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SHORT COMMUNICATION

Development of 11 X-STR loci typing system and genetic analysis in Tibetan and Northern Han populations from China

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Abstract X-chromosomal short tandem repeats (X-STRs) loci are used for forensic practice in recent years which play increasingly important roles in some complex kinship cases. In this paper, a new multiplex polymerase chain reaction (PCR) system which can simultaneously analyze 11 X-STR markers (DXS8378, DXS6795, DXS7132, DXS6803, DXS9898, DXS6801, DXS7133, GATA165B12, HPRTB, DXS8377 and DXS7423) was developed. The samples of 1,605 (742 males and 863 females) unrelated individuals from Tibetan and Northern Han population were successfully analyzed using this multiplex system. A total of 103 alleles for all the loci were observed. Hardy–Weinberg equilibrium tests demonstrated no significant deviation from expected values ($P>0.05$) for all of the 11 X-STR loci in the two studied populations. Polymorphism information contents of the loci were 0.3864–0.9013, and powers of discrimination in females of the loci were 0.6317–0.9845. There were no statistically significant differences between Tibetan and Northern Han populations in allele distribution of the 11 X-STR loci, in line with analysis of molecular variance (AMOVA) results. Our work indicates that this multiplex system is useful for forensic analysis for the two populations in China.

Keywords X-STR · Multiplex PCR · Population · Northern Han · Tibetan

Introduction

Autosomal STRs and Y chromosome STRs have been widely applied in forensic DNA analysis in the past decade. However, in recent years, it is increasingly acknowledged that the application of X chromosome STRs (X-STRs) plays an important role in some complex kinship cases, such as deficiency paternity cases when the disputed child is female, and relationships between putative half-sisters sibling status having the same biological father or paternal grandmother and granddaughter [1, 2]. Population data of X-STRs have been frequently reported, and some reports have presented preliminary panels [3–8]. In this work, we report a new multiplex polymerase chain reaction (PCR) system for the simultaneous analysis of 11 X-STR markers (DXS8378, DXS6795, DXS7132, DXS6803, DXS9898, DXS6801, DXS7133, GATA165B12, HPRTB, DXS8377 and DXS7423), and then investigated the allele frequencies of these loci in Tibetan and Northern Han populations in China.

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Materials and methods

Populations and samples

In this study, 1,605 unrelated individuals were recruited, comprising of 941 Northern Han Chinese individuals (392 males and 549 females) and 664 Chinese Tibetan individuals (350 males and 314 females). Blood samples were obtained from all volunteers, who provided written informed consent prior to their participation in this study. Genomic DNA was extracted using the Chelex-100 protocol as described by Walsh et al. [9].

PCR amplification and typing

PCR Amplification was carried out in a 10-μl PCR reaction volume containing 1–5 ng DNA, 300 μM for each dNTP, 1× GeneAmp PCR Buffer II, 1.5 mM $MgCl_2$, 0.75U AmpliTaq Gold™ DNA polymerase (Applied Biosystems, Foster City, CA, USA). The primer concentrations and detailed information are presented in Table 1. Samples were amplified in GeneAmp PCR System 9700 for 11 min at 94°C followed by 38 cycles of 30 s at 94°C, 50 s at 60°C and 90 s at 72°C, with a final extension cycle of 60 min at 60°C.

The amplified products were separated by capillary electrophoresis on AB PRISM 3730 Genetic Analyzer (Applied Biosystems) according to the manufacture's recommended protocols using filter set G5 and POP4 polymer (Applied Biosystems). The sample run data were analyzed using GeneMapper ID v3.2 software. Allele typing was based on home-made allelic ladder, and 9947A (Promega, Madison, WI, USA) cell lines DNA were typed for calibrating allelic ladder.

Sensitivity testing

9947A (10 ng/μl) with known X-STR genotypes was prepared for the sensitivity experiment which was diluted with quantities of 5, 1, 0.5, 0.25, and 0.125 ng, and each level of DNA was amplified with the multiplex system, respectively.

Sequence analysis

PCR products were purified and sequenced on AB 3130 Genetic Analyzer using a BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions.

Table 1 Information of primer of the11 X-STR loci

Marker	UniSTS id[a]	Chromosomal location[b]	Physical location[b] (Mb)	Primer sequences(5′→3′)	Amount (μM)	Dye
DXS8378	24155	Xp22.31	9.330	AGCGAAACTCCAACTCAA GACAGTTCACCCCTCCTT	0.15	NED
DXS6795	75073	Xp22.11	23.524	TCCATCCCCTAAACCTCT GTTTGACATGGCTTTCTT	0.15	VIC
DXS7132	13344	Centromere	64.572	GCCAGTTCTAAAAATCTTCCA TGTGAGCCCATTTTCATAATA	0.2	JOE
DXS6803	41731	Xq21.2	86.318	ACAGGCAAATGAAAACTAT AATGTGCTTTGACAGGAA	0.2	NED
DXS9898	34403	Xq21.31	87.682	TAGCAAATTAAGTAACCTCC GACAACATTAGGCTCACC	0.15	FAM
DXS6801	16147	Xq21.32	92.378	CTACTCCTGGTATCAAAATCT ACTCTGTTGCCCTCACTAT	0.15	FAM
DXS7133	50709	Xq22.3	108.928	ATACCTGCTTTAGTGTTGAG TGGTCTGCTTTCTTCTGT	0.2	VIC
GATA165B12	63887	Xq25	120.706	TATCATCAATCATCTATCCGTA CATTTTCACTGTGTATGCTTTA	0.4	FAM
HPRTB	511805	Xq26.2	133.443	TGTCTCATGTAAGAGGGCAGTA TCAATAAATAGGAGAAGGGCAT	0.15	NED
DXS8377	154756	Xq28	149.310	ATTATCACCCCGTGCTTGC TGTTCGTATGGACCTTTGG	0.25	FAM
DXS7423	99583	Xq28	149.460	CACATAGTAGGTGCCCAAAA AAGTCTTCCTGTCATCTCCC	0.25	JOE

