Genetic polymorphism and forensic application of 23 autosomal STR loci in the Han population of Panjin City, Liaoning Province, Northeastern China

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ABSTRACT

**Background:** Short tandem repeats (STRs) are consecutive repetition of a repeat motif and widely used in forensic medicine and human genetics because of their high polymorphism.

**Subjects and methods:** In the current study, 23 autosomal STR loci were genotyped from 1263 unrelated healthy individuals living in Panjin City, Liaoning Province, Northeastern China using the VeriFilerTM Express PCR Amplification Kit. The population comparison was performed between the Panjin Han population and the other relevant groups to further explore the structure of Panjin Han and its relationship with the other groups.

**Results:** The results found 316 alleles across the 23 STRs and the corresponding allelic frequencies ranged from 0.5198 to 0.0004. Except for D3S1358, TPOX, TH01, and D3S1358, all STR loci were highly polymorphic (PIC > 0.7), with the Penta E locus having the highest degree of polymorphism (0.9147). For population comparison, the exact test of population differentiation found that no significant difference was observed between the Panjin Han and the other Han populations, except for Guangdong Han and Jiangxi Han.

**Conclusion:** The Panjin Han population showed significant differences with the other ethnic groups in China (Bouyei, Dong, Hui, Miao, Tibetan, and Uygur) and the foreign ethnic groups.

1. Introduction

Short tandem repeats (STRs), also known as microsatellites, are consecutive repetition of a repeat motif with three to six base nucleotides (Rubab et al. 2020). The mean mutation rate of autosomal STRs is $1.8 \times 10^{-2}$ for paternal origin and $0.3 \times 10^{-2}$ for maternal origin (Hamester et al. 2019). On account of their high polymorphism and strong discrimination ability, they can be widely used in forensic medicine and human genetics (Zhu et al. 2015; Yao and Wang 2016). Due to their high mutation rate, they can provide more information for exploring the population genetic structure and revealing the evolutionary relationships between different populations (Chen et al. 2017; Guo 2017; He et al. 2017).

In forensic medicine, STRs have high discrimination ability due to their variable repeat counts among different individuals. Thus, they can be utilised in personal identification and paternity testing (Adnan et al. 2016, 2018; Zhan et al. 2018).

Panjin City, part of Liaoning Province, is located in Northeast China and the centre of the Liaohe Delta. Its land area is 4103 km² with a warm temperate continental semi-humid monsoon climate. It is bordered by Anshan City in the east and northeast, Yingkou City in the southeast across the Daliao River, Jinzhou City in the west and northwest, and Liaodong Bay in the Bohai Sea in the south. Panjin had a population of over 1.44 million in 2019 (www.stats.gov.cn). Han is the most dominant ethnic group of Panjin city followed by other minority groups such as Manchu, Koreans, Mongolians, Hui, and Xibe.

In the current study, we used the VeriFiler™ Express PCR Amplification Kit (Lu et al. 2017) to explore the genetic characteristics of 1263 Han individuals from Panjin City, Northeastern China. Additionally, in order to understand the genetics and structural background of the Panjin Han population, we compared our population with other reference populations. Population comparisons including Reynold’s genetic distance, neighbor-joining tree, and multi-dimensional scaling (MDS) analysis were carried out between the Panjin Han population and different ethnic groups to better understand the genetic background and structure of the Panjin Han population.

2. Subjects and methods

2.1. Ethical compliance

This study was approved by the ethical review board of Shenyang Medical College, Shenyang, Liaoning Province,
2.2. Study population

Blood samples were collected using the FTA cards from 1263 unrelated healthy individuals living in Panjin City, Liaoning Province, Northeastern China (Figure 1).

