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Forensic validation of the STR systems SE 33 and TC11

P. Wiegand[1], B. Budowle[2], S. Rand[1], and B. Brinkmann[1]

[1]Institute of Legal Medicine, Westfälische-Wilhelms-Universität, Von-Esmarch-Strasse 86, W-4400 Münster, Germany
[2]Forensic Science Research and Training Centre, FBI Academy, Quantico, VA 22135, USA

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Summary. Population studies on Caucasians from northwest Germany were carried out using the short tandem repeat (STR) systems SE 33 (Locus: ACTBP2) and TC11 (Locus: 11p15.5). After electrophoresis in PAG 26 alleles could be identified for SE 33 in a sample size of 180 unrelated individuals and 6 alleles were found for TC11 in 110 individuals. The combined mean exclusion chance for both systems was 0.96 and the discrimination index 0.999. No significant deviations from Hardy-Weinberg equilibrium could be demonstrated. In a small sample of families (SE 33 – $n = 21$; TC11 – $n = 30$) no new mutations could be found. Positive and reproducible results for both STRs could be obtained from 50 pg template DNA.

Key words: Short tandem repeats – SE 33 – TC11 – Forensic validation – Population studies

Zusammenfassung. Populationsstichproben nordwestdeutscher Kaukasier wurden mit den beiden Short tandem repeat (STR)-Systemen SE 33 (Locus: ACTBP2) and TC11 (Locus: 11p15.5) untersucht. Nach elektrophoretischer Auftrennung in PAG konnten 26 Allele für SE 33 in einer Bevölkerungsstichprobe von 180 nicht verwandten Personen und 6 Allele für TC11 in einer Stichprobe von 110 Individuen differenziert werden. Der resultierende kombinierte AVACH-Wert für beide Systeme lag bei 0.96, der entsprechende Diskriminationsindex bei 0.999. Eine signifikante Abweichung vom Hardy-Weinberg-Gleichgewicht wurde nicht festgestellt. Erste Familienstudien (SE 33 – $n = 21$; TC11 – $n = 30$) gaben keinen Hinweis auf Neumutation. Die Nachweissensitivität beider STR's lag im Bereich von 50 pg template DNA.

Schlüsselwörter: Short tandem repeats – TC11 – SE 33 – Forensische Validierung – Populationsstudien

Introduction

The first generation of PCR-VNTR systems relevant to the forensic field, i.e. the AmpFLP-systems, were introduced only approximately one year ago (Budowle et al. 1991; Rand et al. 1992; Sajantila et al. 1992). A second generation, the short tandem repeats (STRs) are characterized by repeat lengths of 3 or 4 bp and allele fragment lengths varying between 150–350 bp (Edwards et al. 1992; Polymeropoulos et al. 1992). The aim of this investigation was an evaluation of the potential forensic utility of the STR system SE 33 (repeat: AAAG; Polymeropoulos et al. 1992) and TC11 (HUMTH01, repeat: AATG; Edwards et al. 1992).

Correspondence to: P. Wiegand

Materials and methods

Blood samples were obtained from healthy unrelated Caucasians in the Münster area. DNA extraction was performed as previously described (Brinkmann et al. 1991).

The concentration of DNA was measured fluorimetrically and the degree of degradation of the DNA was assessed by electrophoretical separation in a 1% agarose gel by comparison with known standards (DRIgest III, Pharmacia).

PCR protocol: 1 ng template DNA; 1 U Taq polymerase (Promega, USA) 0.3 µ*M* each primer, 100 µ*M* of each nucleotide, 2 µl buffer (Promega) diluted to a total volume of 25 µl with distilled water. The reaction mixture was overlayed with 2–3 drops of oil.

