Forensic Science International: Genetics 19 (2015) 15–17

Contents lists available at ScienceDirect

Forensic Science International: Genetics

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

Correspondence

Allele frequencies of 20 autosomal STR in the population from Rio Grande do Sul Southern Brazil

Dear Editor,

One of the solutions applied by most of the forensic experts – when faced with unsolved cases of genetic reconstruction of deceased or missing persons using only recommended loci by CODIS or by any other genetic databases system – is to include a larger number of DNA regions [1]. When genetic mutations occur between parents and progeny, by addition or deletion of repeating units in the regions of occurrence of STR, the occurrence of null alleles or even single-parent paternity cases, increasing the number of genetic markers, can also assist in solving cases of paternity/maternity or any genetic link type [2].

One of the objectives of this paper is the proposition for combining two human identification systems to be used in a complementary manner when situations like above appears in the routine of a forensic laboratory.

For this purpose, allele frequencies of twenty STR markers, present in PowerPlex® 16 System (PP16) and PowerPlex® CS7 System (CS7) (Promega Corporation, USA), were obtained from a sample of 760 unrelated individuals undergoing paternity testing in the population of Rio Grande do Sul Southern Brazil (D3S1358, TH01, D21S11, D18S51, PENTA E, D13S317, D5S818, D7S820, D16S539, CSF1PO, PENTA D, vWA, D8S1179, TPOX, FGA, LPL, F13B, FES/FPS, F13A01 and PENTA C). All participants signed a consent form.

Rio Grande do Sul State is one of the 27 federal units in Brazil, located in the extreme south of the country. With a population of more than 10 million inhabitants, it is characterized by descendants from Europeans (especially Portuguese, German, and Italian) and a small number from African and Amerindian origins, mainly [3].

Genomic DNA was purified from dried blood samples preserved in FTA cards (Whatman Bioscience, Cambridge, UK) following the manufacturer's instructions. PCR amplifications were performed by using the PowerPlex® 16 System (PP16) and PowerPlex® CS7 System (CS7) (Promega Corporation, USA) in the GeneAmp 9700 PCR System (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's recommendations. The analysis of the amplified PCR product was performed on the ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, USA) capillary electrophoresis instrument. GeneScan-ILS 600 for PP16 and CS7 were used as internal lane standards. GeneMapper® ID (version 3.2) software enabled data analysis and allele identification.

Calculations of allele frequencies, observed and expected heterozygosity and *p*-values of the Hardy–Weinberg equilibrium tests for all twenty loci, were assessed using CERVUS version 3.0.3 [4]. Bonferroni's correction was used for Hardy–Weinberg equilibrium (HWE) test, which assumes that a 0.05 significance level obtained for twenty tests (one per locus) yields an actual significance threshold of 0.0025 [5]. Power of discrimination (PD) and power of exclusion (PE) were estimated with PowerStats (Promega Corporation) [6]. Analysis of molecular variance (AMOVA) for twelve loci (CSF1PO, TPOX, TH01, vWA, F13A01, FES/FPS, F13B, LPL, D16S539, D7S820, D13S317, and D5S818) were performed with ARLEQUIN version 3.1 [7]. UPGMA trees were built from Nei's genetic distance matrix using the PHYLIP software package version 3.69 [8] and visualized with TreeView software version 1.6.6 [9]. The allele frequencies, statistical parameters and genetic distances (F_{ST} analysis) between the studied sample and other populations are available as an e-component in Supplementary Tables 1 and 2. Our laboratory successfully participated in proficiency testing provided by the GEP-ISFG Working Group.

All the loci analyzed reached the Hardy-Weinberg equilibrium in the population studied ($P > 0.05$), except D16S539 and FGA loci ($P = 0.0281$ and $P = 0.0259$, respectively). When the Bonferroni's correction was employed using the number of loci analyzed, the differences observed were not statistically significant. The observed heterozygosity varies between 0.6750 for TPOX and 0.9066 for Penta E. The polymorphism information content (PIC) values ranged from 0.6437 for FES/FPS to 0.9017 for PENTA E. The power of discrimination (PD) was lowest for LPL:0.8535, and highest for PENTA E:0.9835. The power of exclusion (PE) ranges from 0.3906 for TPOX to 0.8088 for PENTA E. The combined PD and combined PE for PP16 markers were 0.9999999999999999995 and 0.999999577711, respectively. For CS7 markers, combined PD were 0.999999989278 and combined PE were 0.998029431990. For the twenty markers, that compose the two systems for human identification (PP16 and CS7), the combined PD were 0.99999999999999999999993 and the combined PE were 0.999999985366.

