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ORIGINAL ARTICLE

Genetic profile characterization of 10 X-STRs in four populations of the southeastern region of Brazil

Joyce A. Martins · Jeane C. Costa · Greiciane G. Paneto · Raquel F. Figueiredo · Leonor Gusmão · Paula Sánchez-Diz · Ángel Carracedo · Regina M. B. Cicarelli

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Abstract Ten X-chromosomal short tandem repeats (DXS8378, DXS9902, DXS7132, DXS9898, DXS6809, DXS6789, DXS7133, GATA172D05, GATA31E08 and DXS7423) were analyzed in four populations of the southeastern region of Brazil (São Paulo, Rio de Janeiro, Vitória and Belo Horizonte). No deviations from the Hardy–Weinberg equilibrium were observed for any of the analyzed loci in the four populations. The average diversity per locus varied between 68% for DXS8378, DXS7133, and DXS7423 and 83%, for DXS6809, with Rio de Janeiro being the most diverse population. Overall power of discrimination values in females varied between 0.99999999990 and 0.99999999997 and between 0.9999991 and 0.9999995 in males. These high values show the potential of this system for forensic application and relationships' testing in the studied groups. Genetic comparisons (exact tests of population differentiation and pairwise genetic distances) revealed significant differences between Brazilian and other populations from Europe, Latin America and Africa, as well as among different Brazilian populations.

Keywords X chromosome · STRs · Human identification · Genetic population data · Southeastern region · Brazil

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J. A. Martins (✉) · J. C. Costa · G. G. Paneto · R. F. Figueiredo · R. M. B. Cicarelli
Laboratório de Investigação de Paternidade,
Faculdade de Ciências Farmacêuticas,
Universidade Estadual Paulista (UNESP),
Rodovia Araraquara-Jaú, Km 01,
14801-902, Araraquara, São Paulo, Brazil
e-mail: joyce_apa@hotmail.com

L. Gusmão
Institute of Molecular Pathology and Immunology
of the University of Porto (IPATIMUP),
4200-465 Porto, Portugal

P. Sánchez-Diz · Á. Carracedo
Genomics Medicine Group, CIBER for Rare Diseases
(CIBERER), Institute of Legal Medicine,
University of Santiago de Compostela,
Santiago de Compostela,
A Coruña, Spain

Introduction

The markers most commonly utilized in forensic genetics are autosomal short tandem repeats (STRs), followed by Y-chromosome STRs and mitochondrial DNA. The analysis of X-chromosome STRs (X-STRs) has recently become of importance in some complex cases of biological relationships, when the alleged father is not available, in paternity cases involving close blood relatives, in maternity cases involving a son, and in some other identification cases. In these situations, X-STRs may efficiently complement the autosomal analysis as they present higher mean exclusion chances [1]. In view of the wide application of these markers, several X-STRs multiplex PCR systems have been validated for forensic use, including four to 13 markers (e.g., [2–6]). Although, a great variety of markers have been studied, few are common to different works. Recently, a decaplex system was developed and validated in a collaborative work carried out by the Spanish and Portuguese-Speaking Working Group of the International Society For Forensic Genetics (GHEP-ISFG) working group [7] and data were presented for 15 Iberian and Latin American populations. Until now, this is the system with the greatest amount of data on the Brazilian urban population.

Brazilian genetic data on autosomal [8, 9] and Y-chromosome STRs [10–12] are already available, but few studies have been published regarding the X-STRs in Brazil [5, 7, 13–16]. Thus, the aim of the current study was to analyze the 10 X-STRs standardized by the GHEP-ISFG [7] in capital cities of the southeastern region of Brazil (São Paulo, Rio de Janeiro, Vitória and Belo Horizonte).

Materials and methods

DNA samples

After an informed consent, blood samples were collected from 1,001 unrelated individuals living in one of the four capital cities of the southeastern region of Brazil: Rio de Janeiro, Rio de Janeiro State (n= 261, 145 women and 116 men); São Paulo, São Paulo State (n= 250, 164 women and 86 men); Vitória, Espírito Santo State (n= 245, 160 women and 85 men) and Belo Horizonte, Minas Gerais State (n= 245, 108 women and 137 men). Blood samples were collected on flinders technology associates (FTA) cards and genomic DNA was extracted using the FTA method (Whatman, Clifton, NJ, USA).

