SHORT COMMUNICATION

C. Pestoni · M. V. Lareu · J. López-Gómez A. Carracedo

Genetic data on three complex STRs (ACTBP2, D21S11 and HUMFIBRA/FGA) in the Galician population (NW Spain)

Received: 5 October 1998 / Received in revised form: 18 December 1998

Abstract The allele frequency distributions of three complex STRs, ACTBP2 (SE33), D21S11 and HUMFIBRA/ FGA in the population of Galicia were investigated. Analysis was carried out under denaturing conditions and fluorescent detection in the ALF DNA sequencer and typing was made by comparison with sequenced allelic ladders. No significant deviations from Hardy-Weinberg equilibrium were observed. No significant differences were found between our data and other Caucasian population data. ACTBP2 seems to be one of the most informative and polymorphic STRs.

Key words ACTBP2 · D21S11 · HUMFIBRA/FGA · Complex STRs · Galician population

Introduction

Complex STRs such as ACTBP2 (Polymeropoulos et al. 1992), D21S11 (Sharma and Litt 1992) and HUMFI-BRA/FGA (Mills et al. 1992) are gaining increasing attention due to their forensic usefulness. Here we present genetic data from the Galician population from these three systems. Results were compared with other population studies and some statistical parameters indicating the usefulness of these systems in forensics are reported. No deviation from Hardy-Weinberg equilibrium was observed in any of the systems.

Material and methods

The PCR reaction was carried out with 1–5 ng DNA, 0.25 μ M each primer and 1 U Taq DNA polymerase in 25 μ l. Primer sequences and cycling conditions were as previously described for

University of Santiago de Compostela,

E-15705 Santiago de Compostela, Galicia, Spain

e-mail: apimlang@uscmail.usc.es

Tel. +34-981-582327; Fax +34-981-580336

ACTBP2 (Wiegand et al. 1993), D21S11 (Möller et al. 1994), and HUMFIBRA/FGA (Mills et al. 1992).

Separation and detection of the amplified products were carried out under denaturing conditions in an automated laser fluorescent (ALF) DNA sequencer (Pharmacia) (Pestoni et al. 1995). Typing was made by comparison with the sequenced allelic ladders. The ACTBP2 ladder was kindly provided by the Institute of Legal Medicine, Münster, Germany (Möller and Brinkmann 1994) and D21S11 and HUMFIBRA/FGA ladders by the Forensic Science Service, Birmingham, UK (Gill et al. 1996).

Results and discussion

Population data from Galicia for these three systems are shown in Tables 1 and 2. For the ACTBP2 system, the consensus nomenclature based on the number of repeats of the highly variable region proposed by the GEDNAP group was adopted (Schneider et al. 1998) as previously reported by Rolf et al. (1997) and Liu et al. (1997). This

Table 1 ACTBP2 allele frequencies in a population of Galicia (number of individuals: 184)

Allele (bp size)	Frequency	Allele	Frequency
12 (233)	0.005	24.2 (283)	0.035
12.2 (235)	0.011	25.2 (287)	0.014
13 (237)	0.016	26.2 (291)	0.043
13.2 (239)	0.005	27.2 (295)	0.060
14 (241)	0.046	28 (297)	0.003
15 (245)	0.038	28.2 (299)	0.098
16 (249)	0.073	29.2 (303)	0.049
17 (253)	0.071	30.2 (307)	0.052
18 (257)	0.106	31 (309)	0.003
19 (261)	0.079	31.2 (311)	0.016
20 (265)	0.046	32.2 (315)	0.014
20.2 (267)	0.003	33 (317)	0.003
21 (269)	0.030	33.2 (319)	0.003
21.2 (271)	0.008	34.2 (323)	0.003
22 (273)	0.005	36 (329)	0.003
22.2 (275)	0.027	37 (333)	0.005
23.2 (279)	0.027		

C. Pestoni · M. V. Lareu · J. López-Gómez · A. Carracedo (⊠) Institute of Legal Medicine,

 Table 2
 FGA and D21S11 allele frequencies (Galicia)

