Received: 15 September 2020 | Revised: 5 November 2020 | Accepted: 20 November 2020

DOI: 10.1002/mgg3.1572

Molecular Genetics & Genomic Medicine Open Access WILEY

ORIGINAL ARTICLE

Genetic structure and forensic characterization of 36 Y-chromosomal STR loci in Tibeto-Burman-speaking Yi population

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Funding information
Guizhou Medical University Academic Sprout Cultivation Project, Grant/Award Number: [2018]5779-X; Guizhou "Hundred" innovative talents project, Grant/Award Number: [2020] 6012; Guizhou Science Project, Grant/Award Number: [2020]1Y353; Guizhou Province Engineering Technology Research Center Project, Grant/Award Number: [2016]1345; Guizhou Scientific Support Project, Grant/Award Number: [2019] 2825 and [2020] 4Y057

Abstract

Background: Male-specifically inherited Y-STRs have been widely used in population genetics and forensic investigations.

Methods: We genotyped and analyzed Y chromosome haplotypes of 408 unrelated Tibeto-Burman-speaking Yi male individuals from Guizhou using Goldeneye® Y-PLUS kit. Population comparisons between the Guizhou Yi and 67 reference groups were performed via the AMOVA, MDS, and phylogenetic relationship reconstruction.

Results: A total of 389 alleles and 396 haplotypes could be detected, and the allelic frequencies ranged from 0.0025 to 0.9875. The haplotype diversity, random match probability, and discrimination capacity values were 0.9999, 0.0026, and 0.9900, respectively. The gene diversity (GD) of 36 Y-STR loci in the studied group ranged from 0.0248 (DYS645) to 0.9601 (DYS385a/b). Our newly genotyped Yi samples show a close affinity with other Tibeto-Burman speaking groups in China and Southeast Asia.

Conclusions: The population stratification was almost consistent with the geographic distribution and language-family, both among Chinese and worldwide ethnic groups. Our data may provide useful information for paternal lineage in the forensic application and population genetics, as well as evidence for archaeological and historical research.

KEYWORDS
forensic, haplotypes, Y chromosome, Yi ethnicity, Y-STRs

Zhengyang Song and Qian Wang contributed equally to this work.

1 | INTRODUCTION

Genomic studies of the human Y chromosome began in the 1980s, and its complete sequence was published in 2003 (Skaletsky et al., 2003). The complete sequencing of the male-specific region of the Y-chromosome (MSY) that have a father-to-son inheritance as a haplotype (Hughes & Rozen, 2012) have been widely applied in population genetics and genetic genealogy (Kayser, 2017). The Y-chromosome also played a crucial role in forensic genetics, such as inferring the biological sex of a crime scene trace donor, singles out male DNA components in mixtures and inferring paternal genetic ancestry for judicial and investigative purposes (Jobling & Gill, 2004; Prinz et al., 1997). Y-chromosomal short-tandem repeat (STR) markers is widely used in forensic genetics, and has many commercially available Y-STR kits, such as Yfiler™ Plus PCR Amplification kit (Thermo Fisher Scientific Corporation) and Promega PowerPlex® Y23 (Promega Corporation).

However, the Goldeneye® Y-PLUS kit (Peoplespot Technology Ltd., Beijing, China), which was a recently developed amplification system, contained 36 Y-STR loci that covered all the markers from Yfiler Plus and PowerPlex 23 system, including 32 single copy loci, namely DYS19, DYS460, DYS389 I, DYS389 II, DYS390, DYS391, DYS392, DYS518, DYS393, DYS437, DYS438, DYS439, DYS448, Y-GATA H4, DYS449, DYS456, DYS458, DYS481, DYS533, DYS570, DYS627, DYS635, DYS576, DYS388, DYS549, DYS444, DYS643, DYS447, DYS557, DYS596, DYS593, DYS645, and four multiple copy loci, namely DYS385, DYF387S1, DYS527 and DYF404S1. Among them, DYF387S1, DYF404S1, DYS449, DYS570, DYS576, and DYS627 were reported as rapidly mutating (RM) Y-STR (Ballantyne et al., 2010).

