ELSEVIER

International Congress Series 1288 (2006) 447–449

ICS

www.ics-elsevier.com

Validation study and population data of 15 "new" STR loci: A highly discriminating set for paternity and kinship analysis

Petra Grubwieser, Bettina Zimmermann, Harald Niederstätter, Marion Pavlic, Walther Parson*

Institute of Legal Medicine, Innsbruck Medical University, Austria

Abstract. We investigated 15 polymorphic STR loci (D1S1656, D7S1517, D8S306, D8S639, D9S304, D10S2325, D11S488, D12S391, D14S608, D16S3253, D17S976, D18S1270, D19S253, D20S161, D21S1437) which are not included in the standard sets of forensic loci (ISSOL, CODIS). The markers were selected according to the complexity of the polymorphic region: 7 of the 15 investigated loci showed a simple repeat structure (D9S304, D10S2325, D14S608, D16S3253, D18S1270, D19S253, D21S1437), 3 loci (D7S1517, D12S391, D20S161) consisted of compound repeat units and 5 loci (D1S1656, D8S306, D8S639, D11S488, D17S976) showed a complex and hypervariable polymorphic region. A population study on a sample of 270 unrelated west Eurasian persons from Austria was carried out. We did not observe significant deviation from Hardy–Weinberg expectations. The combined PE for the 15 loci was 0.99999998. In combination with the traditional set of STR markers included in commercially available kits (no linkage was observed between these 15 loci and the PowerplexTM 16 System loci) these markers approve as highly discriminating forensic tool, also suitable for the analysis of difficult paternity and kinship constellations. © 2005 Elsevier B.V. All rights reserved.

Keywords: STR; Population data; Sequence variant; Forensic

* Corresponding author. Muellerstrasse 44,6020 Innsbruck, Austria. Tel.: +43 512 507 3303; fax: +43 512 5072764.

E-mail address: walther.parson@uibk.ac.at (W. Parson).

0531-5131/ © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ics.2005.09.028

1. Introduction

For more difficult constellations such as complex kinship analysis, paternity testing in deficiency cases or within related individuals the application of multiple brands of the commercial kits does not always provide the desired discriminatory power as the majority of loci are shared. In order to extend the set of loci we screened the literature for candidate STR markers regarding variability and compatibility for integration in a multiplex design. After careful consideration the following 15 markers were selected from the literature: D1S1656 [1], D7S1517 [2], D8S306 [3], D8S639 [4], D9S304 [5], D10S2325 [6], D11S488 [7], D12S391 [8], D14S608 [9], D16S3253 [9], D17S976 [10], D18S1270 [9], D19S253 [11], D20S161 [12], D21S1437 [9]. We performed a population study and evaluated these loci regarding potential linkage also with respect to commercially available loci (Powerplex 16 system).

2. Materials and methods

DNA samples from 270 healthy unrelated west Eurasian persons from Austria (135 men and 135 women) and 135 children thereof were used in this study. The 15 STR loci plus amelogenin were amplified in three PCR multiplexes generating amplicons in a size range from 100 bp to 400 bp, labelled with FAM, TET and HEX. Primers were designed with the aid of the Primer Express software package v1.5 (Applied Biosystems, AB, Foster City, CA, USA) and Primer3 software (Whitehead Institute for Biomedical Research; http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) or taken from the literature (see Table 1). The markers were amplified in 25 μl reactions on a GeneAmp PCR System 9600 (Perkin Elmer, Norwalk, CT, USA), subjected to capillary electrophoresis on an ABI Prism 3100 Genetic Analyzer using POP 4, 36 cm capillary arrays and default instrument settings. The data were analyzed using GeneScan Analysis Version 3.7 and Genotyper Version 2.5 (both AB). Homozygous alleles of each STR locus were sequenced. Statistical evaluations were facilitated using a computer program kindly provided by Bruce Budowle (FBI Academy, Quantico, VA, USA).

3. Results

The allele nomenclature of the 15 STR markers was described in accordance with previously published studies and the recommendations of the International Society of Forensic Genetics (ISFG)

