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ORIGINAL ARTICLE

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Evaluation of an extended set of 15 candidate STR loci for paternity and kinship analysis in an Austrian population sample

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Abstract We investigated 15 polymorphic short tandem repeat (STR) loci (D1S1656, D7S1517, D8S306, D8S639, D9S304, D10S2325, D11S488, D12S391, D14S608, D16S3253, D17S976, D18S1270, D19S253, D20S161, and D21S1437) which are not included in the standard sets of forensic loci. The markers were selected according to the complexity of the polymorphic region: Of the 15 investigated loci, 7 loci showed a simple repeat structure (D9S304, D10S2325, D14S608, D16S3253, D18S1270, D19S253, and D21S1437), 3 loci (D7S1517, D12S391, and D20S161) consisted of compound repeat units, and 5 loci (D1S1656, D8S306, D8S639, D11S488, and 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. A population study on a sample of 270 unrelated persons from Austria was carried out. We did not observe significant deviations from Hardy–Weinberg expectations. The combined probability of exclusion for the 15 loci was 0.99999998. In combination with the conventional set of STR markers included in commercially available kits (no linkage was observed between these 15 loci and the Powerplex 16 System loci), these markers are approved as highly discriminating forensic tools, also suitable for the analysis of difficult paternity and kinship constellations.

Keywords Non-core STR loci · Population data · Austrian Caucasian · Sequence variants · Forensic

Introduction

National DNA intelligence databases have been recognized as emerging tools in forensic molecular medicine for combating crime. To ensure a broad applicability of these databases, an international comparison of short tandem repeat (STR) profiles is inevitable. Therefore, it was necessary to define core loci, as was done, for example, by the FBI (Combined DNA Index System) and Interpol (Interpol Standard Set of Loci). As a consequence, these core loci are now part of the most commonly used commercially available STR typing kits. For more difficult constellations, such as a complex kinship analysis, paternity testing in deficiency cases or within related individuals, even the application of multiple brands of the commercial kits does not provide the desired discriminatory power, as the majority of the loci are shared. To extend the set of loci, we screened the literature for candidate STR markers regarding chromosomal location, variability, and compatibility for integration in a multiplex design [1, 2]. The 15 selected loci were analyzed in an Austrian Caucasian population sample to examine allele frequencies and distributions. Furthermore, the new loci were also evaluated regarding potential linkage with respect to commercially available loci (Powerplex 16 system), thus enabling the calculation of a (common) power of exclusion and verifying the loci's suitability for the analysis of difficult paternity and kinship constellations.

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. Total DNA was extracted from peripheral venous blood using the Qiagen blood kit (Qiagen, Hilden, Germany).

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The 15 STR loci were amplified in singleplex reactions, and subsequently in three operational polymerase chain reaction (PCR) multiplexes including amelogenin. Primers for D8S639 (forward primer), D12S391 (forward primer), D21S1437 (forward primer), and D7S1517 (forward and reverse primer) were designed with the aid of the Primer Express software package version 1.5 (Applied Biosystems, AB, Foster City, CA, USA) and Primer3 software (Whitehead Institute for Biomedical Research; http://www.frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). All other primer sequences were taken from the literature (see Table S1). Oligonucleotides were screened for potential cross-hybridization and hairpin formation with the computer program AutoDimer (version 1) [3].

