Forensic Science International: Genetics 4 (2010) e95–e100

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Announcement of population data

An X-chromosome pentaplex in two linkage groups: Haplotype data in Alagoas and Rio de Janeiro populations from Brazil

Iede H. Ferreira da Silva [a], Adriana G. Barbosa [a], Dalmo A. Azevedo [a], Paula Sánchez-Diz [b], Leonor Gusmão [c], Celso C. Tavares [d], Elizeu F. Carvalho [d], Luiz Antonio Ferreira da Silva [a,*]

[a] Institute of Biological and Health Sciences, Forensic DNA Laboratory, Federal University of Alagoas, Brazil
[b] Institute of Legal Medicine, Genomics Medicine Group, University of Santiago de Compostela, CIBER for Rare Diseases (CIBERER), Santiago de Compostela, Spain
[c] Institute of Molecular Pathology and Immunology of University of Porto (IPATIMUP), Porto, Portugal
[d] Institute of Biology, DNA Diagnostic Laboratory, University of Rio de Janeiro State, Brazil

ARTICLE INFO

Article history:
Received 15 June 2009
Accepted 6 August 2009

Keywords:
STRs
X-chromosome
Brazil
Forensic
Population genetics

ABSTRACT

Genotype data were obtained for five X-Chr STRs (DXS10079, DXS10074, DXS10075, DXS7424 and DXS101) in two different populations from Brazil, namely Alagoas and Rio de Janeiro. Observed genotype distributions in female samples for each locus do not show deviations from Hardy–Weinberg equilibrium expectations. Gametic association was tested for all pairs of loci in male samples. Significant association values were found between all pairs including DXS10079, DXS10074 and DXS10075, as well as between DXS7424 and DXS101, proving that these two groups of markers must be treated as haplotypes. No significant association could be found between markers from the two groups (DXS10079, DXS10074 or DXS10075 vs. DXS7424 or DXS101), although distances between them varied from 24 to 25 cM. When comparing haplotype frequencies in Alagoas, Rio de Janeiro, Germany and Ghana, significant differences were found between the Brazilian and the Germany sample and that from Ghana. Nevertheless, no significant differences were found between Alagoas and Rio de Janeiro, as well as between these two populations and Germany. The combined power of discrimination in males and females were high in both Brazilian populations ($\geq$0.9996 and $\geq$0.9999998, respectively), showing the utility of these markers for human identification and paternity testing.

Population: Blood samples were collected from unrelated individuals from Alagoas (203 males and 201 females) and Rio de Janeiro (220 males and 122 females), under informed consent.

DNA extraction: Chelex method [1].

PCR: Amplification was performed in a single multiplex reaction for DXS10079, DXS10074, DXS10075, DXS7424 and DXS101. The reaction was carried out in a 25 μl volume containing 1–2 ng of DNA, 1× PCR buffer [200 mM Tris–HCl (pH 8.4), 500 mM KCl], 1.5 mM $MgCl_2$, 200 μM of each dNTPs, 1.0 U platinum Taq DNA polymerase (Invitrogen). DXS10079 [2], DXS10074 [2], DXS10075 [2], DXS7424 [3] and DXS101 [4] primers were labeled with 6-FAM, VIC, 6-FAM, 6-FAM and NED, respectively. In the PCR reaction, primers concentration was 0.28 μM for DXS10079, DXS10075 and DXS7424, 0.2 μM for DXS10074 and 1 μM for DXS101.

PCR thermocycling conditions were optimized in a TC-512 Gradient Thermal Cycler (Techne, UK) or an ABI 9700 (Applied Biosystems, Forster City, CA, USA) and consisted of a initial denaturation at 95 °C for 2 min; followed by 30 cycles at 94 °C for 1 min, 59.5 °C for 1 min, 72 °C for 1 min; and a final extension step at 60 °C for 60 min.