Table 1
Primer sequences

Marker	Primer/label	Primer sequence (5′–3′)	Reference
D7S1517	D7S1517_F	CACCTTCTGGATAAATGATGATGTTC	This study
	D7S1517_R_HEX	TGTTGCTATTGGGCCATCTTG	This study
D8S639	D8S639_F_HEX	AGACCTTGTATCTTTAGGAGTGATGGA	This study
	D8S639_R	CTCAACCAAAAAATGTAAAGTCAGG	[4]
D12S391	D12S391_F	AGAAAGAATCAACAGGATCAATGGA	This study
	D12S391_R_TET	TGGCTTTTAGACCTGGACTG	[8]
D21S1437	D21S1437_F	GTACATGTGTCTGGGAAGGAGG	This study
	D21S1437_R_FAM	TTCTCTACATATTTACTGCCAACA	[9]

for the nomenclature of human STRs [13,14]. Sequencing of homozygote alleles was done in order to approve the classification by determining the true nucleotide length of some of the alleles and also to detect sequence variants. The loci were grouped into 3 classes according to the complexity of the polymorphic region. Seven of the 15 investigated loci showed a simple repeat structure (D9S304, D10S2325, D14S608, D16S3253, D18S1270, D19S253, D21S1437), 3 loci (D7S1517, D12S391, D20S161) consisted of compound repeat units and 5 loci (D1S1656, D8S306, D8S639, D11S488, D17S976) showed a more complex polymorphic region partly including different repeat blocks and incomplete repeat units, which resulted in a relatively high proportion of intermediate alleles. Six mutations in different loci (D19S253, D9S304, D11S488, D8S306, D8S639, D7S1517) were observed in the 135 investigated confirmed father/mother/child triplets. The forensic usefulness of the STR markers was evaluated with respect to their discrimination power. None of the loci showed significant deviation from Hardy–Weinberg expectations. Pair-wise interclass correlation tests were performed for all possible two-locus combinations including the loci D3S1358, TH01, D21S11, D18S51, PentaE, D5S818, D13S317, D7S820, D16S539, CSF1PO, PentaD, vWA, D8S1179, TPOX and FGA (all included in the Powerplex 16 system, Promega) and 16 deviations were detected in 435 pair-wise comparisons, which is well within expectations. For Powerplex 16 data for the same sample set see [15]. Overall, no linkage was observed between the 15 loci described here and the Powerplex 16 system loci, thus enabling the calculation of a common power of exclusion. The combined PE for the 15 loci was 0.99999998.

References

[1] M.V. Lareu, et al., Sequence variation of a hypervariable short tandem repeat at the D1S1656 locus, Int. J. Leg. Med. 111 (1998) 244–247.

[2] P. Wiegand, M. Klintschar, Population genetic data, comparison of the repeat structure and mutation events of two short STRs, Int. J. Leg. Med. 116 (2002) 258–261.

[3] M. Benecke, et al., Short tandem repeat (STR) locus HUMD8S306 in a large population sample from Germany, Electrophoresis 19 (1998) 2396–2397.

[4] L. Nelson, et al., Tetranucleotide repeat polymorphism at the D8S639 locus, Hum. Mol. Genet. 3 (1994) 1209.

[5] K. Fujii, et al., Multiplex PCR amplification of THO1, D9S304, and D3S1744 loci, J. Hum. Genet. 45 (2000) 303–304.

[6] P. Wiegand, et al., D18S535, D1S1656 and D10S2325: three efficient short tandem repeats for forensic genetics, Int. J. Leg. Med. 112 (1999) 360–363.

[7] D. Browne, et al., Tetranucleotide repeat polymorphism at the D11S488 locus, Hum. Mol. Genet. 2 (1993) 89.

[8] M.V. Lareu, et al., Sequence variation of a hypervariable short tandem repeat at the D12S391 locus, Gene 182 (1996) 151–153.

[9] M. Choi, et al., Frequency data on four tetrameric STR loci D18S1270, D14S608, D16S3253 and D21S1437 in a Korean population, Int. J. Leg. Med. 113 (2000) 179–180.

[10] M. Klintschar, et al., Genetic variation and sequence studies of a highly variable short tandem repeat at the D17S976 locus, Int. J. Leg. Med. 112 (1998) 50–54.

[11] M. Gene, et al., Catalonian population study of the tetranucleotide repeat loci D3S1358, D8S1179, D18S51 and D19S253, Int. J. Leg. Med. 112 (1999) 75–77.

[12] Y.P. Hou, et al., D20S161 data for three ethnic populations and forensic validation, Int. J. Leg. Med. 112 (1999) 400–402.

[13] A. Urquhart, et al., Variation in short tandem repeat sequences—a survey of twelve microsatellite loci for use as forensic identification markers, Int. J. Leg. Med. 107 (1994) 13–20.

[14] P. Gill, et al., Report of the European DNA profiling group (EDNAP)—towards standardisation of short tandem repeat (STR) loci, Forensic Sci. Int. 65 (1994) 51–59.

[15] M. Steinlechner, et al., STR loci Penta D and Penta E: Austrian Caucasian population data, Int. J. Leg. Med. (2002) 174–175.