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Allele frequencies of nine STR systems in the Flemish population and application in parentage testing

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Abstract In order to apply a set of nine STR loci in parentage testing, we performed a population genetic study on a sample of the Flemish population. Genotypes for HUMHPRTB, HUMFABP, HUMCD4, HUMCSF1PO, HUMTH01, HUMPLA2A, HUMPLA2A1, HUMF13A01, HUMCYAR04 and HUMLIPOL were determined using three triplex PCR reactions and silver staining. Allele frequencies showed no deviation from Hardy-Weinberg equilibrium. The frequency distribution agreed well with other Caucasian populations but three intermediate fragments, not previously found in Caucasians, were observed. We then resolved a series of 151 parentage disputes of which 103 were exclusions. In six cases, evidence for exclusion was obtained by only one informative STR locus out of eight for male children or out of nine for female children. These exclusions were confirmed with additional polymorphic markers. In one case of inclusion, a paternal allele expanded with one repeat unit of HUMHPRTB. This observation illustrates that STRs do not differ from other genetic systems in the fact that more than one excluding locus is required before exclusion is demonstrated.

Key words DNA polymorphism · Short tandem repeats (STRs) · Paternity testing

Introduction

The application of the nine STR loci previously described (Alford et al. 1994; Edwards et al. 1992; Hammond et al. 1994) for identity and parentage testing is attractive for several reasons: (1) data from nine loci can be obtained from three triplex reactions, (2) alleles of the three simultaneously applied loci are well separated on polyacrylamide gels, (3) alleles are unambiguously identified by the number of repeats and (4) PCR can be used for minute samples such as buccal swabs.

With the number of alleles ranging from 6 to 12, these STR loci are known to be highly informative and the combined use of nine such systems should be highly discriminatory for application in individual identification and parentage testing.

Here we analyse a population sample from Flanders (Dutch speaking part of Belgium) for HUMHPRTB, HUMFABP, HUMCD4, HUMCSF1PO, HUMTH01, HUMPLA2A1, HUMF13A01, HUMCYAR04 and HUMLIPOL and report on our experience with these nine STRs in paternity testing.

Materials and methods

Population and family studies

Peripheral blood samples were collected with informed consent and according to the ethical standards of the Center.

Techniques

DNA was extracted according to Miller et al. (1988). PCR amplification of the nine STR loci was as published (Alford et al. 1994). After electrophoresis on a 6% (w/v) (39:1 acrylamide:bisacrylamide, 7 M urea) 0.4 mm thick gel, alleles were revealed by silver staining (Nelis et al. 1996). On the gel, each paternity trio (mother, child, alleged father) was flanked by a complete allelic ladder.

Conformity of the observed genotype frequencies with Hardy-Weinberg expectations was tested based on the exact (E) test from Guo and Thompson (1992) and using the GENEPOP computer program (Raymond and Rousset 1995). Comparison of the allelic frequency profiles was done using $R \times G$ contingency tables calculated by statistical software (StatMost 2.01, DataMost Corp., Salt Lake City, UTA, USA). A P-value of less than 0.05 was considered significant. The power of exclusion for individual loci was determined according to Garber and Morris (1983) and for combined loci according to Morris (1983).

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Table 1 Allele frequencies for nine STR loci in the Flemish population

