Int J Leg Med (1994) 107:34–36

ORIGINAL ARTICLE

M. N. Hochmeister · J. M. Jung · B. Budowle
U. V. Borer · R. Dirnhofer

Swiss population data on three tetrameric short tandem repeat loci – VWA, HUMTHO1, and F13A1 – derived using multiplex PCR and laser fluorescence detection

Received: 25 January 1994 / Received in revised form: 4 April 1994

Abstract Allele and genotype frequencies for 3 tetrameric short tandem repeat loci VWA, HUMTHO1, and F13A1 were determined in a Swiss population sample using multiplex PCR and subsequent electrophoresis in DNA sequencing gels processed by automated laser fluorescence detection. The technique allows single base pair resolution and rapid typing, with a concomitant reduction in the potential for human transcriptional typing errors. All loci meet Hardy-Weinberg expectations. In addition, there is little evidence for association of alleles among the 3 loci. The allelic frequency data can be used in forensic analyses and paternity tests to estimate the frequency of a multiple STR locus DNA profile in the Swiss population.

Key words PCR · Multiplex · VWA · HUMTHO1 F13A1 · Fluorescence detection · Hardy-Weinberg expectations · Short tandem repeat loci

Zusammenfassung An einer Schweizer Populationsstichprobe wurden mittels Multiplex-PCR und automatisierter Fluoreszenzdetektion (Applied Biosystem 373A DNA Sequencer) die Allel- und Genotypfrequenzen der Short tandem repeat (STR) loci VWA, HUMTHO1 und F13A1 bestimmt. Die Daten wurden umfangreichen statistischen Analysen unterzogen. Alle Loci erfüllen die Hardy-Weinberg-Kriterien. Die ermittelten Daten erlauben die Abschätzung der Häufigkeit des Vorkommens eines Multiplex-STR-Profils in der Schweizer Bevölkerung zur Anwendung in forensischen DNA-Analysen und Vaterschaftsuntersuchungen.

Schlüsselwörter PCR · Multiplex$_2$ · VWA · HUMTHO1 F13A1 · Fluoreszensdetektion · Hardy-Weinberg-Gleichgewicht · Short tandem repeat loci

M. N. Hochmeister (✉) · U. V. Borer · R. Dirnhofer
Department of Forensic Medicine, Institut für Rechtsmedizin, University of Bern, Bühlstraße 20, CH-3012 Bern, Switzerland

J. M. Jung · B. Budowle
Forensic Science Research and Training Center, FBI Academy, Quantico, VA 22135, USA

Introduction

Typing polymorphic loci at the DNA level has become a routine procedure in the paternity and identity testing fields. Originally, highly polymorphic variable number of tandem repeats (VNTR) loci were characterized by restriction fragment length polymorphism (RFLP) analysis. A subgroup of these VNTR loci is the short tandem repeats (STR) loci, which are highly polymorphic and are abundant in the human genome (Edwards et al. 1991, 1992). Moreover, STR loci, which are generally less than 350 base pairs in length, are amenable to amplification by the polymerase chain reaction (PCR) (Saiki et al. 1985; Edwards et al. 1991). Therefore, STRs can by typed with a high degree of specificity and sensitivity, in a relatively short time period, and without the need for isotopic detection methods (Edwards et al. 1991, 1992; Gill et al. 1992; Sullivan et al. 1992). Moreover, the amplified products of STRs can be resolved to single bases by separation on denatured polyacrylamide gels (Edwards et al. 1991). Thus, more discrete allelic data can be obtained for the loci than was possible with VNTRs typed by RFLP analysis.

Currently, there are little data on the STR allele frequencies and genotype distributions in various populations for forensically useful STR loci. For the use of genetic markers, such as STRs in identity testing, it is desirable to collect allele/genotype data from relevant population(s) so that the forensic scientist can provide a guideline or estimate of the rarity of a genetic profile. This paper presents allele/genotype frequency data in a Swiss population sample for 3 tetrameric STR loci, VWA (Kimpton et al. 1992), HUMTHO1 (Edwards et al. 1991; Polymeropoulos et al. 1991 a), and F13A1 (Polymeropoulos et al. 1991 b), typed using multiplex PCR and subsequent electrophoresis in DNA sequencing gels with automated laser fluorescence detection (Edwards et al. 1991, 1992; Gill et al. 1992; Sullivan et al. 1992). The data demonstrate that these loci can be useful for providing estimates of the frequency of a DNA profile in identity testing cases.

