Int J Legal Med (2002) 116:258–261
DOI 10.1007/s00414-002-0291-1

ORIGINAL ARTICLE

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Population genetic data, comparison of the repeat structure and mutation events of two short STRs

Received: 17 October 2001 / Accepted: 27 December 2001 / Published online: 17 April 2002

Abstract The short tandem repeat (STR) systems D3S1545 and D7S1517, both small size STRs (fragment lengths <160 bp), were investigated in a population sample of German Caucasoids. New primer sequences more closely flanking the repetitive region were designed for D3S1545. For D3S1545, 7 alleles could be found (heterozygosity 0.68) while 11 alleles could be typed for D7S1517 (heterozygosity 0.83). Additionally, sequencing of selected alleles was carried out to establish the allele nomenclature and to clarify the structure of the tandem repeat arrays. D3S1545 showed a uniform GATA repeat structure but, in contrast, the repeat stretch of D7S1517 showed a compound structure characterised by different numbers of GAAA and CAAA repeats. Two isolated cases of a new mutation could be confirmed for D7S1517. The alleles of these two family constellations were characterised by sequencing and the probable mutational events were demonstrated.

Keywords Short tandem repeats · Small amplicons · Sequencing data · Mutations

Introduction

The short tandem repeat (STR) systems D3S1545 and D7S1517, small size STRs characterised by PCR fragment lengths less than 160 bp, were investigated in a population sample of German Caucasoids from the area around Halle/Saale (Germany). Moreover, selected alleles were sequenced to establish the allele nomenclatures and to clarify the structure of the tandem repeat arrays. Additionally, new primer sequences closely flanking the repetitive region were designed for D3S1545.

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Materials and methods

Blood and saliva samples (D7S1517 *n*=400 individuals, D3S1545 *n*=206 individuals) were extracted using 5% Chelex 100 [1]. Out of 200 µl extraction volume, 1–2 µl was used for PCR.

PCR protocol 1 involved 94°C for 60 s, 60°C for 60 s, 72°C for 60 s over 30 cycles using the primers selected by Dupuy et al. [2] for D7S1517 and newly designed primers for D3S1545 as follows:

- D7S1517
  - P1: 5' FAM – GTG ACC AAC TGA ATT ATG TTT TG
  - P2: 5' – CAT CTT GCC AGC TGC CT

Table 1 Allele frequencies and forensic efficiency values for D3S1545 and D7S1517. The allele nomenclatures are based on the number of repeats (*Het.* heterozygosity, *Disc.* power of discrimination, *Excl.* power of exclusion, *HWE* Hardy-Weinberg equilibrium)

D7S1517		D3S1345	
Allele	Frequency	Allele	Frequency
17	0.001	9	0.005
18	0.042	10	0.017
19	0.114	11	0.340
20	0.096	12	0.284
21	0.139	13	0.255
22	0.085	14	0.087
23	0.104	15	0.012
24	0.123	–	–
25	0.245	–	–
26	0.041	–	–
27	0.012	–	–
Het.	0.83	0.68	–
Disc.	0.94	0.85	–
Excl.	0.64	0.55	–
HWE	0.30	0.58	–

allele	bp	repeat sequence
17	115	5' – $(GAAA)_{10}$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
18	119	5' – $(GAAA)_{11}$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
19	123	5' – $(GAAA)_{12}$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
20	127	5' – $(GAAA)_{13}$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_{11}$ $(CAAA)_4$ $(GAAA)_3$ $(CAAA)_1$ $(GAAA)_1$ – 3'
21	131	5' – $(GAAA)_{14}$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_{13}$ $(CAAA)_4$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
22	135	5' – $(GAAA)_{15}$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_{14}$ $(CAAA)_4$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_9$ $(CAAA)_3$ $(GAAA)_3$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
23	139	5' – $(GAAA)_{16}$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_{10}$ $(CAAA)_3$ $(GAAA)_3$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_9$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_4$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_7$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_3$ $(GAAA)_3$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
24	143	5' – $(GAAA)_{16}$ $(CAAA)_4$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_{16}$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_{10}$ $(CAAA)_4$ $(GAAA)_2$ $(CAAA)_4$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_{10}$ $(CAAA)_4$ $(GAAA)_3$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_{11}$ $(CAAA)_4$ $(GAAA)_2$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_{11}$ $(CAAA)_3$ $(GAAA)_3$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
25	147	5' – $(GAAA)_{12}$ $(CAAA)_3$ $(GAAA)_3$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_{12}$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_4$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_8$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_4$ $(GAAA)_2$ $(CAAA)_4$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_8$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
26	151	5' – $(GAAA)_{13}$ $(CAAA)_3$ $(GAAA)_3$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_9$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_4$ $(GAAA)_2$ $(CAAA)_4$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
		5' – $(GAAA)_9$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'
27	155	5' – $(GAAA)_{14}$ $(CAAA)_3$ $(GAAA)_3$ $(CAAA)_3$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ – 3'