Allele number, number of alleles, frequency ± standard error								
HUMHPRTB (*n* = 315)			HUMFABP (*n* = 426)			HUMCD4 (*n* = 432)		
9	3	0.9 ± 0.5	10	250	49.2 ± 2.2	7	138	31.8 ± 2.1
10	1	0.3 ± 0.3	11	72	14.2 ± 1.5	8	175	34.2 ± 2.1
11	39	12.5 ± 1.8	12	26	5.1 ± 1.0	11	1	0.2 ± 0.2
11A	1	0.3 ± 0.3	13	140	27.6 ± 2.0	12	156	30.5 ± 2.0
12	114	36.4 ± 2.6	14	17	3.3 ± 0.8	13	15	2.9 ± 0.7
12A	1	0.3 ± 0.3	15	3	0.6 ± 0.3	14	2	0.4 ± 0.3
13	90	28.4 ± 2.4						
14	50	16.5 ± 2.0						
15	13	4.3 ± 1.1						
16	3	0.9 ± 0.5						
HUMCSF1PO (*n* = 492)			HUMTH01 (*n* = 490)			HUMPLA2A1 (*n* = 510)		
8	5	1.0 ± 0.5	5	1	0.2 ± 0.2	10	12	2.4 ± 0.7
9	15	3.0 ± 0.8	6	116	23.7 ± 1.9	11	230	45.1 ± 2.2
10	111	22.6 ± 1.9	7	66	13.5 ± 1.5	12	70	13.7 ± 1.5
11	157	31.9 ± 2.1	8	66	13.5 ± 1.5	13	6	1.2 ± 0.5
12	170	34.6 ± 2.1	9	71	14.5 ± 1.6	14	72	14.1 ± 1.5
13	30	6.1 ± 1.1	9.3	166	33.9 ± 2.1	15	63	12.4 ± 1.5
14	4	0.8 ± 0.4	10	4	0.8 ± 0.4	16	56	11.0 ± 1.4
						17	1	0.2 ± 0.2
HUMF13A01 (*n* = 486)			HUMCYAR04 (*n* = 518)			HUMLIPOL (*n* = 518)		
3.2	39	8.0 ± 1.2	5	186	35.9 ± 2.1	9	9	1.7 ± 0.6
4	26	5.3 ± 1.0	6	79	15.3 ± 1.6	10	237	45.8 ± 2.2
5	92	18.9 ± 1.8	6A	1	0.2 ± 0.2	11	141	27.2 ± 2.0
6	132	27.2 ± 2.0	7	50	9.7 ± 1.3	12	103	19.9 ± 1.8
7	171	35.2 ± 2.2	8	7	1.4 ± 0.5	13	26	5.0 ± 1.0
8	8	1.6 ± 0.6	9	8	1.5 ± 0.5	14	2	0.4 ± 0.3
9	3	0.6 ± 0.4	10	171	33.0 ± 2.1			
10	5	1.0 ± 0.5	11	16	3.1 ± 0.8			
11	5	1.0 ± 0.5	12	1	0.2 ± 0.2			
12	2	0.4 ± 0.3						
14	1	0.2 ± 0.0						
15	2	0.4 ± 0.3						

Results

Allele frequencies of nine STRs in the Flemish population are summarised in Table 1 and experimentally obtained heterozygosity and power of exclusion for each locus are presented in Table 2. The combined calculated power of exclusion for the eight loci without HUMHPRTB is 99.58% and for the nine loci 99.81%. P-values for concordance between the observed frequency and the Hardy-Weinberg expectation were calculated for each locus with the exact (E) test and ranged between 0.141 ± 0.016 and 0.612 ± 0.063 (Table 3). For HUMHPRT, intermediate alleles between 11 and 12 and between 12 and 13 repetitions of [AGAT] were found. Another allele between 6 and 7 repetitions of [AAAT] was found for HUMCYAR04 (Table 1). The combination of the nine STRs was applied in 151 paternity disputes of which 103 turned out to be exclusions. Figure 1 shows the frequency distribution of the number of excluding informative STR loci in the 103 cases of excluded paternity.

Table 2 Statistics for parentage studies in the Flemish population Het: heterozygosity %; Pcalc: calcualted power of exclusion; Pexp: experimetally observed power of exclusion

Locus	Het (%)	Pcalc	Pexp
HUMHPRTB	71.8	55.3	66.0
HUMFABP	65.3	44.2	31.7
HUMCD4	67.7	44.0	45.5
HUMCSF1PO	75.2	42.1	44.9
HUMFTHO1	76.5	59.0	51.5
HUMPLA2A1	70.7	54.6	60.4
HUMF13AO1	74.1	54.5	49.5
HUMCYARO4	71.5	49.8	54.5
HUMLIPOL	66.0	45.8	33.7

Table 3 Empirical levels of significance of E-test for hardy-Weinberg. For the X-linked locus HPRTB, only genotypes from females were included

Locus	*P*-value	Standard error
HPRTB	0.302	0.028
FABP	0.500	0.025
CD4	0.311	0.022
CSF1PO	0.612	0.063
THO1	0.407	0.030
PLA2A1	0.525	0.041
F13A01	0.365	0.027
CYAR04	0.141	0.016
LIPOL	0.246	0.029

