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Report:

Allelic frequency distributions of 21 non-combined DNA index system STR loci in a Russian ethnic minority group from Inner Mongolia, China*

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Abstract: We studied the allelic frequency distributions and statistical forensic parameters of 21 new short tandem repeat (STR) loci and the amelogenin locus, which are not included in the combined DNA index system (CODIS), in a Russian ethnic minority group from the Inner Mongolia Autonomous Region, China. A total of 114 bloodstain samples from unrelated individuals were extracted and co-amplified with four fluorescence-labeled primers in a multiplex polymerase chain reaction (PCR) system. Using capillary electrophoresis, the PCR products of the 21 STR loci were separated and genotyped. A total of 161 alleles were observed in the Russian ethnic minority group, and corresponding allelic frequencies ranged from 0.0044 to 0.5965. The 21 non-CODIS STR loci of the Russian ethnic minority group were characterized by high genetic diversity and therefore may be useful for elucidating the population's genetic background, for individual identification, and for paternity testing in forensic practice.

1 Introduction

As common polymorphic DNA genetic markers, short tandem repeats (STRs) have applications in many areas including population genetics studies, allogeneic bone marrow transplantation engraftment

diagnostic tests, and individual discrimination in forensic science (Hammond *et al.*, 1994; Zhu *et al.*, 2005). The AmpFLSTR® Identifiler® polymerase chain reaction (PCR) kit (Applied Biosystems by Life Technologies, USA) and the PowerPlex® 16 System kit (Promega, USA) are widely employed in forensic science and anthropological research. Both of these multiplex systems include 13 core STR loci designated by the combined DNA index system (CODIS) and can meet the general requirements for forensic identification. However, in paternity identification cases without mother or father samples, or those with STR loci mutation events, more STR loci are needed.

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In this study, we used an AGCU 21+1 fluorescence amplification assay kit (Zhongde-Meilian ScienTech Inc., China) to investigate the allelic frequencies of 21 non-CODIS autosomal STR loci (D1GATA113, D1S1627, D1S1677, D2S441, D2S1776, D3S4529, D4S2408, D5S2500, D6S474, D6S1017, D9S1122, D10S1248, D10S1435, D11S4463, D12ATA63, D14S1434, D17S1301, D18S853, D19S433, D20S482, and D22S1045) in 114 unrelated healthy Russian individuals residing in northeastern Inner Mongolia, China.

The Russian ethnic group exists mainly in Europe and is one of the largest groups in the world. After the Russian Revolution in 1917, many Russians belonging to the White Army moved to China. Nowadays, this Russian minority is an ethnic group officially recognized by China. About 15 600 people of the minority live mostly in Inner Mongolia Province, and others in the northern provinces of Heilongjiang and Xinjiang.

We studied the genetic polymorphism of a new panel of STR markers (21 STR loci) which are different from the 13 core STR loci of CODIS. We evaluated, in this Russian population, their detection effectiveness for forensic testing and anthropological research and calculated forensic statistical parameters of the 21 new STR loci. Some researchers have studied the characteristics and genetic diversity of 15 STR loci, including the 13 core STR loci from CODIS, in the Russian population residing in China (Lu *et al.*, 2005; 2006; Zhu *et al.*, 2010). Therefore, we also present a comparison between the forensic parameters of the previous study (13 CODIS loci) and those of the present study (21 new non-CODIS loci).

2 Materials and methods

2.1 Sample collection and DNA extraction

We randomly selected 114 healthy unrelated individuals from the Russian population residing in northeastern Inner Mongolia, China, following the guidelines of the human and ethical research principles of School of Medicine, Xi'an Jiaotong University, China. Before sample collection, all the participants were asked to give their informed written consent and to complete the required questionnaires under the guidance of medical staff assistants. Indi-

viduals whose ancestors had been living in the region for more than three generations were selected from among participants and individuals having no common ancestry tracing back more than three generations were selected for the study. Genomic DNA was extracted using the Chelex-100 procedure from bloodstain samples as described by Walsh *et al.* (1991).

