Int J Legal Med (1998) 111 : 180–182

ORIGINAL ARTICLE

P. Wiegand · H. R. Schneider · M. Schürenkamp
M. Kleiber · B. Brinkmann

Tetranucleotide STR system D8S1132: sequencing data and population genetic comparisons

Received: 20 August 1997 / Received in revised form: 17 October 1997

Abstract In the present investigation of the D8S1132 locus 31 selected alleles were sequenced. In total there were 9 distinguishable alleles found to increase in size by regular 4 bp increments from 134 to 170 bp with a repeat array following the pattern $(TCTA)_n$ TCA $(TCTA)_n$. One-third of the sequenced alleles exhibited an altered repeat sequence TCT**G** TCTA at the 3′ flanking region of the repeat array. A nomenclature for the designation of D8S1132 alleles is proposed on the basis of this sequence data and in accordance with the ISFH recommendations. The allele distribution of the D8S1132 locus has been investigated in three German populations (Halle-, Münster-, and Wiesbaden area) with frequencies ranging from 0.004 to 0.24. No deviation from Hardy-Weinberg equilibrium could be observed. The heterozygosity was 0.83 and the discrimination power 0.96 for the Halle population.

Key words Short tandem repeat D8S1132 · Sequencing data · Allele designation · Population genetic comparisions

Introduction

The short tandem repeat (STR) system D8S1132 (also known as G08685, CHLC, GATA26E03, D8S1132 – Genebank information) was examined in three German population samples. Allele frequency data were calculated and an allelic ladder containing 10 alleles was established by sequencing selected alleles.

P. Wiegand (✉) · M. Kleiber
Institut für Rechtsmedizin,
Martin-Luther-Universität Halle-Wittenberg, Franzosenweg 1,
D-06112 Halle/Saale, Germany
FAX: +49 345 557 1587

H. R. Schneider
Hessisches Landeskriminalamt, Hölderlin-Strasse 5,
D-65187 Wiesbaden, Germany

M. Schürenkamp · B. Brinkmann
Institut für Rechtsmedizin,
Westfälische Wilhelms-Universität Münster,
von-Esmarch-Strasse 62, D-48129 Münster, Germany

Material and methods

Three population samples: unrelated German Caucasians from the areas around Halle/Saale, Münster and Wiesbaden.

DNA was Chelex-extracted as described previously (Wiegand et al. 1993a).

DNA amplification and electrophoresis: From 200 µl Chelex-extracted DNA, 1–2 µl were used for PCR in a buffer consisting of 1 U of Taq polymerase (Promega, USA), 0.2 µM each primer, 100 µM of each nucleotide in 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 0.01% gelatine and 0.1% Triton-X 100 adding 10 µg BSA (Sigma 3350, München, Germany) in a total volume of 25 µl.

PCR primers and conditions: D8S1132 primer 1: 5′-GGC TAG GAA AGG TTA GTG GC, primer 2: 5′-CCC TCT CTC TTT CGA GCA AT; amplification protocol: 94° C – 1 min, 61° C – 1 min, 72° C – 1 min; 30 cycles. Biometra PC Thermocycler (Göttingen, Germany) – Halle population sample and Münster population sample; Perkin Elmer 480 (ABI, Weiterstadt, Germany) – Wiesbaden population sample.

The amplified alleles were resolved by high resolution polyacrylamide gel electrophoresis using previously described methods (Rand et al. 1992; Wiegand et al. 1993a, b; Schneider and Rand 1996).

Isolation of silver stained fragments, subsequent Taq-cycle-sequencing and sequence analysis was performed as described previously (Brinkmann et al. 1996).

Statistical analysis: Test for heterogeneity between populations: R × C contingency table (G. Carmody, Ottawa, Canada). Discrimination power according to Jones (1972); Hardy-Weinberg equilibrium: exact test (Guo and Thompson 1992, HWE 3.0 software from C. Puers, Münster, Germany).

Results and discussion

The frequency profiles in the three populations appeared very similar (Table 1) and no significant differences were found carrying out the test simulation for all 3 populations (Table 2; $P > 0.05$; R × C contingency table). In contrast, if pairwise comparisons were performed, a significant deviation was found between the Wiesbaden and the Halle data. Because usually no significant deviations within ethnically closely related population groups such as German

Caucasians have been found in STR systems (Huckenbeck et al. 1996), this deviation may be caused by sampling which may be compensated if the sample sizes were larger.