2.3. DNA extraction, PCR amplification, and genotyping

Genomic DNA was extracted from FTA cards using Chelex-100 method (Walsh et al. 1991). A total of 23 autosomal STR loci (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) were amplified simultaneously using the VeriFiler™ Express PCR Amplification Kit (ThermoFisher SCIENTIFC, MA, USA). The 23 autosomal STR loci satisfied the requirements of the Chinese National autosomal DNA database as well as expanded Combined DNA Index System (Green et al. 2021). Multiplex amplification was conducted on a ProFlex™ PCR thermal cycler (ThermoFisher SCIENTIFIC), following the manufacturer’s recommendations. Separation and detection were performed using the Applied Biosystems™ 3500 Genetic Analyser (ThermoFisher SCIENTIFIC). Internal controls (H2O as a negative control and 9947A DNA as a positive control) were genotyped along with each batch of samples to ensure that the results were reproducible and accurate. The raw data was analysed using GeneMapper ID-X v1.2 software (ThermoFisher SCIENTIFIC).

2.4. Statistical and phylogenetic analysis

The allele frequencies of samples, exact tests of Hardy-Weinberg equilibrium (HWE), and pair linkage disequilibrium (LD) tests were calculated with the PowerMarker v3.25 (Liu and Muse 2005). The values for matching probability (MP), power of discrimination (PD), polymorphism information content (PIC), power of exclusion (PE), typical paternity index (TPI), gene diversity (GD), and heterozygosity (He) were calculated using the PowerStats software v1.2 (Promega, Madison, WI, USA) (Tereba 1999), which was modified from Silva, et al. in order to support and manage the large number of samples (Cabezas Silva et al. 2016). Reynold’s genetic distance, based on allele frequencies across the 15 autosomal loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA) shared by the included compared populations, and exact test p values were generated using PowerMarker v3.25. Nei’s standard genetic distance between populations was calculated using the allele frequencies by Phylip 3.69 package (Felsenstein 2009) and visualised by MEGA v7.0.26 software (Kumar et al. 2016). Finally, MDS analyses on the basis of Reynold’s genetic distance were performed using GeneMarker ID-X v1.2 software (ThermoFisher SCIENTIFIC).
distance matrix were performed using SPSS 26.0 software (IBM Corp., Armonk, NY).

3. Results and discussions

3.1. Allele frequencies and forensic parameters of the 23 autosomal STR loci

The allele frequencies for the 23 autosomal STRs from 1263 Panjin Han individuals are listed in Supplementary Table S1. A total of 316 alleles were observed with the allele frequencies ranging from 0.5198 (TPOX) to 0.0004 (D3S1358, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, Penta E, D2S441, D19S433, FGA, D5S818, D7S820, D6S1043, D15S1656, D12S391, and Penta D). Forensic efficiency and statistical parameters across the 23 STR loci are shown in Table 1. A high degree of genetic variation was observed by all STR loci in the Panjin Han population. The values of MP, PD, and PE ranged from 0.2042 (TPOX) to 0.0122 (Penta E), 0.9878 (Penta E) to 0.7958 (TPOX), and 0.9147 (Penta E) to 0.5647 (TPOX), respectively. The ranges of GD and He spanned from 0.9204 (Penta E) to 0.6246 (TPOX) and from 0.9074 (Penta E) to 0.6358 (TPOX), respectively. Except for D3S1358 (0.6770), TPOX (0.5647), TH01 (0.5960), and D3S1358 (0.6770), all STR loci were highly polymorphic (PIC > 0.7), with the Penta E locus having the highest degree of polymorphism (0.9147).

3.2. Hardy-Weinberg equilibrium (HWE)

Initially nineteen loci were in HWE, with D8S1179, TH01, D12S391, and Penta D not in HWE (p < 0.05). However, when

Table 1. Forensic efficiency and statistical parameters of 23 autosomal STR loci in the Panjin Han population (n = 1263).