Primer sequences
SE 33 (Polymeropoulos et al. 1992)
5′ – AAT CTG GGC GAC AAG AGT GA – 3′
5′ – ACA TCT CCC CTA CCG CTA TA – 3′

TC11 (Edwards et al. 1992)
5′ – GTG GGC TGA AAA GCT CCC GAT TAT – 3′
5′ – ATT CAA AGG GTA TCT GGG CTC TGG – 3′

Amplification conditions
SE 33: 93°C – 1 min, 61°C – 1 min, 72°C – 1.5 min; 28 cycles
(Thermocycler: Biometra, Triothermoblock, FRG)
TC11: 1) 94°C – 20 s, 64°C – 20 s
70°C – 60 s; 10 cycles
2) 90° – 20 s, 64°C – 20 s
70°C – 60 s; 20 cycles
(Thermocycler: Perkin Elmer Cetus 9600)

Gels. Polyacrylamide (SE 33: 5% T, 3% C – TC11: 6% T, 3% C – 750 µm), piperazine diacrylamide as cross-linker, 80 m*M* formate; 18 cm separation distance, 2% agarose plugs, 28 m*M* CHES (Allen et al. 1989).

Electrophoresis. Initial 1000 V, 40 mA and 3 W with ramping every 10 min up to 15 W which was continued until the bromophenol