Table 1 Allele frequency data of 3 German populations (*n* number of individuals. *H* heterozygosity rate)

Allele	Halle $n = 100$	Münster $n = 134$	Wiesbaden $n = 97$
16	0.010	0.004	0.015
17	0.085	0.093	0.124
18	0.230	0.194	0.237
19	0.105	0.145	0.180
20	0.160	0.131	0.124
21	0.140	0.142	0.113
22	0.145	0.112	0.098
23	0.070	0.142	0.062
24	0.040	0.022	0.046
25	0.015	0.015	
H	0.83	0.79	0.79

The allele frequency distribution was unimodal with 5 common alleles and 4 rarer alleles in the Halle population sample (Table 1) leading to a heterozygosity rate of 0.83 and a discrimination power of 0.96. The mean exclusion chance of 0.71 showed that this STR is also very useful for paternity analysis. No deviation from Hardy-Weinberg equilibrium was found.

Sequence data from the repeat regions of 31 arbitrarily chosen D8S1132 alleles show a complex sequence composition which is related to the HumVWFA31 (VWA) repeat unit (Möller et al. 1994; Brinkmann et al. 1996) (Table 3). On the basis of this sequence data the repeat structure is designated as $(TCTA)_{5–11}$ TCA $(TCTA)_{9–13}$ $(TCTG)_{0–1}$ (TCTA).

As we know from other STRs with a more complex sequence composition (e.g. VWA), one can expect that sequencing of further alleles will lead to the detection of new variants.

However, one-third of the totally sequenced alleles exhibited an altered repeat sequence TCT**G** TCTA at the 3′ flanking region of the repeat array. The occurrence of the TCTG motif can be explained by a A to G transition, sim-

Table 2 Population comparisions using an R × C contingency table (G. Carmody software) with 1000 simulations (*S.E.* = standard error, significant deviation: *P*-value < 0.05)

Population comparison	Chi-square	*P*-value	G-statistic	*P*-value
Halle – Wiesbaden	17.53	0.035 ± 0.0074 (S.E.)	18.96	0.035 ± 0.0068 (S.E.)
Halle – Münster	11.15	0.265 ± 0.0058 (S.E.)	11.42	0.294 ± 0.0058 (S.E.)
Münster – Wiesbaden	15.10	0.089 ± 0.0090 (S.E.)	16.70	0.073 ± 0.0082 (S.E.)
Halle – Münster – Wiesbaden	27.53	0.067 ± 0.0079 (S.E.)	30.20	0.052 ± 0.0070 (S.E.)

Table 3 Allele nomenclature, sequence composition and fragment length of 31 alleles

Allele designation	Alleles sequenced	Sequence composition	Fragm. length
16	1	$(TCTA)_5$ TCA $(TCTA)_9$ TCTG TCTA	134 bp
17	2	$(TCTA)_5$ TCA $(TCTA)_{10}$ TCTG TCTA	138 bp
	1	$(TCTA)_6$ TCA $(TCTA)_{10}$ TCTA	
18	1	$(TCTA)_5$ TCA $(TCTA)_{11}$ TCTG TCTA	142 bp
	6	$(TCTA)_6$ TCA $(TCTA)_{11}$ TCTA	
19	1	$(TCTA)_8$ TCA $(TCTA)_9$ TCTG TCTA	146 bp
	5	$(TCTA)_6$ TCA $(TCTA)_{12}$ TCTA	
20	1	$(TCTA)_5$ TCA $(TCTA)_{13}$ TCTG TCTA	150 bp
	1	$(TCTA)_6$ TCA $(TCTA)_{13}$ TCTA	
	1	$(TCTA)_9$ TCA $(TCTA)_{10}$ TCTA	
21	1	$(TCTA)_{10}$ TCA $(TCTA)_{10}$ TCTA	154 bp
	1	$(TCTA)_9$ TCA $(TCTA)_{10}$ TCTG TCTA	
	1	$(TCTA)_8$ TCA $(TCTA)_{11}$ TCTG TCTA	
22	2	$(TCTA)_{10}$ TCA $(TCTA)_{11}$ TCTA	158 bp
	1	$(TCTA)_9$ TCA $(TCTA)_{11}$ TCTG TCTA	
	1	$(TCTA)_9$ TCA $(TCTA)_{12}$ TCTA	
23	1	$(TCTA)_{11}$ TCA $(TCTA)_{11}$ TCTA	162 bp
	1	$(TCTA)_{11}$ TCA $(TCTA)_{10}$ TCTG TCTA	
24	1	$(TCTA)_{11}$ TCA $(TCTA)_{12}$ TCTA	166 bp
25	1	$(TCTA)_{11}$ TCA $(TCTA)_{13}$ TCTA	170 bp