Allele	Frequency (n: 189)	Allele	Frequency (n: 188)
HUMFIBRA		D21S11	
18	0.011	< 26	0.003
19	0.068	27	0.035
19.2	0.008	28	0.125
20	0.132	29	0.229
20.2	0.011	30	0.271
21	0.167	30.2	0.045
21.2	0.003	31	0.048
22	0.214	31.2	0.106
22.2	0.013	32	0.003
23	0.140	32.2	0.090
23.2	0.003	33.2	0.035
24	0.087	34.2	0.003
24.2	0.005	35	0.005
25	0.093		
26	0.034		
27	0.008		
28	0.003		

FGA nomenclature: Gill et al. (1996); D21S11 nomenclature: Möller et al. (1994). n: number of individuals

nomenclature together with the nomenclature based on the size of the fragments (Gill et al. 1997; Dupuy and Olaisen 1997) are shown in Table 1. For D21S11 and

Table 3 Comparison of allele frequencies for FGA, D21S11 andSE33 between the Galician population and other European Caucasian populations. Two Asian populations (Chinese and Japan-

No significant differences (P < 0.05) were found between our data and other European Caucasian populations using an exact test (Raymond and Rousset 1995) (Table 3). Separation under denaturing conditions and the use of standard sequenced allelic ladders are essential in order to compare the results of the ACTBP2 system (Lareu et al. 1992, 1998; Schneider et al. 1998). For this reason, the comparison of populations for ACTBP2 was only carried out between our data and the data obtained following the conditions described above.

Some statistical parameters showing the forensic usefulness of these three systems are shown in Table 4. No deviations from Hardy-Weinberg equilibrium were observed in any of the systems using the exact test (Guo and Thompson 1992). Application of interpretative guidelines such as the \pm 0.5 bp rule and the shift calculation (Gill et al. 1996) for detecting intermediate alleles has proved to be useful when assigning alleles but some of the samples of the ACTBP2 system had to be re-run on a different gel to confirm the results.

Paternity cases where parenthood had been confirmed were used to search for mutations but none were observed in a total of 469 meioses (ACTBP2: 161; D21S11: 156; HUMFIBRA/FGA: 152 total paternal and maternal meioses).

ese) are also included. P: p value of the exact test of population differentiation (n° steps in the Markov chain: 10000; n° of dememorization steps: 1000); n: number of individuals

1 1		1 1			-			
	France ^a	Germany ^{b, c}	Holland ^d	Hungary ^e	Slovenia ^f	UK ^g	China ^{b, c}	Japan ^{b, c}
FGA								
Galicia	P: 0.9185 n: 232	P: 0.061 n: 453	P: 0.5851 n: 205	P: 0.7209 n: 127	P: 0.5092 n: 237	P: 0.0784 n: 602	P: 0.3397 n: 95	P: 0.3827 n: 136
D21S11								
Galicia	P: 0.9934 n: 232	P: 0.6308 n: 408	P: 0.3376 n: 205	P: 0.8332 n: 127	P: 0.2917 n: 210	P: 0.4606 n: 602	P: 0.0000 n: 97	P: 0.0000 n: 133
	Catalonia ^h	Valer	ncia ⁱ	Portugal ^j	Portugal ^j Switzerland ^k		ina ¹	Japan ¹
SE33								
Galicia	P: 0.9938 n: 154	P: 0.8 n: 18	3354 5	P: 0.9905 n: 144	P: 0.973 n: 197	1 P: n:	0.0217 179	P: 0.0307 n: 164

^aRousselet et al. 1997; ^bRolf et al. 1998; ^cBrinkmann et al. 1996; ^dOvington et al. 1997; ^eKozma et al. 1998; ^fZupanic et al. 1998; ^gEvett et al. 1997; ^bGené et al. 1996; ⁱPestoni et al. 1998; ^jSouto and Vide 1996; ^kDimo-Simonin et al. 1998; ¹Liu et al. 1997