Fig. 1 D7S1517 – sequencing data of selected alleles. In total 58 alleles have been sequenced

- D3S1545
  - P1: 5' FAM – TGC CTT ATT ACT TTA GGA ATA ACC
  - P2: 5' – CCT GGG TGA CAC AGA GAA AT

Electrophoresis was carried out using (1) non-denaturing gel conditions with subsequent silver staining [3] and (2) high resolution capillary electrophoresis combined with fluorescence dye labelled primer detection. Each forward primer was 5' FAM labelled. Fragment length detection was carried out using an ABI 310 Genetic Analyser.

Statistics

Possible deviations from Hardy-Weinberg equilibrium were tested using an exact test (Genepop software: http://wbiomed.curtin.edu.au/genepop), additionally the values for the power of discrimination and the power of exclusion were calculated.

Sequencing of selected alleles

After electrophoresis and silver staining, individual alleles were cut out from the gel and transferred to microfuge tubes. Elution of DNA was carried out using the „crush and soak „ method [4]. Sequencing was performed using the Big Dye Terminator Cycle Sequencing kit according to the instructions of the manufactor (Applied Biosystems, Foster City, Calif.). Both strands were sequenced for each fragment.

The alleles were designated based on the number of repeats according to the recommendations of the International Society of Forensic Haemogenetics [5]. Allelic ladders were constructed based on the sequenced alleles.

Results and discussion

Allele frequencies and forensic efficiency values

For D3S1545 we differentiated 7 alleles (heterozygosity 0.68) while 11 alleles could be typed for D7S1517 (heterozygosity 0.83) (Table 1). Both systems showed no deviation from Hardy-Weinberg equilibrium. Studies on other STRs have shown that the reduction of the STR am-

Table 2 Distribution of age of the mothers and fathers at the time of conception

Age at conception (years)	Numbers of mothers (%)	Numbers of fathers (%)
15–19	31.0	10.7
20–24	37.9	39.3
25–29	19.1	19.6
30–34	10.3	10.7
35–40	–	5.4
40–44	1.7	3.6
45–49	–	8.9
50–55	–	1.8

plicon length can improve the typing of highly degraded DNA [2, 6, 7]. Additionally, a better electrophoretic separation can be obtained. The two STRs studied here, especially D3S1545, are among those with the smallest fragment sizes (size of the smallest alleles 80 bp for D3S1545 and 115 bp for D7S1517). We therefore argue that the new STRs might be of special interest for samples of low quality and quantity. Both STRs enable a PCR detection sensitivity of less than 100 pg DNA.

Because of the high number of alleles and the highly informative forensic efficiency parameters, D7S1517 typing could also be very useful for the analysis of mixed stains and in paternity cases.

Sequencing data

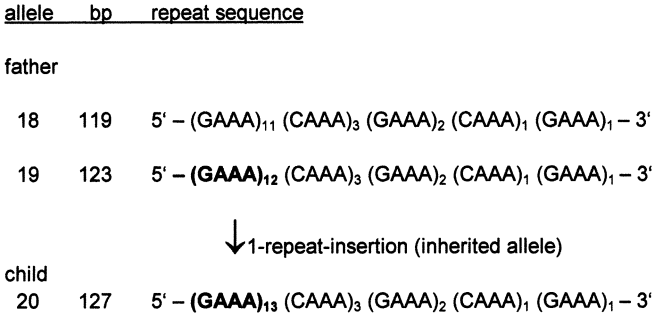
D3S1545 shows a uniform repeat structure with a 4 bp array containing $(GATA)_{9–15}$ as the conserved repeat motif. In constrast, the repeat stretch of D7S1517 shows a compound structure characterised by alternations of different numbers of GAAA and CAAA repeats (Fig. 1): while the shorter alleles of this STR (alleles 17–21) contain a more conserved structure of GAAA/CAAA alternations, a variant repeat stucture was found in alleles >21 repeats [8] which may have been generated by a duplication event of $(CAAA)_3$.

For alleles with 25 repeats, the conserved repeat structure $(GAAA)_n$ $(CAAA)_n$ $(GAAA)_2$ $(CAAA)_1$ $(GAAA)_1$ was not found, although 14 alleles >24 repeats have been sequenced.