2.2 PCR amplification and STR typing

We used the AGCU 21+1 fluorescence amplification assay kit (Zhongde-Meilian ScienTech Inc., China) to co-amplify the 21 autosomal STR loci and the Amelogenin locus in a multiplex PCR system. Multiplex PCR was performed in a 25-µl volume consisting of 0.5-2.0 ng of genomic DNA, 0.5 µl HS-Taq DNA polymerase, 5 µl 21+1 primer sets, 10 μl reaction mix, and 8.5 μl double-distilled water (ddH₂O). PCR thermocycling was programmed under the following conditions: (1) an initial hot start at 95 °C for 11 min; (2) 10 cycles of 60 s at 94 °C, 60 s at 62 °C, and 60 s at 72 °C; (3) 20 cycles of 90 °C for 60 s, 60 s at 60 °C, and 60 s at 72 °C; (4) a final extension of 60 min at 60 °C. The whole process was performed on a GeneAmp PCR 9600 (Applied Biosystems by Life Technologies, USA) thermal cycler. The mixture for capillary electrophoresis included 1 μl PCR product, 0.5 μl AGCU Marker SIZ-500 internal lane standard (Zhongde-Meilian ScienTech Inc., China), and 12 µl Hi-Di formamide. Before capillary electrophoresis, the mixture was denatured at 95 °C for 3 min, and then immediately chilled on ice for 3 min. Electrophoretic separation and allelic detection of all the PCR-STR products were achieved using an ABI3130XL DNA genetic analyzer (Applied Biosystems by Life Technologies, USA), according to the manufacturer's instructions. Allelic identification was determined by comparing the sample PCR-STR fragments with the AGCU Marker SIZ-500 internal lane standard and the allelic ladders provided by the kit, using GeneMapper ID V4.0 software. The alleles of all STR loci were named using the number of repeat units, as recommended by the DNA Commission of the Society for Forensic Genetics (Bär et al., 1997). Positive control DNA (9947A) included in the kit and negative control (ddH₂O) were genotyped for quality control in all experiments. All experimental steps were performed according to the experimental control standards of the Laboratory.

2.3 Statistical analysis

Allelic frequencies, forensic parameters including the observed heterozygosity (HO), expected heterozygosity (HE), typical paternity index (PI), matching probability (MP), power of discrimination (PD), polymorphism information content (PIC), probability of paternity exclusion (PE), and Hardy-Weinberg equilibrium tests of these 21 STR loci were computed using the modified PowerStat (Version 1.2) spreadsheet (Promega, USA) as described by Tereba (1999). Locus-by-locus allelic frequencies were compared between our population data and those of previously published studies using the analysis of molecular variance method (AMOVA; based on F-statistics) in GENEPOP software V4.0.10 (http:// genepop.curtin.edu.au/), which was also used to estimate the linkage disequilibrium (LD) tests for each two STR loci.

3 Results and discussion

Clear gene typing profiles of the 21 autosomal STRs were obtained from all 114 samples from healthy unrelated Russian individuals in northeastern Inner Mongolia, China. The allelic frequency data and the statistical forensic parameters of the 21 autosomal STRs, based on the genotypes of the 114 samples, are listed in Table 1. For the 21 STR loci examined, the combined probability of exclusion was 0.999 999 377, the total power of discrimination was 0.999 999 999 999 999 963 546, and the combined probability of matching was 3.6454×10⁻²⁰. The parameters of these new loci were compared with those of the 13 core STR loci from CODIS from the same Russian population (Zhu et al., 2010). The heterozygosity parameters of most of the 21 STR markers are comparable to those of the CODIS loci. Only the

Table 1 Allele frequencies and statistical parameters of 21 STR loci from Russian population of northeastern Inner Mongolia, China (n=114)

CTD 1:		Allele frequency												
STR loci	4	5	7	8	9	10	11	11.3	12	12.2	13	13.2		
D6S474														
D12ATA63									0.2939		0.0175			
D22S1045							0.0263		0.1754		0.0044			
D10S1248							0.0044		0.0833		0.3509			
D1S1677							0.0044		0.0263		0.1009			
D11S4463							0.0044		0.0526		0.2763			
D1S1627						0.0526	0.0219	0.0658	0.0658		0.5965			
D3S4529									0.0044		0.1579			
D2S441				0.0044	0.0219	0.2018	0.4167	0.0219	0.1754		0.0219			
D6S1017			0.0044	0.2412	0.0307	0.3860	0.0614		0.2237		0.0439			
D4S2408			0.0044	0.1930	0.2632	0.3377	0.1711		0.0307					
D19S433						0.0044			0.0526	0.0132	0.3070	0.0219		
D17S1301				0.0088	0.0175	0.0526	0.1886		0.4430		0.2237			
D1GATA113			0.4386	0.0132		0.0088	0.1535		0.3246		0.0614			
D18S853						0.0044	0.4079		0.0746		0.2105			
D20S482						0.0395	0.0088		0.0482		0.2763			
D14S1434						0.0789	0.1535		0.0570		0.2456			
D9S1122					0.0088	0.0746	0.1754		0.3421		0.3421			
D2S1776				0.0088	0.1009	0.0921	0.2982		0.3553		0.1184			
D10S1435	0.0044	0.0044		0.0132		0.0482	0.1404		0.3991		0.2719			
D5S2500														

