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SHORT COMMUNICATION

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D1S1171: a new highly variable short tandem repeat polymorphism

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Abstract This study reports the evaluation of the STR locus D1S1171 (GDB: 312934) for forensic purposes, which was investigated by PCR amplification and native polyacrylamide gel electrophoresis in 141 unrelated Austrians. No deviations from Hardy-Weinberg expectations were observed. The mean exclusion chance (MEC) was 0.677, the discriminating power (DP) was 0.951 and the observed heterozygosity rate was 0.853. An allelic ladder consisting of 10 sequenced alleles (96–132 bp) was constructed. Sequence analysis revealed a GAAA repeat motif. According to the number of tetranucleotide repeats the smallest allele was designated 9 and the largest allele 18.

Keywords STR · PCR · Sequencing · Allelic ladder · D1S1171

Introduction

Individual differences are based on repetitive DNA sequences in the human genome. In the case of short tandem repeat loci (STRs) these sequences consist of di- to pentameric repeats [1, 2] with fragment lengths usually smaller than 300 bp. These properties make STRs highly suitable for forensic purposes such as stain analysis and paternity testing. In general STRs range from extremely complex to the most simple polymorphisms [3, 4, 5]. As these loci are abundant in the human genome [6] and only a small number has been evaluated up to now, interest should focus on the evaluation of new, more efficient STRs. The aim of this study was to determine the forensic parameters of the tetrameric repeat locus D1S1171 (GDB ID 312934) which have not been investigated so far. Therefore we evaluated the genetic variation of this STR in an Austrian population sample to test its sensitivity and to construct a sequenced allelic ladder.

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Material and methods

Reagents

Taq polymerase, reaction buffer and dNTPs were obtained from Finnzymes (Espoo, Finland) and oligonucleotide primers were obtained from MWG Biotech (Ebersberg, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany), Serva (Heidelberg, Germany) and Sigma (St. Louis, Mo., USA).

DNA preparation

DNA was extracted from blood samples of 141 unrelated Austrians following a modified alkaline lysis protocol [7]. For testing the applicability to casework DNA was extracted from 10 blood stains, 5 postage stamps and 3 samples of chewing gum following the same protocol [7].For the sensitivity study K562 cell line DNA was step-wise diluted down to 10 pg/µl.

PCR assay

Amplification was performed using 2.5 µl DNA extracts without previous quantification in a 12.5 µl reaction volume. The reaction mixture contained 1×buffer (10 mM Tris-HCl, pH 8.8, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100), 0.75 mM $MgCl_2$, 0.5 µM each primer, 200 µM dNTPs and 0.2 U Taq polymerase. Primers were selected from the GDB entry (ID GDB: 312934) as folows:

1. D1S1171 I: 5'-GGG CAA CAA AGT AAG ACC C-3'
2. D1S1171 II: 5'-TTT CCC ATA GCC CCT GTG C-3'

For PCR, a protocol was applied utilising 30 cycles of denaturation at 94 C for 1 min, annealing at 60 C for 1 min and extension at 72 C for 2 min.

Electrophoresis

DNA was separated on native horizontal discontinuous 7.5% polyacrylamide gels as described [8] and bands were visualised by silver staining [9].

Sequence analysis

PCR fragments were purified using Centricon 100 concentrators (Amicon, Witten, Germany). Cycle sequencing was performed using the BigDye Terminator Cycle sequencing kit FS (Perkin Elmer/Applied Biosystems, USA) according to the manufacturers recommendations. Both the sense strand and the antisense strand were subjected to sequence analysis. Electrophoresis and detection of the sequencing reaction products were done on the capillary electrophoresis system ABI Prism 310 genetic analyser using POP (performance optimised polymer) 6, with a capillary length of 61 cm and diameter of 50 µM.

Allele designation and allelic ladder

Alleles were designated based on the number of GAAA repeats in the variable region according to the recommendations of the DNA Commision of the International Society for Forensic Haemogenetics [10, 11, 12]. The 10 alleles sequenced in this study were used to construct an allelic ladder.

Statistics

For evaluating Hardy-Weinberg expectations an exact test [13] using the computer programme GENEPOP, version 1.2 (M. Raymond and F. Rousset, Montpellier) was performed. The mean exclusion chance (MEC) was calculated according to Krüger et al. [14] and the discriminating power was calculated as $1-\Sigma$ (expected phenotype frequencies)^2.

Results

Sequence structure of D1S1171 alleles

In 141 Austrian individuals 10 different alleles were distinguished using native PAGE as described above (Fig. 1). No interalleles have been observed. One of each of the alleles was sequenced and used to construct an allelic ladder. As the sequence given in the GDB entry includes several undetermined bases, we propose the sequence given in Fig. 2 as a new consensus sequence. Comparisons of the 12 bp 5′ flanking region and the 10 bp 3′ flanking region showed absolute length and sequence conformity in the alleles sequenced. The repeat composition of the D1S1171 alleles with fragment lengths ranging between 96 and 132 bp is displayed in Table 1. The locus D1S1171 consists of a varying number of GAAA repeats which were used for allelic designation. The repeat region of each allele starts with an non-repeated CAAAAAA motif which was therefore not included in the allelic designation.