China is a multi-ethnic country with 56 officially recognized ethnic groups from 34 administrative divisions. Chinese Yi, as the seventh sizable minority, which has a population of 8,714,393 according to China's sixth national population census in 2010, mainly distributed in the provinces of Sichuan, Guizhou, and Yunnan. Guizhou province locating in the southwestern China has 49 ethnic groups. Among them, Yi is the sixth largest ethnic group with a population of 834,461. The Yi people have their own language belonging to the Tibeto-Burman family, and the Yi language has its own characters/writing system. Because of the geographical features and their unique ethnic language and culture, the Guizhou Yi rarely intermarried with other ethnic groups and relatively is isolated from other populations. It is necessary to explore the origin and the Chinese Yi people, and the genetic relationship and population stratification with other ethnic groups as well.

Thus, in order to investigate the original genetic polymorphism data and explore the genetic structure of the Chinese Yi minority residing in the Guizhou province, we used Goldeneye® Y-PLUS kit to genotyped 36 Y-STR from 408 Yi unrelated male individuals, and combined our Y-STR data with other 67 populations data from YHRD database, which are divided by geographical distribution, ethnic administrative, and national boundaries. This study can replenish the genetic database of Yi ethnic groups in Guizhou for forensic, and provide data for research.

2 | MATERIALS AND METHODS

2.1 | Subjects and sample collection

Peripheral blood samples were collected from 408 unrelated healthy Yi people with at least three generation residence in Guizhou province (Southwest China). We conducted this study strictly followed the human and ethical research principles, which was approved by the Medical Ethics Committee of Guizhou Medical University (NO. 2019.74). Informed consents were obtained from all of the participating individuals.

2.2 | Multiplex amplification and genotyping

Thirty-six Y-STR loci were analyzed according to Goldeneye® Y-PLUS kit (Peoplespot China). All the Y-STRs were directly co-amplified in one multiplex PCR reaction on the GeneAmp PCR System 9700 (Thermo Fisher) from the FTA card according to the manufacturer's instruction, using 10 μl of reaction volume which contains 2 ul of reaction mix, 2 μl of primers, 1 of μl A-Taq DNA polymerase, and 6 of μl sdH_2O. PCR conditions were 95°C for 2 min, followed by 30 cycles of 94°C for 1 min, 60°C for 45 s, 72°C for 45 s, and a final extension at 60°C for 45 min and a final soak at 4°C. The PCR products were separated by the capillary electrophoresis with the POP-7 polymer by the ABI 3500 Genetic Analyzer (Thermo Fisher, Scientific). The electrophoretic sampling mixture included 1 μl of amplified product, 10 of μl Hi-Di formamide and 1 of μl ORG500 size standard. 9947A was analyzed for positive control, and sdH_2O for negative control as well. Allele nomenclature was conducted using the GeneMapper ID-X v.1.4 software.

2.3 | Quality control

This study strictly followed ISFG recommendations on the analysis of the DNA polymorphisms and nomenclature (Gill et al., 2001) and guidelines for publication of population data (Carracedo et al., 2014). Our lab also has accredited with the China National Accreditation Service for Conformity Assessment (CNAS).