To be continued

Table 1

CTD looi	Allele frequency											
STR loci	13.3	14	14.2	14.3	15	15.2	16	16.2	17	17.2	18	19
D6S474		0.4035		0.0044	0.2632		0.1579		0.1447		0.0263	
D12ATA63		0.0395			0.0175		0.1623		0.3860		0.0746	0.0044
D22S1045					0.0746		0.2632		0.2763		0.1579	0.0219
D10S1248		0.2719			0.2061		0.0658		0.0175			
D1S1677		0.4912			0.2807		0.0789		0.0044		0.0132	
D11S4463		0.2982			0.2237		0.1272		0.0175			
D1S1627		0.2544			0.0088							
D3S4529		0.2895			0.3333		0.1579		0.0570			
D2S441	0.0044	0.1140			0.0175							
D6S1017		0.0088										
D4S2408												
D19S433		0.2237	0.0921		0.0789	0.1272	0.0132	0.0482		0.0175		
D17S1301		0.0658										
D1GATA113												
D18S853		0.2325			0.0702							
D20S482		0.3596			0.1974		0.0702					
D14S1434		0.3640			0.0833		0.0175					
D9S1122		0.0482			0.0044				0.0044			
D2S1776		0.0219			0.0044							
D10S1435	0.0132	0.0877			0.0175							
D5S2500		0.4605							0.2544		0.2018	0.0044
D352300		0.4005							0.20		0.2010	0.0011
			equency		MP	PD	PIC	DE		НО		
STR loci	20		requency 23	24	- MP	PD	PIC	PE	PI	НО	HE	Р
		Allele fr		24	0.1248	0.8752	0.6761	0.3786	PI 1.5000	HO 0.6667		P 0.1668
STR loci D6S474 D12ATA63	20	Allele fr		24	0.1248 0.1079	0.8752 0.8921	0.6761 0.6884	0.3786 0.5023	PI 1.5000 1.9655	0.6667 0.7456	HE 0.7213 0.7306	P 0.1668 0.7751
STR loci D6S474 D12ATA63 D22S1045		Allele fr		24	0.1248 0.1079 0.0786	0.8752 0.8921 0.9214	0.6761 0.6884 0.7609	0.3786 0.5023 0.4447	PI 1.5000 1.9655 1.7273	0.6667 0.7456 0.7105	HE 0.7213 0.7306 0.7919	P 0.1668 0.7751 0.0246
STR loci D6S474 D12ATA63 D22S1045 D10S1248		Allele fr		24	0.1248 0.1079 0.0786 0.1167	0.8752 0.8921 0.9214 0.8833	0.6761 0.6884 0.7609 0.7083	0.3786 0.5023 0.4447 0.6287	PI 1.5000 1.9655 1.7273 2.7143	0.6667 0.7456 0.7105 0.8158	HE 0.7213 0.7306 0.7919 0.7488	P 0.1668 0.7751 0.0246 0.1155
STR loci D6S474 D12ATA63 D22S1045 D10S1248 D1S1677		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560	0.8752 0.8921 0.9214 0.8833 0.8440	0.6761 0.6884 0.7609 0.7083 0.6133	0.3786 0.5023 0.4447 0.6287 0.2972	PI 1.5000 1.9655 1.7273 2.7143 1.2667	0.6667 0.7456 0.7105 0.8158 0.6053	HE 0.7213 0.7306 0.7919 0.7488 0.6626	P 0.1668 0.7751 0.0246 0.1155 0.1728
STR loci D6S474 D12ATA63 D22S1045 D10S1248		Allele fr		24	0.1248 0.1079 0.0786 0.1167	0.8752 0.8921 0.9214 0.8833	0.6761 0.6884 0.7609 0.7083	0.3786 0.5023 0.4447 0.6287	PI 1.5000 1.9655 1.7273 2.7143	0.6667 0.7456 0.7105 0.8158	HE 0.7213 0.7306 0.7919 0.7488	P 0.1668 0.7751 0.0246 0.1155
STR loci D6S474 D12ATA63 D22S1045 D10S1248 D1S1677		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560	0.8752 0.8921 0.9214 0.8833 0.8440	0.6761 0.6884 0.7609 0.7083 0.6133	0.3786 0.5023 0.4447 0.6287 0.2972	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571	0.6667 0.7456 0.7105 0.8158 0.6053	HE 0.7213 0.7306 0.7919 0.7488 0.6626	P 0.1668 0.7751 0.0246 0.1155 0.1728
STR loci D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553
D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463 D1S1627		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043 0.2658	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957 0.7342	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269 0.5193 0.7111 0.7055	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958 0.3305	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571 2.5909 1.7813	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982 0.6316	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654 0.5675	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553 0.2165
D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463 D1S1627 D3S4529		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043 0.2658 0.1183	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957 0.7342 0.8817	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269 0.5193 0.7111	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958 0.3305 0.6121	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571 2.5909	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982 0.6316 0.8070	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654 0.5675 0.7520	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553 0.2165 0.1988
STR loci D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463 D1S1627 D3S4529 D2S441		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043 0.2658 0.1183 0.1065	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957 0.7342 0.8817 0.8935	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269 0.5193 0.7111 0.7055	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958 0.3305 0.6121 0.4587	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571 2.5909 1.7813	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982 0.6316 0.8070 0.7193	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654 0.5675 0.7520 0.7401	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553 0.2165 0.1988 0.5562