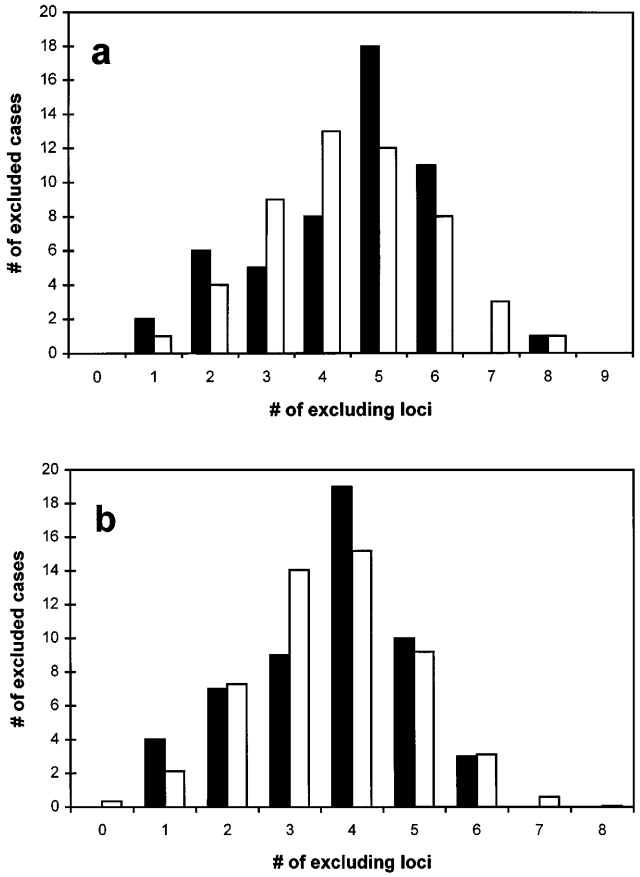

Fig. 1 Number of excluding STR loci for 53 female (**a**) and 51 male (**b**) children with excluded paternity. The expected number of excluding loci was calculated by the normal law (**a** mean 4.41, σ 1.53; **b** mean 3.63, s 1.31). Both distributions show an excess of cases with a low number of excluding loci. ■, no. of excluded cases; □, expected no. of excluded cases

Discussion

Allele frequencies

The experimental frequencies of genotypes do not significantly differ from the Hardy-Weinberg expectation (Table 3; all P-values higher than 0.05).

For eight out of nine of the STR loci, the allelic frequency distribution in this Flemish population corresponds well with the American Caucasians published by Edwards et al. (1992) and Hammond et al. (1994). For HUMHPRTB, HUMFABP, HUMCD4, HUMCSF1PO, HUMTH01, HUMPLA2A1, HUMCYAR04 and HUMLIPOL the *P*-values are 0.85, 0.19, 0.40, 0.12, 0.64, 0.84, 0.64 and 0.094 respectively. Only for F13A01 did we observe a significant difference in allele profile ($P = 0.0053$). The American Caucasian population can be considered as a mixture of European populations. For HUMTHO1, a thoroughly studied locus, there were no significant differences in comparison to the Dutch (Sjerps et al. 1995) ($P = 0.18$), Swiss (Hochmeister et al. 1995) ($P = 0.58$), Hungarian (Füredi et al. 1996) ($P = 0.11$), Croatian (Kubat et al. 1995) ($P = 0.62$) and Basque populations (Alonso et al. 1995) ($P = 0.43$). The HUMTHO1 allelic profile of the Turkish population (Alper et al. 1995) however, showed a clear difference from the Flemish ($P = 0.000002$). This corresponds with the results of the study by Alper et al. (1995), revealing significant frequency differences for HUMTHO1 alleles between the German and Turkish population but not between two Turkish subpopulations, one residing in Turkey, the other in Belgium. For HUMCSF1PO, no significant differences were demonstrated in comparison with the Swiss (Hochmeister et al. 1995) ($P = 0.45$) and the Hungarian populations (Füredi et al. 1996) ($P = 0.069$). For HUMHPRTB, the frequency distribution agreed well with the Hungarian population (Füredi et al. 1996) ($P = 0.79$). For the studied STR loci, the Caucasian populations thus appear to be a relatively homogeneous group.

Intermediate alleles

Intermediate allele 6A of locus HUMCYAR04 was formerly reported in Blacks (Hammond et al. 1994)) and was also found here in the Flemish Caucasian population. Allele 11A has been found before (Edwards et al. 1992) for locus HUMHPRTB in the Mexican-American population and was also found in Flemish Caucasians. Furthermore, for the same locus, we also found a 12A intermediate allele. It would be of interest to sequence these alleles in order to define the variation or mutation mechanism concerning these repeat sequences.

Mutation

A full-length repeat addition at locus HUMHPRTB was found in one paternity case. The child had a HUMHPRTB allele, not inherited from the mother and also not present in the alleged father. The eight other STR loci did not exclude paternity and gave a probability of paternity of W = 99.951%. Additional evidence for inclusion was gathered by typing for HLA DRB1 and HLA DQB1 loci and for the VNTRs D17S5 and D1S80. These supplementary typings did not exclude paternity. Combining the STR and VNTR data gave a probability of inclusion of W = 99.998%. Expansions or insertions of small oligonu-

cleotide (2–4) repeats seem to be mainly of paternal origin (Weber and Wong 1993), while a more locus dependent behaviour has been observed for VNTR loci (Henke and Henke 1995). Until now, the mechanism of mutation is not well understood and complex mechanisms have been proposed (Jeffreys et al. 1994). Since HUMHPRTB is only included in paternity analysis in cases of female children, we have examined only 48 meioses. The observed mutation thus yields a mutation rate of 2.1%, a misleadingly elevated estimate caused by the small sample size. Mutation incidences of 0.2 and 0.7% have been reported for other STR systems (Brinkmann et al. 1995). Probably only by accumulating data from parentage cases from different laboratories, can adequate estimates be made.