| Loci          | MP     | PD     | PIC     | PE     | TPI    | GD     | He     | p       |
|---------------|--------|--------|---------|--------|--------|--------|--------|---------|
| D3S1358       | 0.1248 | 0.8752 | 0.6770  | 0.4933 | 1.9253 | 0.7247 | 0.7403 | 0.9242  |
| vWA           | 0.0701 | 0.9299 | 0.7207  | 0.6088 | 2.5671 | 0.8007 | 0.8052 | 1.0000  |
| D16S539       | 0.0784 | 0.9216 | 0.7560  | 0.5737 | 2.3389 | 0.7883 | 0.7862 | 0.8352  |
| CSF1PO        | 0.1136 | 0.8864 | 0.6902  | 0.4801 | 1.8683 | 0.7332 | 0.7324 | 1.0000  |
| TPOX          | 0.2042 | 0.7958 | 0.5647  | 0.3360 | 1.3728 | 0.6246 | 0.6358 | 0.2510  |
| D8S1179       | 0.0437 | 0.9563 | 0.8246  | 0.6738 | 3.1108 | 0.8438 | 0.8393 | 0.0231  |
| D21S11        | 0.067 | 0.9393 | 0.7821  | 0.6043 | 2.5361 | 0.8071 | 0.8029 | 0.4350  |
| D18S51        | 0.0402 | 0.9598 | 0.8346  | 0.7239 | 3.6930 | 0.8573 | 0.8646 | 0.9122  |
| Penta E       | 0.0122 | 0.9878 | 0.9147  | 0.8105 | 5.3974 | 0.9204 | 0.9074 | 0.7972  |
| D25S41        | 0.0911 | 0.9089 | 0.7277  | 0.5327 | 2.1120 | 0.7625 | 0.7633 | 0.0948  |
| D19S433       | 0.0573 | 0.9427 | 0.7934  | 0.6677 | 3.0507 | 0.8168 | 0.8361 | 0.3633  |
| TH01          | 0.1743 | 0.8257 | 0.5960  | 0.3403 | 1.3849 | 0.6459 | 0.6390 | 0.0143  |
| FGA           | 0.0379 | 0.9621 | 0.8355  | 0.6862 | 3.2385 | 0.8517 | 0.8456 | 0.6974  |
| D22S1045      | 0.0960 | 0.9040 | 0.7282  | 0.5637 | 2.2798 | 0.7678 | 0.7807 | 0.2382  |
| D5S818        | 0.0881 | 0.9119 | 0.7373  | 0.5453 | 2.1776 | 0.7718 | 0.7704 | 0.1081  |
| D15S317       | 0.0687 | 0.9313 | 0.7743  | 0.6147 | 2.6905 | 0.8032 | 0.8084 | 0.9508  |
| D7S820        | 0.0793 | 0.9207 | 0.7491  | 0.5411 | 2.1553 | 0.7814 | 0.7680 | 0.8775  |
| D6S1043       | 0.0503 | 0.9697 | 0.8590  | 0.7318 | 3.8042 | 0.8725 | 0.8686 | 0.2802  |
| D10S1248      | 0.1062 | 0.8938 | 0.7019  | 0.4947 | 1.9312 | 0.7416 | 0.7411 | 0.8362  |
| D15S1656      | 0.0546 | 0.9454 | 0.7942  | 0.6703 | 2.5567 | 0.8155 | 0.8044 | 0.8285  |
| D12S391       | 0.0474 | 0.9526 | 0.8178  | 0.6924 | 3.3063 | 0.8376 | 0.8488 | 0.0112  |
| D21S1386      | 0.0361 | 0.9639 | 0.8450  | 0.7081 | 3.4890 | 0.8604 | 0.8567 | 0.2923  |
| Penta D       | 0.0574 | 0.9426 | 0.7967  | 0.6312 | 2.7338 | 0.8198 | 0.8171 | 0.0343  |
| D3S1358       | 0.1248 | 0.8752 | 0.6770  | 0.4933 | 1.9253 | 0.7247 | 0.7403 | 0.9242  |

Note: MP: matching probability; PD: power of discrimination; PIC: polymorphism information content; PE: power of exclusion; TPI: typical paternity index; GD: gene diversity; He: expected heterozygosity; p: probability values of exact tests for Hardy-Weinberg equilibrium (HWE).
a sequential Bonferroni’s correction was applied, all 23 loci were found to be in Hardy-Weinberg equilibrium (Table 1).