STR loci D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463 D1S1627 D3S4529 D2S441 D6S1017		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043 0.2658 0.1183 0.1065 0.1285	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957 0.7342 0.8817 0.8935 0.8715	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269 0.5193 0.7111 0.7055 0.6945	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958 0.3305 0.6121 0.4587 0.4875	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571 2.5909 1.7813 1.9000	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982 0.6316 0.8070 0.7193 0.7368	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654 0.5675 0.7520 0.7401 0.7361	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553 0.2165 0.1988 0.5562 0.9520
STR loci D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463 D1S1627 D3S4529 D2S441 D6S1017 D4S2408		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043 0.2658 0.1183 0.1065 0.1285 0.1136	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957 0.7342 0.8817 0.8935 0.8715	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269 0.5193 0.7111 0.7055 0.6945 0.7064	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958 0.3305 0.6121 0.4587 0.4875 0.4730	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571 2.5909 1.7813 1.9000 1.8387	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982 0.6316 0.8070 0.7193 0.7368 0.7281	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654 0.5675 0.7520 0.7401 0.7361 0.7492	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553 0.2165 0.1988 0.5562 0.9520 0.5450
D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463 D1S1627 D3S4529 D2S441 D6S1017 D4S2408 D19S433		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043 0.2658 0.1183 0.1065 0.1285 0.1136	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957 0.7342 0.8817 0.8935 0.8715 0.8864 0.9354	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269 0.5193 0.7111 0.7055 0.6945 0.7064 0.7974	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958 0.3305 0.6121 0.4587 0.4730 0.5958	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571 2.5909 1.7813 1.9000 1.8387 2.4783	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982 0.6316 0.8070 0.7193 0.7368 0.7281 0.7982	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654 0.5675 0.7520 0.7401 0.7361 0.7492 0.8186	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553 0.2165 0.1988 0.5562 0.9520 0.5450 0.5042
STR loci D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463 D1S1627 D3S4529 D2S441 D6S1017 D4S2408 D19S433 D17S1301		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043 0.2658 0.1183 0.1065 0.1285 0.1136 0.0646 0.1200	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957 0.7342 0.8917 0.8935 0.8715 0.8864 0.9354 0.8800	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269 0.5193 0.7111 0.7055 0.6945 0.7064 0.7974 0.6693	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958 0.3305 0.6121 0.4587 0.4875 0.4730 0.5958 0.3662	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571 2.5909 1.7813 1.9000 1.8387 2.4783 1.4615	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982 0.6316 0.8070 0.7193 0.7368 0.7281 0.7982 0.6579	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654 0.5675 0.7520 0.7401 0.7361 0.7492 0.8186 0.7107	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553 0.2165 0.1988 0.5562 0.9520 0.5450 0.5042 0.1865
STR loci D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463 D1S1627 D3S4529 D2S441 D6S1017 D4S2408 D19S433 D17S1301 D1GATA113		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043 0.2658 0.1183 0.1065 0.1285 0.1136 0.0646 0.1200 0.1620	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957 0.7342 0.8817 0.8935 0.8715 0.8864 0.9354 0.8800 0.8380	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269 0.5193 0.7111 0.7055 0.6945 0.7064 0.7974 0.6693 0.6176	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958 0.3305 0.6121 0.4587 0.4730 0.5958 0.3662 0.4447	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571 2.5909 1.7813 1.9000 1.8387 2.4783 1.4615 1.7273	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982 0.6316 0.8070 0.7193 0.7368 0.7281 0.7982 0.6579 0.7105	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654 0.5675 0.7520 0.7401 0.7361 0.7492 0.8186 0.7107 0.6747	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553 0.2165 0.1988 0.5562 0.9520 0.5450 0.5042 0.1865 0.4530