2.4 | Statistical analysis

Allele and haplotype frequencies were calculated by direct counting. Forensic statistical parameters as gene diversity (GD) and haplotype diversity (HD) were calculated using the Nei's formula (Nei, 1978; Nei & Tajima, 1981): $GD = (Na/Na - 1)(1 - \Sigma Pai^2)$, and $HD = (Nh/Nh - 1)(1 - \Sigma Phi^2)$, where Na and Nh, respectively, denote the total number of the tested samples and haplotypes, and Pa*i* and Ph*i* denote the frequency of the *i*th allele and *i*th haplotype, respectively. Haplotype match probability (HMP) and the haplotype discrimination capacity (DC) were determined as described by Josephine Purps (Purps et al., 2014): $HMP = \Sigma Phi^2$, where Ph*i* was the frequency of the haplotype, and DC = A/Nh, where A and Nh, respectively, means the number of different haplotypes in one population and total observed haplotypes. In order to investigate genetic similarities and differences based on the same 17 Y-STR loci (Y-filer set) and 27 Y-STR loci (Y-filer Plus set haplotypes between our subjected group and 22 populations, including 22 Chinese ethic groups (17 Y-STR loci) (three Hans and 19 minorities), 45 worldwide populations (27 Y-STR loci; 12 Asian populations, 15 European populations, eight American populations, seven African populations and three Oceanian populations) in Y Chromosome Haplotype Reference Database (YHRD) (http://www.yhrd.org; Willuweit & Roewer, 2007, 2015). Pairwise genetic distances (Rst) were calculated by analysis of molecular variance (AMOVA), and displayed in multidimensional scaling (MDS) plot using the YHRD online tool. A neighbor-joining (NJ) phylogenetic tree was constructed on the basis of the Rst genetic matrixes using the Molecular Evolutionary Genetics Analysis Version 6.0 (MEGA 6.0) (Tamura et al., 2013). The 67 reference groups included 22 Chinese populations (Fan et al., 2019; Gayden et al., 2012; Hu et al., 2017; Ji et al., 2017; Kim et al., 2001; Liu et al., 2019; Luo et al., 2019; Song et al., 2020; Sun et al., 2019; Tang et al., 2020; Xie et al., 2019; Yang et al., 2014; Yin et al., 2020; Zhang et al., 2017; Zhou et al., 2016) and 45 worldwide populations (Aliferi et al., 2018; D'Atanasio et al., 2019; Füredi et al., 1999; García et al., 2016; Hallenberg et al., 2005; Henry et al., 2019; Jankauskiene et al., 2017; Jankova et al., 2019; Pickrahn et al., 2016; Purps et al., 2014; Rapone et al., 2016; Shonhai et al., 2020; Spólnicka et al., 2017; Watahiki et al., 2019; Zgonjanin et al., 2017; Zhabagin et al., 2019) and the information about these groups are listed in Table S1.

3 | RESULTS

3.1 | Genetic polymorphisms and forensic characteristics of 36 Y-STRs in Guizhou Yi population

In total, 408 individuals were successfully genotyped, the alleles of frequencies and genetic diversity of 36 Y-STR loci are presented in Table S2, including a total of 389 alleles with corresponding frequencies ranging from 0.0025 to 0.9730, and GD values spanning from 0.0528 (DYS645) to 0.9540 (DYS385). As shown in Figure 1, seven single copy loci (DYS391 (0.4099), DYS437 (0.3609), DYS438 (0.3437), DYS456 (0.4816), DYS596 (0.4889), DYS645 (0.0528), DYS388 (0.4612)) revealed low level of genetic polymorphism with the GD values less than 0.5. GD values of multicopy loci and RM loci ranged from 0.7997 to 0.9540, which reflected that these loci had higher levels of genetic polymorphism compared with other frequently used single copy loci. From 408 male individuals, 404 different haplotypes were observed, among which 400 ones were unique, and four were observed twice. The HD, HMP and DC were 0.99990, 0.0025, and 0.9902, respectively. In order to assess the capacity of different systems of Y-STR loci, we also evaluated the forensic statistical parameters of Y-filer set, Y-filer Plus set. It turned out that the HD, HMP and DC values of Y-filer plus set were 0.99988, 0.0026, 0.9755, singly, which was less than the values of 36 Y-STR loci. Y-filer set group owned the minimum value, showed as 0.99827, 0.0042, and 0.8529, respectively.

