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SHORT COMMUNICATION

Genetic diversities of 21 non-CODIS autosomal STRs of a Chinese Tibetan ethnic minority group in Lhasa

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Abstract In the present study, we investigated 21 short tandem repeat (STR) loci (D6S474, D12ATA63, D22S1045, D10S1248, D1S1677, D11S4463, D1S1627, D3S4529, D2S441, D6S1017, D4S2408, D19S433, D17S1301, D1GATA113, D18S853, D20S482, D14S1434, D9S1122, D2S1776, D10S1435, D5S2500), which are not included in the Combined DNA Index System and Amelogenin locus in 104 randomly selected healthy autochthonous individuals from the Tibetan ethnic minority group residing in the Lhasa region, Tibet Autonomous Region of China. Allelic frequencies, common forensic statistical parameters, and the Hardy–Weinberg equilibrium in this population were calculated with a modified PowerState V12.xls. A total of 143 alleles were found in the Tibetan group with corresponding allelic frequencies ranging from 0.005 to 0.582. The observed heterozygosity, the expected heterozygosity, the power of discrimination, the power of exclusion, and the polymorphic information content ranged from 0.615 to 0.817, 0.559 to 0.787, 0.727 to 0.926, 0.310 to 0.632, and 0.488 to 0.760, respectively. Chi-square tests of the observed genotype frequencies and expected genotype frequencies in the samples showed no departure from the Hardy–Weinberg equilibrium at all loci except for D5S2500. Our results demonstrate that these 21 STRs are highly polymorphic and suitable for anthropological research, population genetics, and forensic paternity testing and human individual identification in this region, and can enrich Chinese ethnical genetic informational resources.

Keywords Short tandem repeat (STR) · Chinese Tibetan ethnic minority group · Genetic polymorphisms · AGCU 21+1 STR kit

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Introduction

Short tandem repeat (STR) is one of the more ideal DNA genetic markers because of its numerous alleles, highly genetic polymorphisms, and stable heredity in the human genome. Moreover, STR is suitable for analyzing degraded, outmoded, and minute amounts of human DNA samples [1, 2]. As a result, STR typing using multiplex polymerase chain reaction (PCR) has become the standard technique in routine forensic application [3]. In the recent years, more and more Y-chromosomal and X-chromosomal and autosomal STRs were extensively explored in the population genetics and forensic application. In the People's Republic of China, autosomal STRs are widely used in forensic DNA database with commercially available multiplexes of STRs genotyping kits such as the AmpFℓSTR Identifiler PCR kit (Applied Biosystems, Foster City, CA, USA), PowerPlex 16 System Kit (Promega, Madison, WI, USA), and AGCU 17+1 fluorescence testing kit (AGCU ScienTech Incorporation, Wuxi, Jiangsu, China); however, additional new autosomal STRs can provide more highly polymorphic markers and more valuable information for human individual identification, paternity testing, forensic DNA database, and population genetics. In the present study, we investigated the distributions of allelic frequencies and forensic statistical parameters of new 21 STRs loci in a Chinese Tibetan ethnic group living in Lhasa city, China, using a new STR multiplex PCR system.

Material and methods

Sample collection and DNA extraction

All the participants provided their written informed consent before the sample collection and the subsequent analysis, and the investigation was conducted in accordance with the humane and ethical research principles of Xi'an Jiaotong University, Xi'an, Shaanxi, People's Republic of China. One hundred and four unrelated healthy individuals were randomly chosen from the Tibetan ethnic minority group living in the Lhasa area. All participants were randomly chosen among individuals whose ancestors had lived in the region for at least three generations, and they were also interviewed to ensure no individuals share common ancestry. Genomic DNA was extracted using the Chelex-100 protocol as described by Walsh et al. [4].

PCR amplification and STR typing

The amplification of 21 autosomal STR loci, namely D6S474, D12ATA63, D22S1045, D10S1248, D1S1677, D11S4463, D1S1627, D3S4529, D2S441, D6S1017, D4S2408, D19S433, D17S1301, D1GATA113, D18S853, D20S482, D14S1434, D9S1122, D2S1776, D10S1435, D5S2500 loci, and Amelogenin locus were performed in a single PCR system using the AGCU 21+1 fluorescence amplification reagents (AGCU ScienTech Incorporation, Wuxi, Jiangsu, China). Multiplex PCR of the AGCU 21+1 amplification reagent was performed using 0.5–2 ng of genomic DNA in a 25-μl volume, including a 1-μl template DNA, 10 μl of Reaction Mix, 5 μl of Primers 21+1 primer set, 0.5 μl of HS-Taq G-Taq DNA polymerase, and 8.5 μl of ddH_2O. The PCR thermocycling condition was pre-incubation at 95°C for 11 min, followed by ten cycles at 94°C for 1 min, 62°C for 1 min, 72°C for 1 min; subsequently, 20 cycles at 90°C for 1 min, 60°C for 1 min, 72°C for 1 min, and a final extension at 60°C for 60 min. All amplifications were done on a GeneAmp PCR 9700 (Applied Biosystems, Foster City, CA, USA) thermal cycler.