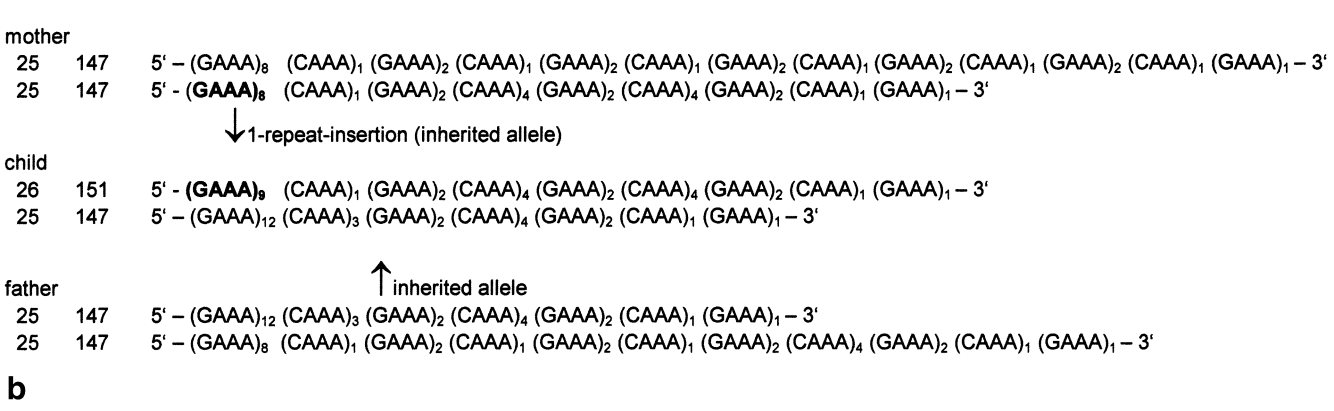
Mutation rates

Up to now we have investigated 100 meioses for D3S1545 but no mutation was detected. In contrast, 500 meioses analysed for D7S1517 led to 2 isolated exclusions (paternity index >10,000), confirming new mutations in these two families. Additionally, the distribution of age of both parents at the time of conception is given in Table 2 [9, 10, 11].

Fig. 2a, b D7S1517 – two cases of new mutations and characterisation of the probable mutational events. **a** Case 1 and **b** case 2



a



b

In paternity case (1) the mother (age at the time of conception 36 years) showed alleles 20, 25, the child alleles 20, 25 and the putative father (age at the time of conception 44 years) alleles 18 and 19. Both alleles of the putative father and allele 20 of the child were sequenced. Based on the assumption of the most probable mutation event, a single step mutation [12], one can postulate a 1-repeat insertion of a 5' GAAA motif (putative father allele 19 to allele 20 of the child) (see Fig. 2a).

In paternity case (2) the mother (age at the time of conception: 25 years) showed allele 25 the child alleles 25, 26 and the putative father (age at the time of conception: 19 years) allele 25. Sequencing revealed that both putative father and mother were homozygous for the number of repeats, but heterozygous for the sequence of the repeat array. The sequences given in Fig. 2b were constructed taking into account the non-mutated allele of the child, the assumption of a single-step mutation in the longest homogenous repeat stretch as the most probable mutational event [12, 13], and the structure of other alleles sequenced for the present study. Following these considerations, the mutation occurred most probably during oogenesis rather than spermatogenesis.

Studies of autosomal STRs in humans and microsatellites in yeast have shown a positive correlation between the mutation rate and the number of uniform repeats [12, 13]. In the study of Brinkmann et al. [12] all of the mutations (n>20) have been identified in uniform repeat arrays >10 repeats. In our first mutation case the repeat array consists of >10 uniform repeats, but in the second case, under the hypothesis of a single step mutation event, an allele starting with 5' $(GAAA)_8$ has mutated. This may indicate that not only the number of homogeneous repeats but also the total number of different sequence motifs of the same repeat size (4 bp) could influence the mutation probability [14]. It may be worth noticing that the parents in case 1 (but not in case 2) were considerably older at conception than the median age of all mothers (22 years old) and fathers (24 years old). This observation supports the results of the study by Brinkmann et al [12], who found an age-dependent mutation rate in a larger sample.

In conclusion both STRs allow easy genotyping. Due to the relatively high heterozygosity, D7S1517 can be also efficiently applied to paternity analysis. The short amplicons of the D3S1545 system combined with the new selected primer sequences, enables a highly sensitive typing of a very small amount of DNA.

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