The markers were amplified in 25-µl reactions including 1× PCR Buffer II (AB), 6.25 µg nonacetylated bovine serum albumin (Serva, Heidelberg, Germany), 200 µM each deoxyribonucleotide triphosphate, 1.5 mM $MgCl_2$, and 2.5 units AmpliTaq Gold DNA polymerase (AB) and primer in the following amounts: D11S488 and D19S253: 40 nM; D1S1656, D12S391, D17S976, and Amelo: 80 nM; D9S304 and D14S608: 160 nM; D7S1517, D8S306, D8S639, D16S3253, and D21S1437: 200 nM; D10S2325: 240 nM; and D18S1270 and D20S161: 400 nM. Amplification was performed on a GeneAmp PCR System 9600 (Perkin Elmer, Norwalk, CT, USA), comprising initial denaturation at 95°C for 11 min, 28 cycles at 94°C for 45 s, 59°C for 1 min, and 72°C for 1 min, followed by final incubation at 72°C for 60 min. Aliquots (1 µl) of the PCR products were combined with 20 µl deionized formamide containing 0.4 µl internal size standard (Genescan-500, TAMRA, AB), denatured at 95°C for 3 min, snap-cooled on ice, and subjected to laser-induced fluorescence capillary electrophoresis on an ABI Prism 3100 Genetic Analyzer using POP 4, 36-cm capillary arrays, and default instrument settings as recommended by the manufacturer. The data were analyzed using GeneScan Analysis version 3.7 and Genotyper Version 2.5 (both from AB).

Sequencing of alleles

Homozygote alleles of all markers were amplified using unlabelled forward and reverse primers (Table S1) in singleplex reactions with a cycler protocol consisting of initial denaturation at 95°C for 11 min, 30 cycles at 94°C for 30 s, 58 or 61°C for 1 min, and 72°C for 1 min, followed by final incubation at 72°C for 30 min. The amplicons were treated with ExoSAP-IT (Amersham Biosciences, Uppsala, Sweden) and sequenced using the amplification primers (160 nM) and BigDye Terminator sequencing reagents (version 1.1; AB) according to the manufacturer's recommendations. The cycler protocol consisted of an initial incubation at 96°C for 1 min, followed by 30 cycles of 10 s at 96°C, 5 s at 50°C, and 4 min at 60°C. The cycle sequencing products were purified by centrifugation over AutoSeq G-50 columns (Amersham Biosciences). Laser-induced fluorescence capillary electrophoresis was carried out on an ABI Prism 3100 Genetic Analyzer using POP6, 50-cm capillary arrays, and default instrument settings. Data were analyzed using Sequencing Analysis version 3.7 (AB) and Sequencher version 4.1 (Gene Codes, Ann Arbor, MI, USA).

Statistical analysis

Allele frequencies were calculated from the numbers of each genotype obtained in the sample set. Unbiased estimates of expected heterozygosity were combined as described by Edwards et al. [4]. Possible divergence from Hardy–Weinberg expectations was tested by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies [5], the likelihood ratio test [4–6], and the exact test [7]. An interclass criterion was used for detecting disequilibrium between the loci [8]. The power of exclusion was calculated according to Garber and Morris [9] and the power of discrimination was calculated according to Fisher [10]. Statistical evaluations were facilitated using a computer program kindly provided by Bruce Budowle (FBI Academy, Quantico, VA, USA).

Results and discussion

After careful consideration, the following 15 markers were taken from the literature: D1S1656 [11–13], D7S1517 [14], D8S306 [15, 16], D8S639 [17, 18], D9S304 [19], D10S2325 [12, 20], D11S488 [18, 21], D12S391 [22–25], D14S608 [2, 26], D16S3253 [26], D17S976 [27], D18S1270 [26], D19S253 [28], D20S161 [29], and D21S1437 [26]. The chromosomal locations of these 15 loci, as well as those of STR markers used in commercially available PCR multiplexes (e.g., Powerplex 16 system), were determined by searching the May 2004 human genome assembly using BLAT [30] (http://www.genome.ucsc.edu). The performance of the markers for routine analyses was tested in singleplex reactions. Results of Control DNA 007 (AB) are shown in Table 1. Furthermore, the coamplification of the 15 STRs under study in three preliminary PCR multiplex designs was tested. These operational multiplexes were found to be suitable for high-quality DNA genotyping; however, work on a final tailor-made multiplex design, taking the statistical results of the study into account, is ongoing.