3.2 | Population structure and phylogenetic relationships among Chinese populations

To investigate the degree of differentiation between the Yi and Chinese reference populations, Multidimensional scaling plots (MDS) and phylogenetic relationship reconstruction based on haplotypes formed by 17 Y-STRs were carried out. The Pairwise Rst values between Guizhou Yi and 22 Chinese reference populations are listed in Table S3. The Rst values demonstrate that Guizhou Yi are most closely related to Youyang Tujia from Chongqing province (Rst = 0.0076), followed by the Guizhou Gelao (Rst =0.0079) and Chongqing Han (Rst = 0.0132), whereas the Sichuan Tibetan populations had the largest genetic distance of 0.2768 with Guizhou Yi. Genetic homogeneity and heterogeneity on the basis of the Rst distance matrix were further explored via multidimensional scaling plots (MDS), is shown in Figure 2A. The Guizhou Yi clustered closely with three Hans (Guizhou, Yunnan and Chongqing). We can also find that several minority groups, including Bouyei, Tujia, and Gelao from Guizhou province, Yi and Hui from Sichuan province, Bai and Hui from Yunnan province and Youyang Tujia from Chongqing province are intermingled with Guizhou Yi population. As expected, other 11 minority ethnic populations are scattered located in the MDS: Guizhou Shui, Yunnan Miao and Guizhou Miao are located in the top left quadrant; Yunnan Hani and Lijiang Mosuo in the top right quadrant; Xishuangbanna Dai and Nujiang Lisu are being assigned in the lower left quadrant; Yunnan Yi, Beichuan Qiang, Tibet Tibetan and Sichuan Tibetan are located in the bottom right quadrant. Phylogenetic relationship among studied