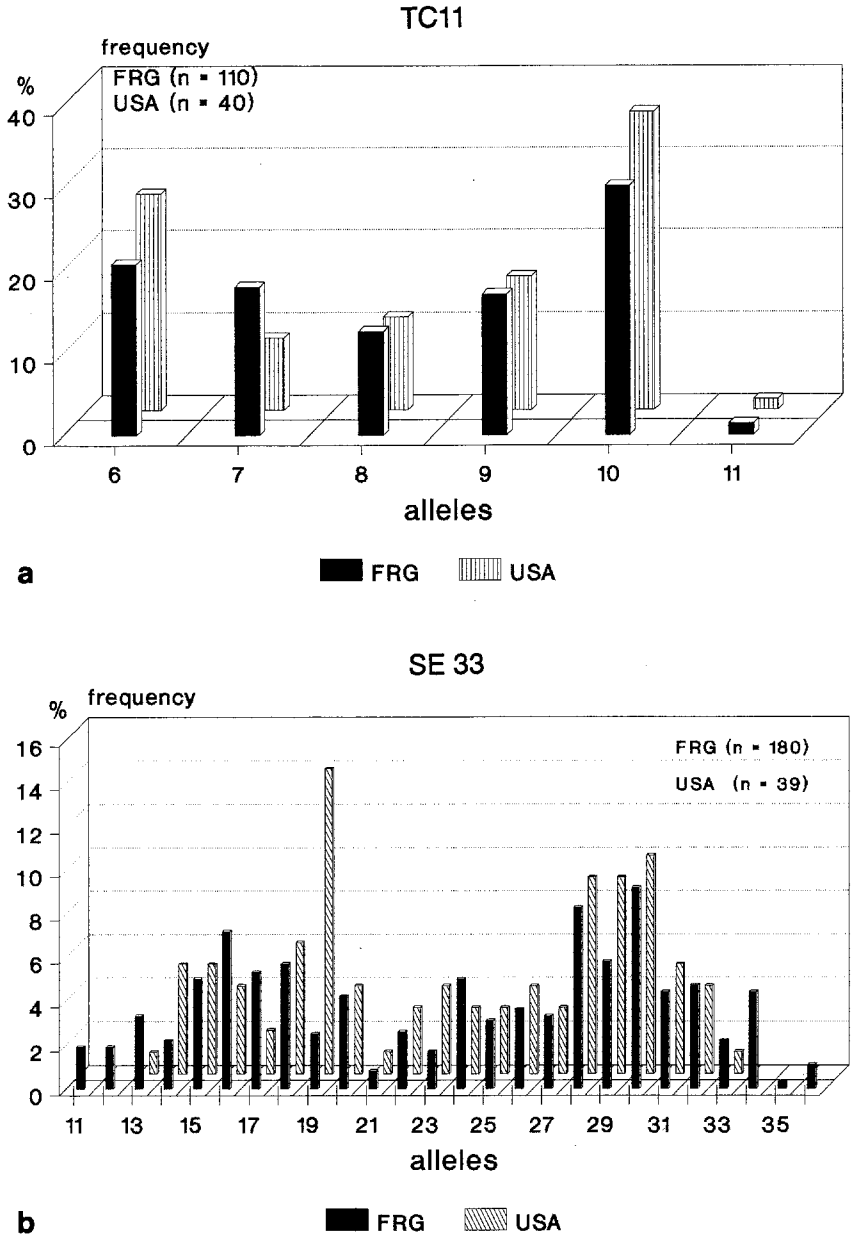

Fig. 1a, b. Comparison of the allele frequencies from this study and USA (Edwards et al. 1992) for TC 11 (**a**) and SE 33 (**b**) – (Polymeropoulos et al. 1992)

blue marker front had reached the anode. Bands were visualized by silver staining (Budowle et al. 1991).

Assignment of alleles. In the initial phase of this investigation for both systems we used preliminary allelic ladders consisting of human alleles. The smallest allele in the SE 33 cocktail was therefore arbitrarily designated 11 and the others numbered upwards consecutively towards the cathode, taking into consideration the repeat sizes of 4 bp (see Fig. 3), while for TC11 the smallest allele was designated 6 as suggested by Edwards et al. (1992).

Results

A total of 26 discrete alleles was observed for SE 33 and 6 for TC11 (Fig. 1b, Table 1). Both systems demonstrated a highly polymorphic allele distribution leading to a high forensic efficiency (Fig. 2a, b; Table 2). Hardy-Weinberg equilibrium was tested for SE 33 by the formation of groups of alleles (Rand et al. 1992) and for TC11 using the individual alleles. No significant deviations were found in either system (Table 3; observed phenotypes see Table 4).

Family studies

Families which had been validated serologically and by RFLP analyses were also tested using both STRs. A total of 21 families (54 meioses) were tested with SE 33 and 30 families (76 meioses) with TC11. The segregations followed Mendelian inheritance and no new mutation was observed using the described electrophoresis system (see Materials and methods).

Sensitivity and DNA quality

In dilution studies, using cell line DNA (K 562, Promega) the limit of detection for both systems was approx. 100

Table 1. Frequency values for SE33 and TC11 (see Fig. 1a, b)

SE33			TC11		
Allele	FRG	USA	Allele	FRG	USA
11	0.019	–	6	0.207	0.262
12	0.019	–	7	0.180	0.088
13	0.033	0.01	8	0.126	0.113
14	0.022	0.05	9	0.171	0.162
15	0.050	0.05	10	0.302	0.362
16	0.072	0.04	11	0.014	0.013
17	0.053	0.02			
18	0.058	0.06			
19	0.025	0.14			
20	0.042	0.04			
21	0.008	0.01			
22	0.025	0.03			
23	0.017	0.04			
24	0.050	0.03			
25	0.031	0.03			
26	0.036	0.04			
27	0.033	0.03			
28	0.083	0.09			
29	0.058	0.09			
30	0.092	0.10			
31	0.044	0.05			
32	0.047	0.04			
33	0.022	0.01			
34	0.044	–			
35	0.003	–			
36	0.011	–			

Table 2. Mean exclusion chance (Krüger et al. 1968*), discrimination index – DI (Sensabaugh 1982) and heterozygosity – H for SE33 and TC11

	Mean excl. chance	DI	Heterozy.
SE33	0.89	0.99	0.93
TC11	0.59	0.91	0.73
Comb.	0.96	$1:10^3$	

* Mean exclusion chance $W = \sum_i p_i^3(1-p_i)^2 + \sum_i p_i(1-p_i)^3 + \sum_{i<j} p_i p_j (p_i+p_j)(1-p_i-p_j)^2$ where pi, pj = corresponding gene frequencies of the alleles A_i and A_j

pg (Fig. 4). By increasing the amplification by 2 cycles, a further reduction down to 50 pg could be achieved without affecting the quality of the results (results not shown). – Heavily degraded DNA was obtained from 2 sources: (a) 12 experimental stains exposed to different aging conditions with regards to time and temperature, (b) case work stains (for example see Fig. 5). – In both series the DNA was severely degraded (generally less than 1000 bp in length) so that typing using the RFLP and AmpFLP systems gave negative results. However, with SE33 and TC11, all stains were typeable (further examples see Brinkmann 1992).