D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463 D1S1627 D3S4529 D2S441 D6S1017 D4S2408 D19S433 D17S1301 D1GATA113 D18S853		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043 0.2658 0.1183 0.1065 0.1285 0.1136 0.0646 0.1200 0.1620 0.1214	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957 0.7342 0.8817 0.8935 0.8715 0.8864 0.9354 0.8380 0.8380	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269 0.5193 0.7111 0.7055 0.6945 0.7064 0.7974 0.6693 0.6176 0.6816	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958 0.3305 0.6121 0.4587 0.4730 0.5958 0.3662 0.4447 0.5023	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571 2.5909 1.7813 1.9000 1.8387 2.4783 1.4615 1.7273 1.9655	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982 0.6316 0.8070 0.7193 0.7368 0.7281 0.7982 0.6579 0.7105 0.7456	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654 0.5675 0.7520 0.7401 0.7361 0.7492 0.8186 0.7107 0.6747 0.7248	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553 0.2165 0.1988 0.5562 0.9520 0.5450 0.5042 0.1865 0.4530 0.6718
D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463 D1S1627 D3S4529 D2S441 D6S1017 D4S2408 D19S433 D17S1301 D1GATA113 D18S853 D20S482		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043 0.2658 0.1183 0.1065 0.1285 0.1136 0.0646 0.1200 0.1620 0.16214 0.1086	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957 0.7342 0.8817 0.8935 0.8715 0.8864 0.9354 0.8800 0.8380 0.8786 0.8914	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269 0.5193 0.7111 0.7055 0.6945 0.7064 0.7974 0.6693 0.6176 0.6816 0.7063	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958 0.3305 0.6121 0.4587 0.4730 0.5958 0.3662 0.4447 0.5023 0.548	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571 2.5909 1.7813 1.9000 1.8387 2.4783 1.4615 1.7273 1.9655 2.1923	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982 0.6316 0.8070 0.7193 0.7368 0.7281 0.7982 0.6579 0.7105 0.7456 0.7719	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654 0.5675 0.7520 0.7401 0.7361 0.7492 0.8186 0.7107 0.6747 0.7248 0.7465	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553 0.2165 0.1988 0.5562 0.9520 0.5450 0.5042 0.1865 0.4530 0.6718 0.5846
D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463 D1S1627 D3S4529 D2S441 D6S1017 D4S2408 D19S433 D17S1301 D1GATA113 D18S853 D20S482 D14S1434		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043 0.2658 0.1183 0.1065 0.1285 0.1136 0.0646 0.1200 0.1620 0.1214 0.1086 0.0922	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957 0.7342 0.8817 0.8935 0.8715 0.8864 0.9354 0.8380 0.8380 0.8786 0.8914 0.9078	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269 0.5193 0.7111 0.7055 0.6945 0.7974 0.6693 0.6176 0.6816 0.7063 0.7343	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958 0.3305 0.6121 0.4587 0.4730 0.5958 0.3662 0.4447 0.5023 0.548 0.473	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571 2.5909 1.7813 1.9000 1.8387 2.4783 1.4615 1.7273 1.9655 2.1923 1.8387	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982 0.6316 0.8070 0.7193 0.7368 0.7281 0.7982 0.6579 0.7105 0.7456 0.7719 0.7281	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654 0.5675 0.7520 0.7401 0.7361 0.7492 0.8186 0.7107 0.6747 0.7248 0.7465 0.7669	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553 0.2165 0.1988 0.5562 0.9520 0.5450 0.5042 0.1865 0.4530 0.6718 0.5846 0.2846
STR loci D6S474 D12ATA63 D22S1045 D10S1248 D1S1677 D11S4463 D1S1627 D3S4529 D2S441 D6S1017 D4S2408 D19S433 D17S1301 D1GATA113 D18S853 D20S482 D14S1434 D9S1122		Allele fr		24	0.1248 0.1079 0.0786 0.1167 0.1560 0.1043 0.2658 0.1183 0.1065 0.1285 0.1136 0.0646 0.1200 0.1620 0.1214 0.1086 0.0922 0.1196	0.8752 0.8921 0.9214 0.8833 0.8440 0.8957 0.7342 0.8817 0.8935 0.8715 0.8864 0.9354 0.8380 0.8380 0.8786 0.8914 0.9078 0.8804	0.6761 0.6884 0.7609 0.7083 0.6133 0.7269 0.5193 0.7111 0.7055 0.6945 0.7064 0.7974 0.6693 0.6176 0.6816 0.7063 0.7343	0.3786 0.5023 0.4447 0.6287 0.2972 0.5958 0.3305 0.6121 0.4587 0.4730 0.5958 0.3662 0.4447 0.5023 0.548 0.473	PI 1.5000 1.9655 1.7273 2.7143 1.2667 2.4783 1.3571 2.5909 1.7813 1.9000 1.8387 2.4783 1.4615 1.7273 1.9655 2.1923 1.8387 1.7813	0.6667 0.7456 0.7105 0.8158 0.6053 0.7982 0.6316 0.8070 0.7193 0.7368 0.7281 0.7982 0.6579 0.7105 0.7456 0.7719 0.7281 0.7281 0.7281	HE 0.7213 0.7306 0.7919 0.7488 0.6626 0.7654 0.5675 0.7520 0.7401 0.7361 0.7492 0.8186 0.7107 0.6747 0.7248 0.7465 0.7669 0.7271	P 0.1668 0.7751 0.0246 0.1155 0.1728 0.4553 0.2165 0.1988 0.5562 0.9520 0.5450 0.5042 0.1865 0.4530 0.6718 0.5846 0.2846 0.7903