3.3. Linkage equilibrium (LE)

Linkage disequilibrium (LD) indicates the association between qualitative random variables corresponding to alleles at different STRs. Measuring the levels of linkage disequilibrium is important for gene mapping and it helps in the understanding of genome structure. Exact tests for LE between 253 pairs showed that the values of only 23 pairs were below 0.05 and thus displaying LD (Supplementary Table S2). When sequential Bonferroni’s correction was applied, there were no pairs of loci displaying LD.
3.4. Population comparison with the other populations

In order to study the genetic structure of the Panjin Han population and its relationship with the other populations, the Reynold’s genetic distance was calculated based on the allele frequencies across the 15 autosomal loci shared by the 21 included compared populations (Tie et al. 2006; Zuniga et al. 2006; Yan et al. 2007; Montelius et al. 2008; Wu et al. 2008; Gomes et al. 2009; Deng et al. 2011; Yoo et al. 2011; Chen et al. 2012; Tong et al. 2013; Zhang et al. 2015; Zhang 2015a, 2015b; Xiao et al. 2016; Yao, Wang, et al. 2016; Yao, Xing, et al. 2016; Hongdan et al. 2017; Li et al. 2017; Xu, Feng, et al. 2017; Xu, Xu, et al. 2017; He et al. 2018). The results showed that the Panjin Han had the nearest genetic distance with Liaoning Han (0.000289), followed by Heilongjiang Han (0.000325), Jilin Han (0.000325), Jiangsu Han (0.000377), Henan Han (0.000417), Hubei Han (0.000546), Jiangxi Han (0.000713), Shaanxi Han (0.000958), Sichuan Han (0.000966), China Hui (0.000989), Guangdong Han (0.002234), China Tibetans (0.003198), Koreans (0.003261), China Dong (0.003851), China Bouyei (0.003574), Japanese (0.005528), China Miao (0.007613), China Uygur (0.008692), Americans (0.024125), Europeans (0.033369), and Africans (0.042489) (Table 2). The exact test of population differentiation found that no significant difference was observed between the Panjin Han and the other Han populations, except for Guangdong Han (p < 0.0001) and Jiangxi Han (p = 0.0190). Moreover, the Panjin Han population was significantly different to the other ethnic groups in China (Bouyei, Dong, Hui, Miao, Tibetan, and Uygur) and the foreign ethnic groups (Japanese, Korean, African, American, and European). The phylogenetic neighbor-joining (N-J) tree was generated to reflect the historical and geographical background of the compared populations (Figure 2). The N-J tree exhibited significant differences between the Han population and the other minority groups. We also observed variations between Han populations from north to south, which was intricately sub-structured and clustered into three subgroups (northern Han, central Han and southern Han) in the previous study (Xu et al. 2009). Clearly, the Panjin Han population belongs to the northern Han subgroup, which also includes Heilongjiang, Jilin, Liaoning, Henan, and Hubei Han populations. These northern Han populations were also distributed in the close clusters in our N-J tree. In addition, the analysis of population differentiation found that The Panjin Han had major differences with the other Chinese minority ethnic populations and the foreign ethnic groups, which was also simultaneously mirrored in the N-J tree. The MDS plot was generated using the Reynold’s genetic distance among the 22 compared populations (Figure 3). The Panjin Han and other Han groups were gathered together. The Chinese ethnic minorities, Korean and Japanese populations were scattered around the periphery. Additionally, the distribution of African, American, and European populations was more discrete. The location of the compared populations in the MDS plot was consistent with the results in the N-J tree. Geographically, Panjin city is located in Liaoning Province. The Liaoning Han integrated gradually with natives, such as Manchu, following geographical migration (Yao, Wang, et al. 2016). Consequently, the Liaoning Han population has its own unique genetic characteristics that are different from Han populations from other provinces. Similarly, the Panjin Han population belongs to the northern Han with its own unique structure characteristics.

4. Conclusions

In summary, 23 autosomal STR loci were genotyped in 1263 Panjin Han individuals using the VeriFiler™ Express PCR Amplification Kit. Population comparison was performed between the Panjin Han population and the other relevant groups to further explore the structure of Panjin Han and its relationship with the other groups. The results showed that the Panjin Han belong to the subgroup of northern Han and have major differences with the other Chinese minority ethnic populations and the foreign ethnic groups.

Author contributions

Hongbo Wang and Jun Yao developed the idea. Hongbo Wang analysed the results and wrote the manuscript. Bao-jie Wang and Cai-rui Xin conducted the experiment. All authors reviewed the manuscript.

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