beijing Han, Hunan Han, Yunnan Bai, Yunnan Han, Zhejiang She, Yunnan Hani, Yunnan Miao, Mongolian, Kazakh, and Tibetan populations (Sun et al. 2019; Yang et al. 2019; Chen et al. 2020; Tao et al. 2020; Zhang et al. 2021; Liu, Yao et al. 2022; Liu, Yuan et al. 2022; Qing et al. 2022).

AMOVA results for 16 STR loci in the Jining Han population and other ethnic groups are summarised in Table S6. Locus-by-locus F_{ST} and corresponding p -values revealed significant differentiation between the Jining Han and Yunnan Miao (fourteen loci), Yunnan Hani (seven loci), Zhejiang She (four loci), Hunan Han, Tibetan, and Kazakh (two loci each), and Yunnan Bai (one locus), after Bonferroni adjustment ($p < 0.05/16 = 0.003$).

A PCA (Figure 2) revealed the genetic structure of 11 populations based on normalised allele frequencies of 16 X-STR loci. The first principal component explained 91.761% of the total variance, while the second accounted for 4.155%. In the PCA plot, the Tibetan and Kazakh groups were in the upper quadrant. Jining Han was clustered in the lower right quadrant, close to Beijing Han, Hunan Han, and Yunnan Bai



Figure 1. Allele frequency distributions for 16 X-STR loci in the Jining Han population (N=527).

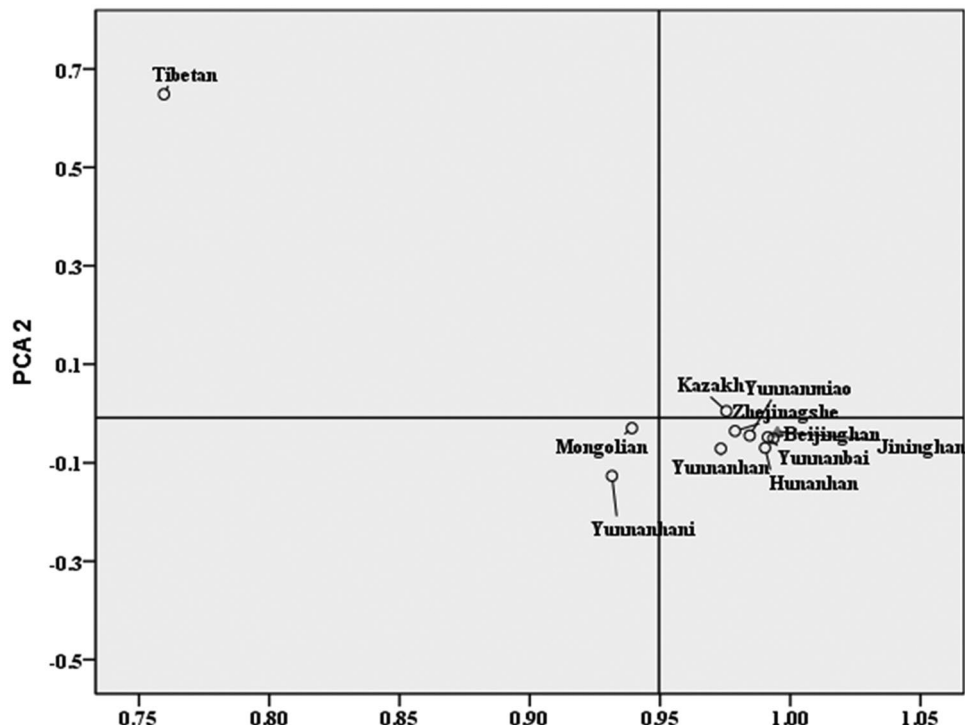


Figure 2. Principal component analyses (PCA) based on 16 overlapping X-STR loci between the Jining Han (Red) and 10 reference Chinese populations (Black).

populations, indicating close genetic relationships among these populations. A MDS plot (Figure 3) showed that the Tibetan, Kazakh, and Yunnan Miao groups were isolated, while the Jining Han population clustered together with other populations, except the Yunnan Han. These results are

consistent with those of the PCA and demonstrate that Chinese Han populations from different administrative divisions are closely related.

