



# Genetic distribution analyses and population background explorations of Gansu Yugur and Guizhou Miao groups via InDel markers

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## Abstract

Insertion and deletion markers (InDels) have gained considerable attentions in population genetics and forensic research. In this study, we investigated genetic distributions of 30 InDels in Gansu Yugur and Guizhou Miao groups and evaluated their forensic application values. Genetic relationship analyses between Gansu Yugur, Guizhou Miao groups and other published populations were conducted based on these 30 InDels. Power of discrimination and power of exclusion in trio and duo cases of 30 InDels ranged from 0.3528 to 0.6247, 0.0937 to 0.1873, and 0.0219 to 0.1247 in Gansu Yugur group; and they ranged from 0.2579 to 0.6247, 0.0671 to 0.1874, and 0.0105 to 0.1247 in Guizhou Miao group. Obtained cumulative power of discrimination values indicated these InDels could be used for forensic individual identifications in both ethnic groups. Principal component analysis and phylogenetic reconstruction revealed that Gansu Yugur and Guizhou Miao groups had close affinities with their neighboring populations. Genetic structure analyses among these populations also indicated that studied Gansu Yugur and Guizhou Miao groups showed similar genetic structure with their neighboring populations. Further analyses of Y-STR, mtDNA, and ancestry informative markers should be conducted to better understand genetic backgrounds of Gansu Yugur and Guizhou Miao groups in the future.

## Introduction

Short tandem repeats (STRs) are commonly used genetic markers in forensic investigation since they are highly polymorphic [1]. However, STR profiles of some samples

like highly degraded or dated biological materials may not be obtained. The presence of stutter peaks of STR profiles may mislead the interpretation of mixed samples [2]. Besides, high mutation rate of STRs may induce adverse effects in paternity testing [3]. Contrary to STRs, insertion and deletion (InDel) polymorphisms possess some advantages including low mutation rate [4] and small amplicons [5], which has been paid considerable attentions by forensic geneticists during the recent years. For examples, some researchers constructed InDels panels for ancestral origin estimation [6–8]; multiplex InDels systems for forensic individual identification have been presented [9, 10].

As the first commercial InDels kit (Investigator DIPplex Kit) having been released, an army of population data regarding these InDel markers were reported [11–23]. Previous validation analyses of the kit have shown that the kit could type low amount DNA samples (62 pg) and degraded casework samples; their results stated that the assay could be viewed as a useful tool for human individual identification [2, 24]. As genetic variations of the same genetic markers in various populations exist, it is vital for us to evaluate their

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genetic distributions in the studied population before these markers being used for forensic applications [25].

Yugur group is one of 55 ethnic groups in China, which mainly distributes in Gansu province. The language of Yugur people belongs to the branch of the Altaic language family. Although Yugur group has relatively small population size, it has a long history. For ethnic origins of Yugur group, frequent interactions between Yugur group and its neighboring populations exert certain impacts on the formation of Yugur group [26]. Accordingly, research on genetic background of Yugur group should be performed, which will benefit for revealing its ethnic origin. Genetic distributions of autosomal STRs [27], X-STRs [25], and HLA alleles [28] in Yugur group have been conducted, but no report on InDel markers is published. Likewise, Miao group is also an old ethnic group in China, which mainly dwells in south China regions like Guangxi Zhuang Autonomous region and Yunnan, Guizhou, and Sichuan provinces. Miao people cultivated millet and buckwheat with the method of the slash-and-burn before modernization of farming methods have been putting into practice. Their religion beliefs include nature and ancestor worship and Christianity. The language of Miao people is part of the Hmong-Yao branch of Sino-Tibetan language family (<http://www.paulnoll.com/China/Minorities/min-Miao.html>). Although research on InDels in Miao group has been conducted [12], further analysis in Miao samples from different regions may contribute to revealing its genetic background better.

To enrich genetic data of Gansu Yugur and Guizhou Miao groups, genetic diversities of 30 InDels in both populations were assessed on the basis of Investigator DIPplex Kit (Qiagen, Hilden, Germany). Forensic efficiencies of these markers were evaluated. Moreover, genetic relationship analyses between the studied Gansu Yugur, Guizhou Miao groups, and other published populations [11–23, 29–31] were performed.

