RESEARCH PAPER



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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

ARTICLE HISTORY

Background: Short tandem repeats (STRs) are consecutive repetition of a repeat motif and widely Received 29 January 2022 Revised 15 June 2022 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 unre-Accepted 21 June 2022 lated healthy individuals living in Panjin City, Liaoning Province, Northeastern China using the VeriFileTM Express PCR Amplification Kit. The population comparison was performed between the Han population; autosomal Panjin Han population and the other relevant groups to further explore the structure of Panjin HanSTR; population genetics; and its relationship with the other groups. forensic medicine 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

Liaodong Bay in the Bohai Sea in the south. Panjin had a

Short tandem repeats (STRs), also known as microsatellites Han is the most dominant ethnic group of Panjin city folare consecutive repetition of a repeat motif with three to six lowed by other minority groups such as Manchu, Koreans,

are consecutive repetition of a repeat most with a solution lowed by other minority groups such as manager, base nucleotides (Rubab et al. 2020). The mean mutation Mongolians, Hui, and Xibe. rate of autosomal STRs is 1.8 10³ for paternal origin and In the current study, we used the VeriFilerTM Express PCR 0.3 10⁻³ for maternal origin (Hamester et al. 2019). On Amplification Kit (Lu et al. 2017) to explore the genetic characcount of their high polymorphism and strong discrimin-ateristics of 1263 Han individuals from Panjin City, ation ability, they can be widely used in forensic medicine Northeastern China. Additionally, in order to understand the and human genetics (Zhu et al. 2015; Yao and Wang 2016), genetics and structural background of the Panjin Han popu-Due to their high mutation rate, they can provide more lation, we compared our population with other reference information for exploring the population genetic structure populations. Population comparisons including Reynold's and revealing the evolutionary relationships between differ-genetic distance, neighbor-joining tree, and multi-dimensional genetic distance, neighbor-joining tree, and multi-dimensional ent populations (Chen et al. 2017; Guo 2017; He et al. 2017). scaling (MDS) analysis were carried out between the Panjin In forensic medicine, STRs have high discrimination ability Han population and different ethnic groups to better underdue to their variable repeat counts among different individu-stand the genetic background and structure of the Panjin als. Thus, they can be utilised in personal identification and Han population. 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 2. Subjects and methods area is 4103 km² with a warm temperate continental semihumid monsoon climate. It is bordered by Anshan City in².1. Ethical compliance the east and northeast, Yingkou City in the southeast across This study was approved by the ethical review board of the Daliao River, Jinzhou City in the west and northwest, and Shenyang Medical College, Shenyang, Liaoning Province,

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People's Republic of China, and in accordance with the standards of the Declaration of Helsinki (SYMC-20211015-15). All participants gave their informed consent either orally and with thumb prints (in case they could not write) or in writing after the study aims and procedures were carefully explained to them.

2.2. Study population

Blood samples were collected using the FTA cards from 1263 unrelated healthy individuals living in Panjin City, Liaoning The allele trequencies or samples, court terms were concerned using the trequencies of samples, court terms were concerned using the trequencies of samples, court terms were concerned using the trequencies of samples, court terms were concerned using the trequencies of samples, court terms were concerned using the trequencies of samples, court terms were concerned using the trequencies of samples, court terms were concerned using the trequencies of samples, court terms were concerned using the trequencies of samples, court terms were concerned using the trequencies of samples, court terms were concerned using the trequencies of terms and terms were concerned using the trequencies of terms and te

Analyser (ThermoFisher SCIENTIFIC). Internal controls (H O as a negative control and 9947 A 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

