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SHORT COMMUNICATION

Development of two multiplex PCR systems for the analysis of 14 X-chromosomal STR loci in a southern Brazilian population sample

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Abstract We developed two multiplex systems for the coamplification of X-chromosomal short tandem repeats (STRs). X-Multiplex 1 consisted of DXS6807, DXS6800, DXS7424, DXS101, GATA172D05 and HPRTB and X-Multiplex 2 consisted of DXS8378, DXS9898, DXS6801, DXS6809, DXS6789, DXS7133, DXS8377 and DXS7423. In addition, we present allele frequencies for these loci in a south Brazilian population comprising 124 females and 141 males and haplotype frequencies of linked markers for males. Hardy–Weinberg equilibrium (HWE) was tested in the female sample and no significant deviations were found after applying Bonferroni's correction. Linkage disequilibrium (LD) tests were performed for all pairs of loci and three significant results, out of 91 pairwise comparisons, were obtained. We did not find any evidence of linkage disequilibrium between close or linked markers. The power of discrimination in females (PD_F) varied between 0.832 for DXS6801 and 0.987 for DXS8377. DXS6801 was the least informative marker (PIC=0.605), while DXS8377 was the most polymorphic (PIC=0.911), followed by DXS101 (PIC=0.872). Genetic distances were estimated for each STR marker applying the calculation of F_{ST} between our total sample and other studies from Brazil, Europe, Asia and Africa. The most distant populations were Japan, Korea, China, Ghana and Uganda.

Keywords X-chromosome · STRs · Population data · Rio Grande do Sul · Brazil

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Introduction

Autosomal and Y-chromosome short tandem repeat (STR) markers have been studied and widely applied as an effective tool for forensic analysis and human identification [1, 2]. In the last few years, X-chromosome has been the focus of several population studies. Since a male's X-chromosome is transmitted only to his female offspring, X-STRs are helpful in some deficient and complex kinship tests such as paternity cases when the disputed child is female, maternity testing and cases where parents are not available but some relatives are [1–5]. An advantage of X-STRs is that in trios involving daughter, the mean exclusion

chance (MEC) proposed by Desmarais et al. [6] is higher than for autosomal loci with similar polymorphism information content (PIC) [3, 7–10].

Most studies of X-STRs have been conducted with Asian and European populations, while there are just a few with African and Latin American populations. Therefore, the aim of this study was to develop two multiplex PCR systems to investigate a population in the south of Brazil with an overall of 14 X-STRs markers, which includes the following loci: DXS6807, DXS8378, DXS6800, DXS9898, DXS6801, DXS6809, DXS6789, DXS7424, DXS101, DXS7133, GATA172D05, HPRTB, DXS8377 and DXS7423. Population data of several places, including Latin America, are scarce and allele frequency is essential to establish population databases, an important tool in forensic cases and anthropological research. Thus, we present our allele frequencies and compare it with other population groups.

Materials and methods

Samples and DNA extraction

DNA was extracted according to manufacturer's instructions from FTA® cards containing blood spots collected from fingertip puncture of 265 (124 females and 141 males) unrelated individuals living in Rio Grande do Sul, a state located in the south of Brazil. This study was approved by the Research Ethics Board of Hospital de Clínicas de Porto Alegre (IRB0000921).

PCR amplification and fragment analysis

Two discs of 2.0 mm containing DNA were amplified in a 7.5μL reaction mix that included 6.25μL of Qiagen Multiplex PCR Kit and 1.25μL of primer mix. Primer sequences, dye labeling, concentrations, fragment size and references are shown in Table 1. The X-Multiplex 1 amplification conditions consisted of an initial denaturation of 15 min at 95°C, followed by 30 cycles of 1 min at 95°C, annealing at 63°C for 1 min and 1 min at 72°C, with a final extension step at 72°C for 90 min. The X-Multiplex 2 amplification conditions were an initial denaturation of 15 min at 95°C, followed by 30 cycles of 30s at 94°C, annealing at 59°C for 90s and 60s at 72°C, with a final extension step at 72°C for 60 min. Electrophoresis was performed with an ABI 3100-Avant Genetic Analyzer (Applied Biosystems). Fragment size was determined using GeneMapper ID software (v.3.2). Allele typing was based on the comparison to the alleles of the cell line K562. We also amplified 2008 and 2009 GEP-ISFG quality control samples to adjust and test the quality of the typing protocol for both X-Multiplex.