## Materials and methods

### Sample collections and DNA extraction

The study was in conformity with the human and ethical research principles of Xi'an Jiaotong University Health Science Center, and was warranted by the ethics committee of Xi'an Jiaotong University Health Science Center. Prior to sample collections, written informed consent of each participant was obtained. Blood-stain samples of 139 Yugur individuals living in Sunan Yugur Autonomous County, Gansu province and 274 Miao individuals residing in Guizhou province were gathered. DNA samples of the studied individuals were extracted according to the Chelex-100 method [32]. Supplementary Fig. 1 showed

geographical distributions of Gansu Yugur, Guizhou Miao, and other 22 reference populations.

### Multiplex PCR amplification and InDel genotyping

Referring to the specification provided by the kit, multiplex PCR amplification of 30 InDels and the Amelogenin gene was conducted. Next, the cocktail including 1  $\mu$ L PCR product and the mixture of 12  $\mu$ L Hi-Di formamide and 0.5  $\mu$ L DNA Size Standard 550 (BTO) was initially denatured at 95 °C for 3 min, then immediately chilled at 4 °C for 5 min. Allelic assignments of 30 InDels and the Amelogenin gene were conducted on the ABI 3500xL Genetic Analyzer instrument (Applied Biosystems, USA) according to the parameters recommended by the kit. Finally, alleles were determined on GeneMapper ID software v3.2 (Applied Biosystems, USA) by comparing with the allele ladder of the kit. During the experimental process, control DNA 9948 and nuclear-free water were used as positive and negative controls, respectively. The genotyping results of 30 InDels and the Amelogenin gene in the studied Gansu Yugur and Guizhou Miao groups were given in Supplementary Table 1.

### Statistical analysis

STR\_Genotype tool [33] was used to calculate allele frequencies and forensic relevant parameters of 30 InDels. Hardy–Weinberg equilibrium (HWE) analyses of 30 InDels were performed by Arlequin software v3.5 [34]. Linkage disequilibrium (LD) analyses (correlation coefficient,  $r^2$ ) of pairwise InDels were estimated by SHEsis program [35]. A heatmap of insertion allele frequencies for 30 InDels in the studied Gansu Yugur, Guizhou Miao groups and other compared populations was plotted by pheatmap program in R software v3.3 [36]. Average heterozygosity values and genetic distances ( $D_A$ ) of Gansu Yugur, Guizhou Miao groups and other reference populations were calculated by DISPAN program (<https://www.winsite.com/Multimedia/3D-Modeling-CAD/DISPAN/>) based on allele frequencies of 30 InDels. To explore genetic affinities among Gansu Yugur, Guizhou Miao groups and other reference populations, principal component analysis (PCA) was performed by XLSTAT program (<https://www.xlstat.com/en/>). Based on  $D_A$  values among these populations, a phylogenetic tree was constructed by MEGA software v6.0 [37] based on the Neighbor-Joining method. Assuming admixture, LOC-PRIOR and correlated allele frequencies model, genetic structure analyses of 24 populations were run five replicates for  $K = 2–5$  with the 10,000 burnins and 10,000 MCMC by STRUCTURE software v2.3 [38]. Estimated individuals' genetic components were displayed in the bar plot by dis-struct program [39].

