Int J Legal Med (1995) 107:283–290

ORIGINAL ARTICLE

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The use of the STRs HUMTH01, HUMVWA31/A, HUMF13A1, HUMFES/FPS, HUMLPL in forensic application: validation studies and population data for Galicia (NW Spain)

Received: 24 October 1994 / Received in revised form: 14 February 1995

Abstract The 5 tetranucleotide short tandem repeats, HUMTH01, HUMVWA31/A, HUMF13A1, HUMFES/FPS and HUMLPL were studied using different electrophoretic methods and PCR amplification conditions in order to optimize the typing conditions. A genetic population study in the population of Galicia was carried out and the allele and genotype frequencies are given. Compliance with the Hardy-Weinberg equilibrium was tested using different statistical parameters, with clear advantages resulting in favor of using the exact test (Guo-Thompson method) instead of conventional chi-square methods. Some statistical parameters of forensic interest (PD, CE, h) were also calculated. There were no mutations found in a total of 73 paternal meioses and 101 maternal meioses. Abnormal electrophoretic mobility was found in the AT-rich STR HUMF13A1 under non-denaturing conditions and, therefore, the use of denaturing conditions is absolutely necessary. No "stutter" bands were found, although double peaks in the HUMFES/FPS system were observed in some samples. The advantage of using automated sequencers with fluorescent technology is also reported.

Key words Short Tandem Repeats · HUMTH01 · HUMVWA31/A · HUMF13A1 · HUMFES/FPS · HUMLPL · PCR · Population studies · Forensic validation

Introduction

Short tandem repeat (STR) systems are highly polymorphic loci consisting of simple tandemly repeated sequences 1–6 bp in length. Because of their small sizes (< 300 bp) they are amenable to PCR amplification with the additional advantage of making the successful typing of forensic material containing highly degraded DNA more likely. Also, STR typing in polyacrylamide gels allows precise allele sizing, thus avoiding the problems of the continuous allele distribution models.

Since this technology has recently been introduced as a method for individual identification, appropriate standardization protocols and validation studies are required. One of the more important areas of concern is the electrophoretic mobility of some STR systems, which has been demonstrated to be a great problem in typing AT-rich STRs (Lareu et al. 1994a; Gill et al. 1994; Möller and Brinkmann 1994). In addition, the assessment of these genetic markers in forensic identification and paternity testing requires the existence of appropriate databases for the populations where the systems are going to be used. Bearing in mind all these points, the specific aims of this study were:

- To obtain allele frequency estimates for a Galician (NW Spain) population sample for the following STRs:
  - HUMTH01: Human Tyrosine Hydroxylase gene, intron 1 (Edwards et al. 1991) Location:11p15.5–p15; Repeat: (AATG)
  - HUMVWA31/A: Human VWF gene, intron 40 (Kimpton et al. 1992) Location: 12p12–12pter; Repeat: (TCTA, TCTG)
  - HUMF13A1: Human Coagulation Factor XIII A subunit gene (Polymeropoulos et al. 1991a) Location: 6p24–p25; Repeat: (GAAA)
  - HUMFES/FPS: Human C-FES/FPS Proto-oncogene (Polymeropoulos et al. 1991b) Location: 15q25–qter; Repeat: (ATTT)
  - HUMLPL: Human Lipoprotein Lipase gene (Zuliani and Hobbs 1990) Location: 8q22; Repeat: (TTTA)

The results were also compared with other population studies and the allele frequencies were tested for deviation from Hardy-Weinberg equilibrium using different statistical parameters.

- To calculate some of the statistical parameters of medico-legal interest, such as heterozygosity value, dis-

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crimination power and chances of exclusion in paternity cases. Family data from paternity cases from our institute are also reported thus providing additional information about mutations.

- To study the electrophoretic behaviour of these STRs under different electrophoretic conditions, including the use of denaturing gels in an automatic sequencer.

Materials and methods

Blood samples were obtained from healthy unrelated individuals from Galicia (NW Spain). DNA was extracted from EDTA blood using a phenol-chloroform procedure (Valverde et al. 1993). DNA was quantified using a Perkin Elmer 552 UV/VIS spectrophotometer.

Primer sequences

HUMTH01 (Edwards et al. 1991)
*5′GTGGGCTGAAAAGCTCCCGATTAT3′
5′ATTCAAAGGGTATCTGGGTCTTGG3′

HUMVWA31/A (Kimpton et al. 1992)
*5′CCCTAGTGGATGATAAGAATAATC3′
5′GGACAGATGATAAATACATAGGATGGATGG3′

HUMF13A1 (Polymeropoulos et al. 1991a)
5′GAGGTTGCACTCCAGCCTTT3′
*5′ATGCCATGCAGATTAGAAA3′

HUMFES/FPS (Polymeropoulos et al. 1991b)
*5′GGGATTTCCCTATGGATTGG3′
5′GCGAAAGAATGAGACTACAT3′

HUMLPL (Williamson et al.1990)
*5′AAATCTGACCAAGGATAGTGGG3′
5′CGAGTGACGTTGGAGACGAAAG3′
(* Primer 5′end labeled with fluorescent dye)

Oligonucleotides were synthesized following the phosphoramidite method in a 380A DNA synthesizer and purified through an OPC column (Applied Biosystems, Foster City, Calif.). Selected primers were 5′end labeled with fluorescent dye (Pharmacia, Sweden).

