

## TYPING OF THE HUMVWA MICROSATELLITE POLYMORPHISM: ALLELE FREQUENCIES AND SEQUENCING DATA

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### SYSTEM AND LOCUS

tetranucleotide polymorphism in intron 40 of the human von Willebrand factor gene 12p12-12pter; Genbank Accession No.25858 [Kimpton 1992]

**POPULATION AND SAMPLE SIZE:** Vienna region (Austria). N=354

### METHODS

#### PCR

0.4µM primer VWA1 5'-ccc tag tgg atg ata aga ata atc-3'

0.4µM primer VWA2 5'-gga cag atg ata aat aca tag gat gga tgg-3' [Kimpton 1992]

40ng template DNA, 2U polymerase (Dynazyme™, Finn Zymes Oy), 200µM each dNTP, 1x PCR buffer, final reaction volume 50µl, layer with 50µl paraffine oil

amplification: 1.cycle: 98°C 5min 58°C 10 min  
30 cycles: 94°C 1min 50°C 1min 72°C 110sec  
last cycle: 72°C 10min

thermocycler: Hybaid OmniGene

#### AMPFLP Typing

PAGE and silver staining was carried out on 7% native polyacrylamide gels in 120mM Tris/Acetic Acid and 200mM Tris/Tricine in horizontal mode (ramp: 1200V/7mA/30W; final: 1200V/15mA/30W) as previously described [Schwartz 1994]. Typing was performed by side-to-side comparison with a sequenced allelic ladder, composed of distinct alleles as described elsewhere in this issue.

#### Sequencing

Automated single strand sequence determination of alleles corresponding to the ladder alleles was performed on an automatic DNA-Sequencer (ALF™, Pharmacia LKB Technology) according to the protocol of the Pharmacia AutoRead™ Sequencing Kit (dye primers, T7 polymerase) on a 6% sequencing gel. For each sample, the sense and the antisense strand were sequenced.

### RESULTS AND COMMENTS

A total of 12 alleles could be resolved by AMPFLP. At this level, one new allele (11) and two interalleles (14s and 15 f) could clearly be identified. Allele 13 and 22, that have been described by other workers [Moeller1994, Sajantila 1994] were not detected in our population. Frequencies, together with the forensic statistical efficiency values are listed in Tab.1. The phenotype distribution showed no significant deviation from Hardy-Weinberg expectations.

Sequencing showed the allele 11 to conform with the basic repeat structure that has been described by Moeller[1994]. Allele 14s showed a variation in the core repeat region (c→t transition in the third of 9 tcta repeats). In allele 15f the same variation of the core repeat region as in allele 14 [Moeller 1994] could be detected and an additional variation in the 3'-constant region.

In conclusion, HUMVWA shows a complex sequence polymorphism as already described [Moeller 1994]. Comparison of phaenotype distribution shows significant differences between different populations. AMPFLP typing is usually without ambiguities and with good forensic efficiency. HUMVWA can be recommended for the use in forensic applications.

Table 1: HUMVWA allele frequencies (n=354)

allele designation	frequency (%)
11	0.1
13	---
14	8.3
14s	0.1
15f	0.1
15	12.6
16	20.5
17	24.9
18	21.2
19	10.5
20	1.6
21	0.1
22	---

 $\chi^2=33.13; df=55; 0.990 < p < 0.995$ 

mean paternity exclusion chance (MEC) 0.64646

mean exclusion probability (MEP) 0.63402

polymorphism information content (PIC) 0.79214

Table 2: HUMVWA microsatellite sequence polymorphism

allele	size (bp)	5'constant region (43bp)	3'constant region (39bp)		
C11 <sup>2</sup>	126	..... tcta	(tctg)3	(tcta)7	.....
13 <sup>1</sup>	134	..... tcta	(tctg)4	(tcta)8	.....
C14 <sub>2</sub>	138	..... tcta(tctg)(tcta)	(tctg)4 (tcta)3	(tcca) (tcta)3	.....c....
14 <sup>2</sup>	138	..... tcta	(tctg)4 (tcta)2	(tcta)6	.....
15 <sup>2</sup>	142	..... tcta(tctg)(tcta)	(tctg)4 (tcta)3	(tcca) (tcta)3	(tcca)3 t .....
C15 <sub>1</sub>	142	..... tcta	(tctg)3	(tcta)11	.....
15 <sup>1</sup>	142	..... tcta	(tctg)4	(tcta)10	.....
16 <sup>1</sup>	146	..... tcta	(tctg)3	(tcta)12	.....
C16	146	..... tcta	(tctg)4	(tcta)11	.....
C17 <sub>3</sub>	150	..... tcta	(tctg)4	(tcta)12	.....
C18 <sub>3</sub>	154	..... tcta	(tctg)4	(tcta)13	(tcca) (tcta) t .....
C19	158	..... tcta	(tctg)4	(tcta)14	.....
C20	162	..... tcta	(tctg)4	(tcta)15	.....
C21 <sub>1</sub>	162	..... tcta	(tctg)4	(tcta)16	.....
22 <sup>1</sup>	170	..... tcta	(tctg)4	(tcta)17	.....

5' constant region: 5'- [ ccc tag tgg atg ata aga ata atc ] agt atg tga ctt gga ttg a -3'

3' constant region: 5'- tcca tcta t [cca tcc atc cta tgt att tat cat ctg tcc] - 3'

C11,14,... Alleles included in allelic cocktail for AMPFLP typing

[] denotes primer target sequences

<sup>1</sup> alleles and variants described by Moeller [1994]<sup>2</sup> alleles and variants described by Schwartz [this study]<sup>3</sup> EMBL M25858 sequence

## REFERENCES

- Kimpton C, Walton A, Gill P: A further tetranucleotide repeat polymorphism in the vWF gene. *Hum.Mol.Genet.* 1: 287 (1992)
- Moeller A, Meyer E, Brinkmann B: Different types of structural variation in STRs: HumFES/FPS, HumVWA and HumDS21S11. *Int.J.Leg.Med.* 106: 319-323 (1994)
- Sajantila A, Pacek P, Lukka M, Syaenen AC, Nokelainen P., Sistonen P, Peltonen L, Budowle B: A microsatellite polymorphism in the von Willebrand factor gene: comparison of allele frequencies in different population samples and evaluation for forensic medicine. *Forensic Sci.Int.* 65: 169-175 (1994)
- Schwartz DWM, Jungl EM, Krenek OR, Mayr WR: Simple and rapid typing for VNTR polymorphisms using high resolution electrophoresis of PCR products on rehydratable polyacrylamide gels. *Advances in Forensic Haemogenetics* 5: 170-172, Springer, Berlin, FRG (1994)

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