One microliter of PCR product or 21+1 allelic ladder was mixed with 12 μl of Hi-Di Formamide and 0.5 μl of AGCU Marker SIZ-500 internal lane standard (AGCU ScienTech Incorporation, Wuxi, Jiangsu, China) denatured at 95°C for 3 min, followed by chilling on ice for 3 min immediately. The separation, detection, and genotyping of all PCR products were accomplished using an ABI3130XL DNA Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Allele designations were determined by comparing the sample PCR fragments with the allelic ladders provided with the kit using a genotype analysis software.

The alleles of all STR loci were named according to the number of repeat units present as recommended by the DNA Commission of the Society for Forensic Genetics [5]. The control DNA (9947A) was genotyped as standard reference in all experiments. All experimental steps were carried out according to the laboratory internal control standards and kit controls.

Statistical analysis

Allelic frequencies, forensic parameters, including the observed heterozygosity, expected heterozygosity, power of discrimination, polymorphism information content, matching probability, probability of paternity exclusion, and probability values of the Hardy–Weinberg equilibrium of the 21 STR loci were computed using the modified PowerStat (version 1.2) spreadsheet (Promega, Madison, WI, USA) as described previously [6]. The R×C contingency test was employed for pairwise interpopulation comparisons using the SPSS 13.0 [7]. Linkage disequilibrium (LD) tests for marker pairs comprising of each two STR loci were estimated by Genepop version 4.0.10 (http://genepop.curtin.edu.au/).

Table 1 Allelic frequencies and forensic statistical parameters regarding 21 STR loci of Chinese Tibetan ethnic group in Lhasa city

Allele	D17S1301	D18S853	D19S433	D1GATA113	D1S1627	D10S1248	D10S1435	D11S4463	D12ATA63	D14S1434	D20S482	D22S1045	D2S1776	D2S441	D3S4529	D5S2500	D6S1017	D6S474	D9S1122	D4S2408	D1S1677
7				0.457																	
8				0.034		0.005	0.005										0.250			0.207	
9	0.048									0.005			0.168	0.010					0.005	0.327	
10	0.024	0.010		0.019	0.048		0.010			0.077	0.014		0.067	0.264			0.236		0.067	0.361	0.029
11	0.245	0.466		0.188			0.144	0.005	0.005	0.082		0.375	0.264	0.341			0.048		0.130	0.077	
12	0.481	0.096	0.038	0.284	0.053	0.077	0.346	0.077	0.260	0.019	0.063		0.365	0.125	0.005		0.346		0.298	0.029	0.038
12.2			0.014																		
13	0.178	0.231	0.245	0.019	0.582	0.380	0.260	0.192	0.005	0.322	0.250		0.106	0.053	0.322		0.101		0.409		0.111
13.2			0.029																		
14	0.024	0.159	0.341		0.313	0.183	0.221	0.269	0.048	0.442	0.476	0.019	0.029	0.202	0.168	0.351	0.005	0.385	0.058		0.466
14.2			0.120																		
15		0.038	0.072			0.202	0.005	0.327	0.053	0.048	0.154	0.178		0.005	0.293		0.014	0.370	0.019		0.308
15.2			0.115																		
16			0.005		0.005	0.144	0.010	0.115	0.077	0.005	0.043	0.269			0.163			0.125	0.014		0.048
16.2			0.019																		
17						0.010		0.014	0.495			0.135			0.048	0.322		0.091			
18									0.053			0.019				0.216		0.024			
19									0.005			0.005						0.005			
20																0.053					
21																0.010					
23																0.043					
24																0.005					
MP	0.170	0.141	0.074	0.176	0.273	0.100	0.116	0.105	0.165	0.164	0.143	0.117	0.102	0.106	0.117	0.115	0.114	0.142	0.117	0.138	0.165
DP	0.830	0.859	0.926	0.824	0.727	0.900	0.884	0.895	0.835	0.836	0.857	0.883	0.898	0.894	0.883	0.885	0.886	0.858	0.883	0.862	0.835
PIC	0.625	0.650	0.760	0.619	0.488	0.719	0.698	0.727	0.632	0.635	0.636	0.693	0.714	0.710	0.711	0.672	0.708	0.636	0.676	0.662	0.619
EPP	0.493	0.431	0.560	0.526	0.310	0.543	0.493	0.632	0.477	0.446	0.388	0.374	0.526	0.477	0.560	0.322	0.560	0.402	0.374	0.388	0.334
HE	0.674	0.693	0.787	0.674	0.559	0.755	0.714	0.764	0.673	0.685	0.681	0.736	0.752	0.755	0.753	0.721	0.749	0.690	0.719	0.714	0.671
HO	0.740	0.702	0.779	0.760	0.615	0.769	0.740	0.817	0.731	0.712	0.673	0.663	0.760	0.731	0.779	0.625	0.779	0.683	0.663	0.673	0.635
P	0.166	0.906	0.755	0.072	0.268	0.797	0.887	0.233	0.239	0.613	0.800	0.075	0.919	0.518	0.595	0.022	0.542	0.806	0.180	0.319	0.389