Table 4 Statistical parameters showing the high forensic use-fulness of the systems. The power of discrimination (PD) was calculated following Fisher's method (Fisher 1951), heterozygosity value (*h*) and chance of exclusion (CE) were calculated as described by Nei and Roychoudhury (1974) and Ohno et al. (1982), respectively

	ACTBP2 (n: 184)	D21S11 (n: 188)	HUMFIBRA/FGA (n: 189)
h obs.	0.913	0.819	0.825
h exp.	0.944 ± 0.0207	0.834 ± 0.02808	0.869 ± 0.0276
PD	0.995	0.949	0.968
PD combined		0.99999	
CE	0.882	0.670	0.733
CE combined		0.990	

Acknowledgements This work was supported by a grant from *A Xunta de Galicia* (XUGA20806B97). We also appreciate the technical assistance of Amelia Rodríguez.

References

- Brinkmann B, Meyer E, Junge A (1996) Complex mutational events at the HumD21S11 locus. Hum Genet 98:60–64
- Dino-Simonin N, Grange F, Kratzer A, Brandt-Casadevall C, Mangin P (1998) Forensic validation of the short tandem repeat HUMACTBP2 using capillary electrophoresis. Electrophoresis 19:256–261
- Dupuy BM, Olaisen B (1997) A dedicated internal standard in fragment length analysis of hyperpolymorphic short tandem repeats. Forensic Sci Int 86:207–227
- Evett IW, Gill PD, Lambert JA, Oldroyd N, Frazier I, Watson S, Panchal S, Connolly A, Kimpton C (1997) Statistical analysis of data for three British ethnic groups from a new STR multiplex. Int J Legal Med 110:5–9
- Fisher RA (1951) Standard calculations for evaluating a blood group system. Heredity 5:95–102
- Gené M, Huguet E, Luna M, Moreno P, Lareu. MV, Carracedo A (1996) Study of HUMACTBP2 STR polymorphism, performed by PCR and automated laser fluorescence (ALF) sequencer in a population sample of Catalonia. In: Carracedo A, Brinkmarm B, Bär W (eds) Advances in forensic haemogenetics 6. Springer, Berlin Heidelberg New York, pp 635–636
- Gill P, Urquhart A, Millican E, Oldroyd N, Watson S, Sparkes R, Kimpton CP (1996) A new method of STR interpretation using inferential logic-development of a criminal intelligence database. Int J Legal Med 109:14–22
- Gill P, Brinkmann B, d'Aloja E, Andersen J, Bär W, Carracedo A, Dupuy B, Eriksen B, Jangblad M, Johnsson V, Kloosterman AD, Lincoln P, Morling N, Rand S, Sabatier M, Scheithauer F, Schneider P, Vide MC (1997) Considerations from the European DNA profiling group (EDNAP) concerning STR nomenclature. Forensic Sci Int 87:185–192
- Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48 :361–372
- Kozma Z, Nagai A, Woller J, Füredi S, Sétáló J, Ohya I, Nishi K (1998) Fluorescence based co-amplification and automated detection of the STR loci HUMFIBRA and HUMD21S11 in a Hungarian Caucasian population sample. Int J Legal Med 111: 103–104
- Lareu MV, Phillips CP, Pestoni C, Barros F, Muñoz J, Carracedo A (1992) Anomalous electrophoretic behaviour of HUMACTBP2 (SE33) In: Bär W, Fiori A, Rossi U (eds). Advances in forensic haemogenetics 5. Springer, Berlin Heidelberg New York pp 121–123
- Lareu V, Pestoni C, Phillips C, Barros F, Syndercombe Court D, Lincoln P, Carracedo A (1998) Normal and anomalous electrophoretic behavior of polymerase chain reaction-based DNA polymorphisms in polyacrylamide gels. Electrophoresis 19: 1566–1572
- Liu C, Harashima N, Katsuyama Y, Ota M, Arakura A, Fukushima H (1997) ACTBP2 gene frequency distribution and sequencing of the allelic ladder and variants in the Japanese and Chinese populations. Int J Legal Med 110:208–212
- Mills KA, Even D, Murray JC (1992) Tetranucleotide repeat polymophism at the human alpha fibrinogen locus (FGA). Hum Mol Genet 1(9):779