Amplification conditions

PCR amplification for all 5 systems was performed using 5–25 ng of genomic DNA in a 50 µl reaction volume. Each locus was amplified initially in an individual reaction consisting of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin, 1.5 mM $MgCl_2$, 200 µM each dNTP, 0.25 µM each primer and 1.25 units AmpliTaq DNA Polymerase (Cetus, Emeryville, Calif.).

For PCR duplex reactions (HUMTH01-HUMFES/FPS and HUMVWA31/A-HUMF13A1), identical buffers, dNTPs and enzyme concentrations were employed with the only differences being the primer concentrations: 0.125 µM each HUMTH01 primer, 0.0625 µM each HUMFES/FPS primer, 0.15 µM each HUMVWA31/A primer, 0.08 µM each HUMF13A1 primer.

PCR cycling (individual and duplex reaction) conditions were 94°C-45 sec, 54°C-1 min, 72°C-1 min; 30 cycles in a programmable heatblock (DNA Thermal Cycler 9600, Perkin-Elmer/Cetus).

Detection systems

Non-denaturing gel electrophoresis: electrophoretical separation of the amplified fragments was carried out in polyacrylamide gels (T:10%, C:3.3%) with piperazine diacrylamide as crosslinker, 0.035 M tris sulfate (pH 9), 17 cm separation distance, and 2% agarose plugs in 0.52 M tris borate (pH 8.5). The separation conditions were 450 V, 30 mA, 50 W. Electrophoresis was stopped when the bromophenol blue front reached the anodal end of the gel. Bands were visualised using silver staining (Heukeshoven and Dernik 1985). HUMTH01 and HUMVWA31/A were routinely typed using this method.

Denaturing gel electrophoresis: 5 µl of loading buffer (5 mg/ml dextran blue/formamide) were added to 1 µl of each PCR product and combined with internal lane standards (Pharmacia). The samples were heat denatured at 94°C for 2 min before being loaded onto a standard 6% polyacrylamide denaturing sequencing gel (7.0 M urea, 5.7% acrylamide, 0.3% bisacrylamide, 100 mM tris-borate pH 8.3 and 1 mM Na_2EDTA). The gels were run for 4 h at constant power (45 W), 1600 V and 42 mA on the Automatic Laser Fluorescent (ALF) DNA sequencer (Pharmacia). Fragment sizes were determined automatically using the "Fragment Manager" software, and typed by comparison with a sequenced allelic ladder. HUMF13A1, HUMFES/FPS and duplex systems were routinely typed with this method.

Allelic designation

Allelic designation of the STRs systems was made according to the repeat number in line with the recommendations of the DNA Commission of the International Society for Forensic Haemogenetics (1992). The 173 bp allele at the HUMTH01 locus was found and designated as allele 9.3, and the 181 bp allele at the HUMF13A1 locus was designated as allele 3.2.

Sequenced allelic ladders for each STR system included most of the alleles of each particular STR.

Statistical analysis

Hardy-Weinberg equilibrium was tested for with conventional Pearson's chi-square methods (χ^2), and with the two exact tests proposed by Guo and Thompson (1992) based on conventional Monte Carlo methods and the Markov chain approach, respectively. For the Monte Carlo method the number of batches was 100 and the size of each batch 170. For the Markov chain method the number of batches was 20 and the size of each batch 5000. For the Markov chain method the dememorization period was 1000 steps.

Comparison of population data was carried out using a 2-way RxC contingency table test comparing allele distributions for population sample homogeneity and using chi-square as statistical parameters.

Other statistical parameters of genetic and medico-legal interest that were used: power of discrimination (PD) was calculated following Fisher's method (Fisher 1951), heterozygosity value (h) was calculated as described by Nei and Roychoudhury (1974) and chance of exclusion (CE) was calculated as described by Ohno et al. (1982).

Results and discussion

Allele and genotype frequencies

The allele and genotype frequencies for the 5 systems are shown in Tables 1–5.

The most frequent alleles were: HUMTH01: 9.3; HUMVWA31/A: 16; HUMF13A1: 7; HUMFES/FPS: 11; HUMLPL: 2.