Table 3. Chi^2-test to test for deviations from Hardy-Weinberg equilibrium for TC11 (6 alleles) and SE33 (4-allele-group-model; according to Rand et al. 1992)

Alleles (groups)	6 Alleles	4-Allele-model
I	Allele 1	Allele 1– 7
II	Allele 2	Allele 8–15
III	Allele 3	Allele 16–20
IV	Allele 4	Allele 21–26
V	Allele 5	
VI	Allele 6	
Chi^2	23.98	1.42
P	0.2–0.3 ($df = 20$)	0.999–0.995 ($df = 9$)

Table 4. Phenotype frequencies for TC11 ($n = 110$ ind.) and SE33 ($n = 180$ ind.)

Phenotype	Obs. alleles	Frequency
TC11 phenotypes (obs. in n = 110 ind.)		
6/ 6	6	0.054
6/ 7	7	0.063
6/ 8	9	0.081
6/ 9	7	0.063
6/10	11	0.099
6/11	0	0
7/ 7	4	0.036
7/ 8	3	0.027
7/ 9	1	0.009
7/10	19	0.171
7/11	2	0.018
8/ 8	2	0.018
8/ 9	6	0.054
8/10	6	0.054
8/11	0	0
9/ 9	8	0.072
9/10	8	0.072
9/11	0	0
10/10	10	0.09
10/11	1	0.009
11/11	0	0
SE33 phenotypes (obs. in n = 180 ind.)		
11/16	2	0.011
11/14	1	0.006
11/18	1	0.006
11/20	1	0.006
11/22	2	0.011
12/15	2	0.011
12/18	1	0.006
12/20	2	0.011
12/30	1	0.006
13/15	1	0.006
13/20	1	0.006
13/24	2	0.011
13/25	2	0.011
13/29	1	0.006
13/30	5	0.028
13/32	1	0.006
13/34	1	0.006

Table 4 (continued)

Phenotype	Obs. alleles	Frequency
14/16	1	0.006
14/17	1	0.006
14/22	1	0.006
14/28	1	0.006
14/30	2	0.011
14/31	2	0.011
15/17	1	0.006
15/18	1	0.006
15/25	1	0.006
15/26	1	0.006
15/28	2	0.011
15/29	2	0.011
15/31	3	0.017
15/32	1	0.006
15/36	1	0.006
16/16	2	0.011
16/18	1	0.006
16/20	1	0.006
16/22	2	0.011
16/23	1	0.006
16/25	2	0.011
16/28	4	0.022
16/29	1	0.006
16/30	2	0.011
16/31	2	0.011
16/33	1	0.006
16/34	2	0.011
17/18	3	0.017
17/20	3	0.017
17/23	1	0.006
17/25	1	0.006
17/26	1	0.006
17/27	3	0.017
17/28	1	0.006
17/30	2	0.011
17/32	1	0.006
17/34	1	0.006
18/19	1	0.006
18/20	2	0.011
18/22	1	0.006
18/29	3	0.017
18/30	1	0.006
18/32	3	0.017
18/34	1	0.006
19/22	1	0.006
19/25	1	0.006
19/28	1	0.006
19/29	2	0.011
19/30	2	0.011
19/34	1	0.006
20/24	2	0.011
20/26	1	0.006
20/28	2	0.011
20/29	1	0.006
20/30	1	0.006
21/28	1	0.006
21/30	1	0.006
21/31	1	0.006
22/24	1	0.006
22/25	1	0.006
22/27	1	0.006
22/32	1	0.006

Table 4 (continued)