## Results and discussion

### HWE and LD analyses for 30 InDels

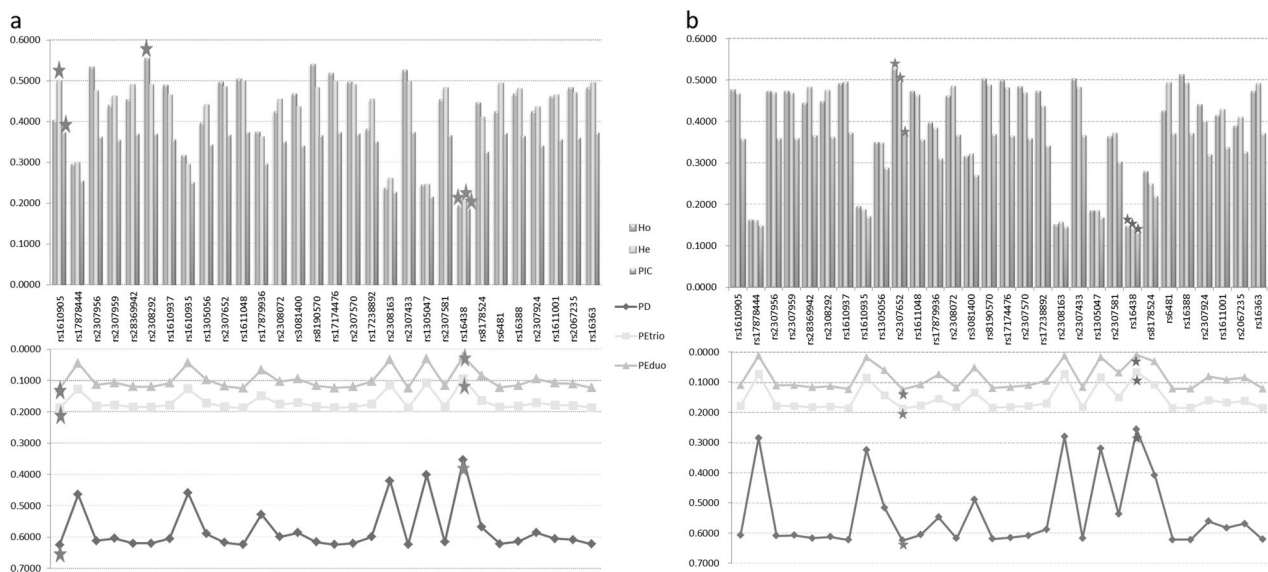
The  $P$ -values for HWE tests of 30 InDels in Gansu Yugur and Guizhou Miao groups were given in Supplementary Tables 2–3. Although the  $P$ -values of rs1610905 locus in Gansu Yugur group and rs6481 locus in Guizhou Miao group were less than 0.05,  $P$ -values of all loci were not statistically significant after applying Bonferroni correction (the significant level =  $0.05/30 = 0.0017$ ), implying these loci conformed to HWE in Gansu Yugur and Guizhou Miao groups. Therefore, these 30 InDels could be employed for the further analysis.

LD indicated that alleles at different loci were in the state of non-random associations. In forensic research, LD analyses of pairwise loci should be evaluated prior to product law being employed. Correlation coefficient ( $r^2$ ) could be utilized to evaluate the level of LD between pairwise loci: pairwise loci will have strong correlations when the  $r^2$  value between them is more than 0.8 [40]. The  $r^2$  values of pairwise 30 InDels in Gansu Yugur and Guizhou Miao groups were displayed in Supplementary Figs. 2 and 3. These  $r^2$  values were magnified 100 times so that we could display them easily. Results showed that  $r^2$  values of all pairwise loci were less than 0.1, indicating relatively weak correlations among these pairwise loci. Consequently, these loci could be viewed as independent loci from each other in Gansu Yugur and Guizhou Miao groups.

### Allele frequencies and forensic parameters of 30 InDels in Gansu Yugur and Guizhou Miao groups

Allele frequencies of 30 InDels were shown in Supplementary Fig. 4. For biallelic markers, the markers can be used as valuable loci for forensic individual identification as long as their minor allele frequencies are more than 0.2 [10]. In Gansu Yugur group, the minor allele frequencies of five InDels (rs17878444, rs1610935, rs2308163, rs1305047, and rs16438) were less than 0.2, demonstrating these loci were less valuable in Gansu Yugur group (Supplementary Fig. 4a). For Guizhou Miao group, rs17878444, rs1610935, rs2308163, rs1305047, rs16438, and rs8178524 loci showed less valuable (Supplementary Fig. 4b). Therefore, more loci which are suitable for forensic individual identifications in Chinese populations should be selected in the future.