Alleles consisting of 11 repeats in HUMTH01 and 13, 21 and 22 repeats in HUMVWA31/A that have been previously reported in the literature (Edwards et al. 1992; Wiegand et al. 1993; Hochmeister et al. 1994; Möller et al. 1994) were not detected in our study. A total of 12

Table 1 Allele and genotype frequencies of the HUMTH01 system in the Galician population ($n = 234$)

Genotype	Observed (%)	Expected (%)
5–7	1 (0.4)	0.2 (0.1)
6–6	9 (3.9)	10.5 (4.5)
6–7	18 (7.7)	17.1 (7.3)
6–8	15 (6.4)	13.1 (5.6)
6–9	22 (9.4)	17.6 (7.5)
6–9.3	26 (11.1)	30.0 (12.8)
7–7	6 (2.5)	7.0 (2.9)
7–8	9 (3.9)	10.7 (4.6)
7–9	14 (6.0)	14.4 (6.1)
7–9.3	27 (11.5)	24.6 (10.5)
8–8	4 (1.7)	4.1 (1.7)
8–9	10 (4.3)	11.0 (4.7)
8–9.3	20 (8.5)	18.8 (8.1)
9–9	6 (2.5)	7.4 (3.1)
9–9.3	25 (10.8)	25.2 (10.9)
9.3–9.3	22 (9.4)	21.5 (9.3)
Others	0 (0.0)	0.8 (0.3)

Allele	Frequency
5	0.0021
6	0.2115
7	0.1731
8	0.1325
9	0.1774
9,3	0.3034

$\chi^2 = 8.0679$; $P = 0.7133$; df = 11; Exact test: $P = 0.928$

Table 2 Allele and genotype frequencies of the HUMVWA31/A system in the Galician population ($n = 158$)

Genotype	Observed (%)	Expected (%)
14–14	3 (1.9)	2.6 (1.7)
14–15	7 (4.4)	4.5 (2.9)
14–16	10 (6.3)	10.6 (6.7)
14–17	7 (4.4)	9.2 (5.8)
14–18	10 (6.3)	9.2 (5.8)
14–19	1 (0.6)	1.7 (1.1)
15–15	1 (0.6)	1.9 (1.2)
15–16	9 (5.7)	9.1 (5.7)
15–17	7 (4.4)	7.9 (5.0)
15–18	9 (5.7)	7.9 (5.0)
15–20	1 (0.6)	0.3 (0.2)
16–16	13 (8.3)	10.6 (6.7)
16–17	19 (12.0)	18.4 (11.7)
16–18	15 (9.5)	18.4 (11.7)
16–19	3 (1.9)	3.4 (2.2)
17–17	8 (5.1)	8.0 (5.1)
17–18	17 (10.8)	16.0 (10.1)
17–19	4 (2.6)	2.9 (1.8)
17–20	1 (0.6)	0.7 (0.4)
18–18	8 (5.1)	8.0 (5.1)
18–19	4 (2.6)	2.9 (1.8)
19–20	1 (0.6)	0.1 (0.1)
Others	0 (0.0)	3.5 (2.2)

Allele	Frequency
14	0.1297
15	0.1108
16	0.2595
17	0.2247
18	0.2247
19	0.0411
20	0.0095

$\chi^2 = 16.3799$; $P = 0.4529$; df = 16; Exact test: $P = 0.857$

alleles were found for HUMF13A1, including alleles 10 and 12 that have not been detected in other caucasian population studies (Kimpton et al. 1993). The allele size ranges found were: HUMTH01 (154–173 bp), HUMVWA31/A (139–163 bp), HUMF13A1 (181–235 bp), HUMFES/FPS (213–233 bp) and HUMLPL (171–191 bp).

Hardy-Weinberg equilibrium

A prerequisite for the application of any genetic marker in forensic cases is that the system be tested for deviation from Hardy-Weinberg equilibrium. The methods proposed so far for testing H-W equilibrium can be categorized into 2 groups. One consists of large-sample goodness-of-fit tests such as Pearson's χ^2, the likelihood ratio statistics G^2 or the conditional χ^2 test (Li 1955). The χ^2 is the test most commonly used in population genetic studies. The other approach involves exact tests (Levene 1949; Haldane 1954; Chapco 1976) and is clearly more appropriate for large numbers of alleles. In such cases, even if the sample size is only moderately large, the number of genotypes is so large that many genotype frequencies will be zero, especially when the corresponding allele frequencies are low. Therefore, the adequacy of applying a classical χ^2 test is questionable.

Exact tests (or probability tests) for H-W equilibrium are based on Fisher's exact test for independence in contingency tables. These tests depend on the discrete multinomial distribution for multiple-allele systems and require the enumeration of all possible samples which keep the same gene frequencies and sample sizes as in the observed sample. However enumeration becomes a problem as the sample size or the number of alleles increases and the gene frequencies become close to each other. For this reason, classical exact tests involve many calculations and are difficult to apply to highly polymorphic systems.

The only solution seemed to be the use of binning approaches and classical χ^2 tests (Brenner and Morris 1990; Rand et al. 1992; Lareu et al. 1993), which are less sensitive but still informative to some degree.

