INTRODUCTION

Jining is a prefecture-level city in southwestern Shandong province. It covers an area of 11,187 km² and has a population of 8.37 million. Jining is situated on the canal northeast of a long string of lakes (Zhaoyang, Weishan, Nanyang, and Dushan) and is a key crossing place, linking to the road system of the plain to the west. Jining has several distinctive associations in Chinese history and culture. In antiquity, it was the birthplace and home of Confucius, along with many of his more famous disciples, including Mencius, and it was later established as the center of Confucianism.

Previous studies showed that the Huaxia™ Platinum kit (Thermo Fisher Scientific) can be used for important forensic identification and paternity testing in this population.
genetic science applications, particularly for investigating paternity and genealogy (He, Wang, Liu, Hou, & Wang, 2018; Wang et al., 2016); however, to date, no data has been published on the STR loci included in the Huaxia™ Platinum kit derived from Jining Han population samples. In this study, we determined the allele frequency and forensic parameters of the 23 STR loci included in the Huaxia™ Platinum kit (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, Penta E, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, D6S1043, D10S1248, D1S1656, D12S391, D2S1338, and Penta D) in the Jining Han population. Following this investigation of the applicability of the Huaxia™ Platinum kit to the Jining Han population, we intend to obtain data on related forensic parameters.

2 | MATERIALS AND METHODS

2.1 | Study population

Peripheral blood samples were collected from 1,215 unrelated, healthy individuals randomly chosen from the Jining Han population after obtaining informed consent. The ancestors of these individuals had lived in Jining for at least three generations. Samples were obtained and used with the ethical approval of the Medical Ethics Committee of Jining Medical University.

2.2 | DNA extraction and PCR

Blood samples were obtained using standard procedures, and DNA was extracted from blood samples stored on FTA cards, according to the Chelex-100 protocol, as previously described (Walsh, Metzger, & Higushi, 2013). Multiplex PCR was performed using the Huaxia™ Platinum kit and the GeneAmp® PCR System 9700 system (Thermo Fisher Scientific).

2.3 | Genotyping and quality control

Amplification products were separated by capillary electrophoresis on an Applied Biosystems® 3500 Genetic Analyzer (Thermo Fisher Scientific). Allele designations were determined by comparison of sample PCR fragments with allelic ladders provided with the kit. All steps were conducted using laboratory internal control standards and kit controls. For genotype assignment, raw data were analyzed using GeneMapper ID-X v.1.4 software (Thermo Fisher Scientific). All experiments were conducted at the Forensic Science Center of Jining Medical University, which is an accredited laboratory (ISO 17025), in accordance with quality control measures. Additionally, the laboratory has been accredited by the China National Accreditation Service for Conformity Assessment (CNAS L9338).

3 | RESULTS AND DISCUSSION

The allele frequencies and other genetic parameters for the 23 STR loci in the Jining Han population are shown in Table S1. In total, we observed 321 alleles, with frequencies ranging from 0.00041 to 0.52222, in the Jining Han group. The observed heterozygosity (He) ranged from 0.64856 (TH01) to 0.91934 (Penta E). We detected a discrimination power of at least 0.9 for all loci, other than D3S1358 (0.86619), CSF1PO (0.88159), TPOX (0.79192), TH01 (0.82266), and D10S1248 (0.89672). The combined discrimination power (PD), power of exclusion (PE), matching probability (MP), typical paternity index (TPI), observed heterozygosity (Ho), and expected heterozygosity (He), for the 23 autosomal STR loci, as well as Hardy–Weinberg equilibrium (HWE) p-values. Principal component analysis (PCA) was implemented in SPSS. PHYLIP was used to analyze the genetic distances among the Jining Han and other populations (Sichuan Han, Xinjiang Uygur, Sichuan Tibetan, Hainan Han, Guizhou Han, Yunnan Han, Wenzhou Han, Ningbo Han, Beijing Han, Dongbei Korean, Hunan Han, ShanxiTaiyuan Han, Anhui Han, Guangdong Han, Shanghai Han, ShanxiYuncheng Han, Huai’an Han, Henan Han, Inner Mongolia Mongolian, Chengde Manchu, Yunnan Yi, Gansu Hui, Xinjiang Kazakh, Fujian Han, Hebei Han, Jiujiang Han, Hubei Han, and Xiamen Han) (Figure S1), based on published data (Chen, Wang, Gao, Bai, & Jia, 2018; Han & Zhang, 2016; He et al., 2018; Hu et al., 2016; Hu, Zhao, & Wang, 2015; Jin et al., 2015; Kang et al., 2016; Liang et al., 2015; Liu, Song, & Jiang, 2015; Liu, Li, Guo, Li, & Shi, 2019; Lu, Song, Huang, & Wu, 2017; Meng, Cai, & Lu, 2018; Si et al., 2018; Sun et al., 2017; Wang, Han, et al., 2018; Wang, Wang, et al., 2018; Wang et al., 2016; Xiao, Zhang, Wei, Pan, & Huang, 2016; Xie et al., 2014; Yang et al., 2018, 2019; Yao, Xiong, & Zhang, 2017; Zhang, Zhao, & Liu, 2015; Zhang et al., 2017; Zhao et al., 2016; Zou et al., 2016). FST and p-values were estimated for 18 STR loci to determine differentiation among Jining Han populations and other groups, using Arlequin 3.11 (http://cmpg.unibe.ch/software/arlequin3). A phylogenetic tree was reconstructed for these groups using Mega 7.0 (http://www.megasoftware.net) with the neighbor-joining method. A p-value <.05 was considered significant.
**Figure 1** Principal component (PC) map of the 29 populations based on normalized allele frequencies.