Forensic relevant parameters including observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), polymorphism information content (PIC), power of discrimination (PD), power of exclusion in trio ( $PE_{trio}$ ), and power of exclusion in duo ( $PE_{duo}$ ) were shown in Fig. 1. Detailed values of these parameters were given in Supplementary Tables 2 and 3. For Gansu Yugur group, the highest  $H_e$ , PIC, PD,  $PE_{trio}$ , and  $PE_{duo}$  values were observed at rs1610905 locus with the values of 0.4994, 0.3747, 0.6247, 0.1873, and 0.1247, respectively; whereas the lowest  $H_e$ , PIC, PD,  $PE_{trio}$ , and  $PE_{duo}$  values were at rs16438 locus (Fig. 1a). For Guizhou Miao group (Fig. 1b), the highest  $H_o$ ,  $H_e$ , PIC, PD,  $PE_{trio}$ , and  $PE_{duo}$  values were observed at rs2307652 locus, and the



**Fig. 1** Forensic relevant parameters of 30 InDels in the Gansu Yugur (a) and Guizhou Miao (b) groups. The abbreviations including  $H_o$ ,  $H_e$ , PIC, PD,  $PE_{trio}$ , and  $PE_{duo}$  denote observed heterozygosity, expected heterozygosity, polymorphism information content, power of

discrimination, power of exclusion in trio case, power of exclusion in duo case, respectively. Red and blue stars denote the highest and smallest values, respectively. (color figure online)





**Table 1** Average heterozygosities in the studied Gansu Yugur, Guizhou Miao, and other reference populations based on allele frequencies of 30 InDel loci

Populations	Average heterozygosities	Populations	Average heterozygosities
Guangxi Dong	0.3867	Tibet Tibetan	0.4219
Yunnan Yi	0.3944	Qinghai Tibetan	0.4234
Guangxi Miao	0.3976	Qinghai Salar	0.4320
<b>Guizhou Miao</b>	<b>0.3987</b>	<b>Gansu Yugur</b>	<b>0.4356</b>
Hubei Tujia	0.4082	Gansu Dongxiang	0.4413
Guangdong Han	0.4101	Xinjiang Kyrgyz	0.4595
Henan Han	0.4125	Xinjiang Kazak	0.4643
Shanghai Han	0.4129	Xinjiang Uygur	0.4764
Guangxi Zhuang	0.4136	Basque	0.4794
Beijing Han	0.4163	Dane	0.4876
Chengdu Han	0.4172	Central Spanish	0.4879
Xinjiang Xibe	0.4213	Hungarian	0.4910

Populations in bold were studied populations

High heterozygosity values were observed in Basque, Dane, Central Spanish, and Hungarian groups, whereas lower heterozygosity values were observed in most Chinese populations, implying that these 30 InDels showed lower polymorphisms in Chinese populations in comparison with European populations. Moreover, heterozygosity value in Gansu Yugur group was 0.4356, indicating relatively high heterozygosity in Gansu Yugur group. However, heterozygosity value in Guizhou Miao (0.3987) reflected low genetic heterozygosity, which was similar with the previous result (0.3976) in Miao group [12]. Lifestyle of dwelling tightly in community might lead to low heterozygosity of Miao group [43].