(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),

2.3. DNA extraction, PCR amplification, and genotyping

Genomic DNA was extracted from FTA cards using Chelexpower of exclusion (PE), typical paternity index (TPI), gene 100 method (Walsh et al. 1991). A total of 23 autosomal STR diversity (GD), and heterozygosity (He) were calculated using loci (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, the PowerStats software v1.2 (Promega, Madison, WI, USA) ['] (Tereba 1999), which was modified from Silva, et al. in order to D21S11, D18S51, Penta E, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, D6S1043, D10S1248, support and manage the large number of samples (Cabezas D1S1656, D12S391, D2S1338, and Penta D) were amplified Silva et al. 2016). Reynold's genetic distance, based on allele PCR frequencies across the 15 autosomal loci (D8S1179, D21S11, simultaneously using the VeriFiler[™] Express Amplification Kit (ThermoFisher SCIENTIFIC, MA, USA). The 297S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, autosomal STR loci satisfied the requirements of the ChineseD19S433, vWA, TPOX, D18S51, D5S818, and FGA) shared by National autosomal DNA database as well as expanded the included compared populations, and exact test p values Combined DNA Index System (Green et al. 2021). Multiplex were generated using PowerMarker v3.25. Nei's standard genamplification was conducted on a ProFlexTM PCR thermal etic distance between populations was calculated using the cycler (ThermoFisher SCIENTIFIC), following the manufac- allele frequencies by Phylip 3.69 package (Felsenstein 2009) turer's recommendations. Separation and detection were per- and visualised by MEGA v7.0.26 software (Kumar et al. 2016). formed using the Applied BiosystemsTM 3500 Genetic Finally, MDS analyses on the basis of Reynold's genetic



Figure 1. In this study, Panjin City was the area of sample collection (Map from Wikipedia, https://en.wikipedia.org/wiki/).

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, D1S1656, D12S391, D2S1338, 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 D8S1197, TH01, D12S391, and Penta D not in HWE (p < 0.05). However, when

Table 1. For ensic efficiency and statistical parameters of 23 autosomal STR loci in the Panjin Han population (n ½1263).

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Loci	MP	PD	PIC	PE T	'PI G	D He	e p		
D3S1358	0.1248	0.8752	0.6770	0.4933	1.9253	0.7247	0.7403	0.92	42
vWA	0.0701	0.9299	0.7707	0.6088	2.5671	0.8007	0.8052	1.000	0
D16S539	0.0784	0.9216	0.7560	0.5737	2.3389	0.7883	0.7862	0.83	52
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	0.251	0
D8S1179	0.0437	0.9563	0.8246	0.6738	3.1108	0.8438	0.8393	0.023	31
D21S11	0.0607	0.9393	0.7831	0.6043	2.5361	0.8071	0.8029	0.435	D
D18S51	0.0402	0.9598	0.8346	0.7239	3.6930	0.8513	0.8646	0.912	2
Penta E	0.0122	0.9878	0.9147	0.8105	5.3974	0.9204	0.9074	0.797	2
D2S441	0.0911	0.9089	0.7277	0.5327	2.1120	0.7625	0.7633 (0.0948	
D195433	0.0573	0.9427	0.7934	0.6677	3.050	7 0.8168	0.8361	0.36	B3
TH01	0.1743	0.8257	0.5960	0.3403	1.3849	0.6459	0.6390	0.014	В
FGA	0.0379	0.9621	0.8355	0.6862	3.2385	0.8517	0.8456	0.697	4
D22S1045	0.0960	0.9040	0.7282	2 0.5637	7 2.279	8 0.767	8 0.7807	' 0.23	82
D5S818	0.0881	0.9119	0.7373	0.5453	2.1776	0.7718	0.7704	0.1081	
D13S317	0.0687	0.9313	0.7743	0.6147	2.6095	0.8032	0.8084	0.950	8
D7S820	0.0793	0.9207	0.7491	0.5411	2.1553	0.7814	0.7680	0.877	Б
D6S1043	0.0303	0.9697	0.8590	0.7318	3.8042	2 0.8725	0.8686	0.28	02
D10S1248	0.1062	0.8938	0.7019	0.4947	1.9312	0.7416	0.7411 ().8362	
D1S1656	0.0546	0.9454	0.7942	0.6073	2.5567	0.8155	0.8044	0.82	85
D12S391	0.0474	0.9526	0.8178	0.6924	3.3063	0.8376	0.8488	0.01	2
D2S1338	0.0361	0.9639	0.8450	0.7081	3.4890	0.8604	0.8567	0.29	23
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	0.7803	0.7953	0.92	42
			1.111. 0.0	~	C 11		DIC	• .	

Note. MP: matching probability; PD: power of discrimination; PIC: polymorph ism 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).