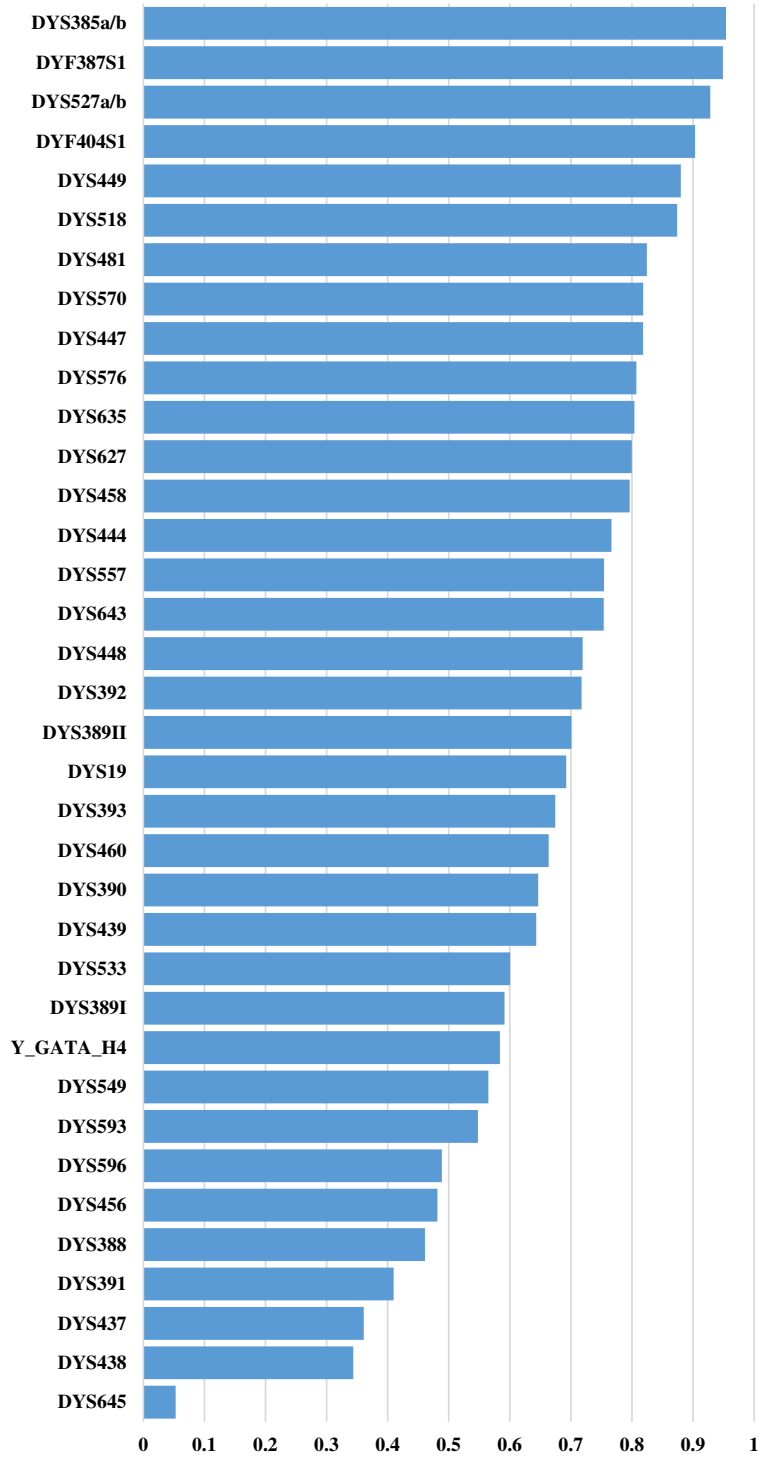

FIGURE 1 Genetic diversity (GD) of 36 Y-STR in Guizhou Yi. The GD values of these Y-STR loci were distributed from 0.0528 (DYS645) to 0.9540 (DYS385)

population and reference populations in China was shown in Figure 2B. Two main clusters can be clearly identified in the phylogenetic tree, the corresponding population compositions and distributions are congruent with the findings in the MDS. Guizhou Yi cluster with Youyang Tujia population, while Sichuan Yi clustered with Sichuan Hui and Yunnan Yi clustered with Tibet Tibetan.

3.3 | Genetic differentiation along national or continental geographical divisions

To further explore the genetic homogeneity and heterogeneity among the Yi population and other worldwide populations, we employed 12 Asian populations, 15 European populations, eight American populations, seven African populations and