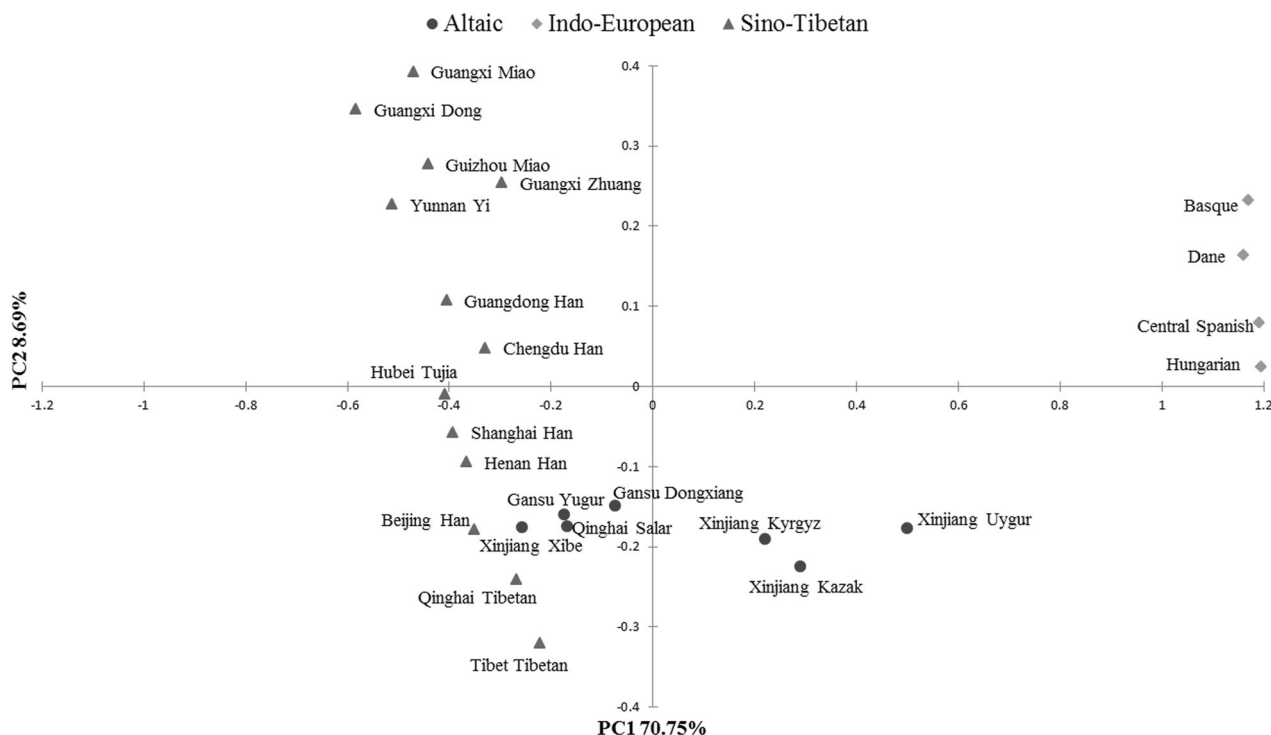
### Genetic relationship explorations among Gansu Yugur, Guizhou Miao groups, and other reference populations

Based on allele frequencies of 30 InDels, PCA plot among Gansu Yugur, Guizhou Miao groups and other reference populations was conducted (Fig. 3). A total of 79.44% of the variance can be explained by the first two principal components. Different colors in Fig. 3 denote different language families. Results showed that some populations with the same language families did not cluster closely, like Hubei Tujia, Yunnan Yi, and Tibetan groups. Geographical isolation may contribute to genetic differentiations of these populations. Moreover, we found that three Eurasian populations (Xinjiang Kazak, Xinjiang Uygur, and Xinjiang Kyrgyz) located in the lower right part; most southern China populations including Guizhou Miao, Guangxi Dong, Yunnan Yi, Guangxi Zhuang, and Guangxi Miao positioned in the upper left corner; northwest China populations including Gansu Yugur group situated in the lower left part; four European populations located in the upper right part. In short, populations from different regions exhibited certain

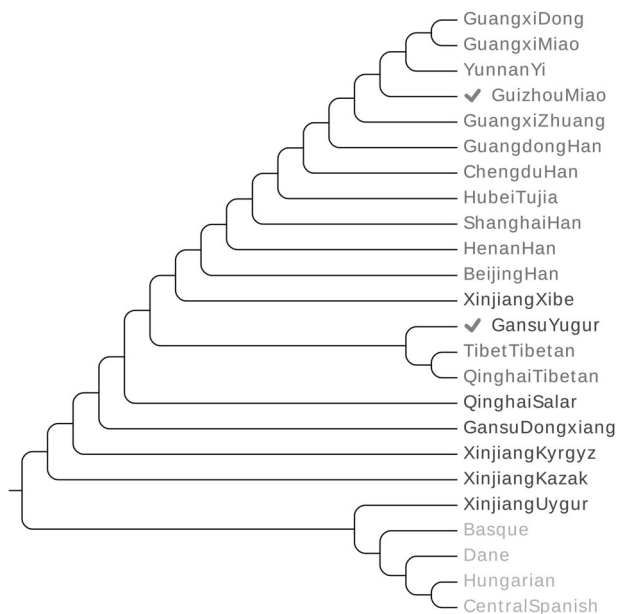
genetic variations, which lead to their different distribution patterns in the PCA plot.