- Möller A, Brinkmann B (1994) Locus ACTBP2 (SE33) sequencing data reveal considerable polymorphism. Int J Legal Med 106:262–267
- Möller A, Meyer E, Brinkmann B (1994) Different types of structural variation in STRs: HumFES/FPS, HumVWA and HumD21S11. Int J Legal Med 106:319–323
- Nei M, Roychoudhury AK (1974) Sampling variances of heterozygosity and genetic distance. Genetics 76:379–390
- Ohno Y, Sebetan IM, Akaishi S (1982) A simple method for calculating the probability of excluding paternity with any number of codominant alleles. Forensic Sci Int 19:93–98
- Ovington A, Daselaar P, Sjerps M, Kloosterman A (1997) A Dutch population study of the STR loci D21S11 and HUMFIBRA. Int J Legal Med 110:14–17
- Pestoni C, Lareu MV, Rodríguez MS, Muñoz I, Barros F, Carracedo A (1995) The use of the STRs HUMTH01, HUMVWA31/A, HUM13A1, HUMFES/FPS, HUMLPL in forensic application: validation studies and population data from Galicia (NW Spain). Int J Legal Med 107:283–290
- Pestoni C, López-Gómez J, Aler M, Lareu MV, Rodríguez-Calvo MS, Carracedo A (1998) Complex STRs (ACTBP2, D21S11 and FIBRA/FGA) in two Spanish populations: new intermediate alleles and population genetic peculiarities in ACTBP2. In: Olaisen B, Brinkmann B, Lincoln PJ (eds) Progress in forensic genetics 7. Elsevier, Amsterdam Lausanne New York Oxford, pp 324–325
- Polymeropoulos MH, Rath DS, Xiao H, Merril CR (1992) Tetranucleotide repeat polymorphism at the human beta-actin related pseudogene H-beta-Ac-psi (ACTBP2). Nucleic Acids Res 20(6):1432
- Raymond M, Rousset F (1995) An exact test for population differentiation. Evolution 49:1280–1283
- Rolf B, Schürenkamp M, Junge A, Brinkmann B (1997) Sequence polymorphism at the tetranucleotide repeat of the human betaactin related pseudogene H-beta-Ac-psi (ATCBP2) locus. Int J Legal Med 110:69–72
- Rolf B, Waterkamp K, Hühne J (1998) Allele frequency data for the FGA locus in eight populations. Int J Legal Med 111:55– 56
- Rousselet F, Pfitzinger H, Mangin P (1997) A pentaplex automated fluorescent typing system for forensic identification and French caucasian population data. J Forensic Sci 42(3):500– 503
- Schneider HR, Rand S, Schmitter H, Weichhold G (1998) ACTBP2nomenclature recommendations of GEDNAP. Int J Legal Med 111:97–100
- Sharma V, Litt M (1992) Tetranucleotide repeat polymorphism at the D21S11 locus. Hum Mol Genet 1:67
- Souto L, Vide MC (1996) Allele frequency distribution of the STR system ACTBP2 (SE33) in a population of Portugal (Central Area). In: Carracedo A, Brinkmann B, Bär W (eds) Advances in forensic haemogenetics 6. Springer, Berlin Heidelberg New York, pp 650–651
- Wiegand P, Budowle B, Rand S, Brinkmann B (1993) Forensic validation of the STR systems SE33 and TC11. Int J Legal Med 105:315–320
- Zupanic I, Balazic J, Komel R (1998) Analysis of nine short tandem repeat (STR) loci in the Slovenian population. Int J Legal Med 111:248–250