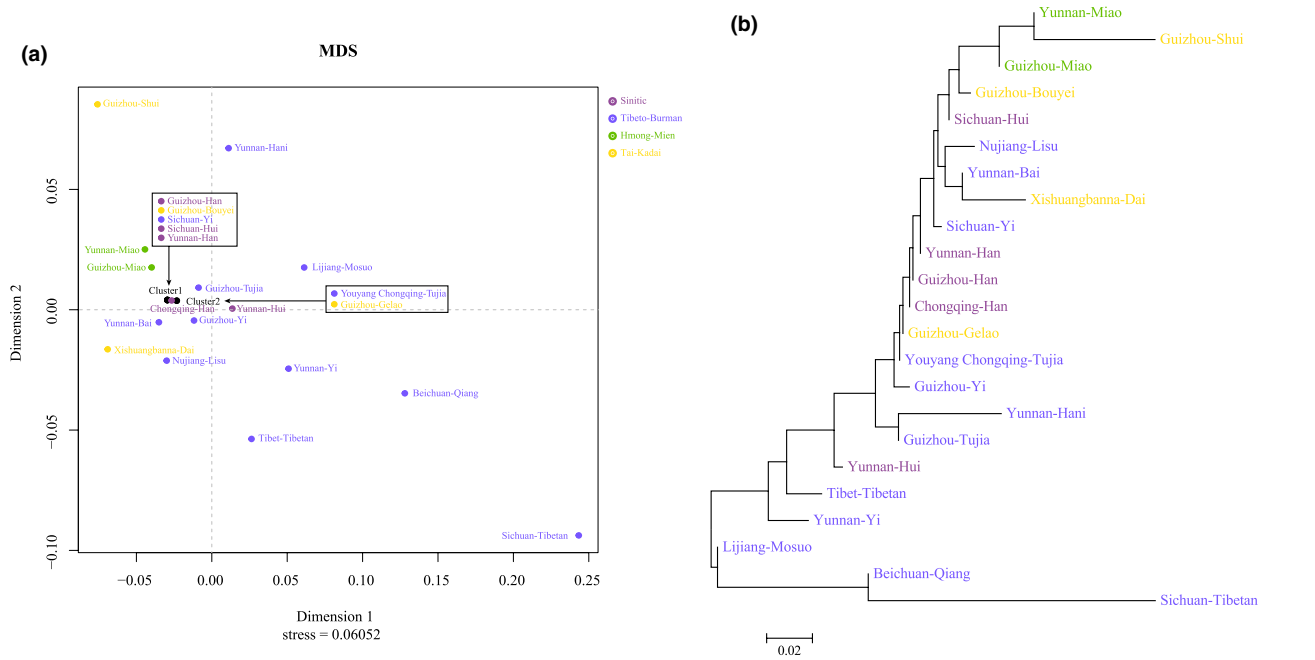

FIGURE 2 Genetic affinity among studied population and reference populations. (a) Multidimensional scaling plots show the genetic correlation between our subject and 22 Chinese populations; (b) Phylogenetic relationship between our target and 22 Chines populations

three Oceanian populations into the comprehensive population comparisons based on 27 Y-STRs. Pairwise Rst standard genetic distances among 45 populations are calculated in Table S4. California Asian American was genetically closest to Guizhou Yi (Rst = 0.0186), followed by Philippines Cebuano (Rst = 0.222), Ecuador Mestizo (Rst = 0.0739) and United Arab Emirates Arab (Rst=0.0747). Consistent patterns of genetic affinity among 45 populations were reconstructed in the neighbor-joining tree (Figure 3). There were four clear genetic affinity clusters could be discerned: Asian cluster, American cluster, European cluster, and African cluster. And the investigated Yi population was next to Southeast Asian populations, which kept the furthest genetic relationship with South Asian.

4 | DISCUSSION

Y-STRs can be divided into two main kinds as its mutation rates: Rapidly Mutating (RM) Y-STRs with high mutation rates approximately 10^{-2} per locus per generation and slowly mutating (SM) Y-STRs with low mutation rates approximately 10^{-3} (Willems et al., 2016). SM Y-STRs was suitable for phylogenetic studies and providing investigative leads in the family searches (Baeta et al., 2018), however, RM Y-STRs are more suitable for forensic paternal lineage identification or other complex kinship identification (Ballantyne et al., 2014). Y-STRs also can be divided into single-copy Y-STRs and multi-copy (MC) Y-STRs, and MC Y-STRs with higher genetic polymorphism (Ballantyne et al., 2012). Goldeneye® Y-PLUS system include all markers included in the previously developed systems (Minimal haplotype, PowerPlex® Y, PowerPlex® Y23, AmpFlSTR® Yfiler and AmpFlSTR® Yfiler Plus) and other nine SM Y-STRs (DYS388, DYS549, DYS444, DYS643, DYS447, DYS557, DYS596, DYS593, DYS645), one RM Y-STRs (DYF404S1) included in the recently selected 13 RM Y-STRs (Ballantyne et al., 2014) and two MC Y-STRs (DYS527 and DYF404S1). In present study, we investigated the genetic polymorphisms/haplotype diversity and forensic characteristics of 36 Y-STRs in 408 unrelated Guizhou Yi individuals using the Goldeneye® Y-PLUS kit. The DC value (0.9902) of the system our study is much higher than the YfilerPlus haplotypes containing 27 Y-STR loci, indicating that these 36 Y-STR loci have higher polymorphism and systematic efficiency in Guizhou Yi population is a useful forensic marker set. However, there were seven loci have low polymorphisms, including DYS391 (0.4099), DYS437 (0.3609), DYS438 (0.3437), DYS456 (0.4816), DYS596 (0.4889), DYS645 (0.0528), and DYS388 (0.4612), which seemed not to be suitable for forensic purpose in this population. The Y-chromosome is male specific inheritance, this gives rise to different forensic genetic applications such as paternity tests, identification of suspects, familial searching and constructing regional effective forensic reference database. Although Chinese male genetic landscape was revisited by 38,000 17-Y-STR haplotypes study (Nothnagel et al., 2017), the analysis of population stratification and genetic relationships among Chinese Han and minorities based on only 17 loci may not be accurate