Statistical analysis

Arlequin Software (v.3.01) [18] was used to determine allele frequencies, haplotype frequencies, exact tests of population differentiation between allele frequencies of males and females, genetic distances (F_{ST}), Hardy–Weinberg equilibrium (HWE) test in female samples and linkage

Table 1 Dye labeling, primer sequences, concentrations and PCR sizes for both multiplex systems

Locus	Dye labeling	Forward primer (5′–3′)	Reverse primer (5′–3′)	Concentration[a] (μM)	PCR size (bp)	Ref.
X-Multiplex 1						
DXS6807	FAM	GAGCAATGATCTCATTTGCA	AAGTAAACATGTATAGGAAAAAGCT	5	247–275	[11]
DXS6800	NED	GTGGGACCTTGTGATTGTGT	CTGGCTGACACTTAGGGAAA	1	194–218	[12]
DXS7424	FAM	CTGCTTGAGTCCAGGAATTCAA	GAACACGCACATTTGAGAACATA	1.5	145–175	[9]
DXS101	VIC	ACTCTAAATCAGTCCAAATATCT	AAATCACTCCATGGCACATGTAT	7	180–220	[13]
GATA172D05	PET	TAGTGGTGATGGTTGCACAG	ATAATTGAAAGCCCGGATTC	3	108–136	[12]
HPRTB	VIC	TCTCTATTTCCATCTCTGTCTCC	TCACCCCTGTCTATGGTCTCG	2	144–176	[14]
X-Multiplex 2						
DXS8378	VIC	TTAGGCAACCCGGTGGTCC	ACAAGAACGAAACTCCAACTC	5	110–134	[12]
DXS9898	FAM	CGAGCACACCTACAAAAGCT	TCGATTAGGTTCAGTTCCCA	4.5	188–215	[15]
DXS6801	PET	CATTTCCTCTAACAAGTCTCC	CAGAGAGTCAGAATCAGTAG	1	113–137	[16]
DXS6809	NED	TGAACCTTCCTAGCTCAGGA	TCTGGAGAATCCAATTTTGC	5	235–279	[17]
DXS6789	PET	GTTGGTACTTAATAAACCCTCTTT	AAGAAGTTATTTGATGTCCTATTGT	4	154–198	[8]
DXS7133	FAM	GCTTCCTTAGATGGCATTCA	CTTCCAAGAATCAGAAGTCTCC	2	104–132	[12]
DXS8377	PET	CACTTCATGGCTTACCACAG	GACCTTTGGAAAGCTAGTGT	5	207–252	[12]
DXS7423	NED	GTCTTCCTGTCATCTCCCAAC	TAGCTTAGCGCCTGGCACATA	2	175–199	[12]

[a] Final concentration in the primer mix

disequilibrium (LD) in male samples. F_{ST} p values, obtained when comparing our population sample and literature data, were corrected by the Finner's procedure using WinPepi [19]. Finner's procedure [20], which is based on the familywise error rate, is more powerful than Bonferroni's procedure, which in effect adjusts the p value by multiplying it by the number of tests. Values of p less than or equal to 0.05 were regarded as significant.

Several forensic statistical parameters were calculated according to earlier papers: PIC [21], heterozygosity (HET) [22], power of discrimination in males (PD_M) and females (PD_F) [6], mean exclusion chance in father/daughter (MEC_{duos}) and mean exclusion chance in trios involving daughters (MEC_{trios}) [6].

