Int J Legal Med (1999) 112 : 139–141 © Springer-Verlag 1999

SHORT COMMUNICATION

R. Pawłowski

HUMFIBRA allele distribution in northern Poland using capillary electrophoresis

Received: 8 May 1998 / Received in revised form: 24 August 1998

Abstract Amplification products of the HUMFIBRA locus were analysed in a population from Northern Poland using capillary electrophoresis. The most frequent alleles were 21, 22 and 20 with a frequencies of 0.201, 0.179 and 0.167 respectively, and five rare variants were observed with frequencies less than 0.015. A homogeneous allele distribution was found between Polish and Dutch populations and significant differences between Poles and Italians. The high discrimination power (0.963), polymorphic information content (0.849) and heterozygosity confirm the usefulness of HUMFIBRA locus for forensic identification purposes. The application of capillary electrophoresis offers the possibility of precise identification of rare variants.

Key words HUMFIBRA · PCR · Capillary electrophoresis · Population genetics · Poland

Introduction

The HUMFIBRA locus (FGA) [1] is situated in the third intron of the gene for the alpha fibrinogen chain (4q28; access number M64982 in the Gene Bank). Most HUMFIBRA alleles posses a regular 4 bp repeat unit, but there are also variants differing by 2 bp. According to Barber et al. [2] the repeat region of HUMFIBRA alleles ranging from 15 to 28 is made up of the sequence (TTTC)3 TTTT TTCT (CTTT)n CTCC (TTCC)2 and variants show a deletion of the dinucleotide CT in the sequence TTCT. Allele 29 and those up to 46.2 have a more complex nature, but they are observed very rarely.

The aim of this work was to test the usefulness of the system of capillary electrophoresis (CE) for detecting alleles at the locus HUMFIBRA and to assess the usefulness of this system in forensic haemogenetics. No data on frequency distribution of HUMFIBRA alleles have yet been published for the Polish population and the present study was carried out in a population from northern Poland.

R. Pawłowski (✉)
Institute of Forensic Medicine, Medical University of Gdañsk, PL 80–210, Gdañsk, Debinki 7, Poland
e-mail: Richard@amg.gda.pl
Fax +48-58-341-04-85

Materials and methods

DNA was isolated from 201 blood samples from unrelated individuals living in northern Poland (Gdansk region). The extraction and quantification were carried out according to Pawlowski et al. [3]. The amplification was carried out with a previously published pair of primers [1, 2]. The PCR reaction was carried out in 15 µl and the reaction mixture was made up of 0.35 U Taq DNA polymerase (Eurogentec, The Netherlands), 200 µM dNTP (Promega, USA), 0.5 µM of each primer (IDT, USA), 1.5 µl 10 × concentrated PCR buffer (Eurogentec, The Netherlands) and 3 ng template DNA. Amplification was carried out through 30 cycles of 94°C for 1 min, 60°C for 1 min and 70°C for 1 min 30 s using the Perkin Elmer (PE) ABI 877 PCR robotic station or the Trithermoblock (Biometra, Germany)

Capillary electrophoresis (CE)

Of the PCR products 1 µl was mixed with 12 µl of deionised formamide and 1 µl of internal DNA size standard (GS500, labelled with TAMRA). Before electrophoresis, the samples were denatured for 3 min at 95°C and subsequently snap-cooled on ice-water bath. The electrophoretic separation was conducted in a ABI Prism 310 Genetic Analyser (PE Applied Biosystems). The samples were electrokinetically injected for 10 s at 15 kV and run on a 47 cm (36 cm length to detector), 50 µm ID capillary filled with the denaturing polymer POP4 (Performance Optimised Polymer 4). The separation was conducted for 24 min at 15 kV, 9 mA, and 10 µW and 60°C. The raw data were analysed with GeneScan software v. 2.1 (GS500 peaks 250 bp and 340 bp were not used to size samples).

In order to assess the precision of the CE analysis, multiple injections of a sequenced HUMFIBRA allelic ladder (alleles 16–29, without 2 bp variants) obtained from Professor B. Brinkmann (Institute of Forensic Medicine, Muenster, Germany) were carried out. The same sample was injected many times in one series, with all other parameters being maintained at an identical level and also over a long period, when the only constant parameter was the DNA sample (HUMFIBRA ladder).