Markers genotyping

Amplification of the 10 X-STRs loci was achieved in a single PCR multiplex reaction using a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA). Multiplex PCR was performed as proposed by Gusmão et al. [7] with minor changes: one disk (1.2 mm) of FTA paper with DNA adsorbed, 5 μL of Gold Star Buffer 2X (Promega, Madison, WI, USA), 1 μL of 10X primer mix at 2 μM each primer and 1.5 U of Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA); the solution was made up to a 10 μL of final reaction volume with ddH_2O. Separation and detection were performed in an ABI377 automated sequencer (Applied Biosystems) and genotyping was performed by comparison with DNA control reference samples (9947A and 9948, Promega) and allelic ladders provided by the GHEP-ISFG collaborative study [7] using the GeneScan 2.1 software (Applied Biosystems).

Statistical analysis

The allele frequencies, gene diversities, exact test of the Hardy-Weinberg equilibrium for female samples, pairwise exact test of linkage disequilibrium for male samples, analysis of molecular variance (AMOVA), population pairwise genetic distances (F_{ST}) and pairwise exact test of population differentiation were calculated using the ARLEQUIN software version 3.1 [17]. Statistics for forensic efficiency evaluation of each locus, namely mean exclusion chance in trios involving daughters (MECT) as well as in father/daughter duos (MECD), power of discrimination in females (PDF) and in males (PDM) were computed as proposed by Desmarais et al. [18].

Results and discussion

Genetic variations

The exact test for population differentiation, after applying the Bonferroni correction (significance level of 0.005), revealed no significant differences between allele distributions of men and women (Supplementary Table S1), so samples were pooled and allele frequencies and gene diversities in each population are presented in Table 1. Gene diversities for all markers were above 64%. The average diversity per loci varied between 68% for DXS8378, DXS7133, and DXS7423 and 83% for DXS6809. Rio de Janeiro showed the highest average gene diversity (above 76.2%).

No identical haplotype-like allelic combinations of the 10 X-STRs markers were found between males within or among the four Brazilian groups studied (Supplementary Table S2). For a significance level of 0.005 (after the Bonferroni correction) no deviations from the Hardy–Weinberg equilibrium were observed for any of the analyzed loci (Table 2).

Forensic efficiency parameters

Statistical parameters of forensic interest are shown in Table 2. The DXS6809 was the most informative marker in São Paulo, Rio de Janeiro and Vitória, whereas, the GATA172D05 was the most polymorphic in Belo Horizonte. The DXS8378 was the less discriminating locus in Rio de Janeiro and Belo Horizonte, and the DXS7423 was the less discriminating locus in São Paulo and Vitória. Overall, values for the power of discrimination in females varied between 0.99999999990 and 0.99999999997 and between 0.9999991 and 0.9999995 in males. The combined mean exclusion chance values were between 0.999996 and 0.999998 in trios, and between 0.99983 and 0.99989 in duos.

Most markers were more discriminative in the Brazilian samples analyzed here than in Iberian and Latin American populations from Portugal, Spain, Argentina, Costa Rica and Colombia [7]. On the other hand, these parameters were higher in African populations from Angola, Mozambique and Uganda [19, 20] than in the four Brazilian groups.

Table 1 Allele frequency distribution and gene diversity in capital cities of the southeastern region of Brazil