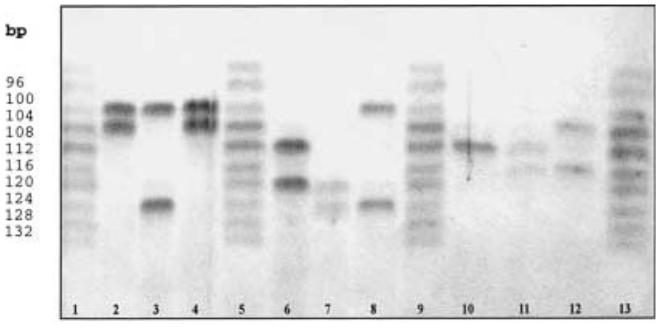

Fig. 1 Electropherogram showing different genotypes of the locus D1S1171 (*Lanes* 1, 5, 9 and 13 allelic ladder containing alleles 9–18, *lane* 2: 11/12, *lane* 3: 11/16, *lane* 4: 11/12, *lane* 6: 13/15, *lane* 7: 15/16, *lane* 8: 11/16, *lane* 10: 13/13, *lane* 11: 13/14, *lane* 12: 12/14)

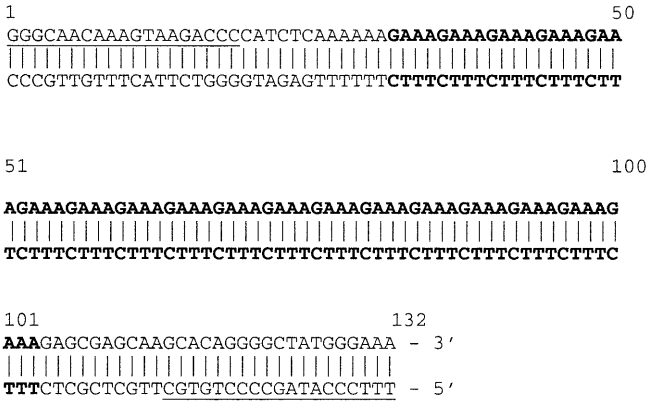

Fig. 2 Consensus sequence of allele 18 at the D1S1171 locus (repeat region in bold print, primer sequences underlined)

Table 1 Sequence structure and characteristics of the alleles 9–18 of the D1S1171 locus

Alleles	Sequence	Length (bp)	Frequency (f)
9	5′-CAAAAAA-$(GAAA)_9$-3′	96	0.04
10	5′-CAAAAAA-$(GAAA)_{10}$-3′	100	0.07
11	5′-CAAAAAA-$(GAAA)_{11}$-3′	104	0.17
12	5′-CAAAAAA-$(GAAA)_{12}$-3′	108	0.23
13	5′-CAAAAAA-$(GAAA)_{13}$-3′	112	0.38
14	5′-CAAAAAA-$(GAAA)_{14}$-3′	116	0.52
15	5′-CAAAAAA-$(GAAA)_{15}$-3′	120	0.36
16	5′-CAAAAAA-$(GAAA)_{16}$-3′	124	0.14
17	5′-CAAAAAA-$(GAAA)_{17}$-3′	128	0.06
18	5′-CAAAAAA-$(GAAA)_{18}$-3′	132	0.03

The repeat region sequence structure of each allele is shown in the middle, the fragment length and frequency are given on the right

Population data

Allelic frequencies of the D1S1171 locus in Austria are shown in Table 1 and a total of 34 genotypes was found in the 141 Austrian probants tested. No significant deviations from Hardy-Weinberg expectations were observed ($P>0.5$). The heterozygosity rate was 0.853, the mean exclusion chance (MEC) amounted to 0.677 and the discriminating power (DP) was 0.951.

Sensitivity studies

Correct typing was possible with as little as 100 pg DNA per 12.5 µl assay (data not shown). Applying D1S1171 to casework strong signals were obtained (data not shown).

Discussion

We have analysed the STR locus D1S1171 in an Austrian population sample to obtain allelic and genotype frequencies, furthermore its usefulness in forensic casework and paternity testing was demonstrated. In addition we have studied the sequence variation at this locus and an allelic ladder consisting of sequenced alleles was constructed. According to the guidelines published by Urquhart et al. [3] D1S1171 seems to be a simple locus, as sequence analysis of the 10 alleles revealed a simple repeat structure. However, further sequence studies have to be performed to confirm this assumption. The polymorphism of the locus proposed in this study is slightly lower than FGA [15], but higher than that of most other STRs commonly used in forensic practice [2, 3]. The sensitivity of this locus is thus comparable to that of other STRs [5, 16]. We suggest that this locus is suitable for typing severely degraded stains, as the fragment length of the alleles is well below 150 bp. No mutation studies have been performed, nevertheless it can be assumed from the uninterrupted repeat length of this locus that the mutation rate should be comparable to that of other STRs used for paternity testing [4, 17, 18, 19]. Since typing the D1S1171 locus proved to be easy, we suggest this locus as a very useful system for both forensic stain analysis and paternity testing.

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