**Figure 2** Multidimensional scaling (MDS) plot based on pairwise Rst values among Jining Han and other populations.
the 23 autosomal STR loci exhibited favorable characteristics for use in forensic applications, including individual identification and paternity testing, in our studied population.

The results of an AMOVA analysis for 18 STR loci in the Jining Han population and other ethnic groups are summarized in Table S2. Calculation of locus-by-locus $F_{ST}$ and corresponding p-values showed that there were statistically significant differences between the Jining Han and Xinjiang Kazakh at six loci; between the Yunnan Han and Xinjiang Uygur at two loci; and among the Sichuan Tibetan, Guizhou Han, Henan Han, Inner Mongolia Mongolian, and Xiamen Han at one locus, after Bonferroni adjustment ($p < .00033$).

The results of PCA (Figure 1) indicated the genetic structure in 29 populations, based on normalized allele frequencies of 18 autosomal STR loci. The first principal component explained 97.998% of the total variance, while the second accounted for 0.595%. The Xinjiang groups (Uyghur and Kazakh), which have a similar culture and language, were located together in the upper left quadrant. As shown in the PCA plot, Mongolian and Tibetan groups were in the upper right quadrant, while the Jining Han were part of a cluster in the lower right quadrant. A multidimensional scaling plot (Figure 2) showed that the Xinjiang groups (Uyghur and Kazakh) and the Mongolian and Tibetan groups were isolated, while the Jining Han population clustered together with other populations, except the Guizhou Han. These results are consistent with those of PCA. The large genetic distance of the Guizhou Han from other Chinese Han populations may be attributable to a long history of intermarriage within the Guizhou ethnic minority (Chen et al., 2019). These distributions demonstrate the close genetic relationships among Chinese Han populations from different administrative divisions.

The genetic distances among the Jining Han and 28 other ethnic groups are presented in Table S3. In addition, we constructed a phylogenetic tree of these populations using the neighbor-joining method (Figure 3). In the phylogenetic tree, based on genetic distances, all 29 ethnic groups were divided into two main clusters, which can be explained by differences in their ethnic origins. The Jining Han population exhibited the greatest distance from the Xinjiang Uygur population ($Rst = 0.0939$), followed by Xinjiang Kazakh ($Rst = 0.0390$). We detected the shortest distances between the Jining Han and the Anhui Han population ($Rst = 0.0013$), followed by the Hebei Han ($Rst = 0.0022$).

4 | CONCLUSION

In conclusion, after investigating the allele frequencies and forensic parameters of 23 autosomal STR loci in the

**FIGURE 3** Phylogenetic tree showing the relationships among the Jining Han population and 28 reference populations. The phylogenetic tree was constructed using the neighbor-joining method based on 18 overlapping STR loci with Mega 7.0 software.
Chinese Han population from Jining city, Shandong province, eastern China, our results suggest that these 23 STR loci can provide highly informative polymorphic data for paternity testing, individual identification, and genetic population studies.

CONFLICT OF INTEREST
We declared that we have no conflicts of interest to this work.

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

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