The 15 STR markers were typed in a population sample of 270 unrelated persons from Austria and their children (135). The observed fragment lengths of the amplification products were binned into one-nucleotide-broad categories. Sequencing of homozygote alleles was done to approve the classification by determining the true nucleotide length of some of the alleles and also to detect sequence variants (see Table S2). Wherever feasible, the allele nomenclature was described in accordance with previously published studies and the recommendations of the International Society of Forensic Genetics for the nomenclature of human STRs [31–35].

Table 1 Control DNA 007 (AB)—genotypes of the investigated loci

Marker	Apparent lengths [bp]	Alleles
D1S1656	131.83/144.7	13/16
D7S1517	244.41/256.60	21/24
D8S306	264.87/276.54	22/25
D8S639	350.67/358.70	24/26
D9S304	129.50/151.09	8/13
D10S2325	140.66/146.24	12/13
D11S488	244.33/271.39	28/35
D12S391	230.95/234.95	18/19
D14S608	197.09/213.04	7/11
D16S3253	180.91/184.74	10/11
D17S976	243.68/252.46	21.3/24
D18S1270	266.54/285.67	7.1/12
D19S253	218.77/226.63	10/12
D20S161	182.13	17
D21S1437	115.82/127.95	12/15

The loci were grouped into three classes according to the complexity of the loci's polymorphic regions (Table S3) [31]. Of the 15 investigated loci, seven showed a simple repeat structure (D9S304, D10S2325, D14S608, D16S3253, D18S1270, D19S253, and D21S1437). Allele designation was easy for these loci. For the marker D14S608, a single 6.3 variant was observed in our population sample. The locus D18S1270 showed some intermediate alleles (N.1 and N.2 alleles) and also had insertions of a guanine nucleotide in the repeat-flanking regions of some alleles (see Table S2, sequence data for allele 11 and 13). The marker D10S2325 is the only pentameric marker investigated in this study. Three loci (D7S1517, D12S391, and D20S161) consisted of compound repeat units. Single base-pair length variants (N.3 alleles) were observed for the loci D7S1517 and D12S391. The other five of the 15 investigated loci (D1S1656, D8S306, D8S639, D11S488, and 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 (N.2 and N.3 alleles). The loci D8S306, D8S639, and D11S488 showed constant regions within the polymorphic regions. For the locus D1S1656, a single base substitution affecting the sequenced allele 12 was detected (see Table S2, sequence data for allele 12).

For most of the STR markers used in this study, population data for Europeans (often from Germany or Austria) are available; in these cases our allele frequencies were similar to those found in the literature. For some of the STRs with these population data, we found additional alleles, for example, the D10S2325 allele 19 (n=1); the D7S1516 alleles 15 (n=1), 16 (n=6), and 26.3 (n=4); and the D12S391 allele 27 (n=2). Notably, in the complex and hypervariable STR loci, we observed new alleles and additional variants, such as in D1S1656 (9.3, n=1), D8S639 (19, n=1; 26.3, n=3; 29.2, n=2; 30.2, n=2; and 32.3, n=1), D11S488 (27.2, n=1; 37, n=20; 40, n=5; and 42, n=1), and D17S976 (19, n=3; 20, n=3; 20.3, n=13; 22.3, n=3; 23.3, n=2; 24.3, n=4; 25.3, n=6; 26.3, n=22; 28, n=1; 29, n=1; 33.3, n=6; 34, n=1; 35, n=1; and 38, n=1). For the locus D10S2325, point mutations concerning the alleles 9–16 were reported [12], which could be affirmed by our data (see Table S2, sequence data for allele 10). For D19S253, our observed allele frequency distribution differed distinctly from that published for a Catalonian population sample [28]. For the loci D16S3253, D14S608, D21S1437, D18S1270, and D9S304, mainly data from non-European populations were available; in these cases, allele frequencies expectedly differed in some cases from our population data. For example, for D18S1270, no variant alleles were reported for a Korean population sample [26], whereas in our population sample, numerous N.1 and N.2 alleles were detected (see Table S4). Furthermore, the dominating allele 7 (30%) in the locus D14S608 in our sample, and also in a German sample [2], was not detected in the Korean population sample (see Table S4).