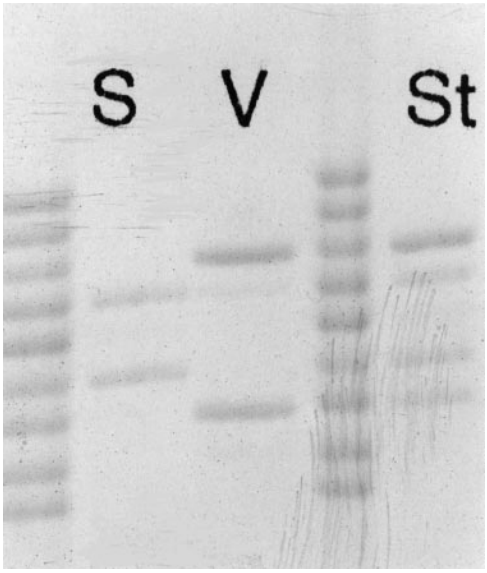

Fig. 1 D8S1132 result of epithelial cells which were removed from the neck of the victim (*V*), *S* = suspect; the stain pattern (*St*) shows a mixture which corresponds to the combined phenotypes of the suspect and the victim. *Lanes 1,4* = allelic ladder (VWA-Ladder was used for typing which comigrated to the D8S1132 alleles; allele 16 matched the VWA-allele 14, 17 machted VWA-allele 15 etc.; non-denaturing PAGE [Allen et al. 1989; Wiegand et al. 1993 a, b])

ilar to the sequence variants which were found in VWA (Brinkmann et al. 1996). So far we identified 10 distinguishable alleles which increase in size by regular 4 bp increments ranging from 134 to 170 bp. In accordance with the previously reported recommendations of the International Society of Forensic Haemogenetics (Bär et al. 1997), alleles are designated according to the total number of repeat units each allele contains. Therefore, the proposed designation for D8S1132 alleles range from 16 for an 134 bp allele to 25 for an 170 bp allele. The obviously conserved TCA-motif within the repeat array was not included into the allele nomenclature.

To estimate the sensitivity of the D8S1132 system and its relevance for stain analysis, we investigated problematical stain extracts in which the DNA amount was the limited parameter. For example, DNA typing was carried out on epithelial cells which were transferred from the hands of the suspect onto the neck of the victim in a strangulation case (Wiegand and Kleiber 1997). The DNA patterns of the victim and the suspect could be successfully amplified (Fig. 1) demonstrating that the sensitivity of this STR is in range of 20–50 pg template DNA.

References

Allen RC, Graves G, Budowle B (1989) Polymerase chain reaction amplification products separated on rehydratable polyacrylamide gels and stained with silver. BioTechniques 7:736–744

Bär W, Brinkmann B, Budowle B, Carracedo A, Gill P, Lincoln P, Mayr W, Olaisen B (1997) DNA recommendations – Further report of the DNA Commission of the ISFH regarding the use of short tandem repeat systems. Int J Legal Med 110:175–176

Brinkmann B, Sajantila A, Goedde HW, Matsumoto H, Nishi K, Wiegand P (1996) Population genetic comparisons among eight populations using allele frequency and sequence data from three microsatellite loci. Eur J Hum Genet 4:175–182

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

Huckenbeck W, Scheil HG, West S, Demir K, Kanja J, Kaiser A, Hess V, Meyer W, Scholten D, Stancu V, Bronczek M, Bonte W (1996) German data on the PCR based loci HumVWA31, HumTH01, HumFES/FPS, HumF13B and D1S80. In: Carracedo A, Brinkmann B, Bär W (eds) Advances in Forensic Haemogenetics 6, Springer Verlag, Berlin Heidelberg New York, pp 523–525

Jones DA (1972) Blood samples: probability of discriminations. J Forensic Sci Soc 12:355–359

Möller A, Meyer E, Brinkmann B (1994) Different types of structural variation in microsatellites: HumFES/FPS, HumVWA and HUMD21S11. Int J Legal Med 106:319-323

Rand S, Puers C, Skowasch K, Wiegand P, Budowle B, Brinkmann B (1992) Population genetics and forensic efficiency data of 4 AMPFLP's. Int J Legal Med 104:329–333

Schneider HR, Rand S (1996) High-resolution vertical PAGE: an alternative electrophoretic system with multiple forensic applications. Int J Legal Med 108:276–279

Wiegand P, Bajanowski T, Brinkmann B (1993 a) DNA typing of debris from fingernails. Int J Legal Med 106:81-84

Wiegand P, Budowle B, Rand S, Brinkmann B (1993 b) Forensic validation of the STR systems SE33 and TC11. Int J Legal Med 105:315-320

Wiegand P, Kleiber M (1997) DNA typing of epithelial cells after strangulation. Int J Legal Med 110:181–183