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SHORT COMMUNICATION

B. Glock · D. W. M. Schwartz · E. M. Schwartz-Jungl
G. H. Thorwartl · E. M. Dauber · W. R. Mayr

A new allele at the short tandem repeat locus HumF13A01

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Abstract We investigated the (AAAG)n short tandem repeat (STR) polymorphism HumF13A01 an Austrian Caucasoid population sample (n = 674). PCR amplified fragments were detected on an automatic A.L.F. DNA sequencer using laser-induced fluorescence. A total of 14 alleles could be identified, including a new 179 bp allele which was designated allele 3. Sequence determination of allele 3 confirmed the typing results by revealing three continuous copies of the core repeat, whereas in sequencing of 54 additional alleles no further variants or microheterogeneities could be observed. The population data showed no significant deviation from Hardy-Weinberg equilibrium.

Key words Short tandem repeat (STR) · Sequencing · HUMF13A01 · Population genetics

Introduction

The (AAAG)n STR polymorphism in the 5′ untranslated region of the human coagulation factor subunit A gene on 6p24–p25, designated HUMF13A01 (GenBank Accession No. M21986; Polymeropoulos et al. 1991) has been investigated in an Austrian population sample (Vienna region) in order to build up a local frequency data base and to evaluate its usefulness for genetic and forensic applications.

B. Glock (✉) · D. W. M. Schwartz · E. M. Schwartz-Jungl
E. M. Dauber · W. R. Mayr
Clinical Department for Blood Group Serology,
University of Vienna, Währinger Gürtel 18-20/4I,
A-1090 Vienna, Austria
Fax: +43(1) 40 400 5321

G. H. Thorwartl
Institute of Forensic Medicine, University of Salzburg,
Ignaz-Harrer Strasse 79, A-5020 Salzburg, Austria

Materials and methods

Genomic DNA extraction from peripheral blood of 674 healthy, unrelated Austrian individuals, elution of alleles after horizontal polyacrylamide electrophoresis, sequencing of the 11 ladder alleles and 44 additional alleles and construction of the allelic ladder was conducted as described elsewhere (Glock et al. 1996a, b). Amplification of the population samples by PCR was performed employing 0.4 μ*M* each 5′ fluorescent labelled reverse primer and unlabelled forward primer (Polymeropoulos et al. 1991) and 8–20 ng DNA in 28–30 cycles of the protocol according to Wall et al. (1993). For sequencing, eluted alleles were reamplified with 0.1 μ*M* biotinylated forward primer and 0.2 μ*M* reverse primer.

Typing was carried out using 1 μl of the amplification products, 7.5 fmol each of the size standards 150 bp and 250 bp (Pharmacia Biotech) and 3 μl loading buffer (Pharmacia Biotech). After denaturing, the samples were run on 0.5 mm thick, 6% denaturing polyacrylamide gels at 50° C, 1500 V, 38 mA, 34 W in 0.6 × Tris-borat EDTA for 200 min on an A.L.F. DNA sequencer (Pharmacia LKB Technology AB). Lanes 1, 13, 26 and 40 of the 40-well gel contained the sequenced allelic ladder as external size standard.

Sizing was performed employing Fragment manager software (Pharmacia Biotech, version 1.1). Allele assignment was made by size comparison with the allelic ladder (+/– 0.5 bp range). Evaluation of Hardy-Weinberg expectations using the exact test (Guo and Thompson 1992) and determination of further statistical parameters of forensic interest (mean exclusion chance, Krüger et al. 1968; polymorphism information content, Botstein et al. 1980; probability of match and discrimination power, Jones 1972) was done using the computer programme HWE-analysis, version 3.0 (Christoph Puers, Institute for Legal Medicine, University of Münster).

Results and dicussion

Laser fluorescent typing of the short tandem repeat polymorphism HUMF13A01 in an Austrian population sample of 674 healthy, unrelated individuals revealed 14 different alleles, including a 179 bp allele, which is 2 bp shorter than the smallest reported allele 3.2. It was designated allele 3 in accordance with the recommendations of the International Society for Forensic Haemogenetics (1994). Sequence determination of allele 3 revealed three continuous copies of the (AAAG) core repeat, which confirmed the allele assignment. Additional sequencing of 54

Table 1 HUMF13A01-Allele frequencies, allele sizes, number of sequenced alleles and further statistical parameters of forensic interest in an Austrian Caucasian population sample (n = 674)

Allele designation	Allele frequency (%)	Number of sequenced alleles	Allele size (bp)
3	0.07	1	179
3.2	8.16	5	181
4	3.19	5	183
5	18.32	6	187
6	31.45	6	191
7	33.38	5	195
8	0.30	3	199
11	0.15	2	211
12	0.07	1	215
13	0.37	5	219
14	1.34	4	223
15	1.93	5	227
16	1.12	5	231
17	0.15	2	235

Mean paternity exclusion chance (MEC): 0.524
Polymorphism information content (PIC): 0.707
Probability of match (pM): 0.108
Discrimination power (D): 0.892

different alleles, including the ladder alleles, produced results which are in perfect agreement with those published by Puers et al. (1994). In side-by-side comparisons of the 5′ and 3′ sequence regions of all 55 alleles no further microheterogeneities (with the exception of the known variant allele 3.2) were observed.

Allele sizes, number of sequenced alleles, the resulting allele frequencies and additional parameters of forensic interest are shown in Table 1. The observed heterozygosity was 0.7671 and the expected value was 0.7482. No significant deviations from Hardy-Weinberg equilibrium could be detected (exact test: $P = 0.857$). In comparison of populations no significant differences could be observed between Austrian and Galician data (Pestoni et al. 1995), whereas allele frequencies and distribution in a Japanese population sample (Nagai et al. 1996) were significantly different (data not shown).

In another series of samples no mutations could be observed in a total number of 333 meioses.

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