A total of six mutations were observed in the 135 confirmed father/mother/child triplets (paternity index >50,000): one isolated exclusion in each of the systems D19S253, D9S304, D11S488, D8S306, D8S639, and D7S1517 (see Table 2). Although the number of investigated trios is not large enough to make definitive conclusions, the mutation rate for the investigated loci seems to be reasonably low. The median age of the mothers was 24 (minimum 15; maximum 37) and the median age for the fathers was 27 (minimum 16; maximum 62); for frequency distribution see Table 3. In Table 2, the respective ages of the parents of children with the novo mutations can be found. Mann–Whitney U testing revealed no statistical significance for the observed differences of median ages at conception (mothers: Δ=4; fathers: Δ=0.5) for meioses with and without mutations (mothers: P=0.228; fathers: P=0.649).

The forensic usefulness of the STR markers was evaluated with respect to their discrimination power. Allele frequencies and a summary of statistical analysis of the 15

Table 2 Observed mutations in 135 confirmed paternity trios (father/mother/child), mothers and fathers including the age at conception

	D19S253	D9S304	D11S488	D8S306	D8S639	D7S1517
Mother [age[a]]	12/14 [19]	8/13 [32]	29/33 [32]	19/23 [24]	23/26 [22]	19/24 [35]
Child	12/**13**	8/**12**	29/**35**	**18**/23	23/**25**	19/**25**
Father [age[a]]	7/12 [26]	8/13 [27]	29/36 [34]	19/23 [26]	24/26 [21]	24/26 [43]

Bold data refer to affected alleles
[a]Age at conception in years

Table 3 Distribution of ages of mothers and fathers at the time of conception in 135 confirmed paternity trios

Age at conception (years)	Number of mothers (%)	Number of fathers (%)
15–19	26 (19,3)	9 (6,7)
20–24	51 (37,8)	39 (28,9)
25–29	35 (25,9)	41 (30,4)
30–34	18 (13,3)	22 (16,3)
35–39	5 (3,7)	14 (10,4)
40–44		6 (4,4)
45–49		1 (0,7)
50–54		1 (0,7)
55–59		1 (0,7)
60–64		1 (0,7)

STR loci are shown in Table S4. None of the loci showed significant deviation from Hardy–Weinberg expectations. Based on the result of the exact test, the locus D10S2325 showed a departure from Hardy–Weinberg expectation. However, after employing a Bonferroni correction [36] for the number of loci analyzed, this observation is not likely to be significant, as homozygosity and likelihood test results were within Hardy–Weinberg expectations (Table S4).

Pairwise interclass correlation tests were performed for all possible two-locus combinations, and two deviations were detected in 105 pairwise comparisons (D20S161/D16S3253, P=0.003 and D8S306/D11S488, P=0.041), which is well within expectations. The power of discrimination and the power of exclusion for the Austrian population sample are listed in Table S4. The combined probability of exclusion for the 15 loci was 0.99999998.

Pairwise interclass correlation tests were also 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 (including the two mentioned above) were detected in 435 pairwise comparisons (D3S1358/PentaE, P=0.021; TH01/CSF1PO, P=0.047; D18S51/D8S1179, P=0.011; PentaE/D7S1517, P=0.035, D13S317/D8S306, P=0.015; D7S820/D8S1179, P=0.042; D16S539/CSF1PO, P=0.013; D16S539/D8S1179, P=0.010; CSF1PO/TPOX, P=0.026; CSF1PO/D18S1270, P=0.044; CSF1PO/D16S3253, P=0.015; PentaD/D1S1656, P=0.003; D8S1179/FGA, P=0.00; D8S1179/D1S1656, P=0.10), which is well within expectations. For Powerplex 16 data for the same sample set, see Steinlechner et al. [37]. 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.