The genetic distances between the Jining Han and 10 other ethnic groups are presented in Table S7. In addition,

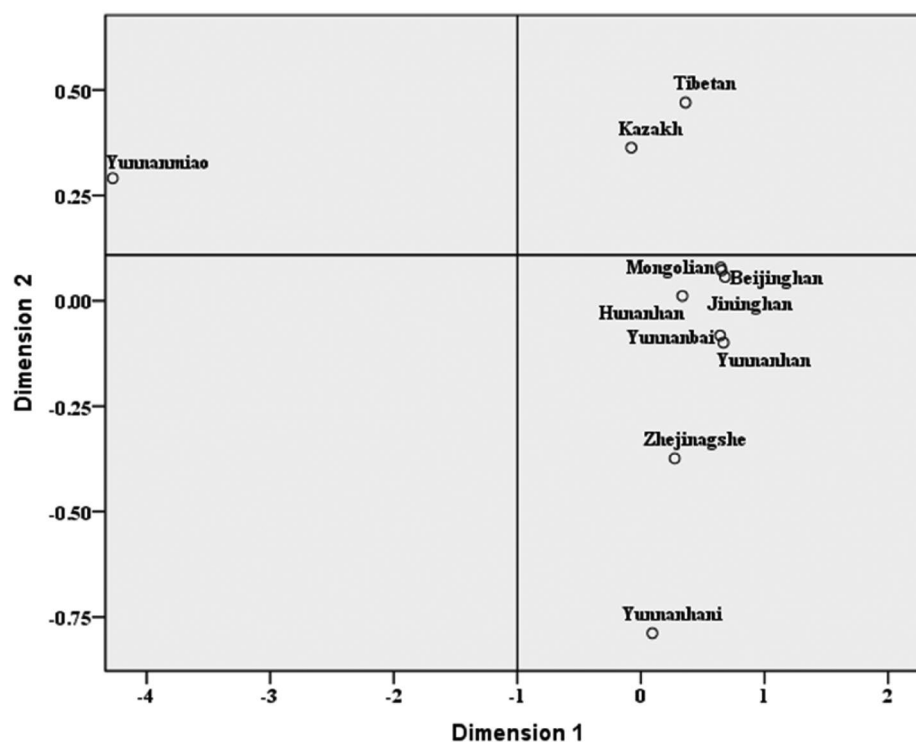


Figure 3. Multidimensional scaling (MDS) plot based on pairwise F_{ST} values among Jining Han and other populations.

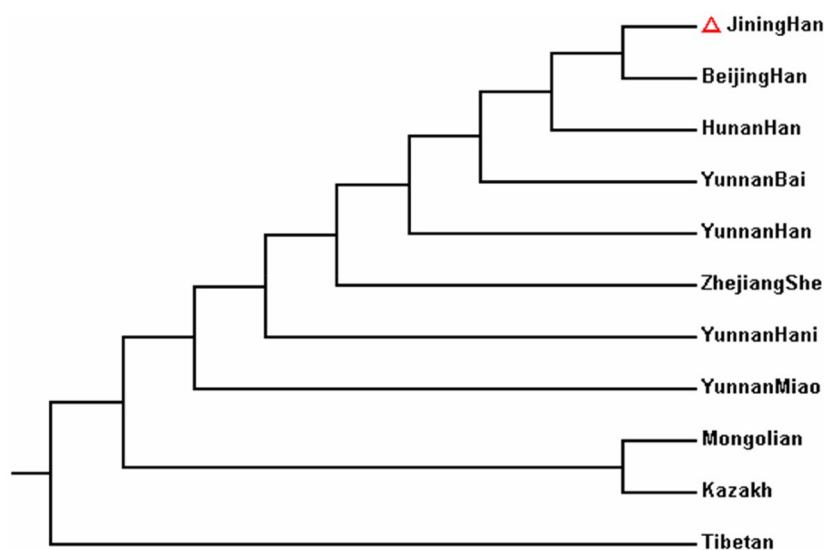


Figure 4. Phylogenetic analysis of the relationships among the Jining Han population and 10 reference populations. The phylogenetic tree was constructed using the neighbor-joining method based on 16 overlapping STR loci using Mega 7.0.

we constructed a phylogenetic tree of these populations using the neighbor-joining method (Figure 4). In the phylogenetic tree based on genetic distances, all 11 ethnic groups were divided into two main clusters corresponding to ethnic origins. The Jining Han population exhibited the greatest distance from the Tibetan population ($R_{ST} = 5.9437$). We detected the shortest distances between the Jining Han and the Beijing Han population ($R_{ST} = 0.0017$). The relationships based on genetic distances were similar to those based on the PCA and phylogenetic analysis.

In conclusion, the 16 X-STR loci had abundant polymorphisms in the Han population in Jining City and can be used

in forensic and genetic research. Furthermore, we found that populations with the same ethnic origin or a close geographical origin exhibit relatively high genetic relatedness. This study expands the available data for X chromosome genetic polymorphisms in the Chinese Han population. Finally, interested researchers can contact the corresponding author to obtain data.

Disclosure statement

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

Funding

This work was supported by the Student's Platform for Innovation and Entrepreneurship Training Program [S202010443001, cx2020001], The Project of Teaching Reform Research for Shandong Province Undergraduate University [M2020069], Supporting Fund for Teachers' Research of Jining Medical University [JYFC2018FY003].

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