Allelic frequencies for twelve loci in Rio Grande do Sul (Southern Brazil) population were compared with other population samples: Three ethnic groups of São Paulo, Brazil ($n = 708$) [10]: European-derived ($n = 312$), African-derived ($n = 337$), and Asian-derived ($n = 59$); Belgium ($n = 198$) [11]; Kuwait ($n = 500$) [2] and four U.S. population samples ($n = 1036$) [12]: African American ($n = 342$), Caucasian ($n = 361$), Hispanic, ($n = 236$), and Asian ($n = 97$). Based on genotypic frequencies of twelve STRs (CSF1PO, TPOX, TH01, vWA, F13A01, FES/FPS, F13B, LPL, D16S539, D7S820, D13S317 and D5S818) to which data are available [2,10,11,12], pairwise genetic distances were calculated

http://dx.doi.org/10.1016/j.fsigen.2015.05.004
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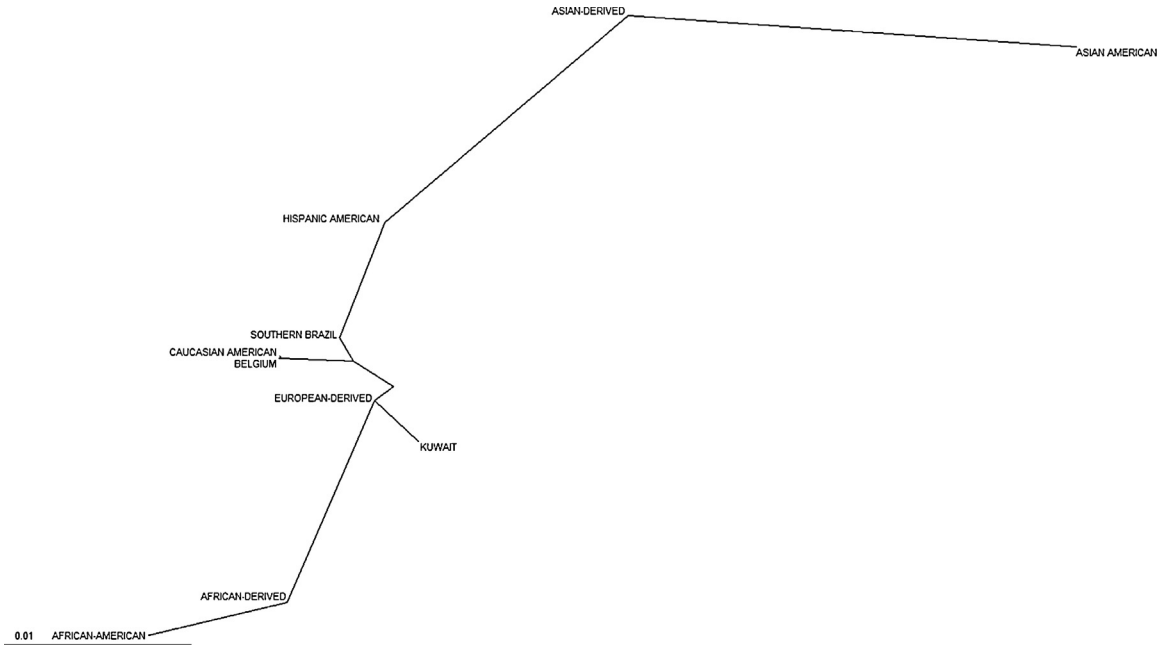

Fig. 1. Neighbour-joining tree based on pairwise Nei genetic distances calculated between the ten populations. References for the population data used: Southern Brazil [this study]; three ethnic groups of São Paulo, Brazil [10]: European-derived, African-derived, and Asian-derived; Belgium [11]; Kuwait [2] and four U.S. population samples [12]: African American, Caucasian, Hispanic, and Asian.

between populations, using Nei's formulas implemented in PHYLIP software. The analysis (see Fig. 1) showed a clear separation between Southern Brazil and other populations studied, although Southern Brazil is closer to European-derived, Caucasian American and Belgian populations than other populations (Asian American and African American, mainly). This paper presents the allele frequencies of seven STR loci (LPL, F13B, FES/FPS, F13A01, PENTA C, PENTA D and PENTA E) that have not been published yet in the population of Rio Grande do Sul. The presentation of the allele frequencies of locus PENTA C, in this work, is one of the first publications in the scientific community, since there are few published articles that used this marker [2,12,13]. In conclusion, combining those two human identification systems (PP16 and CS7) in a complementary manner could be a powerful auxiliary tool for forensic identification and paternity testing, especially to assist in solving complex cases like paternity cases with mutations, single-parent paternity cases and family reconstructions that cannot be sufficiently resolved with STR kits used routinely in a forensic lab.

This paper follows the guidelines for publication of population data requested by the journal [14] and ISFG recommendations concerning STR nomenclature [15].

Acknowledgements

This work was supported by Laboratório de Investigação de Paternidade of FEPPS,Promega Corporation, Laboratório de Genética Humana e Molecular of PUCRS and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). We would like to thank Dr. Rodrigo Soares Moura-Neto (Laboratório de Biologia Molecular Forense – Instituto de Biologia/UFRJ, Brazil), Dr. Ronny Decorte (Laboratory for Forensic Genetics and Molecular Archaeology - Leuven, Belgium) and Dr. Mohammad Al-Enizi (Ministry of Interior, General Department of Criminal Evidences, Identification Department, Kuwait) for providing the population samples.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fsigen.2015.05.004.

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Received 28 January 2015
Received in revised form 16 April 2015
Accepted 6 May 2015