	São Paulo	Rio de Janeiro	Vitória	Belo Horizonte
DXS8378				
N	414	406	405	353
9	0.0121	0.0099	0.0049	0.0027
10	0.3986	0.3128	0.3062	0.3514
11	0.3333	0.3941	0.3704	0.3286
12	0.2367	0.2488	0.2889	0.2919
13	0.0193	0.0320	0.0272	0.0198
14		0.0025	0.0025	0.0057
GD	0.6751	0.6855	0.6866	0.6849
DXS9898				
N	414	406	405	353
7	0.0024	0.0025	0.0049	0.0085
8.3	0.1305	0.1798	0.2049	0.1726
9			0.0025	
10	0.0266	0.0394	0.0346	0.0454
11	0.1908	0.1970	0.1852	0.1871
12	0.3430	0.2611	0.2790	0.3115
13	0.2077	0.2315	0.1975	0.2125
13.3	0.0024	0.0074	0.0074	0.0055
14	0.0845	0.0764	0.0617	0.0453
15	0.0121	0.0049	0.0222	0.0115
GD	0.7796	0.8016	0.8032	0.7909
DXS7133				
N	412	406	401	351
7	0.0072	0.0049	0.0025	
8	0.0097	0.0025	0.0050	0.0057
9	0.4999	0.3103	0.3666	0.3250
10	0.1408	0.2069	0.1746	0.1679
11	0.2913	0.3892	0.3766	0.4243
12	0.0291	0.0591	0.0474	0.0456
13	0.0097	0.0172	0.0150	0.0171
14	0.0122	0.0099	0.0075	0.0144
15			0.0050	
GD	0.6457	0.7073	0.6925	0.6855
GATA31E08				
N	414	406	405	353
7	0.0024	0.0074	0.0074	0.0115
8	0.0048	0.0123	0.0049	0.0084
9	0.1353	0.1626	0.1827	0.2010
10	0.0362	0.0813	0.0543	0.0765
11	0.1715	0.1453	0.1185	0.1162
12	0.2705	0.2488	0.2765	0.2239
13	0.2633	0.2291	0.2296	0.2633
14	0.1039	0.1010	0.1136	0.0823
15	0.0121	0.0123	0.0123	0.0169
GD	0.7994	0.8230	0.8093	0.8158

Table 1 (continued)

	São Paulo	Rio de Janeiro	Vitória	Belo Horizonte
GATA172D05				
N	414	406	405	353
6	0.1473	0.1897	0.1481	0.1531
7	0.0169	0.0222	0.0272	0.0369
8	0.1546	0.1724	0.1531	0.1246
9	0.1256	0.1576	0.1407	0.1843
10	0.2730	0.2685	0.2963	0.2069
11	0.2126	0.1601	0.1605	0.1953
12	0.0701	0.0271	0.0716	0.0965
13		0.0025	0.0025	0.0028
GD	0.8157	0.8125	0.8174	0.8378
DXS7423				
N	414	406	405	353
8	0.0048	0.0074		
10	0.0024			
12		0.0049	0.0049	0.0057
13	0.0555	0.0714	0.0494	0.0595
14	0.3309	0.3596	0.3531	0.3851
15	0.4855	0.3990	0.4321	0.3684
16	0.0918	0.1158	0.1259	0.1190
17	0.0290	0.0419	0.0346	0.0623
GD	0.6440	0.6929	0.6708	0.6963
DXS6809				
N	414	406	405	353
27	0.0024			0.0057
28	0.0072	0.0049	0.0198	0.0142
29	0.0145	0.0320	0.0321	0.0508
30	0.0531	0.0517	0.0716	0.0313
30.1		0.0025		
31	0.1570	0.1502	0.1506	0.1274
31.1	0.0048	0.0049	0.0074	
32	0.1377	0.1429	0.1259	0.1642
32.1	0.0024			0.0057
33	0.3043	0.2586	0.2914	0.2661
33.1		0.0025		
34	0.1932	0.1970	0.1605	0.2154
35	0.0725	0.0985	0.1012	0.0766
35.1		0.0025		
36	0.0362	0.0320	0.0370	0.0227
37	0.0072	0.0123	0.0025	0.0199
38	0.0072	0.0025		
39		0.0049		
GD	0.8187	0.8387	0.8346	0.8313
DXS7132				
N	412	406	405	353
10	0.0048			
11	0.0194	0.0074	0.0222	0.0169

Table 1 (continued)