Table 3 Allele and genotype frequencies of the HUMF13A1 system in the Galician population ($n = 143$)

Genotype	Observed (%)	Expected (%)
3.2– 4	1 (0.7)	0.9 (0.7)
3.2– 5	3 (2.0)	4.3 (3.0)
3.2– 6	10 (7.0)	7.1 (5.0)
3.2– 7	11 (7.7)	9.1 (6.4)
4 – 5	1 (0.7)	1.9 (1.3)
4 – 6	3 (2.1)	3.1 (2.1)
4 – 7	6 (4.2)	4.0 (2.8)
5 – 5	5 (3.5)	4.4 (3.1)
5 – 6	15 (10.5)	14.2 (9.9)
5 – 7	19 (13.3)	18.4 (12.9)
5 –12	1 (0.7)	0.2 (0.1)
5 –15	1 (0.7)	0.9 (0.6)
6 – 6	7 (4.9)	11.5 (8.0)
6 – 7	32 (22.4)	29.7 (20.8)
6 – 8	1 (0.7)	0.6 (0.4)
6 –10	1 (0.7)	0.3 (0.3)
6 –15	2 (1.4)	1.4 (1.0)
6 –16	1 (0.7)	0.6 (0.4)
6 –17	2 (1.4)	0.6 (0.4)
7 – 7	16 (11.2)	19.3 (13.5)
7 – 8	1 (0.7)	0.7 (0.5)
7 –11	1 (0.7)	0.4 (0.3)
7 –15	2 (1.4)	1.8 (1.3)
7 –16	1 (0.7)	0.7 (0.5)
Others	0 (0.0)	6.8 (4.7)

Allele	Frequency
3.2	0.0874
4	0.0385
5	0.1748
6	0.2832
7	0.3671
8	0.0070
10	0.0035
11	0.0035
12	0.0035
15	0.0175
16	0.0070
17	0.0070

$\chi^2 = 24.4307$; $P = 0.0311$; df = 13; Exact test: $P = 0.708$

Recently, new approaches for performing exact tests have been proposed (Louis and Dempster 1987; Hernández and Weir 1989; Guo and Thompson 1992). Here we have used the Guo and Thompson approach (1992). They proposed 2 algorithms (based on the conventional Monte Carlo method and the construction of the Markov chain respectively) to estimate the significance level for a test of Hardy-Weinberg. The advantages and disadvantages of each method are discussed in Guo and Thompson (1992) but they are remarkably simple and easy to compute. Similar results were obtained with both meth-

Table 4 Allele and genotype frequencies of the HUMFES/FPS system in the Galician population ($n = 124$)

Genotype	Observed (%)	Expected (%)
8–10	3 (2.4)	1.0 (0.8)
9–11	2 (1.6)	0.8 (0.6)
10–10	9 (7.3)	13.5 (11.0)
10–11	40 (32.2)	34.4 (27.7)
10–12	15 (12.2)	14.9 (12.1)
10–13	6 (4.8)	4.0 (3.2)
11–11	21 (16.9)	21.8 (17.6)
11–12	18 (14.5)	18.9 (15.2)
11–13	2 (1.6)	5.0 (4.0)
12–12	4 (3.2)	4.1 (3.3)
12–13	4 (3.2)	2.2 (1.8)
Others	0 (0.0)	3.4 (2.7)

Allele	Frequency
8	0.0121
9	0.0081
10	0.3306
11	0.4193
12	0.1815
13	0.0484

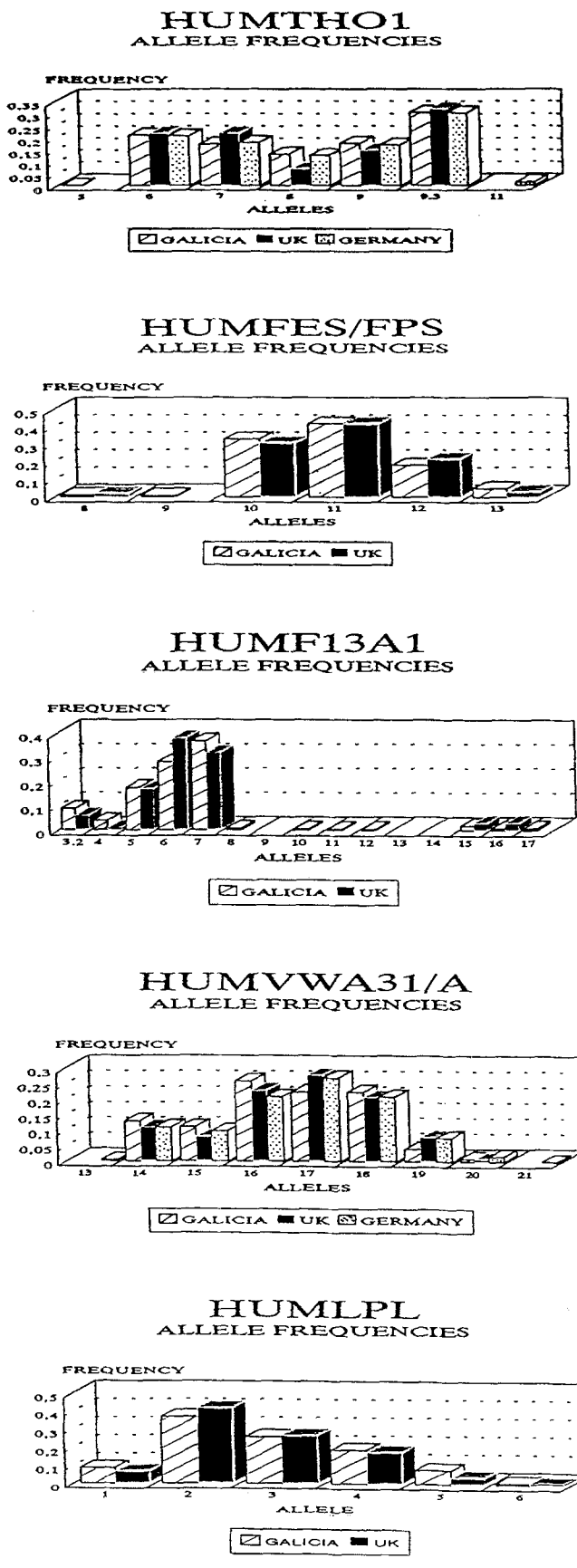
$\chi^2 = 15.9963$; $P = 0.0203$; df = 6; Exact test: $P = 0.7234$