To further analyze genetic relationships of these populations, a phylogenetic tree was constructed based on pairwise  $D_A$  values (Fig. 4). Results indicated that these populations could be classified into two apparent branches: one included four European populations and Xinjiang Uygur group; the other comprised other Chinese populations, of which south to north cline was observed. The studied Gansu Yugur group firstly located in one sub-branch with Tibetan populations from different regions, and then with Qinghai Salar, Gansu Dongxiang, and other compared populations, implying Gansu Yugur group had close genetic affinities with Tibetan, Salar, and Dongxiang groups. The other studied Guizhou Miao group clustered with Guangxi Dong, Guangxi Miao, Yunnan Yi, and Guangxi Zhuang groups, revealing close genetic relationships between the studied Guizhou Miao group and its neighboring populations.

To assess ancestral components of studied Gansu Yugur and Guizhou Miao groups, genetic structure analyses of these 24 populations was conducted at  $K = 2-5$  based on raw data of 30 InDel loci (Fig. 5). At  $K = 2$ , four European populations including Basque, Central Spanish, Dane, and Hungarian displayed orange components; Han populations from different regions, Hubei Tujia, Yunnan Yi, Guangxi Dong, Guangxi Zhuang, Guangxi Miao, and Guizhou Miao showed blue components; Xinjiang Uygur, Xinjiang Kazak, Xinjiang Kyrgyz, Tibet Tibetan, Qinghai Tibetan, Qinghai Salar, Gansu Dongxiang, and Gansu Yugur groups demonstrated the admixed components. As the increase of  $K$  values, populations from different sampling locations showed different genetic component distributions, especially for Chinese populations. For the studied Gansu Yugur group, we found it showed similar ancestral proportions with Gansu Dongxiang populations



**Fig. 3** Principal component analysis among Gansu Yugur, Guizhou Miao, and other reference populations based on allele frequencies of 30 InDels