MP matching probability, *EPP* power of exclusion, *DP* power of discrimination, *PIC* polymorphism information content, *HE* expected heterozygosity, *HO* observed heterozygosity, *p* probability values of exact tests for the Hardy–Weinberg equilibrium

Results and discussion

The distributions of allelic frequencies and forensic statistical parameters in the Tibetan population were presented in Table 1 (Excel version in supplementary Table 1). A sample STR profile and the position information of 21 non-Combined DNA Index System (CODIS) autosomal STRs was displayed in supplementary Figure 1 and Table 2, respectively.

LD tests show that some STRs were located on the same chromosome; linkage disequilibrium between these loci was not observed. Ten deviations, including D18S853/D14S1434 (p=0.0429), D18S853/D1GATA11 (p=0.0103), D18S853/D1S1627 (p=0.0334), D10S1435/D20S482 (p=0.0489), D14S1434/D2S1776 (p=0.0019), D3S4529/D11S4463 (p=0.0229), D3S4529/D19S433 (p=0.0391), D1GATA11/D4S2408 (p=0.0271), D17S1301/D1S1677 (p=0.0387), and D6S1017/D1S1677 (p=0.0410) were detected in 210 pairwise comparisons with p values below the nominal 0.05 threshold. Three pairs of LD are due to the only D18S853 locus, so this locus may be removed from the panel in the future study and other STR results acceptable for forensic data.

An exact test indicated that the agreement with the Hardy–Weinberg equilibrium (p>0.05) was detected at 20 STR loci in the population except for D5S2500 (p=0.022). A total of 143 alleles at these 21 STR loci were found with corresponding allelic frequencies ranging from 0.005 to 0.582 in the Tibetan population. All STR loci showed a high degree of genetic polymorphism, as evidenced by the high values of common forensic parameters listed in Table 1. The values of the observed heterozygosity, the expected heterozygosity, the power of discrimination, the power of exclusion, the matching probability, and the polymorphic information are from 0.615 to 0.817, 0.559 to 0.787, 0.727 to 0.926, 0.310 to 0.632, 0.074 to 0.273, and 0.488 to 0.760, respectively. The values of combined probability of exclusion, power of discrimination, and probability of matching for all 21 STR loci are 0.999998063, 0.9999999999999999999723, and 2.9696×10^{-19}, respectively.

We also compared our data with previously published data from other ethnic groups at the D19S433 locus using the method of the R×C contingency test. The results showed no significant differences between our studied population and the Chinese Mongolian [8], Sherpa and Kathmandu population [9], Tibetan population described by Yan et al. [10], Chinese Dongxiang and Salar [11], Tu [12], Maonan [13], Ewenki [14], Yi [15], Uigur [16], Minnan Han [17], and Xi'an Han [18]. The only statistically significant difference (p=0.045) was found between the studied population and Guangdong Han [19] at the D19S433 locus.

In conclusion, our study demonstrated that the 21 STR loci are highly discriminating and polymorphic. Additional 21 new autosomal STRs, together with the classical STRs of CODIS will obtain additional information for routine forensic casework, especially complex kinship analysis, cases of suspected mutation, paternity testing in deficiency cases or within related individuals [20]. In the future, it can be expected that non-CODIS STR profiles of more Chinese populations, which can further our understanding of the Chinese population genetics and provide valuable information for the paternity testing and human individual identification in forensic science, will be studied and reported.

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