Table 5 Allele and genotype frequencies of the HUMLPL system in the Galician population ($n = 113$)

Genotype	Observed (%)	Expected (%)
1–2	5 (4.4)	7.4 (6.6)
1–3	7 (6.2)	5.2 (4.6)
1–4	7 (6.2)	3.8 (3.3)
1–5	1 (0.8)	1.6 (1.4)
2–2	19 (16.9)	15.6 (13.9)
2–3	23 (20.4)	22.0 (19.5)
2–4	14 (12.5)	16.0 (14.2)
2–5	4 (3.5)	6.7 (5.9)
3–3	5 (4.4)	7.7 (6.8)
3–4	10 (8.9)	11.2 (9.9)
3–5	7 (6.2)	4.7 (4.1)
3–6	2 (1.8)	0.5 (0.5)
4–4	4 (3.5)	4.1 (3.6)
4–5	4 (3.5)	3.4 (3.0)
5–5	1 (0.8)	0.7 (0.6)
Others	0 (0.0)	2.4 (2.1)

Allele	Frequency
1	0.0885
2	0.3717
3	0.2611
4	0.1903
5	0.0796
6	0.0088

$\chi^2 = 15.3769$; $P = 0.1321$; df = 10; Exact test: $P = 0.695$



◀ **Fig. 1** Comparison of data from Galicia with German (HUMTH01, Wiegand et al. 1993; HUMVWA31/A, Möller et al. 1994) and UK (HUMTH01, Lareu et al. 1994b; HUMFES/FPS, HUMF13A1 and HUMVWA31/A, Kimpton et al. 1993; HUMLPL, Wall et al. 1993) populations

Table 6 Comparison of different populations with the Galician data

Population	χ^2	*P* value
HUMTH01		
Galicia-Caucasian UK (Lareu et al. 1994b)	14.88	0.5335
Galicia-Germany (Wiegand et al. 1993)	21.71	0.1962
Galicia-Caucasian USA (Edwards et al. 1992)	11.01	0.9235
Galicia-Black USA (Edwards et al. 1992)	96.22	0.0001
Galicia-Mexican-Amerindian (Edwards et al. 1992)	48.32	0.0001
Galicia-South Spain (Lorente 1994)	18.89	0.4641
HUMVWA31/A		
Galicia-Caucasian UK (Kimpton et al. 1993)	12.42	0.9275
Galicia-Germany (Möller et al. 1994)	20.08	0.8903
Galicia-South Spain (Lorente et al. 1994)	27.40	0.2394
HUMF13A1		
Galicia-Caucasian UK (Kimpton et al. 1993)	16.38	0.8739
Galicia-Greece (Greenhalgh et al. 1994)	10.38	0.4966
Galicia-Northern Europe (Greenhalgh et al. 1994)	23.37	0.0248
Galicia-Gujarati (Greenhalgh et al. 1994)	45.27	0.0001
HUMFES/FPS		
Galicia-Caucasian UK (Kimpton et al. 1993)	6.14	0.8636
Galicia-Asian UK (Kimpton et al. 1993)	16.27	0.2349
Galicia-Afrocaribbean UK (Kimpton et al. 1993)	16.96	0.2013
HUMLPL		
Galicia-Northern Europe (Wall et al. 1993)	4.69	0.9896
Galicia-Greece (Wall et al. 1993)	6.95	0.9367
Galicia-Caucasian USA (Ahn et al. 1992)	20.37	0.1189
Galicia-Gujarati (Wall et al. 1993)	32.96	0.0029

ods (data not shown) but, since the Monte Carlo method is most suitable for data with a large number of alleles but a small sample size (Guo and Thompson 1992), only the results of the Monte Carlo approach are reported here.

All the systems studied, with the exception of the HUMF13A1 and HUMFES/FPS, were in Hardy-Wein-

berg equilibrium for both conventional χ^2 as well as for the Guo and Thompson exact tests. The usefulness of the exact tests is exemplified in these cases. The large number of genotypes affects the χ^2 estimates but the systems are clearly in H-W equilibrium as demonstrated by the Guo and Thompson tests.

Therefore, we strongly recommend the use of these exact tests when the number of alleles is large.