MP: matching probability; PD: power of discrimination; PIC: polymorphism information content; PE: probability of parternity exclusion; HE: expected heterozygosity; PI: typical paternity index; HO: observed heterozygosity; P: probability values of the exact test for Hardy-Weinberg equilibrium

D22S1045 locus was found to have a lower value than the CODIS loci and to present a deviation from Hardy-Weinberg equilibrium (*P*<0.05). El Ossmani *et al.* (2007) suggested that unusual mutations or possible genetic drift could likely change the allelic distribution in some loci but could not change it in all. Matrimonial behavior in this population could also lead to the observed disequilibrium (El Ossmani *et al.*, 2009).

LD tests for marker pairs were estimated using GENEPOP V4.0.10 (Table 2). The LD test is used to test the association of the alleles at different loci, but LD does not completely equate to linkage: loci in disequilibrium in the LD test may not be linked, and linked loci may not be in disequilibrium. Loci on different chromosomes can show LD because of population substructure or natural selection. Hence, LD tests for all marker pairs were estimated, no matter whether they were on the same chromosome or not. The selected loci on the same chromosome in our study are at least 50 Mb apart and there is every chance that they are not linked. From the LD test results, we also found that the marker pairs on the same chromosomes were not in LD. In the 210 pairwise comparisons, 34 interchromosomal pairs showed LD (P<0.05). We found that there was no LD between D5S2500 and the other 20 loci in this population. In the Tibetan population from the Tibetan ethnic minority group living in the Lhase area of China, D5S2500 was also found without LD with the other 20 loci. These findings differ from those of studies of other Chinese populations (Zhu et al., 2011; Shen et al., 2012), in which LD tests were also conducted. In the Bai population from the Dali Bai Autonomous Prefecture in Yunnan Province, China, D5S2500 was found in LD with D18S853. D1GATA113, D4S2408, and D9S1122. These results may be due to different evolutionary pathways and the diversity of the gene frequencies. Therefore, LD testing can give some ethnicity-specific genetic information for the study population.

Population substructure was measured by calculating the single-locus coefficient *F* and *P* values using the AMOVA (Table 3). Differences were found between the Tujia (Yuan *et al.*, 2012) and our study population at five loci (D14S1434, D20S482, D22S1045, D2S441, and D6S474). Between the Chinese Han population in Beijing (Guo *et al.*, 2010) and our study population, differences were found at

loci (D10S1248, D10S1435, D19S433, six D1GATA113, D4S2408, and D9S1122). P values were less than 0.05 at four loci (D10S1248, D10S1435, D1S1627, and D1S1677) when our study population compared with three other populations (Tibetan, Bai, and Salar). Apart from these four loci, the comparison also showed significant differences from the Tibetan population (Zhu et al., 2011) at another 10 loci (D11S4463, D12ATA63, D14S1434, D19S433, D20S482, D22S1045, D2S441, D3S4529, D5S2500, and D6S1017), the Bai population (Shen et al., 2012) at another three loci (D22S1045, D3S4529, and D4S2408), and the Chinese Salar population in the Qinghai Province (Teng et al., 2012) also at another two loci (D2S441 and D5S2500). When calculating the single-locus F and P values for the study population and the other four ethnic minority groups from China, most differences were found in the Tibetan group, with differences at 14 loci. This comparison revealed some genetic differences among populations from China.

In our previous study, the selected STR loci on the same chromosome were at least 50 Mb apart. Moreover, they were all positioned on chromosomes at sites that differ from the 13 CODIS loci, or if on the same chromosome, they were at least 50 Mb from the 13 CODIS loci and therefore considered to be unlinked. Thus, these 21 STR loci will serve as useful complements to the CODIS loci to aid in the forensic analysis of degraded DNA, in missing persons work and in parentage testing with limited next-of-kin reference samples.

