POPULATION DATA



Forensic characteristics and phylogenetic analyses of the Chinese Yi population via 19 X-chromosomal STR loci

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Abstract The demographic characteristics and genetic polymorphism data of 56 Chinese nationalities or 31 administrative divisions in Chinese mainland have repeatedly been the genetic research hotspots. While most genetic studies focused on some particular Chinese populations based on autosomal or Y-chromosomal genetic markers, the forensic characteristics and phylogenetic analyses of the seventh largest Chinese population (Yi ethnicity) on the X-chromosomal genetic markers are scarce. Here, allele frequencies and forensic statistical parameters for 19 X-chromosomal short tandem repeat loci (DXS7424-DXS101, DXS6789-DXS6809, DXS7423-DXS10134, DXS10103-HPRTB-DXS10101, DXS10159-DXS10162-DXS10076, DXS10148-DXS10135-DXS8378, and DXS7132-DXS10079-DXS10074-DXS10075) of 331 Chinese Yi individuals were

GuangLin He and Ye Li contributed equally to these studies.

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obtained. All 19 X-chromosomal short tandem repeat (STR) loci in females were consistent with the Hardy-Weinberg equilibrium test. A total of 214 alleles were identified with the corresponding allele frequencies spanned from 0.0019 to 0.6106. The combined PE, PDF, and PDM were 0.999999214, 0.99999999999999999999999, and 0.999999999998, respectively. The high combined MEC_{Krüger}, MEC_{Kishida}, MEC_{Desmarais}, and MEC_{Desmarais} Duo were achieved as 0.9999999617638, 0.9999999999971, 0.9999999999971, and 0.999999931538, respectively. The findings suggested that the panel of 19 X-STR loci is highly polymorphic and informative in the Yi ethnic population and can be considered to be a powerful tool in forensic complex kinship identification. Population differentiation analyses among 12 populations indicated that significant differences in genetic structure were observed in between the Yi ethnicity and the Chinese Uyghur as well as Kazakh, and genetic homogeneity existed in similar ethno-origin or geographic origin populations.

Keywords X-chromosomal STR · Phylogenetic analyses · Forensic science · Genetic polymorphisms · Yi ethnicity

Autosomal short tandem repeats (STRs), as a common genetic marker in forensic practice, had limitation in addressing deficiency paternity cases [1]. Recently, X-chromosomal short tandem repeats played an important complementary role in forensic parentage test and personal identification, especially in the complicated kinship [1, 2]. In the past decades, several mature multiplex amplification systems including the Mentype® Argus X-UL, Mentype® Argus X-8, Investigator Argus X-12, and X-Decaplex (In-house Kit) PCR amplification kits (Table S1) had been applied in forensic application and explored the population data in different ethnicity groups. The AGCU X19 commercial kit is a new amplification system with 19 X-STR loci (DXS8378, DXS7423, DXS10148, DXS10159, DXS10134, DXS7424, DXS10164, DXS10162, DXS7132, DXS10079, DXS6789, DXS101, DXS10103, DXS10101, HPTRB, DXS6809, DXS10135, DXS10074, and DXS10075). Population genetic data and haplotype analyses of this panel have been investigated in the Chinese Kazakh [3], Uyghur, [4] and Guanzhong Han [5] populations, but genetic polymorphisms for the 19 X-STR loci of the Chinese Yi population are lacking and the population genetic relationships between the Chinese Yi ethnic group and the neighboring populations are unclear.

The Yi ethnic group was scattered in the southwest of China, Vietnam, and Thailand in Southeast Asia. Chinese Yi with a population more than 8 million, ranking seventh largest groups among 56 ethnic groups, mainly distributed in the Liangshan Yi Autonomous Prefecture in Sichuan Province, Honghe Hani and Yi Autonomous Prefecture and Chuxiong Yi Autonomous Prefecture in Yunnan province, and Guangxi and Guizhou province. The Yi language belongs to Loloish language and Sino-Tibetan language which is closely related to the Burmese (https://en.wikipedia.org/wiki/Yi_people). Our previous study [6] had investigated the genetic distribution characteristics of Y-chromosome STRs in the Chinese Yi ethnicity.

Our study design was subject to approval by the Ethics Committee of the Institute of Forensic Medicine, Sichuan University, China. Informed consents were achieved before sample collection. A total of 331 (133 males and 198 females) heparin-treated anticoagulant blood samples was collected from unrelated healthy aboriginal individuals residing in the Liangshan Yi Autonomous Prefecture, Sichuan, China (Fig. S1). Genomic DNA was extracted from 200 μ L peripheral blood samples by the salting-out method described by Miller et al. [7] and quantified on the Nanodrop-1000 spectrophotometry (Thermo Scientific, Waltham, MA).

Nineteen X-chromosomal loci included in the AGCU X19 STR Kit (AGCU ScienTech Incorporation, Wuxi, Jiangsu, China) were co-amplified in accordance with the manufacturer's recommendations on a GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Amplified PCR products were separated and detected on the ABI 3130 Genetic Analyzer (Applied Biosystems). PCR fragments were identified in the GeneMapper® version 3.2 (Applied Biosystems) in comparison with the corresponding allelic ladder. We followed the updated recommendations [8] on the use of Y-STRs in forensic analysis published by the International Society of Forensic Genetics (ISFG) in the present study.