Comparison of populations

Few population studies of STRs have been published thus making population comparison a difficult task. In addition, some population studies only report allele frequencies in graphics, making any comparison impossible. For these reasons, only the Galician and UK populations could be compared in each of the 5 systems studied; a qualitative comparison is shown in Fig. 1. Data from a German population is also included when available.

Table 6 shows a quantitative comparison between some published population studies and the Galician data: χ^2 and p values were calculated using a 2-way RxC contingency table.

Significant differences were observed between the Galician population and the Mexican Amerindian (for HUMTH01), the Black USA population (for HUMTH01), and the population data from Indian Gujaratis (for HUMF13A1 and HUMLPL).

No significant differences with other caucasians populations were observed in any of the STRs studied. As with other DNA polymorphisms, population heterogeneity does not seem to be a problem for these markers, and the distribution of alleles in caucasians is also similar.

Meioses

Germline length mutations in VNTR loci have been encountered in human genealogy, and in some VNTR systems they have been shown to be especially high. Due to this fact, the prerequisite of a number of meioses in a polymorphism that is high enough to give a good estimate of the mutation rate for paternity casework is even more important for DNA polymorphisms.

In our case, no mutations were obtained in a total of 73 paternal meioses and 101 maternal meioses. Although the number of meioses studied until now is too low to make conclusions, the mutation rate seems to be reasonably low.

Parameters of medico-legal interest

Other statistics of genetic and medico-legal interest are shown in Table 7.

The combined chance of exclusion is 0.97801, and the combined discrimination power is 0.99999. Observed heterozygosity values show no significant differences with expected values.

Electrophoretic behaviour

The electrophoretic mobility of the STRs included in this work was tested in non-denaturing and denaturing polyacrylamide gels with different T and C values (T: 6%–20%; C: 2%–5%). A 123 bp ladder and a pBR322/MspI digest were used together with the allelic ladders of each system to study the electrophoretic mobility.

"Normal electrophoretic behaviour" was defined to occur when the relative mobility of the allelic ladder fragments stayed equal or nearly equal using different T and C values. "Anomalous behaviour" was defined to occur when changes in the T and C values were accompanied by changes in the relative mobility of the allelic ladder fragments. Temperature was strictly controlled in the electrophoretic experiments because it influences the absolute mobility but not the relative electrophoretic behaviour.

Normal electrophoretic behaviour was found in all the systems studied with the exception of HUMF13A1 under non-denaturing conditions. In this case, an extremely high anomalous electrophoretic behaviour was found even if we only changed the T proportions, leaving C values constant. An example is shown in Fig. 2. The most plausible explanation for the observed behaviour is that stable, local distorsions of the DNA helix axis results in macroscopic

Table 7 Forensic value of the STRs studied using various statistical parameters in the Galician population

Systems	*h*	PD	CE
HUMTH01	0.7859	0.9195	0.5737
HUMVWA31/A	0.8033	0.9308	0.6052
HUMF13A1	0.7475	0.8956	0.5226
HUMFES/FPS	0.6821	0.8392	0.4172
HUMLPL	0.7465	0.8932	0.5160

h: heterozygosity value, PD: power of discrimination, CE: chance of exlusion

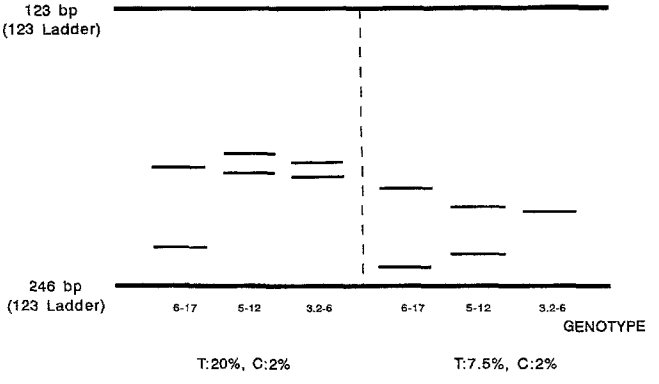


Fig. 2 Relative mobility of three HUMF13A1 genotypes in polyacrylamide gels with different T values under non-denaturing conditions

curvature when such distorsions are propagated in phase with the helix repeat (Hagerman 1985).

The existence of anomalous electrophoretic mobility in some DNA fragments has long been known (Mertz and Berg 1974; Maniatis et al. 1975), especially in AT-rich fragments (Marini et al. 1982; Stellwagen 1983). Anomalous migrations of artificial fragments with periodical distribution of AT was reported by Hagerman (1985). In human STRs, anomalous mobility has been found in the ACTPB2 system (SE33) (Lareu et al. 1994a) and has proved to be a problem for the forensic standardization of this system (Gill et al. 1994; Möller and Brinkmann 1994).

Like SE33, HUMF13A1 is another AT-rich STR, confirming that changes in DNA conformations amenable to producing noticeable changes in mobility are more likely to be produced in AT-rich STRs. The mobility of HUMF13A1 is, nevertheless, normal under denaturing conditions. No significant differences in the relative mobility were found using various gel compositions.