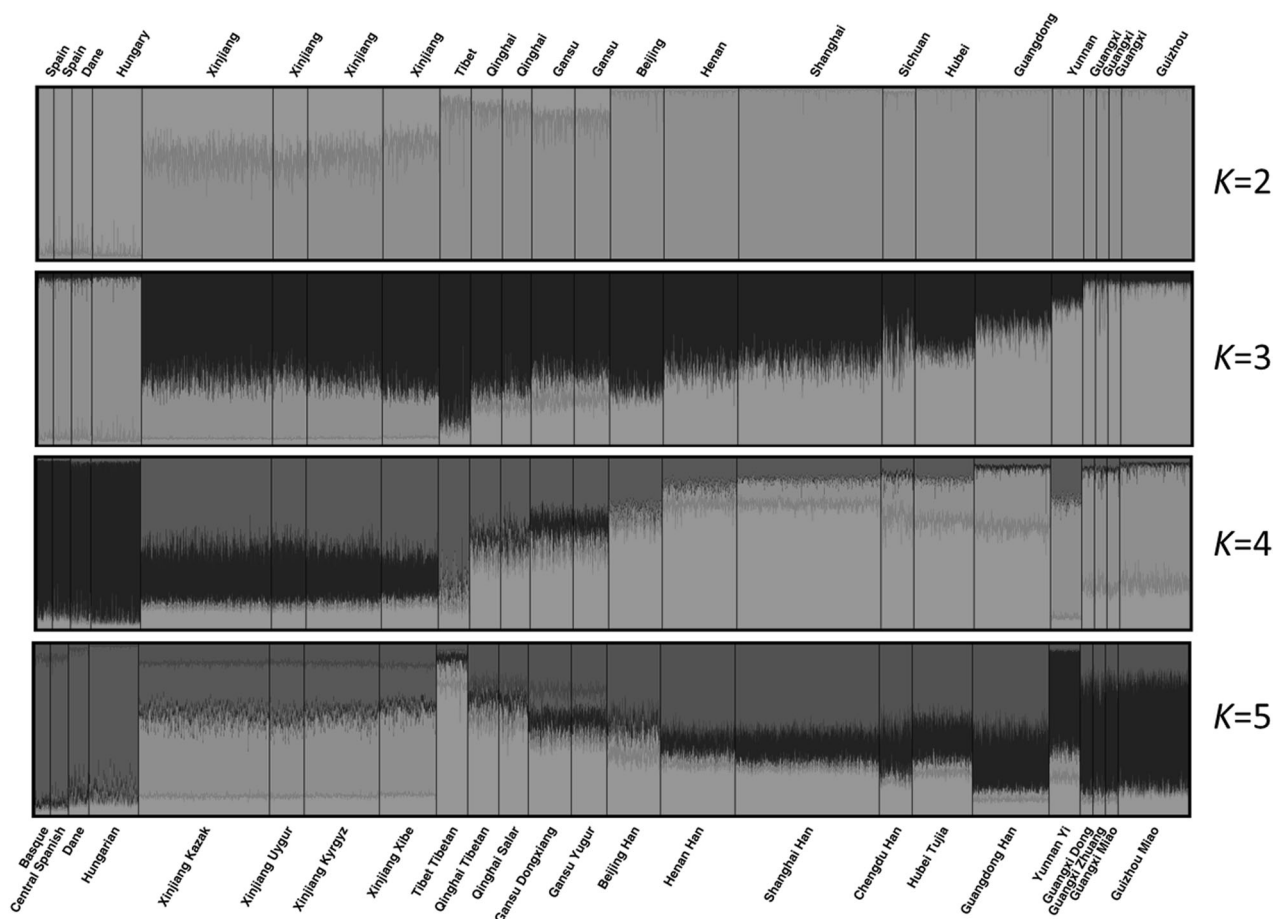


**Fig. 4** A phylogenetic tree of 24 populations by the Neighbor-Joining method based on pairwise  $D_A$  values

at  $K = 2-5$ . Besides, similar genetic structure among the studied Guizhou Miao, Guangxi Miao, Guangxi Zhuang, and Guangxi Dong populations were observed. Based on the present results, we found that the studied Gansu

Yugur and Guizhou Miao groups showed similar ancestral components with their neighboring populations, respectively.

For ethnic origin of Yugur group, most scholars stated that ancient Huihe people living around Erhui River might be ancestors of Yugur group. After Huihe Khanate fell apart during the Tang dynasty, some survivors begun their western migration and settled in regions around Qilian mountain and Hexi corridor. As Hexi corridor being conquered by Mongolian in the 13th century, Huihe individuals living here were ruled by Mongolians. Both ethnic groups (Huihe and Mongolian group) got along with each other swimmingly and gradually formed one new group Yugur. Furthermore, the Tibetan culture and Yugur group’s surrounding ethnic groups like Tu nationality also contributed to the formation of today Yugur group [26, 44]. By assessing genetic distributions of six X-STR loci in Yugur group, Chen et al. found that Yugur group located in one branch with Tibetan, Xi’an Han and Mongolian groups in the phylogenetic tree and they suggested Yugur group was closely related with Tibetan, Mongolian, and Han populations [25]. Research on HLA allele and haplotype distributions demonstrated that Yugur group had close genetic relationships with northern China populations like Tu, Hui, Beijing Han, and Tibetan groups [28]. In this study, based on 30 InDels, close genetic relationships between Gansu Yugur group



**Fig. 5** Genetic structure analyses of the studied Gansu Yugur, Guizhou Miao, and other reference populations by using the LOCprior model. The labels below the figure are 24 populations; and the labels atop the figure are the sampling locations of 24 populations

and Tibetan, Gansu Dongxiang groups were observed, which might attribute to gene and culture interactions of these populations in history. For the other studied Guizhou Miao group, close genetic relationships among the studied Guizhou Miao group and Guangxi Dong, Guangxi Miao and Guangxi Zhuang groups were observed. Previous research on autosomal STRs in Miao group also found similar results: Miao group had relatively close genetic affinities with its neighboring populations like Guangxi Yao [45], Guangxi Dong, and Guangxi Bouyei populations [46]. Further analyses of Y-STRs, mtDNA, and ancestry informative markers in Gansu Yugur and Guizhou Miao groups should be conducted, which might be beneficial for parsing their ethnic histories and population migration process.

In conclusion, we presented genetic data of 30 InDel loci in Gansu Yugur and Guizhou Miao groups, which laid the foundation for forensic and population genetic studies in the future. Obtained CPD values supported that these InDels could be used for forensic personal identification in Gansu Yugur and Guizhou Miao groups.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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