Results and discussion

Allele frequencies of the 14 markers were computed considering male and female data together and separately by gender (Table S1), and no significant differences were observed between males and females at any marker. To our knowledge, for seven loci these are the first population data reported in the literature for Rio Grande do Sul. HWE was tested in females by the exact test and no significance was observed after Bonferroni's correction. Haplotype frequencies were calculated for the linked markers DXS6801, DXS6809 and DXS6789 (Table S2); DXS7424 and DXS101 (Table S3); DXS8377 and DXS7423 (Table S4).

Forensic statistical parameters for the 14 markers investigated in our data are shown in Supplementary Table 5. DXS8377 had the highest heterozygozity (HET=0.917) whereas DXS6801 showed the lowest degree of variability (HET=0.653). Accordingly, DXS8377 showed the highest forensic efficiency, with a PD_F of 0.987 and MEC of 0.911 in trio cases, on the other hand, DXS6801 presented the lowest forensic efficiency, with 0.832 and 0.605, respectively. Overall values obtained for the PD were high in males (PD_M=0.999999998) and females (PD_F=0.99999999999), as well as for the combined MEC in duos (MEC_{duos}= 0.9999980) and trios (MEC_{trios}=0.999999992).

X-linked markers have higher LD compared to autosomal markers, a consequence of low recombination rate of X-chromosome in female germline [17]. LD tests were performed for all pairs of loci. Three significant results, out of 91 pairwise comparisons, were obtained after Bonferroni's correction: DXS101 and DXS8377 ($p<$ 0.001); DXS7133 and DXS6809 ($p<0.001$); DXS7423 and DXS6809 ($p<0.001$). In the sample investigated, we did not find any evidence of linkage disequilibrium between close or linked markers. LD does not only depend on the physical distance between loci and may result from the breeding system, the pattern of geographic subdivision, gene flow, genetic drift, mutation and other forces that cause gene-frequency evolution [23, 24]. Nevertheless, the three significant results obtained are probably a consequence of sampling effects since these associations were not observed in the studies conducted so far.

Genetic distances were estimated for each STR marker applying the calculation of F_{ST} between our total sample and the following studies: DXS6807 [2, 4, 5, 25], DXS8378 [1–5, 7, 14, 17, 25–31], DXS6800 [2, 5, 10, 17, 32, 33], DXS9898 [1–3, 5, 7, 10, 14, 27–33], DXS6801 [16, 17], DXS6809 [3, 4, 7, 17, 27–31], DXS6789 [1–4, 7, 10, 17, 26–33], DXS7424 [1, 4, 5, 17, 25, 26], DXS101 [1–5, 7, 14, 17, 26, 27], DXS7133 [1, 4, 5, 10, 14, 25, 26, 28–33], GATA172D05 [2–4, 7, 17, 27–31, 33], HPRTB [2–5, 7, 10, 14, 17, 26, 32, 33], DXS8377 [2, 3, 5, 7, 14, 17, 25, 27] and DXS7423 [1–5, 7, 10, 25–33]. F_{ST} p values below 0.05, after applying Finner's correction, were regarded as significant (Table S6).

In general, significant genetic distances were obtained comparing our population with Japan [1], Korea [2], Ghana [5], African and Asian American [7], China [26] and Uganda [28], when data from these populations were available for the respective marker being compared.

On the other hand, Rio Grande do Sul (RS) is mainly composed of descendants of Portuguese, German and Italian immigrants and we observed that Portugal [3], Italy [4, 17], Germany [14] and other groups studied in Brazil [10, 25, 29–33] are genetically closer to our population. Few groups studied in Brazil had significant F_{ST} results, probably, due to the miscegenation observed across the country.

In conclusion, the 14 X-linked markers used in this work proved to be highly informative. X-chromosomes seem to have peculiarities in their allelic distribution, which makes the population study extremely important as a tool in forensic activity. Therefore, more studies with such markers are required in order to compile information, which then may complement database of the Brazilian population enhancing its use in the forensic context.

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