4 Conclusions

In summary, the allelic frequency distribution data and the forensic parameters showed that the 21 non-CODIS STR loci had highly genetic polymor phisms in this study population. This 21+1 multiplex PCR system was shown to be an efficient DNA detection system. The 21 STR loci are powerful and reliable for anthropological research, individual identification, and paternity testing. In tests without father or mother samples or in forensic cases with mutation events in paternity testing, they can be used as supplementary loci and can provide valuable data for forensic DNA database improvement and population genetics research. The results of this study are

Table 2 Linkage disequilibrium parameter (P value) for pairs of STR loci in Russian population of northeastern Inner Mongolia, China

~~~	P value										
STR loci	D17S1301	D11S4463	D12ATA63	D10S1248		D14S1434	D9S1122	D6S474	D6S1017	D5S2500	
D11S4463	0.8249										
D12ATA63	0.8270	0.3496									
D10S1248	0.6107	0.5948	0.2858								
D10S1435	0.1747	0.0196	0.0791	0.4598							
D14S1434	0.8710	0.4036	0.3454	0.2956	0.0031						
D9S1122	0.7067	0.1583	0.3138	0.6587	0.0749	0.0012					
D6S474	0.8014	0.5210	0.0000	0.9196	0.0203	0.5353	0.5180				
D6S1017	0.0896	0.6481	0.8656	0.1371	0.5915	0.1844	0.0525	0.6843			
D5S2500	0.4679	0.1234	0.1417	0.2977	0.3398	0.2500	0.9241	0.3394	0.4616		
D4S2408	0.3836	0.4463	0.2030	0.3497	0.3820	0.0101	0.0226	0.7010	0.2703	0.7974	
D1S1677	0.2372	0.0000	0.3776	0.2371	0.0305	0.4054	0.0749	0.4244	0.1265	0.3952	
D1S1627	0.0582	0.8265	0.2896	0.2984	0.4600	0.2584	0.3293	0.1399	0.0442	0.8392	
D1GATA113	0.0000	0.0425	0.1232	0.3236	0.0204	0.2673	0.0685	0.5321	0.0136	0.3409	
D19S433	0.2600	0.6419	0.5609	0.3735	0.2699	0.0069	0.2949	0.6941	0.5041	0.5037	
D18S853	0.1373	0.1459	0.2398	0.3919	0.6687	0.1184	0.0159	0.5994	0.2434	0.2547	
D3S4529	0.2373	0.4520	0.9182	0.9414	0.1507	0.3108	0.3332	0.3975	0.0006	0.1957	
D2S441	0.3634	0.4197	0.0055	0.3019	0.5421	0.3003	0.6200	0.0025	0.0007	0.8772	
D2S1776	0.3034	0.9047	0.0532	0.2863	0.0000	0.1809	0.0842	0.7962	0.6725	0.1826	
D22S1045	0.0406	0.0537	0.0877	0.0002	0.2691	0.0132	0.0770	0.9777	0.2807	0.4359	
D20S482	0.3989	0.2202	0.8767	0.1534	0.9405	0.9887	0.4655	0.2763	0.9905	0.8230	
STR loci	P value										
	D4S2408	D1S1677	D1S1627	D1GATA113	D19S433	D18S853	D3S4529	D2S441	D2S1776	D22S1045	
D11S4463											
D12ATA63											
D10S1248											
D10S1248 D10S1435											
D10S1435											
D10S1435 D14S1434											
D10S1435 D14S1434 D9S1122											
D10S1435 D14S1434 D9S1122 D6S474											
D10S1435 D14S1434 D9S1122 D6S474 D6S1017											
D10S1435 D14S1434 D9S1122 D6S474 D6S1017 D5S2500	0.0590										
D10S1435 D14S1434 D9S1122 D6S474 D6S1017 D5S2500 D4S2408	0.0590 0.9763	0.3780									
D10S1435 D14S1434 D9S1122 D6S474 D6S1017 D5S2500 D4S2408 D1S1677		0.3780 0.4327	0.6432								
D10S1435 D14S1434 D9S1122 D6S474 D6S1017 D5S2500 D4S2408 D1S1677 D1S1627 D1GATA113	0.9763		0.6432 0.3226	0.3546							
D10S1435 D14S1434 D9S1122 D6S474 D6S1017 D5S2500 D4S2408 D1S1677 D1S1627	0.9763 <b>0.0116</b>	0.4327		0.3546 0.8151	0.9576						
D1081435 D1481434 D981122 D68474 D681017 D582500 D482408 D181677 D181627 D1GATA113 D198433	0.9763 <b>0.0116</b> <b>0.0000</b>	0.4327 0.7582	0.3226		0.9576 0.1084	0.4316					