In our opinion, HUMF13A1 should only be used under denaturing conditions for forensic purposes. If non-denaturing conditions were to be used, and the electrophoretic conditions (T and C values, temperature, etc) were not strictly standardized, different results would be expected for the same sample in inter-lab comparisons. For this same reason, population data obtained for this STR using non-denaturing conditions should be considered with caution.

Double peaks

"Stutter" bands produced by slippage during PCR are typical of dinucleotides and have been reported for other STRs but were not observed in this study.

Double peaks in HUMFES/FPS were observed in some samples. The incomplete addition of an extra base by the Taq polymerase enzyme at the end of the extension is the most probable reason for this (Kimpton et al. 1993). Some strategies have been considered for addressing this problem (Kimpton et al. 1993) which, in any event, does not represent a serious problem for typing.

Electrophoretic systems

Reliable results were found using discontinuous PAGE and silver staining, especially for HUMTH01 and HUMVWA31/A and the use of an automated sequencer with fluorescent technology has a number of advantages, mainly sensitivity, enhanced level of precision and accuracy.

Using the ALF sequencer, reliable results were obtained from 0.1 ng of template DNA and 30 cycles. In order to avoid non-specific amplification products and contamination risks, 5 ng and 30 cycles is required for more consistent STRs results. Similar results have been found using other sequencers and different fluorochromes (Kimpton et al. 1993; Frégeau and Fourney 1993).

The level of precision achieved with the sequencer was similar to the data discussed in the literature with SDs between 0.06 and 0.11 in intergel comparisons (data not shown).

Other advantages of this technology have to do with the routine processing of large numbers of DNA samples, not only because of the capacity of the gel (40 samples) but also because of the possibility of re-using the gel. Reliable results were obtained when using the gel a second time with HydroLink matrix (AT Biochem), but although there are no problems with re-using the gels in population studies, more caution is required for forensic samples, and is therefore not recommended. In addition, the sequencer greatly facilitates the simultaneous detection of different STRs after PCR multiplex amplification. Duplex amplification of HUMTH01-HUMFES/FPS and HUMVWA31/A-HUMF13A1 has proved to provide good results. Using duplex amplification no significant differences in peak sizes were observed in 80 samples tested and, although this population study was performed using only single-plex amplification, we believe that these 2 duplexes can be used with accuracy for fresh blood samples (i.e. paternity cases).

Acknowledgments This work was supported by grants from the Ministerio de Educación y Ciencia (DGICYT PB92-0371) and Fondo de Investigación Sanitaria (FISS 94/1053). The technical assistance of Amelia Rodríguez López is also acknowledged with appreciation.

References

Ahn YI, Kamboh MI, Ferrell RE (1992) Two new alleles in the tetranucleotide repeat polymorphism at the lipoprotein lipase (LPL) locus. Hum Genet 90:184

Brenner C, Morris JW (1990) Paternity index calculations in single locus hypervariable DNA probes: validation and other studies. Prodeedings for the International Symposium on Human Identification. Promega Corporation, Madison, USA, pp 21–52

Chapco W (1976) An exact test of the Hardy-Weinberg equilibrium in small samples. Biometrics 33:536–542

DNA recommendations – 1992 report concerning recommendations of the DNA Commission of the International Society for Forensic Haemogenetics relating to the use of PCR-based polymorphisms. Int J Legal Med 105:63–64

Edwards A, Civitello A, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 49:746–756

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

Fisher RA (1951) Standard calculations for evaluating a blood group system. Heredity 5:95–102

Frégeau CJ, Fourney RM (1993) DNA typing with fluorescently tagged short tandem repeats: a sensitive and accurate approach to human identification. BioTechniques 15:100–119

Gill P, Kimpton C, D'Aloja E, Andersen JF, Bär W, Brinkmann B, Holgersson S, Johnson V, Kloosterman AD, Lareu MV, Nellemann L, Pfitzinger H, Phillips CP, Schmitter H, Schneider PM, Stenersen M (1994) Report of the European DNA profiling group (EDNAP) – towards standardisation of short tandem repeat (STR) loci. Forensic Sci Int 65:51–59

Greenhalgh M, Andersen J, Wall W, Williamson R, Parkin B (1994) Letter to the editor. Int J Leg Med 107:162–163

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

Hagerman PJ (1985) Sequence dependence of the curvature of DNA: a test of the phasing hypothesis. Biochemistry 24: 7033–7036

Haldane JBS (1954) An exact test for randomness of mating. J Genet 52:631–635

Hernández JL, Weir BS (1989). A disequilibrium coefficient approach to Hardy-Weinberg testing. Biometrics 45:53–70

Heukeshoven J, Dernik R (1985) Simplified methods for silver staining of proteins in polyacrylamide gels and the mechanism of silver staining. Electrophoresis 6:103–112

Hochmeister MN, Jung JM, Budowle B, Borer UV, Dirnhofer R (1994) Swiss population data on three tetrameric short tandem repeat loci – VWA, HUMTH01, and F13A1 – derived using multiplex PCR and laser fluorescence detection. Int J Legal Med 107:34–36