Conclusion

No linkage between the investigated set of 15 STR loci and the markers included in commercially available kits (e.g., Powerplex 16 system) was observed, enabling the calculation of a common power of exclusion. Therefore, the extended set of markers appears to be a highly discriminating tool in forensic science, especially useful for complex kinship analysis, paternity testing in deficiency cases or within related individuals. A continuative study concerning the establishment of a tailor-made PCR multiplex based on the selection of loci is in progress.

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References

1. Asamura H, Uchida R, Takayanagi K, Ota M, Fukushima H (2005) Allele frequencies of the six miniSTR loci in a population from Japan. Int J Legal Med 13:1–3 (Epub ahead of print)
2. Becker D, Vogelsang D, Brabetz W (2005) Population data on the seven short tandem repeat loci D4S2366, D6S474, D14S608, D19S246, D20S480, D21S226 and D22S689 in a German population. Int J Legal Med 19:1–4 (Epub ahead of print)
3. Vallone PM, Butler JM (2004) AutoDimer: a screening tool for primer-dimer and hairpin structures. Biotechniques 37:226–231
4. Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 12:241–253
5. Chakraborty R, Fornage M, Gueguen R, Boerwinkle E (1991) Population genetics of hypervariable loci: analysis of PCR based VNTR polymorphism within a population. In: Burke T, Dolf G, Jeffreys AJ, Wolff R (eds) DNA fingerprinting: approaches and application. Birkhäuser, Berlin, pp 127–143
6. Weir BS (1992) Independence of VNTR alleles defined as fixed bins. Genetics 130:873–887
7. Guo SW, Thompson EA (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48:361–372
8. Karlin S, Cameron EC, Williams PT (1981) Sibling and parent–offspring correlation estimation with variable family size. Proc Natl Acad Sci U S A 78:2664–2668
9. Garber R, Morris J (1983) General equations for the average power of exclusion for genetic system of n codominant alleles in one-parent case of disputed parentage testing. In: Walker R (ed) International workshop on inclusion probabilities in parentage testing. American Association of Blood Banks, Arlington, pp 277–280
10. Fisher RA (1951) Standard calculations for evaluating a blood-group system. Heredity 5:95–102
11. Lareu MV, Barral S, Salas A, Pestoni C, Carracedo A (1998) Sequence variation of a hypervariable short tandem repeat at the D1S1656 locus. Int J Legal Med 111:244–247
12. Wiegand P, Lareu MV, Schürenkamp M, Kleiber M, Brinkmann B (1999) D18S535, D1S1656 and D10S2325: three efficient short tandem repeats for forensic genetics. Int J Legal Med 112:360–363