Typing: PCR products were separated in an ABI PRISM 310 or 3100 AVANT Genetic Analyzer (Applied Biosystems, Forster City, CA, USA). GeneMapper v.3.5 software (Applied Biosystems) was used to determine fragment sizes, and alleles were typed by comparison with allelic ladders as recommended by the ISFG [5]. Commercial DNA from the cell lines K562 and 9947A (Applied Biosystems) were used as positive controls.

Results: See Fig. 1 and Tables 1 and 2.

Analysis of data: Allele frequencies, observed and expected heterozygosity values, Hardy–Weinberg equilibrium (HWE) exact test, genetic diversity, exact tests of population differentiation between allele frequencies of males and females and for linkage disequilibrium test between all pairs were calculated using Arlequin software version 3.11 [6]. Using the same software, genetic distance analysis was performed assuming the stepwise

* Corresponding author at: Laboratório de DNA Forense, Universidade Federal de Alagoas (UFAL), Rua Aristeu de Andrade, 452 – Farol, 57021-090 Maceió-AL, Brazil. Tel.: +55 82 3336 6558.
E-mail address: laferreirasilva@uol.com.br (L.A. Ferreira da Silva).

1872-4973/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.fsigen.2009.08.002

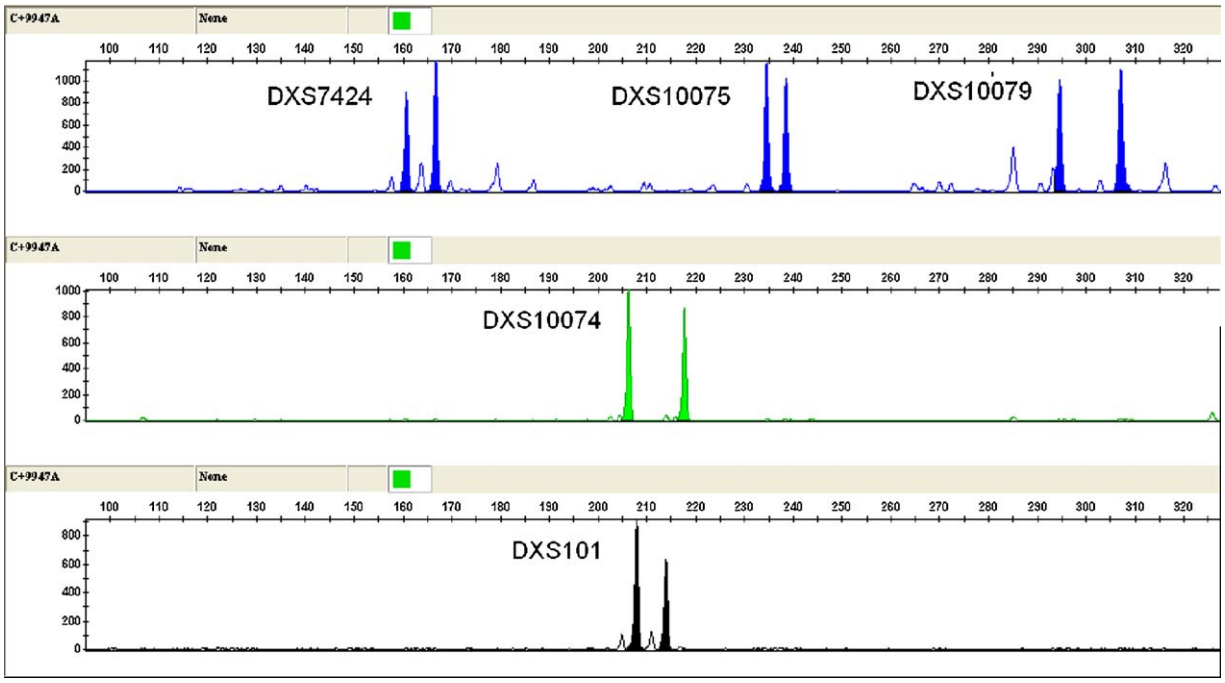

Fig. 1. Electropherogram of a female X-chromosome pentaplex profile, including the STRs DXS7424 (6-FAM), DX10075 (6-FAM), DXS10079 (6-FAM), DXS10074 (VIC) and DXS101 (NED).