Phenotype	Obs. alleles	Frequency
23/29	1	0.006
23/33	1	0.006
23/34	2	0.011
24/24	1	0.006
24/28	1	0.006
24/29	4	0.022
24/31	1	0.006
24/32	2	0.011
24/33	1	0.006
24/34	1	0.006
25/28	2	0.011
25/32	1	0.006
26/26	1	0.006
26/27	1	0.006
26/28	1	0.006
26/30	2	0.011
26/33	1	0.006
26/34	3	0.017
27/28	4	0.022
27/29	1	0.006
27/31	1	0.006
27/33	1	0.006
28/28	3	0.017
28/31	1	0.006
28/32	1	0.006
28/33	1	0.006
28/35	1	0.006
29/30	1	0.006
29/31	1	0.006
29/32	2	0.011
29/33	1	0.006
29/36	1	0.006
30/30	5	0.028
30/31	2	0.011
30/32	1	0.006
31/33	1	0.006
31/43	1	0.006
32/32	1	0.006
34/36	2	0.011

Discussion

A comparison of the frequency profiles for TC 11 from this study showed good agreement with those obtained from Edwards et al. (1992) (Fig. 1a), but minor differences were found for SE 33 compared with data from Polymeropoulos et al. (1992). Allele No. 19 in the German sample shows a striking difference to the corresponding allele in the US population (Fig. 1b). In addition, we observed 2 smaller and 3 larger fragments compared to the US survey. It should be born in mind that the sample sizes in both studies are different ($n = 180$ and $n = 39$). This could explain the differences observed.

SE 33:
26 alleles in 180 individuals, het. = 0.93 (this study)
21 alleles in 39 individuals, het. = 0.93 (Polymeropoulos et al. 1992)

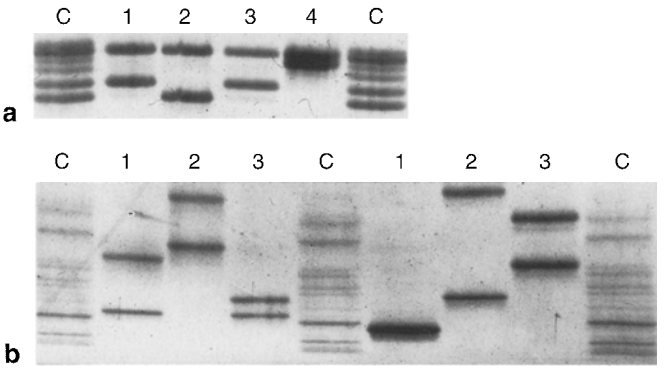

Fig. 2a, b. Amplification patterns from unrelated individuals for TC 11 (**a**) and SE 33 (**b**). PAGE after silver staining (s. Materials and methods). *C* = allelic ladder

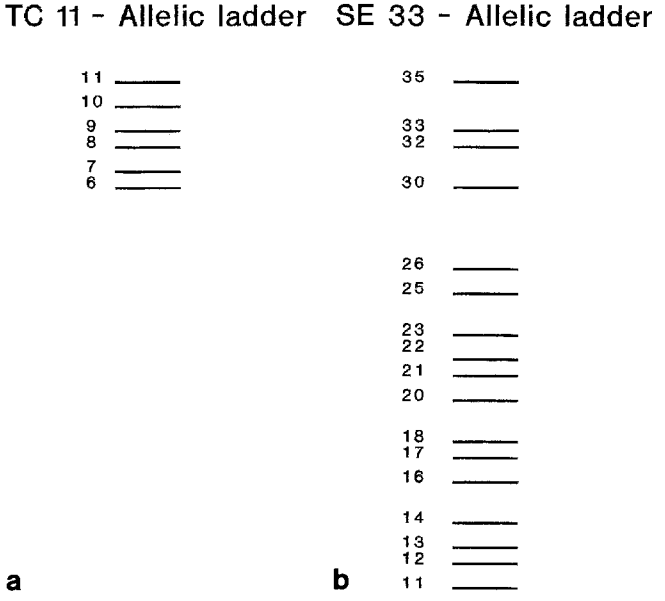

Fig. 3a, b. Schematic presentation of the allelic ladder used for allele typing for TC 11 (**a**) and SE 33 (**b**)

TC11:
6 alleles in 110 individuals, het. = 0.73 (this study)
6 alleles in 40 individuals, het. = 0.76 (Edwards et al. 1992).