Allele frequencies and associated forensic parameters of 19 X-STR loci were estimated using the modified PowerStats and the online tool on the ChrX-STR.org 2.0 database (http://www.chrx-str.org/), respectively. The Hardy-Weinberg equilibrium (HWE), observed heterozygosity (HO), and

expected heterozygosity (HE) in female population were calculated in the Arlequin v3.2 [9]. Haplotype frequencies and haplotype diversity (HD) were calculated as our previous study [6]. Subsequently, population comparisons between the Chinese Yi minority and the 11 neighboring populations [3-5, 10-17] were performed. The Nei's genetic distances were assessed by Phylip3.695 and visualized as the neighboring-joining tree in Mega 6.0.

Detecting departures from the HWE in Yi females were performed. The genotype distribution was in agreement with HWE. No sex difference of allele frequency distributions was identified; consequently, the pooled allele frequencies of 331 Chinese Yi individuals are calculated and submitted in Table S1. A total of 214 alleles were identified with the associating allele frequencies spanned from 0.0019 to 0.6106 among our investigated individuals. The number of alleles at each locus varied from 4 at DXS7423 to 27 at DXS10135. Forensic statistical parameters are listed in Table S3. The locus showed the lowest heterozygosity among 198 individuals was observed in DXS7423 (HO = 0.5101). The PIC of 19 X-STR loci varied from 0.4384 at the locus of DXS7423 to 0.9060 at the locus of DXS10135. The paternity index spanned from 0.0438 to 0.2402. The combined PE, PDF, and PDM are 0.9999999214, 0.999999999999999999999993, and 0.9999999999998, respectively. The combined mean paternity exclusion chance calculated by the formula of $MEC_{Krüger}$, MEC_{Kishida}, MEC_{Desmarais}, and MEC_{Desmarais Duo} are 0.9999999617638, 0.999999999971, 0.9999999999971, and 0.999999931538, respectively. The aforementioned forensic parameters indicated that the 19 X-STRs included in the AGCU X19 PCR amplification kit are highly polymorphic and informative in the Chinese Yi population and can be used as a powerful tool in forensic complex kinship identification.

Nineteen X-chromosomal STR loci can be clustered into seven linkage groups based on previous studies [10, 18–22]. The detailed loci information about each linkage group are listed in Table S1. The haplotype distributions of each cluster and the corresponding haplotype frequencies among 133 Yi ethnic male individuals are presented in Table S4. The number of haplotypes at each cluster varied from 29 in DXS7423-DXS10134 to 108 in DXS7132-DXS10079-DXS10075-DXS10074 with the corresponding haplotype diversities spanned from 0.9332 to 0.9961. The most common haplotypes were 10-25.1-21 with the corresponding frequency of 0.0376 in cluster one, 24-10-19 with the corresponding frequency of 0.0752 in cluster two, 14-20-17-18 with the corresponding frequency of 0.0301 in cluster three, 20-34 with the corresponding frequency of 0.1429 in cluster four, 16-25 with the corresponding frequency of 0.1278 in cluster five, 16-31-14 with the corresponding frequency of 0.0526 in cluster six, and 15-36 with the corresponding frequency of 0.1353 in cluster seven. Our findings demonstrated that the highly polymorphic and informative linkage groups provide a constructive pathway in dealing with the deficiency parentage cases.

Population genetic relationships between the Chinese Yi ethnic group and the five Han Chinese groups [5, 10, 12–14] as well as six different minority ethnic groups [3, 4, 11, 15–17] (Fig. S1) are investigated in the present study. The Fst and the corresponding p value are listed in Table S5. Along ethnic divisions, significant genetic differences between the Chinese Yi and the Uyghur as well as Kazakh ethnic group were identified at four and three loci, respectively. Along Han Chinese populations, no significant genetic differences were observed with the Chinese Yi ethnic group except the Guangdong Han at one locus.

Inter-population genetic variability was further analyzed by phylogenetic analyses. Genetic distances among 12 populations are presented in Table S6. The smallest and largest Nei's standard distances between the Chinese Yi and the reference populations were identified were 0.0038 (Chinese Yi and Henan Han) and 0.0348 (Chinese Yi and Xinjiang Uyghur). A neighboring-joining tree (N-J tree) derived from the Nei's genetic distance is displayed in Fig. S2. Twelve Chinese populations were clustered into two branches. Two Chinese minority populations (Uyghurs and Kazakhs) residing in Xinjiang formed one branch. Additional ten populations were grouped closely. Our studied population and the branch consisting of most Han Chinese populations clustered first and then with the branch comprising three populations living in Guangdong provinces. Population comparisons and phylogenetic analyses indicated that significant differences with the Chinese Yi ethnic group were identified with the Chinese Uyghur and Kazakh, and genetic homogeneity was observed in similar ethno-origin or geography close populations.

In summary, our study presents the first batch of genetic polymorphism data on X-chromosome in the Chinese Yi ethnicity group and enriched Chinese minority ethnic population reference databases. Nineteen X-STR loci included in AGCU X19 amplification system showed highly polymorphic and informative in the Yi ethnicity and can be used as a potential tool in forensic complex kinship identification and population genetic study. Inter-population comparisons and phylogenetic analyses revealed that the Chinese Yi group had close genetic distance with most Han Chinese populations and kept far from Uyghurs and Kazakhs. Besides, our findings indicated that geographic close population possessed the genetic homogeneity in Xchromosome.

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Conflict of interests None.

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