	São Paulo	Rio de Janeiro	Vitória	Belo Horizonte
12	0.1044	0.0887	0.1012	0.0850
13	0.2233	0.2512	0.2642	0.2406
14	0.3277	0.3768	0.3012	0.3854
14.3		0.0025		
15	0.2597	0.1946	0.2198	0.2125
15.3	0.0024	0.0025		
16	0.0388	0.0517	0.0519	0.0426
16.3	0.0072	0.0197	0.0173	0.0058
17	0.0097		0.0173	0.0112
17.3	0.0024	0.0049		
18			0.0025	
18.3			0.0025	
GD	0.7642	0.7478	0.7790	0.7410
DXS9902				
N	414	406	405	353
8	0.0024		0.0025	
9	0.0072	0.0246	0.0222	0.0170
10	0.0314	0.0443	0.0222	0.0395
11	0.3840	0.3251	0.3457	0.3230
11.1		0.0025		
12	0.3285	0.3719	0.3679	0.3400
12.1	0.0072	0.0074	0.0148	0.0055
13	0.2295	0.2044	0.2198	0.2605
13.1	0.0048	0.0049		
14	0.0048	0.0148	0.0049	0.0087
15				0.0028
16				0.0028
GD	0.6925	0.7131	0.6973	0.7122
DXS6789				
N	414	406	405	353
14	0.0121	0.0074	0.0074	0.0028
15	0.0821	0.1034	0.1012	0.1217
16	0.1376	0.0616	0.0568	0.0680
17	0.0097	0.0025	0.0025	0.0058
18	0.0048	0.0049	0.0049	0.0084
19	0.0362	0.0493	0.0593	0.0426
20	0.3019	0.2906	0.3407	0.3114
21	0.2005	0.2438	0.2494	0.2209
22	0.1667	0.1773	0.1309	0.1302
23	0.0362	0.0493	0.0420	0.0766
24	0.0121	0.0074	0.0049	0.0112
25		0.0025		
GD	0.8141	0.8071	0.7877	0.8122

N sample size, *GD* gene diversity

Linkage disequilibrium (LD) analysis

For a significance level of 0.0011 (after the Bonferroni correction for 45 comparisons in each population) only a significant p value was obtained for the DXS9898–DXS9902 pair of loci in Belo Horizonte ($p \leq 0.0000$, Supplementary Table S3), which are quite distant on the chromosome (over 72 Mb). The pairwise LD test for the overall sample from the southeastern region did not confirm associations between any of the maker pairs investigated. LD does not only depend on the distance between marker pairs, but may be associated to a random genetic drift, founder effect, population admixture or stratification, etc. [21]. The Brazilian population is highly heterogeneous; however, the significant DXS9898–9902 LD in Belo Horizonte is thought to be more related to sampling effects than a genetic substructure since this association was not observed in the three other admixed populations studied in this work or in other Brazilian populations [7].

Comparisons between Brazilian populations

Pairwise population comparisons between the studied samples and those published for other Brazilian populations that used the same set of markers [7] were performed at a single locus level (exact test of population differentiation and F_{ST} genetic distance analysis) and for the whole set of markers (AMOVA and F_{ST} genetic distance analysis).

Considering both methodologies, the results (Supplementary Table S4) showed significant differences after the Bonferroni correction ($p<0.005$) for DXS9898, DXS7133 GATA172D05 and DXS7423. For the complete set of markers, significant pairwise genetic distances ($p< 0.005$) were found between Paraná and the four populations from the southeastern region, as well as between São Paulo and the other three southeastern populations (Supplementary Table S5). The overall F_{ST} value found in the AMOVA ($F_{ST}=0.0028$, $p \leq 0.000$) showed a significant differentiation among Brazilian populations and a similar result was obtained when only the southeastern groups were compared.

Comparisons with other populations

The F_{ST} was performed between Brazilian and other populations from Europe, Latin American and Africa [7], those whose data are available for the same markers studied in this work. In most comparisons, significant genetic distances were obtained (Supplementary Table S6). The results revealed that São Paulo, Rio de Janeiro, Vitória and Belo Horizonte are, in general, are more similar to the European populations (Portugal and

Table 2 Forensic parameters in the capital cities of the southeastern region of Brazil