Furthermore, sequencing data and comparative studies are necessary to establish a final control ladder. It has been recommended that ideally a nomenclature based on the number of repeats within an allele should be used (DNA recommendations 1992). However this can only be achieved by sequencing the individual alleles. Until this has been accomplished for SE 33 we suggest that the first allele in the ladder be designated allele number 11 and the others numbered sequentially (11–36) towards the cathode. This nomenclature allows for the occurrence of additional alleles anodal to allele 11 and these can be numbered according to their position. Although these alleles are rare in a Caucasian population sample, other alleles could be found in other populations and could occur more frequently.

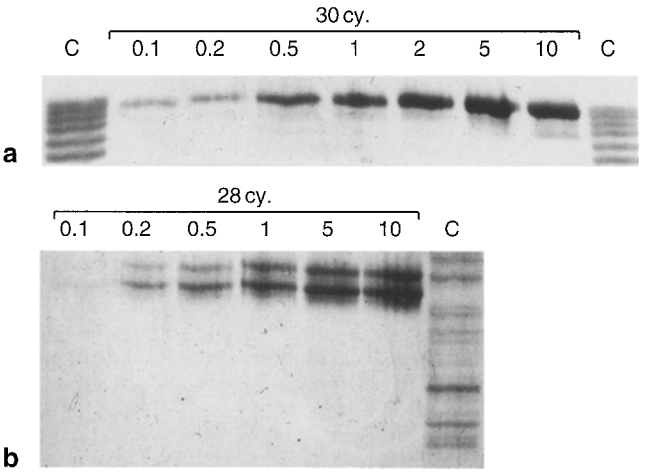

Fig. 4a, b. Sensitivity study for TC 11 (**a**) and SE 33 (**b**) with doubling dilutions of template DNA (cell line K 562; Promega, USA) from 10–0.1 ng: 7 µl out of 25 µl amplification volume of each sample were loaded on PAG (phenotype pattern for TC11: 10/10; SE 33: 28/30). *cy.* = amplification cycles; *C* = allelic ladder

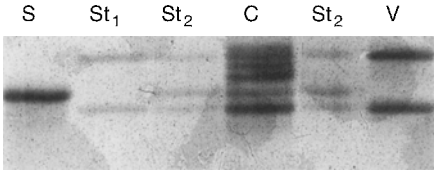

Fig. 5. Murder case with 2-year-old bloodstains amplified with TC 11. *S* = suspect; *St1* = bloodstain from scene of crime which matched with the pattern of the victim; St2 = mixed bloodstains from the fingernail of the suspect with matched with the pattern of the suspect and victim; *C* = allelic ladder; *V* = victim

A new nomenclature based on number of repeats will be submitted when the sequencing data becomes available.

In contrast, the profiles of TC11 are very similar in both studies. As the alleles have already been sequenced the previously used nomenclature has been changed to that of Edwards et al. (1992) as this is based on the number of repeats.

The combination of both STRs leads to high efficiency with a discrimination index of 0.999. Furthermore, there is an approximately tenfold higher sensitivity in comparison to AmpFLP systems (Rand et al. 1992) and in addition, the relatively short fragments are clearly associated with much higher success rates in severely degraded stains. Another advantage of STRs lies in the stability and reproducibility of the 4 bp repeats. A reliable designation of alleles is complicated in some AmpFLP systems by the varying repeat lengths within a single system (e.g. COL2A1, Puers et al. 1992).

Because of the high sensitivity of STR systems the risk of contamination is substantially increased and therefore special consideration must be given to this problem for stain investigations (see DNA recommendations 1992). More extensive family studies are necessary before the mutation rate can be clearly defined. This is obviously a prerequisite before the STR systems can be used in combination with AmpFLPs in paternity cases.

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