Table 1 Average sizes of HUMFIBRA alleles together with the SD values achieved during analysis precision measurement by the CE method

HUMFIBRA alleles (bp)	16	17	18	19	20	21	22	23	24	25	26	27	28	29
True size of allele	172	176	180	184	188	192	196	200	204	208	212	216	220	224
Average allele size	169.48	173.40	177.21	181.05	184.83	188.59	192.32	196.00	199.68	203.51	207.30	211.16	215.11	218.88
SD for within capillary series $n = 12$	0.12	0.13	0.09	0.10	0.11	0.10	0.09	0.07	0.08	0.09	0.11	011	0.13	0.09
SD for between capillaries $n = 28$	0.24	0.24	0.22	0.23	0.22	0.20	0.22	0.21	0.19	0.21	0.20	0.22	0.19	0.19

Statistical calculations

Allele frequencies, expected frequency of heterozygotes assuming HWE, standard error for h, power of discrimination (PD) and the polymorphic information content (PIC) were calculated as previously described [3, 4].

Results and discussion

The introduction of the automatic detection of STR-type systems by CE on the ABI Prism 310 apparatus, requires the definition of analysis precision. To this end multiple injections of sequenced HUMFIBRA ladder were conducted. The average HUMFIBRA allele size was defined and the value of standard deviations for within, and between capillaries was also defined (Table 1). The SD values for within capillary analyses ranged from 0.07–0.13 bp (0.007–0.14 of the SD range), while for between capillaries from 0.19 to 0.24 bp (0–0.45 of the SD range), which gives values and ranges of SD higher than the average values obtained by Ovington et al. [5] for HUMFIBRA using the ABI377 sequencer. Dupuy and Olaisen [6] observed similar values for HUMACTBP2 and HUMAPOA01 using the ABI373A and 377 analysers using GS500 as an internal size standard. Such a comparison does not appear feasible because a dependency seems to exist between the SD values obtained and the type of locus tested [6]. A comparison between the HUMFIBRA allele sizes obtained in our laboratory and those of Ovington et al. [5] indicates that different HUMFIBRA allele sizes are obtained depending on the type of apparatus and internal standard used. The average value of ± 3

Table 2 Distribution of alleles and phenotypes of the HUMFIBRA locus in a population from Poland ($n = 201$)

Alleles	16	17	18	19	19.2	20	21	21.2	22	22.2	23	23.2	24	25	25.2	26	27	28	29	Frequency of alleles
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0.0025
17		0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0.0050
18			0	0	0	1	1	0	4	0	1	0	0	0	0	0	0	0	0	0.0174
19				1	0	5	5	0	7	0	2	0	1	0	0	0	1	0	0	0.0572
19.2					0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0.0025
20						9	14	0	4	1	6	1	8	7	0	2	0	0	0	0.1666
21							8	0	14	2	10	1	9	6	0	2	0	0	0	0.2015
21.2								0	0	0	1	0	0	0	0	0	0	0	0	0.0025
22									5	0	12	0	9	8	0	2	1	0	0	0.1791
22.2										0	0	0	1	1	0	1	0	0	0	0.0149
23											0	0	8	4	0	0	0	0	0	0.1095
23.2												1	1	1	0	0	0	0	0	0.0149
24													2	2	0	1	1	0	0	0.1119
25														2	0	0	0	0	0	0.0821
25.2															0	0	0	0	0	0.0025
26																0	0	0	0	0.0224
27																	0	0	0	0.0075
28																		0	0	0.0000
29																			0	0.0000
Σ																				1.0000

Observed heterozygosity	0.861	PD	0.963
Expected heterozygosity (SE)	0.866 (0.012)	PIC	0.849

standard deviations was accepted as the range of values for particular HUMFIBRA alleles. In this range 99.74% of all measurements of HUMFIBRA fragments can be found. The precision values obtained therefore allow a clear distinction not only of HUMFIBRA alleles differing by 4 bp but also of the variants differing by 2 bp. The average size of HUMFIBRA fragments obtained (sequenced allelic ladder) differed in relation to their true sizes obtained by sequencing by about 2.5 bp for allele 16 to over 5 bp for allele 29 (Table 1). The cause of this observed difference can be the nucleotide content of the analysed fragments [6] and also the type of fluorescence marker used. The size differences were observed not only for different fluorescence markers but also for different alleles [7] and, just as in our experiments, they were larger in direct relation to the greater number of repetitive elements they contained [7]. We observed a greater electrophoretic mobility of alleles also in the native electrophoretic system (according to Budowle and Allen [8]) when the HUMFIBRA fragments labelled with TET were compared to an unlabelled HUMFIBRA allele ladder (data not shown).