13. De Leo D, Turrina S, Marigo M, Tiso N, Danieli GA (2001) Italian population data for D1S1656, D3S1358, D8S1132, D10S2325, VWA, FES/FPS, and F13A01. Forensic Sci Int 123:71–73
14. Wiegand P, Klintschar M (2002) Population genetic data, comparison of the repeat structure and mutation events of two short STRs. Int J Legal Med 116:258–261
15. Nelson L, Riley R, Lu J, Robertson M, Ward K (1993) Tetranucleotide repeat polymorphism at the D8S306 locus. Hum Mol Genet 2:1984
16. Benecke M, Knopf M, Voll W, Oesterreich W, Jacobi Y, Edelmann J (1998) Short tandem repeat (STR) locus HUMD8S306 in a large population sample from Germany. Electrophoresis 19:2396–2397
17. Nelson L, Lu J, Petterson M, Fillmore K, Riley R, Ward K (1994) Tetranucleotide repeat polymorphism at the D8S639 locus. Hum Mol Genet 3:1209
18. Seidl C, Müller S, Jäger O, Seifried E (1999) Sequence analysis and population data of short tandem repeat polymorphisms at loci D8S639 and D11S488. Int J Legal Med 112:355–359
19. Fujii K, Senju H, Yoshida K, Sekiguchi K, Imaizumi K, Kasai K, Sati H (2000) Multiplex PCR amplification of TH01, D9S304, and D3S1744 loci. J Hum Genet 45:303–304
20. Lee DH, Han JS, Lee WG, Lee SW, Rho HM (1998) Quadruplex amplification of polymorphic STR loci in a Korean population. Int J Legal Med 111:320–322
21. Browne D, Gen M, Evans GA, Clark SP, Litt M (1993) Tetranucleotide repeat polymorphism at the D11S488 locus. Hum Mol Genet 2:89
22. Lareu MV, Pestoni C, Schürenkamp M, Rand S, Brinkmann B, Carracedo A (1996) A highly variable STR at the D12S391 locus. Int J Legal Med 109:134–138
23. Schröer KP, Schmitt C, Staak M (2000) Analysis of the co-amplified STR loci D1S1656, D12S391 and D18S51: population data and validation study for a highly discriminating triplex-PCR. Forensic Sci Int 113:17–20
24. Nievas P, Martinez-Jarreta B, Abecia E, Lareu MV (1999) Fluorescence-based amplification of the STR loci D18S535, D1S1656 and D12S391 in a population sample from Aragon (north Spain). Int J Legal Med 113:58–59
25. Klintschar M, Ricci U, al Hammadi N, Reichenpfader B, Ebner A, Uzielli ML (1998) Genetic variation at the STR loci D12S391 and CSF1PO in four populations from Austria, Italy, Egypt and Yemen. Forensic Sci Int 97:37–45
26. Choi M, Kim JH, Lee DH, Lee SH, Rho HM (2000) Frequency data on four tetrameric STR loci D18S1270, D14S608, D16S3253 and D21S1437 in a Korean population. Int J Legal Med 113:179–180
27. Klintschar M, Glock B, Dauber EM, Mayr WR (1998) Genetic variation and sequence studies of a highly variable short tandem repeat at the D17S976 locus. Int J Legal Med 112: 50–54
28. Gené M, Piqué E, Borrego N, Carracedo A, Huguet E, Moreno P (1999) Catalonian population study of the tetranucleotide repeat loci D3S1358, D8S1179, D18S51 and D19S253. Int J Legal Med 112:75–77
29. Hou YP, Jin ZM, Li YB, Wu J, Walter H, Kido A, Prinz M (1999) D20S161 data for three ethnic populations and forensic validation. Int J Legal Med 112:400–402
30. Kent WJ (2002) BLAT—the BLAST-like alignment tool. Genome Res 12:656–664
31. Urquhart A, Kimpton CP, Downes TJ, Gill P (1994) Variation in short tandem repeat sequences—a survey of twelve microsatellite loci for use as forensic identification markers. Int J Legal Med 107:13–20
32. Gill P, Kimpton C, D'Aloja E et al (1994) Report of the European DNA profiling group (EDNAP)—towards standardisation of short tandem repeat (STR) loci. Forensic Sci Int 65:51–59
33. Gill P, Brinkmann B, D'Aloja E et al (1997) Considerations from the European DNA profiling group (EDNAP) concerning STR nomenclature. Forensic Sci Int 87:185–192
34. Gill P, Brenner C, Brinkmann B et al (2001) DNA commission of the International Society of Forensic Genetics: recommendations on forensic analysis using Y-chromosome STRs. Int J Legal Med 114:305–309
35. Bär W, Brinkmann B, Budowle B et al (1997) DNA recommendations. Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. International Society for Forensic Haemogenetics. Int J Legal Med 110:175–176
36. Weir BS (1990) Genetic data analysis. Sinauer Associates, Sunderland, pp 109–110
37. Steinlechner M, Grubwieser P, Scheithauer R, Parson W (2002) STR loci Penta D and Penta E: Austrian Caucasian population data. Int J Legal Med 116:174–175
38. Sullivan KM (1993) A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X–Y homologous gene amelogenin. Biotechniques 15:636–641