Materials and methods

Whole blood was obtained in EDTA Vacutainer tubes by venipuncture from 100 unrelated Caucasian donors from the Swiss Red Cross (Basel, Switzerland). The DNA was extracted according to the non-organic method of Grimberg et al. (1989). The quantity of DNA in each sample was estimated using the slot-blot procedure described by Waye et al. (1989).

The coamplification of VWA, HUMTHO1, and F13A1 was performed using a procedure that was modified from Kimpton et al. (1993). The PCR was carried out in 50 µl reaction volumes containing 5 ng template DNA, 800 ng bovine serum albumin (BSA), 10 m*M* Tris-HCl, pH 8.3, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% gelatin, 6.7 nmol each of the 4 deoxyribonucleoside triphosphates, 10 pmoles of each VWA primer, 9 pmoles of each HUMTHO1 primer, 12.5 pmoles of each F13A1 primer and 1.1 units of Taq DNA polymerase. The reactions were carried out in a Perkin Elmer 9600 thermal cycler and were subjected to 28 cycles of denaturation at 95° C for 1 min, a 95° C to 54° C cooling ramp for 2 mins, primer annealing at 54° C for 1 min, and primer extension at 72° C for 1 min. In the last cycle, the primer extension period was 11 min at 72° C.

Allelic ladders for VWA, HUMTHO1, and F13A1 were kindly provided by Kimpton and Gill (Central Research and Support Establishment, Birmingham, United Kingdom). The ladders were diluted 10^5-fold for VWA, 10^6-fold for HUMTHO1, and 10^3-fold for F13A1 and amplified as described above but without BSA.

Typing of the amplified samples was according to the automated fluorescent detection method of Kimpton et al. (1993). Electrophoresis was carried out on an Applied Biosystems 373A DNA Sequencer. The sizes of the DNA fragments in the samples and the allelic ladders were determined by applying the user-defined feature of the Genescan Analysis Software to the Genescan 2500 standard in each lane. Allele designations were determined by comparison of the base pair sizes of the sample fragments with those of the allelic ladders.

The frequency of each allele at each locus was calculated from the numbers of each genotype in the sample set. Unbiased estimates of expected heterozygosity were computed as described by Edwards et al. (1992). Possible divergence from Hardy-Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (Chakraborty et al. 1988; Nei and Roychoudhury 1974; Nei 1978), the likelihood ratio test (Chakraborty et al. 1991; Edwards et al. 1992; Weir 1992), and the exact test (Guo and Thompson 1992). An inter-class correlation criterion (Karlin et al. 1981) was used for detecting disequilibrium between loci. Independence among more than 2 loci was determined by examining whether the observed variance of the number of heterozygous loci in the population sample was outside its confidence interval under the assumption of independence (Brown et al. 1980). When appropriate, the Bonferroni procedure (Weir 1990) was used to correct for multiple analyses to determine whether or not HWE or equilibrium between loci holds in the population.

Table 1 VWA allele frequencies in a sample of unrelated Swiss Caucasians

Allele	Frequency (n = 100)
14	0.100
15	0.095
16	0.225
17	0.290
18	0.215
19	0.060
20	0.010
21	0.005

a) Observed homozygosity = 0.180
b) Expected homozygosity (unbiased) = 0.200
c) HWE-homozygosity test (P = 0.622), likelihood ratio test (P = 0.466), and exact test (P = 0.538)

Table 2 HUMTO1 allele frequencies in a sample of unrelated Swiss Caucasians

Allele	Frequency (n = 100)
5	0.010
6	0.245
7	0.185
8	0.110
9	0.160
9.3	0.280
10	0.010

a) Observed homozygosity = 0.180
b) Expected homozygosity (unbiased) = 0.207
c) HWE-homozygosity test (P = 0.511), likelihood ratio test (P = 0.317), and exact test (P = 0.450)

Table 3 F13A1 allele frequencies in a sample of unrelated Swiss Caucasians

Allele	Frequency (n = 99)
3	0.020
4	0.045
5	0.202
6	0.323
7	0.354
8	0.000
9	0.005
10	0.000
11	0.015
12	0.000
13	0.005
14	0.000
15	0.010
16	0.020

a) Observed homozygosity = 0.232
b) Expected homozygosity (unbiased) = 0.270
c) HWE-homozygosity test (P = 0.400), likelihood ratio test (P = 0.200), and exact test (P = 0.169)

Results and discussion

All but one of the 100 samples were typed successfully using the protocol described. The one sample was typeable for VWA and HUMTHO1, but not for F13A1. The distributions of observed allelic frequencies for VWA, HUMTHO1, F13A1 are shown in Tables 1–3. All alleles differed in size by one repeat unit (i.e., 4 base pairs) for all loci, except for the HUMTHO1 allele 9.3. The 9.3 allele, which is a relatively common HUMTHO1 allele in the Swiss population sample (f = 0.280) is one base pair smaller in size than the 10 allele. The ability to type the 9.3 allele unequivocally demonstrates the resolving capacity of the electrophoretic system used in our study.