	He	Ho	P-HWE	MECT	MECD	PDM	PDF
São Paulo							
DXS8378	0.6773	0.7012	0.9636	0.6076	0.4606	0.6735	0.8275
DXS9898	0.7789	0.7927	0.4624	0.7458	0.6146	0.7778	0.9186
DXS7133	0.6566	0.5644	0.0057	0.5876	0.4408	0.6442	0.8168
GATA31E08	0.8023	0.7561	0.0062	0.7680	0.6417	0.7975	0.9295
GATA172D05	0.8232	0.8232	0.2342	0.7879	0.6666	0.8137	0.9395
DXS7423	0.6481	0.6890	0.4784	0.5822	0.4365	0.6424	0.8119
DXS6809	0.8170	0.7988	0.0085	0.7940	0.6758	0.8167	0.9438
DXS7132	0.7624	0.7669	0.1530	0.7246	0.5898	0.7624	0.9058
DXS9902	0.6962	0.6829	0.0719	0.6314	0.4858	0.6908	0.8450
DXS6789	0.8254	0.8049	0.3450	0.7880	0.6678	0.8122	0.9405
Rio de Janeiro							
DXS8378	0.6889	0.7034	0.9730	0.6214	0.4749	0.6838	0.8376
DXS9898	0.7990	0.7103	0.1143	0.7696	0.6434	0.7996	0.9298
DXS7133	0.7162	0.6690	0.0096	0.6528	0.5089	0.7055	0.8606
GATA31E08	0.8212	0.8138	0.1755	0.7967	0.6782	0.8209	0.9437
GATA172D05	0.8043	0.7931	0.8085	0.7833	0.6604	0.8105	0.9368
DXS7423	0.6760	0.7103	0.4441	0.6380	0.4943	0.6911	0.8515
DXS6809	0.8414	0.8276	0.7654	0.8169	0.7057	0.8366	0.9536
DXS7132	0.7460	0.6965	0.3787	0.7071	0.5696	0.7460	0.8966
DXS9902	0.7203	0.6207	0.0256	0.6600	0.5171	0.7113	0.8654
DXS6789	0.8031	0.8069	0.9098	0.7790	0.6565	0.8051	0.9359
Vitória							
DXS8378	0.6859	0.6875	0.3611	0.6201	0.4734	0.6849	0.8359
DXS9898	0.7989	0.8062	0.9734	0.7723	0.6472	0.8013	0.9315
DXS7133	0.7039	0.6478	0.1217	0.6342	0.4896	0.6908	0.8478
GATA31E08	0.8094	0.8000	0.2870	0.7803	0.6572	0.8073	0.9358
GATA172D05	0.8131	0.7687	0.1047	0.7911	0.6709	0.8154	0.9417
DXS7423	0.6605	0.6812	0.0264	0.6103	0.4651	0.6691	0.8317
DXS6809	0.8260	0.8062	0.5918	0.8133	0.7009	0.8326	0.9527
DXS7132	0.7760	0.7250	0.0774	0.7430	0.6118	0.7771	0.9162
DXS9902	0.6971	0.6437	0.3349	0.6379	0.4930	0.6956	0.8496
DXS6789	0.7861	0.7750	0.4480	0.7576	0.6303	0.7857	0.9259
Belo Horizonte							
DXS8378	0.6826	0.6296	0.0242	0.6166	0.4696	0.6829	0.8331
DXS9898	0.7941	0.7870	0.8182	0.7575	0.6290	0.7886	0.9242
DXS7133	0.6886	0.6667	0.4038	0.6278	0.4827	0.6836	0.8441
GATA31E08	0.8124	0.8148	0.9898	0.7880	0.6672	0.8135	0.9397
GATA172D05	0.8394	0.8796	0.8630	0.8137	0.6999	0.8355	0.9512
DXS7423	0.6982	0.6574	0.2393	0.6416	0.4980	0.6944	0.8538
DXS6809	0.8428	0.8611	0.9116	0.8079	0.6939	0.8290	0.9497
DXS7132	0.7276	0.7222	0.6497	0.6983	0.5593	0.7389	0.8912
DXS9902	0.7060	0.6852	0.5685	0.6551	0.5111	0.7102	0.8609
DXS6789	0.8198	0.8241	0.3875	0.7861	0.6657	0.8099	0.9401

He expected heterozygosity, *Ho* observed heterozygosity, *P-HWE p* values for Hardy–Weinberg equilibrium test, *MECT* mean exclusion chance in trios involving daughters, *MECD* mean exclusion chance in father/daughter duos, *PDM* power of discrimination in males, *PDF* power of discrimination in females.

Spain), followed by Latin American (Argentina, Costa Rica and Colombia), and more distant to the African population from Uganda.

In conclusion, the data obtained in this work shows that the present X-STR set is highly informative in the southeastern region of the country and that specific databases for this multiplex system should be used in forensic casework and kinship analysis in Brazilian populations. Until this time, including this article, 21 X-STR markers were reported by 17 Brazilian populations, covering ten urban groups and seven Amerindian groups [5, 7, 13–16]. This decaplex system was studied in six of the ten urban groups and six of these markers (DXS7132, DXS9898, DXS6789, DXS7133, GATA31E08, DXS7423) were the most studied, being reported in more than 15 groups. Therefore, the result of this work will contribute to establish this system as standard for the X-STRs in the Brazilian forensic context.

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