[a] Data obtained from NCBI (http://www.ncbi.nlm.nih.gov/sites/entrez)

[b] Data obtained from ChrX-STR.org 2.0 (http://www.chrx-str.org/)

Statistical analysis

Allelic frequencies of 11 X-STRs loci were calculated by single counting method. Hardy–Weinberg equilibrium in females and exact test of pairwise linkage disequilibrium were performed with the software Arlequin 3.1 [10]. Test size α was equal to 0.05; P value was corrected by Bonferroni adjustment, and $P<0.0009$ (0.05/55, 55 = number of comparisons performed) was considered statistically significant in the exact test. Forensic parameters were computed based on allelic frequencies as follows: power of discrimination in females (PD_F), power of discrimination in males (PD_M), mean exclusion chance in trios involving daughters (MEC_T), mean exclusion chance in father daughter duos (MEC_D), polymorphism information content (PIC) [11–14]. Analysis of molecular variance (AMOVA) of the 11 X-STR loci in the Tibetan and Northern Han populations was done with GenAlEx v6.3 software (http://www.anu.edu.au/BoZo/GenAlEx/) [15].

Results

A new 11plex genotyping system for 11 X-STR markers including DXS8378, DXS6795, DXS7132, DXS6803, DXS9898, DXS6801, DXS7133, GATA165B12, HPRTB, DXS8377 and DXS7423 was successfully developed in this study (Fig. S1). Sensitivity testing revealed that DNA of 5, 1, 0.5, 0.25 and 0.125 ng could be amplified with satisfactory results, using this 11plex genotyping system. The results of 9947A control DNA were consistent with the report of Szibor et al. [16]. Sequence analysis of DXS6795 revealed a complex repeat motif and the genotype of 9947A DNA were "12, 13", which have not been reported previously.

All samples were genotyped with the 11plex X-STR genotyping system. Hardy–Weinberg equilibrium tests demonstrated no significant deviation from expected values ($P>0.05$) for all 11 X-STR loci in the Tibetan and Northern Han populations in China. Allele frequencies and forensic parameters of the 11 X-STR loci in the Tibetan and Northern Han populations are shown in Table S1. Of the 11 X-STR loci, DXS8377 shows the highest forensic efficiency, with PD_F of 0.9841 and 0.9845 in the Northern Han and Tibetan, respectively. Contrarily, DXS7133 shows the lowest forensic efficiency, with PD_F of 0.6317 and 0.6539 in the Northern Han and Tibetan populations, respectively.

There were no statistically significant differences between Tibetan and Northern Han populations in allele distribution of the 11 X-STR loci, in line with AMOVA results between the two populations, which indicated that genetic variation within the two populations represents only 1% of the total genetic diversity. Linkage disequilibrium for all pairs of loci was tested in the two studied populations (Tables S2 and S3). After 55 pairwise comparisons were performed in the population, no obvious evidence for LDE among the 11 loci was found with the significant threshold P value of 0.0009 (by Bonferroni adjustment).

Discussion

Han Chinese can be classified as Northern Han, Central Han and Southern Han, and Tibetan populations are more close to Northern Han Chinese from the perspective of human evolution [17–19]. In this study, Han Chinese samples were collected from four different provinces, Gansu, Henan, Shandong and Shanxi province, which were classed into Northern Han Chinese. AMOVA results and allele distribution of 11 X-STR loci among the four geographic populations and that of between Tibetan and Northern Han populations were consistent with those of a previous study [19]. Evaluation of forensic efficiency of the new 11plex X-STR genotyping system indicated that it is a powerful forensic analysis tool in at least the two studied populations.

In this work, data for the DXS6803, GATA165B12, DXS7133, DXS6795, DXS6801 and DXS9898 in the Tibetan and Northern Han populations are reported for the first time. Allele frequencies of Northern Han populations were compared to available data for the same markers in other populations including Guangdong Han [8], Sichuan Han [20], Zhejiang Han [21], Uigur [8], Mongolia [8], Japanese [22], Ghana [23], Algerian [24], Polish [25] and Northern Italy [5] (Table S4). Three alleles which were not included in the Investigator Argus X-12 Kit (Qiagen, Germany) ladder were obtained including allele 8 of DXS8378 and allele 11 and 12 of DXS7423. Compared with Southern Han population [8], allele 14 for DXS8378 and allele 13.2 for HPRTB were not found; however, allele 40 for DXS8377 and allele 9 for HPRTB were observed.

Concerning linkage analysis, a higher linkage disequilibrium would be expected between X-linked markers in comparison to autosomal markers because they have less chance to recombine (only in female meiosis).The exact test for linkage disequilibrium showed that exact P value below 0.0009 was not found, indicating that there is no LDE among the 11 loci in the two studied populations from China, although two (DXS8377 and DXS7423) of the 11 loci was in a same linkage group of X chromosome (www.chrx-str.org). Interestingly, a recently published study [20] also indicated that no evidence for LDE was found among the eight X-STR loci included in Mentype Argus X-8 PCR amplification kit (Biotype AG, Germany) in the Chinese population living in Sichuan province, although the eight loci had been classified into four different linkage groups on X chromosome. Similar with their results, our work also implied that allele frequencies rather than haplotype

frequencies should be applied for forensic practice at least in the two studied Chinese populations.

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