D10S1435 D14S1434 D9S1122 D6S474 D6S1017 D5S2500 D4S2408 D1S1677 D1S1627 D1GATA113 D19S433 D18S853	0.9763 <b>0.0116 0.0000</b> 0.0445	0.4327 0.7582 0.3723	0.3226 0.4555	0.8151		0.4316 0.2129	0.3766				
D10S1435 D14S1434 D9S1122 D6S474 D6S1017 D5S2500 D4S2408 D1S1677 D1S1627 D1GATA113 D19S433 D18S853 D3S4529	0.9763 <b>0.0116 0.0000</b> 0.0445 0.4867	0.4327 0.7582 0.3723 0.2093	0.3226 0.4555 <b>0.0000</b>	0.8151 0.2215	0.1084		0.3766 0.7699	0.2953			
D10S1435 D14S1434 D9S1122 D6S474 D6S1017 D5S2500 D4S2408 D1S1677 D1S1627 D1GATA113 D19S433 D18S853 D3S4529 D2S441	0.9763 <b>0.0116 0.0000</b> 0.0445 0.4867 0.5576	0.4327 0.7582 0.3723 0.2093 0.0522	0.3226 0.4555 <b>0.0000</b> <b>0.0409</b>	0.8151 0.2215 0.6145	0.1084 0.4852	0.2129		0.2953 0.2449	0.9769		

P<0.05 are indicated in bold

Table 3 F-statistic (F) and P values for each analyzed STR loci between our study population and other previously published populations

STR loci	Tibetan		Salar		Bai		Han		Tujia	
STRIOCI	F	P	F	P	F	P	F	P	F	P
D10S1248	0.1151	0.0000	0.1296	0.0000	0.1257	0.0000	0.0106	0.0260	-0.0038	1.0000
D10S1435	0.0836	0.0000	0.0848	0.0000	0.0938	0.0000	0.0157	0.0263	0.0000	0.7292
D11S4463	0.0079	0.0293	0.0047	0.1056	0.0026	0.1681	-0.0010	0.6723	0.0053	0.1496
D12ATA63	0.0164	0.0020	-0.0011	0.5591	-0.0009	0.5425	0.0063	0.4126	-0.0006	0.8807
D14S1434	0.0090	0.0303	0.0025	0.1701	0.0002	0.3861	-0.0048	0.9451	0.0142	0.0127
D17S1301	0.0028	0.1691	0.0039	0.1075	0.0005	0.3109	-0.0013	0.7582	-0.0022	1.0000
D18S853	0.0030	0.1525	-0.0012	0.5562	0.0043	0.1017	-0.0023	0.1505	-0.0035	1.0000
D19S433	0.0078	0.0225	-0.0013	0.6501	-0.0028	0.8837	0.0077	0.0003	0.0023	0.4145
D1GATA113	-0.0006	0.4252	-0.0033	0.8632	0.0028	0.2063	0.0003	0.0217	0.0063	0.1183
D1S1627	0.2188	0.0000	0.1836	0.0000	0.2179	0.0000	-0.0039	0.6937	0.0007	0.5445
D20S482	0.0086	0.0401	-0.0011	0.5328	0.0040	0.1134	-0.0048	0.6244	0.0099	0.0430
D22S1045	0.1145	0.0000	0.0019	0.2287	0.0823	0.0000	-0.0036	0.1237	0.0974	0.0000
D2S1776	-0.0002	0.3871	0.0014	0.2493	-0.0002	0.4027	0.0015	0.0551	-0.0002	0.7683
D2S441	0.0097	0.0108	0.0174	0.0020	0.0010	0.2884	-0.0053	0.9560	0.0241	0.0000
D3S4529	0.0235	0.0000	-0.0014	0.6022	0.0076	0.0430	-0.0042	0.8812	0.0046	0.2141
D4S2408	0.0047	0.0997	-0.0013	0.5758	0.0100	0.0274	0.0007	0.0365	0.0069	0.1085
D5S2500	0.0080	0.0459	0.0163	0.0059	0.0013	0.2630	0.0028	0.1739	-0.0011	0.8397
D6S1017	0.0232	0.0000	0.0066	0.0694	0.0053	0.0919	0.0046	0.1514	-0.0004	0.8006
D6S474	0.0065	0.0753	0.0009	0.2981	-0.0007	0.4956	-0.0032	0.8247	0.0232	0.0049
D9S1122	0.0016	0.2366	0.0009	0.2815	0.0016	0.2385	0.0104	0.0349	0.0000	0.6911
D1S1677	0.2287	0.0000	0.2256	0.0000	0.2338	0.0000	0.0117	0.1521	-0.0033	1.0000

P<0.05 shows statistically significant difference

also expected to enrich Chinese population genetic information resources and provide biological evidence for the origin of the Russian population.

#### Compliance with ethics guidelines

Hong-dan WANG, Chun-mei SHEN, Wen-juan LIU, Yu-dang ZHANG, Guang YANG, Jiang-wei YAN, Hai-xia QIN, and Bo-feng ZHU declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000(5). Informed consent was obtained from all people for being included in the study.

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