Paternity testing

While Alford et al. (1994), in 13 paternity cases examined with the same 9 STR systems, found no exclusion based on only one locus, we observed this event in 6 out of 103 (5.8%) cases of exclusion. This is illustrated by Figure 1, showing a shift of the number of excluding loci to lower numbers. In all these cases, additional evidence was obtained by HLA-typing and/or VNTR-typing. As mentioned before, we have also experienced one case of mutation. Eventually, it must be clear that also the highly acclaimed PCR STR systems do not differ from any other genetic system in the aspect that an isolated exclusion should not be sufficient to exclude paternity.

When only STR systems are used, typing of nine (eight for male children) loci seems a minimal requirement to be able to conclude on included paternity.

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References

Alford RL, Hammond HA, Coto I, Caskey CT (1994) Rapid and efficient resolution of parentage by amplification of short tandem repeats. Am J Hum Genet 55:190–195

Alonso S, Castro A, Fernandez I, Gomez de Cedron M, Garcia-Orad A, Meyer E, Martinez de Pancorbo M (1995) Population study of 3 STR loci in the Basque Country (northern Spain). Int J Legal Med 107:239–245

Alper B, Wiegand P, Brinkmann B (1995) Frequency profiles of 3 STRs in a Turkish population. Int J Legal Med 108:110–112

Brinkmann B, Möller A, Wiegand P (1995) Structure of new mutations in 2 STR systems. Int J Legal Med 107:201–203

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

Füredi S, Budowle B, Woller J, Padar Z (1996) Hungarian population data on six STR loci – HUMVWFA31, HUMTHO1, HUMCSF1PO, HUMFES/FPS, HUMTPOX, and HUMHPRTB – derived using multiplex PCR amplification and manual typing. Int J Legal Med 109:100–101

Garber RA, Morris JW (1983) General equations for the average power of exclusion for genetic systems of n codominant alleles in one parent and no parent cases of disputed parentage. In: Walker RH (ed) Inclusion probability in parentage testing. American Association of Blood Banks, Arlington, Va, pp 277–280

Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361–372

Hammond HA, Jin L, Zhong Y, Caskey CT, Chakraborty R (1994) Evaluation of 13 short tandem repeat loci for use in personal identification applications. Am J Hum Genet 55:175–189

Henke J, Henke L (1995) Recent observations in human DNA-minisatellite mutations. Int J Legal Med 107:204–208

Hochmeister MN, Budowle B, Schumm JW, Sprecher CJ, Borer UV, Dirnhofer R (1995) Swiss population data and forensic efficiency values on 3 tetrameric short tandem repeat loci-HUMTHO1, TPOX, end CSF1PO – derived using a STR multiplex system. Int J Legal Med 107:246–249

Jeffreys AJ, Tamaki K, MacLeod A, Monckton DG, Neil DL, Armour JAL (1994) Complex gene conversion events in germline mutation at human minisatellites. Nat Genet 6:136–145

Kubat M, Wiegand M, Brinkmann B (1995) Population genetic study from the Zagreb area using 3 STR systems. Int J Legal Med 107:219–221

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Morris JW (1983) Relationships between power of exclusion and probability of paternity. In: Walker RH (ed) Inclusion probability in parentage testing. American Association of Blood Banks, Arlington, Va, pp 267–276

Nelis E, Simokovic S, Timmerman V, Löfgren A, Backhovens H, De Jonghe P, Martin JJ, Van Broeckhoven C (1997) Mutation analysis of the Connexin 32 (Cx32) gene in Charcot-Marie-Tooth neuropathy type 1: identification of five new mutations. Hum Mut 9 : 47–52

Raymond M, Rousset F (1995) GENEPOP (ver. 1.2): a population genetics software for exact test and ecumenicism. J Hered 86: 248–249

Sjerps M, Van der Geest N, Pieron C, Gajadher M, Kloosterman A (1995) A Dutch population study of the STR loci HUMTHO, HUMFES/FPS, HUMVWA31/1 and HUMF13A1, concluded for forensic purposes. Int J Legal Med 108:127–134

Weber J, Wong C (1993) Mutation of human short tandem repeats. Hum Mol Genet 3:1–7