There was no evidence for deviation from expected values for the 3 STR loci based on the homozygosity test, likelihood ratio test, and the exact test (Tables 1–3). Analyses also were performed to determine whether or not there were any detectable associations between any of the STR loci. An inter-class correlation test (Karlin et al. 1981) analysis demonstrated that there is little evidence for correlation between the alleles at any of the pairs of loci. The pair-wise comparison of HUMTHO1 and F13A1 showed a marginal deviation from expectation (P = 0.047). There was no evidence for deviation for the other pair-wise comparisons. A Bonferroni procedure (Weir 1990) was used as a correction when multiple tests were

performed on a population sample. After correction, there was no evidence for association between loci ($P = 0.017$ is the rejection level). As an additional test for association, independence among the 3 loci was evaluated by examining whether or not the observed variance (s_k^2) of the number of heterozygous loci in the population sample is outside the confidence interval under the assumption of independence using the procedure described by Brown et al. (1980). There was no evidence of association for the STR loci described in our Swiss sample population using s_k^2 criterion ($s_k^2{}_{OBS} = 0.551$; 95% confidence interval of variance is 0.335–0.604).

In conclusion, a Swiss population database has been established for VWA, HUMTHO1, and F13A1. The data demonstrate that valid estimates of a multiple STR locus profile frequency can be derived for identity testing purposes using the product rule under the assumption of independence.

References

Brown AHD, Feldman MW, Nevo E (1980) Multilocus structure of natural populations of Hordeum spontaneum. Genetics 96: 523–536

Chakraborty R, Smouse PE, Neel JV (1988) Population amalgamation and genetic variation: observations on artificially agglomerated tribal populations of Central and South America. Am J Hum Genet 43: 709–725

Chakraborty R, Fornage M, Guegue 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 applications. Birkhäuser, Berlin, pp 127–143

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 H, Jin L, Caskey CT, Chakraborty R (1992) Genetic variation at five trimeric and tetrameric repeat loci in four human population groups. Genomics 12: 241–253

Gill P, Kimpton CP, Sullivan K (1992) A rapid method for identifying fixed specimens by DNA profiling. Electrophoresis 13: 173–175

Grimberg J, Nawoschik S, Belluscio L, McKee R, Turck A, Eisenberg A (1989) A simple and efficient nonorganic procedure for the isolation of genomic DNA from blood. Nucleic Acids Res 17: 8390

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

Karlin S, Cameron EC, Williams PT (1981) Sibling and parent-offspring correlation estimation with variable family size. Proc Natl Acad Sci USA 78: 2664–2668

Kimpton CP, Walton A, Gill P (1992) A further tetranucleotide repeat polymorphism in the VWF gene. Human Mol Genet 1: 287

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

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590

Nei M, Roychoudhury AK (1974) Sampling variances of heterozygosity and genetic distance. Genetics 76: 379–390

Polymeropoulos MH, Xiao H, Rath DS, Merril CR (1991a) Tetranucleotide repeat polymorphism at the human tyrosine hydrolase gene (TH). Nucleic Acids Res 19: 3753

Polymeropoulos MH, Rath DS, Xiao H, Merril CR (1991b) Tetranucleotide repeat polymorphism at the human coagulation factor XIII A subunit gene (F13A1). Nucleic Acids Res 19: 4036

Saiki RK, Scharf S, Faloona T, Mullis KB, Horn GT, Erlich HA, Arnheim N (1985) Enzymatic amplification of beta-globin genomic sequences and restriction analysis for diagnosis of sickle cell anemia. Science 230: 1350–1354

Sullivan KM, Pope S, Gill P, Robertson JM (1992) Automated DNA profiling by fluorescent labeling of PCR products. PCR Methods Appl 2: 34–40

Waye JS, Presley L, Budowle B, Shutler GG, Fourney RM (1989) A simple method for quantifying human genomic DNA in forensic specimen extracts. Biotechniques 7: 852–855

Weir BS (ed) (1990) Multiple tests. In: Genetic Data Analysis. Sinauer, Sunderland, Massachusetts, pp 109–110

Weir BS (1992) Independence of VNTR alleles defined by fixed bins. Genetics 130: 873–887