Kimpton CP, Walton A, Gill P (1992) A further tetranucleotide repeat polymorphism in the vWF gene. Hum Mol Genet 1:28

Kimpton CP, Gill P, Walton A, Urquhart A, Millican ES, Adams M (1993) Automated DNA profiling employing multiplex amplification of short tandem repeat loci. PCR Methods Appl 3:13–22

Lareu MV, Muñoz I, Pestoni C, Rodriguez MS, Vide C, Carracedo A (1993) The distribution of HLA DQA1 and D1S80 (pMCT118) alleles and genotypes in the populations of Galicia and Central Portugal. Int J Legal Med 106:124–128

Lareu MV, Phillips CP, Pestoni C, Barros F, Muñoz JI, Carracedo A (1994a) Anomalous electrophoretic behaviour of HUMACTBP2 (SE33). In: Bär W, Fiori A, Rossi M (eds) Advances in Forensic Haemogenetics 5. Springer, Berlin Heidelberg New York, pp 121–123

Lareu MV, Phillips CP, Carracedo A, Lincoln PJ, Syndercombe D, Thompson JA (1994b) Investigation of the STR locus HUMTH01 using PCR and two electrophoresis formats: UK and Galician Caucasian population surveys and usefulness in paternity investigations. Forensic Sci Int 66:41–52

Levene H (1949) On a matching problem arising in genetics. Ann Math Stat 20:91–94

Li CC(1955) Population genetics. Chicago: University of Chicago Press

Lorente M (1994) Polimorfismo del ADN e identificación médico-legal: estudio de siete loci (D1S80, D17S5, HUMTH01, HUMVWA, ACTBP2, D21S11 y HLA-DQ-alfa) mediante la reacción en cadena de la polimerasa (PCR). Granada: Doctor Thesis

Lorente JA, Lorente M, Budowle B, Wilson MR, Villanueva E (1994) Analysis of short tandem repeat (STR) HUMVWA in the Spanish population. Forensic Sci Int 65:169–175

Louis EJ, Dempster ER (1987) An exact test for Hardy-Weinberg and multiple alleles. Biometrics 43:805–811

Maniatis T, Jeffrey A, van de Sande H (1975) Chain length determination of small double- and single-stranded DNA molecules by polyacrylamide gel electrophoresis. Biochemistry 14:3787–3995

Marini JC, Levene SD, Crothers DM, Englund PT (1982) Bent helical structure in kinetoplast DNA. Proc Natl Acad Sci USA 79:7664–7668

Mertz JE, Berg P (1974) Viable deletion mutants of simian virus 40: selective isolation by means of a restriction endonuclease from hemophilus parainfluenzae. Proc Natl Acad Sci USA 71:4879–4883

Möller A, Brinkmann B (1994) Locus ACTBP2 (SE33). Sequencing data reveal considerable polymorphism. Int J Legal Med 106:262–267

Möller A, Wiegand P, Grüschow C, Seuchter SA, Baur MP, Brinkmann B (1994) Population data and forensic efficiency values for the STR systems HumVWA, HumMBP and HumFABP. Int J Legal Med 106:183–189

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

Polymeropoulos MH, Rath DS, Xiao H, Merril CR (1991a) Tetranucleotide repeat polymorphism at the human coagulation factor XIII A subunit gene (F13A1). Nucleic Acids Res 19: 4306

Polymeropoulos MH, Rath DS, Xiao H, Merril CR (1991b) Tetranucleotide repeat polymorphism at the human c-fes/fps proto-oncogene (FES). Nucleic Acids Res 19:4018

Rand S, Puers C, Skowasch K, Wiegand P, Budowle B, Brinkmann B (1992) Population genetics and forensic efficiency data of 4 AMPFLP's. Int J Legal Med 104:329–333

Stellwagen NC (1983) Anomalous electrophoresis of deoxyribonucleic acid restriction fragments on polyacrylamide gels. Biochemistry 22:6186–6193

Valverde E, Cabrero C, Cao R, Rodriguez Calvo MS, Díez A, Barros F, Alemany J, Carracedo A (1993) Population genetics of three VNTR polymorphisms in two different Spanish populations. Int J Legal Med 105:251–256

Wall W, Williamson R, Petrou M, Papaioannou D, Parkin B (1993) Variation of short tandem repeats within and between populations. Hum Mol Genet 2:1023–1029

Wiegand P, Budowle B, Rand S, Brinkmann B (1993) Forensic validation of the STR systems SE 33 and TC11. Int J Legal Med 105:315–320

Williamson R, Bowcock A, Kidd K, Pearson P, Schimidtke J, Chan HS, Chiperfield M, Cooper DN, Hewitt TJ, Lewitter F, Maidak B, Quitt M, Ricciuti F, Track R (1990) Report of the DNA Committee and catalogues of cloned and mapped genes and DNA polymorphisms. Cytogenet Cell Genet 55:457–778

Zuliani G, Hobbs HH (1990) Tetranucleotide repeat polymorphism in the LPL gene. Nucleic Acids Res 18:4958