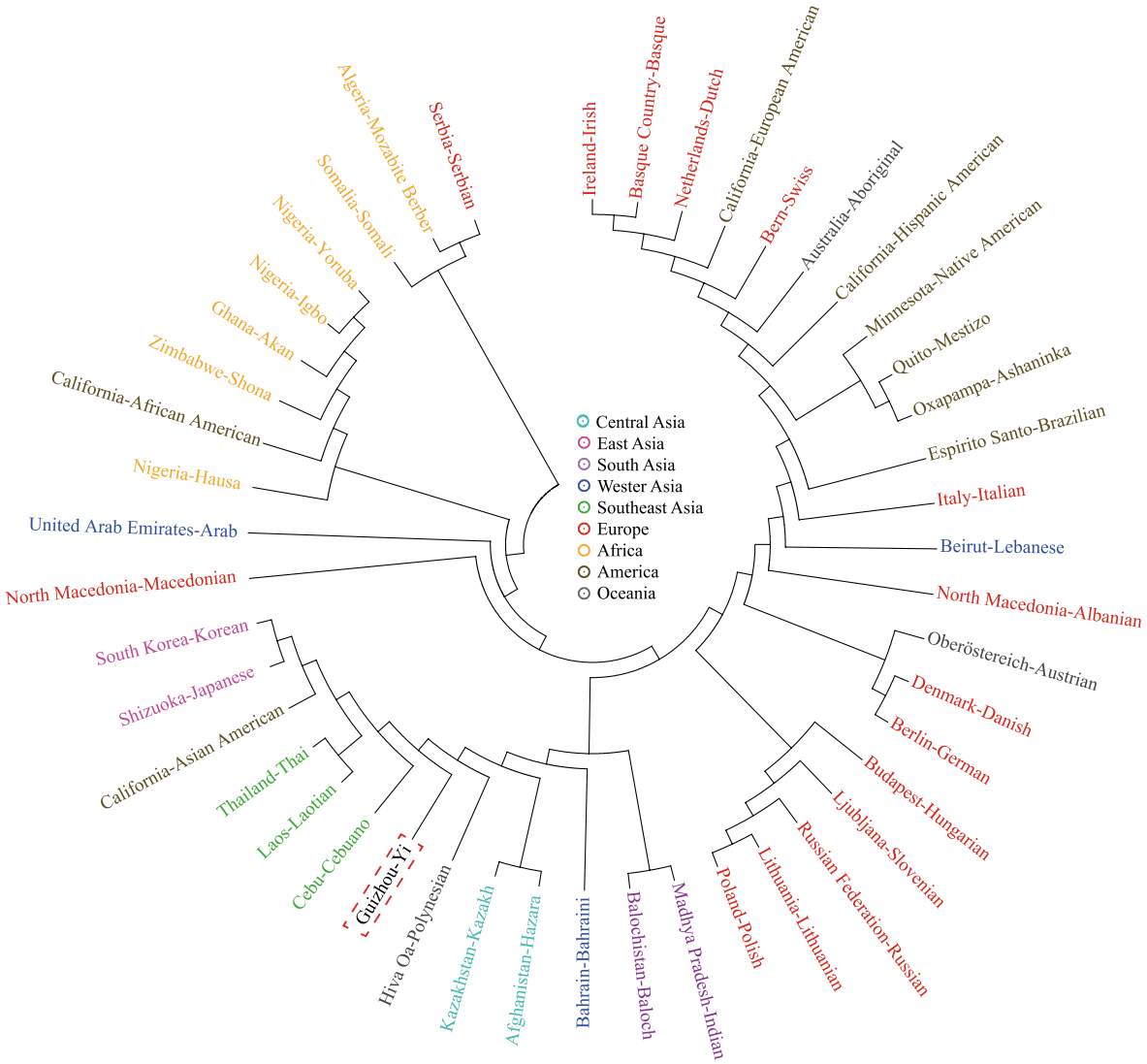

FIGURE 3 Phylogenetic tree constructed by the Neighbor-Joining method using the Mega 6.0 software based on Y-chromosomal STR loci shows the phylogenetic relationship among Guizhou Yi population and 45 reference populations