Table 1
Haplotype frequencies of the linked X-chromosome STRs DXS10079-DXS10074-DXS10075 (Group A) and DXS7424-DXS101 (Group B).

Group A	Alagoas		Rio de Janeiro		Group B	Alagoas		Rio de Janeiro	
	N	Freq.	*N*	Freq.		*N*	Freq.	*N*	Freq.
13, 15,16			1	0.0045	10, 25	1	0.0049		
14, 15, 16	1	0.0049			10, 26	1	0.0049	1	0.0045
14, 16, 16	3	0.0148			10, 27	2	0.0099		
14, 16, 17			1	0.0045	11, 21			1	0.0045
14, 16, 18	1	0.0049			11, 22	1	0.0049	1	0.0045
14, 18, 16	1	0.0049	1	0.0045	11, 23			2	0.0091
15, 15, 16	1	0.0049	1	0.0045	11, 27	1	0.0049	1	0.0045
15, 17, 16			1	0.0045	12, 19	1	0.0049		
15, 18, 16	2	0.0099	1	0.0045	12, 21			1	0.0045
16, 13, 16	1	0.0049			12, 22			1	0.0045
16, 16, 16	1	0.0049	1	0.0045	12,23			2	0.0091
16, 18, 12	1	0.0049			12, 25	1	0.0049		
16, 18, 16	3	0.0148	2	0.0091	12, 26	1	0.0049	4	0.0182
16, 18, 17	1	0.0049			13, 19	3	0.0148	3	0.0136
16, 18, 17.2	1	0.0049			13, 20	1	0.0049	1	0.0045
16,20,16			1	0.0045	13, 21	3	0.0148	3	0.0136
16, 8, 16			1	0.0045	13, 22	2	0.0099		
17, 14, 15			1	0.0045	13,23			1	0.0045
17, 15, 15	1	0.0049			13, 24	1	0.0049	3	0.0136
17,15,16			1	0.0045	13, 25	6	0.0296	8	0.0364
17, 15, 18			1	0.0045	13, 26	6	0.0296	4	0.0182
17, 16, 16	1	0.0049	1	0.0045	13, 27			4	0.0182
17, 16, 17	1	0.0049			13, 28	3	0.0148	1	0.0045
17, 17, 15			1	0.0045	13, 29			3	0.0136
17,17,16			1	0.0045	14, 18	2	0.0099	1	0.0045
17, 17, 17	1	0.0049			14, 19			3	0.0136
17, 18, 12			1	0.0045	14, 20	1	0.0049	1	0.0045
17,19,13			1	0.0045	14, 21			5	0.0227
17, 19, 18	1	0.0049	1	0.0045	14, 22	3	0.0148	2	0.0091
17, 7, 13			2	0.0091	14, 23	3	0.0148	2	0.0091
17,7,14			1	0.0045	14, 24	6	0.0296	4	0.0182
17, 8, 16	2	0.0099	1	0.0045	14, 25	2	0.0099	6	0.0273
17, 8, 17	4	0.0197	1	0.0045	14, 26	7	0.0345	6	0.0273
17, 8, 18			1	0.0045	14, 27	8	0.0394	4	0.0182
17,9,18			1	0.0045	14, 28	2	0.0099	1	0.0045
18, 14, 15	1	0.0049	3	0.0136	14, 29	1	0.0049	1	0.0045
18, 14, 16	1	0.0049			14, 30	2	0.0099		
18, 15, 15			1	0.0045	15, 18	6	0.0296	3	0.0136
18, 15, 16			1	0.0045	15, 19	1	0.0049	3	0.0136
18, 15, 17	1	0.0049	2	0.0091	15, 20	2	0.0099	2	0.0091