In the population sample of 201 unrelated people 17 alleles were found forming 52 out of 153 theoretically possible phenotypes (Table 2). Among the observed alleles 5 variants were detected (19.2,21.2,22.2,23.2 and 25.2) differing by 2 bp from the alleles with exclusively tetranucleotide repetitive units. The most frequent alleles in the sample tested were 21, 22 and 20 (0.2010, 0.1791 and 0.1666 respectively), while the most common phenotypes were 20/21 and 21/22. The rarest of the observed alleles were the variants 19.2, 21.2, 25.2, as well as 16, which appears very rarely in Europe. Allele 16 was not observed in the sample from the Dutch population ($n = 205$) and in the population sample of 201 people from northern Italy it was present in only one person [5, 9]. A comparison of homogeneity of the HUMFIBRA allele distribution between the Polish, Dutch and Italian populations revealed a statistically significant difference between the Polish and Italian populations ($P = 0.016 \pm 0.004$), while in the comparison with the Dutch population there was a highly homogeneous distribution ($P = 0.576 \pm 0.154$). A homogenous distribution was also observed between Italian and Dutch populations ($P = 0.135 \pm 0{,}011$). The χ^2-test did not indicate deviations from HWE ($\chi^2 = 83.65$; $0.250 > p > 0.100$, for 66 d.f.). HUMFIBRA is characterised by a high heterozygosity ($H_{obs.} = 0.861$) in accordance with the value of the calculated expected heterozygosity and with the high power of discrimination (PD) and the PIC value (Table 2). Very similar values for H_{obs}, PD and PIC were observed in population samples of similar numbers from Italy and the Netherlands [5, 9]. All these parameters indicate the very high usefulness of this system in identification tests and is also shown by the comparison with nine other commonly used DNA systems [3, 10] where HUMFIBRA is only exceeded by ACTBP2.

In conclusion CE is fully suitable for the identification of HUMFIBRA alleles, but a direct comparison of allele sizes among different automatic electrophoretic systems gives different values. The correct definition of alleles can therefore only be achieved using an additional standard in the form of a ladder composed of sequenced alleles.

References

1. Mills KA, Even D, Murray JC (1992) Tetranucleotide repeat polymorphism at the human alpha fibrinogen locus (FGA). Hum Mol Genet 1:779
2. Barber MD, McKeown BJ, Parkin BH (1996) Structural variation in the alleles of a short tandem repeat system at the human alpha fibrinogen locus. Int J Legal Med 108:180–185
3. Pawlowski R, Maciejewska A, Paszkowska R, Welz A (1997) Frequencies of the five short tandem repeat systems (STR) in a population from North Poland. Int J Legal Med 110:10–13
4. Pawlowski R, Paszkowska R, Hauser R, Brinkmann B (1996) Population studies of three AMPFLPs systems in a North Polish population. Int J Legal Med 109:155–156
5. 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
6. Dupuy BM, Olaisen B (1997) A dedicated internal standard in fragment length analysis of hyperpolymorphic short tandem repeats. Forensic Sci Int 86:207–227
7. Poeltl R, Luckenbach C, Fimmers R, Ritter H (1997) Typing of the short tandem repeat D8S347 locus with different fluorescence markers. Electrophoresis 18:2871–2873
8. Budowle B, Allen RC (1991) Discontinuous Polyacrylamide Gel Electrophoresis of DNA Fragments. In: Mathew C (ed) Methods in molecular biology, vol 9. Protocols in human molecular genetics. The Humana Press Inc, Clifton, NJ
9. Betti F, Giacomazzo B, Ghio F, Piccinini A (1997) North Italian population genetic data on the STR system HumFGA. Int J Legal Med 110:111
10. Pawlowski R, Maciejewska A, Paszkowska R, Welz A (1997) The current status of forensic DNA analysis in Poland. In: Proceedings from the First European Symposium on Human Identification 1996. Promega Corp, pp 116–125