Figure 2. Neighbor-joining phylogenetic tree based on the allele frequencies of 15 shared STRs between the Panjin Han and the other 21 populations.



Figure 3. MDS plot based on the Reynold's genetic distance between the Panjin Han and the other 21 populations.

a sequential Bonferroni's correction was applied, all 23 loci different STRs. Measuring the levels of linkage disequilibrium

3.3. Linkage equilibrium (LE)

Linkage disequilibrium (LD) indicates the association between qualitative random variables corresponding to alleles at

were found to be in Hardly-Weinberg equilibrium (Table 1). 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.

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3.4. Population comparison with the other populations

2016). Consequently, the Liaoning Han population has its own unique genetic characteristics that are different from In order to study the genetic structure of the Panjin Han Han populations from other provinces. Similarly, the Panjin population and its relationship with the other populations, Han population belongs to the northern Han with its own the Reynold's genetic distance was calculated based on the unique structure characteristics. 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. 4. Conclusions

et al. 2006; Yan et al. 2007, Montenus et al. 2011; 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 Panjin Han individuals using the VeriFilerTM Express PCR 2015a, 2015b; Xiao et al. 2016; Yao, Wang, et al. 2016; Yao, Amplification Kit. Population comparison was performed Xing, et al. 2016; Hongdan et al. 2017; Li et al. 2017; Xu, between the Panjin Han population and the other relevant Feng, et al. 2017; Xu, Xu, et al. 2017; He et al. 2018). The groups to further explore the structure of Panjin Han and its results showed that the Panjin Han had the nearest genetic relationship with the other groups. The results showed that distance with Liaoning Han (0.000289), followed by the Panjin Han belong to the subgroup of northern Han and its Heilongjiang Han (0.000325), Jilin Han (0.000325), Jiangsu have major differences with the other Chinese minority eth-Han (0.000377), Henan Han (0.000417), Hubei Han (0.000546) are major differences with the other Chinese minority eth-liangsi Han (0.000712). Shoopyi Han (0.000550), State are file populations and the fersion with 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 DongAuthor contributions

(0.003851), China Bouyei (0.005347), Japanese (0.005528),

China Miao (0.007613), China Uygur (0.008692), American Hongbo Wang and Jun Yao developed the idea. Hongbo Wang analysed (0.024125), Europeans (0.033369), and Africans (0.042489) he results and wrote the manuscript. Bao-jie Wang and Cairui Xin con-(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 Disclosure statement

Guangdong, Han (p < 0.0001) and Jiangxi Han ($p \frac{1}{4}0.0190$).No potential conflict of interest was reported by the author(s). Moreover, the Panjin Han population was significantly differ-

ent to the other ethnic groups in China (Bouyei, Dong, Hui, Miao, Tibetan, and Uygur) and the foreign ethnic groups Funding

(Japanese, Korean, African, American, and European). The phylogenetic neighbor-joining (N-J) tree was generated toured in this article.

reflect the historical and geographical background of the compared populations (Figure 2). The N-J tree exhibited sig-

nificant differences between the Han population and the ORCID

other minority groups. We also observed variations between Hongbo Wanging http://orcid.org/0000-0002-5030-5529

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 studyReferences

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 Cabezas Silva R, Ribeiro T, Lucas I, Porto MJ, Costa Santos J, Dario P. 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 Chen JG, Pu HW, Chen Y, Chen HJ, Ma R, Xie ST, Zhang LP. 2012. other Han groups were gathered together. The Chinese ethnic minorities, Korean and Japanese populations were scat_{Chen L}, Duan L, Yuan L, Shen Z, He W, Zhai D, Huang Y, Xu B. 2017. tered around the periphery. Additionally, the distribution of Genetic polymorphisms of 19 autosomal STR loci in the China African, American, and European populations was more dis- Burmese immigrants. Forensic Sci Int Genet. 31:e46-e47. crete. The location of the compared populations in the MDS Peng YJ, Zhu BF, Shen CM, Wang HD, Huang JF, Li YZ, Qin HX, et al. plot was consistent with the results in the N-J tree. Geographically, Panjin city is located in Liaoning Province.

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