enough. Therefore, it is necessary to detect as many loci as possible to constructing forensic reference database. Thus, we conducted a more comprehensive population genetic comparisons based on 17 Y-STRs from 408 Guizhou Yi individuals to dissect the genetic relationship of 22 Chinese ethic groups (three Hans and 19 minorities) and 45 worldwide populations (12 Asian populations, 15 European populations, eight American populations, seven African populations, and three Oceanian populations). Our results demonstrated that Guizhou Yi has a close genetic affinity with Youyang Tujia, followed by Guizhou Gelao, Chongqing Han, Guizhou Han and Yunnan Han, all of which were distributed in southern and southwestern China. Additionally, the Guizhou Yi clustered in the phylogenetic tree also belonged to the Tibeto-Burman-speaking with Youyang Tujia, Chongqing Han, Guizhou Han, Yunnan Han and Sichuan Yi. However, three ethnic Yi including our subject, Sichuan Yi and Yunnan Yi from YHRD database possess similar ethno-cultural or geographic origins, they did not exist genetic homogeneity in MDS plot and phylogenetic tree. It is consistent with the conclusion obtained by Fan GY et al (Fan et al., 2019). The analysis of populations structure revealed a strong association between genetic distance and geographical or ethnic affinity. And from the phylogenetic tree among worldwide populations, we found that the worldwide populations were clustered as each continent they located. Our subject with the Southeast Asian populations have closer genetic relationships among each other than other populations.

Considering that the public data of 17 Y-STRs in Yi or other ethnic groups is rare currently, so that it is difficult to analyzing the genetic differentiations of the same ethnic group in different geographical regions to explore the population stratification at paternal lineage. Thus, investigating more Y-STR data to improve the various Yi population database should be taken into consideration.

5 | CONCLUSION

This study investigated the 36 Y-STRs distributions in Guizhou Yi in the southwestern China and released the batch of Y-STR haplotype date of Guizhou Yi (YA004594) for forensic researches. It has highly polymorphic (HD 0.99990) and high power of discrimination (DC 0.9902). Moreover, the genetic relationships between Guizhou Yi and 67 ethnic populations showed that the population stratification was almost consistent with geographic distribution and language-family, both among Chinese and worldwide ethnic groups.

ACKNOWLEDGMENTS

The work was funded by Guizhou Province Engineering Technology Research Center Project (Qian High-Tech of Development and Reform Commission NO. [2016]1345), Guizhou Scientific Support Project (Qian Science Support [2019] 2825), Guizhou "Hundred" innovative talents project (Qian Science Talent Platform [2020] 6012), Guizhou Scientific Support Project (Qian Science Support [2020] 4Y057), Guizhou Science Project (Qian Science Foundation [2020] 1Y353) and Guizhou Medical University Academic Sprout Cultivation Project (Qian Science Platform Talent [2018]5779-X).

CONFLICT OF INTEREST

The authors report no declarations of interest.

AUTHOR CONTRIBUTIONS

JH and YW contributed to the study conception and design. ZYS, QW, JT, JYJ, HLZ, HZ, QYW, and RZ collected samples and analyzed the data. The first draft of the manuscript was written by ZYS and QW. All the authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data to support the findings in the study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Song Z, Wang Q, Zhang H, et al. Genetic structure and forensic characterization of 36 Y-chromosomal STR loci in Tibeto-Burman-speaking Yi population. *Mol Genet Genomic Med*. 2021;9:e1572. https://doi.org/10.1002/mgg3.1572