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vWA STR locus structure and variability

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Abstract. More than 100 vWA alleles were sequenced to detect any possible sequence difference at this locus in Portuguese Caucasian and African populations. Internal variability was detected—the most common structure is TCTA (TCTG)4 (TCTA)n with a TCTA(TCTG)3(TCTA)n structure more frequent in small alleles and a TCTA (TCTG)5–6 (TCTA)n structure predominantly in large ones. Allele 17 has a unique structure in all 20 studied samples and the nonconsensus allele 14 structure has a prevalence of 60% in both populations. Performing more than 3000 paternity investigation cases with multiplex systems, eight vWA mutations were detected with a 0.0024 average mutation rate. Six mutations at the primer-binding site have also been detected when performing ProfilerPlus/SGMPlus versus Powerplex1.2.

Keywords: vWA locus; Sequencing; Mutations; Forensic typing

1. Introduction

Sequence variation of STR alleles is common to encounter specially in compound and complex STR locus [1]. The high primer-binding mutation observed at this locus and the interest in structure analysis of rare alleles observed in certain population groups were the main reasons to perform vWA sequence. In this study, we will present the structure analysis of vWA alleles from allele 11 to allele 22 studied in two main populations—a Portuguese Caucasian population and an African population mainly from Cabo Verde Islands and Angola, to detect any possible sequence difference at vWA locus in both populations.

2. Materials and methods

Blood samples were extracted by the Chelex method and analysed with ProfilerPlus/SGMPlus (Applied Biosystems) and Geneprint PowerPlex1.2 (Promega) according to the

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Table 1
Allele nomenclature, sequenced structure and number of alleles sequenced at vWA locus in Portuguese (Port) and African (Afric) populations—consensus structure TCTA (TCTG)3–6 (TCTA)n

Alleles	Structure	Port	Afric
11	TCTA (TCTG)3 (TCTA) 7	–	3
13	TCTA (TCTG)3 (TCTA) 9	1	–
	TCTA (TCTG)4 (TCTA)8	2	4
14	TCTA (TCTG)3 (TCTA)10	2	–
	TCTA (TCTG)4 (TCTA) 9	2	2
	Nonconsensus structure[a]	5	4
15	TCTA (TCTG)4 (TCTA)10	1	1
16	TCTA (TCTG)3 (TCTA)12	–	3
	TCTA (TCTG)4 (TCTA)11	10	2
17	TCTA (TCTG)4 (TCTA)12	12	8
18	TCTA (TCTG)4 (TCTA)13	11	4
	TCTA (TCTG)5 (TCTA)12	1	–
19	TCTA (TCTG)4 (TCTA)14	8	3
	TCTA (TCTG)5 (TCTA)13	–	2
20	TCTA (TCTG)3 (TCTA)16	1	–
	TCTA (TCTG)4 (TCTA)15	2	–
21	TCTA (TCTG)4 (TCTA)16	2	–
	TCTA (TCTG)5 (TCTA)15	3	–
	TCTA (TCTG)6 (TCTA)14	1	2
22	TCTA (TCTG)5 (TCTA)16	2	–

[a] TCTA TCTG TCTA (TCTG)4 (TCTA)3 TCCA (TCTA)3 TCCA TCCA TCCA TCCA.

manufacturer's recommendations in an ABI Prism 377 with Genescan (V.3.1.2.) and Genotyper (v.2.0.) or in an ABI 3100 with Genescan and Genotyper (v.3.7). More than 100 vWA alleles have been sequenced with DNA Sequencing Kit Big Dye™ v.3.0 Terminator Cycle Sequencing (Applied Biosystems) with forward and reverse primers [2]. Sequencing was performed in an ABI Prism 377 DNA Sequencer and samples were analysed by Sequencing Analysis v.3.4.1. (Applied Biosystems).

3. Results and discussion

Table 1 shows allele nomenclature, sequence structure and number of alleles sequenced at the vWA locus in two populations. Internal variability was detected at allelic level at this

Table 2
Eight vWA mutations obtained in paternity investigation cases

	W without mutation	A Father	Mother	Child	W with mutation
Case 1	W = 99.99998%	16	17–18	17	W = 99.993%
Case 2	W = 99.998%	14–18	17–18–19	14–17	–
Case 3	W = 99.99995%	18–19	16–19	19–20	–
Case 4	W = 99.99999998%	17	15–17	15–16	W = 99.999997%
Case 5	W = 99.99993%	17–18	18–20	18–19	W = 99.98%
	W = 99.999997%	17–18	18–20	18–20	(brother)
Case 6	W = 99.999998%	17–19	15–16	15–18	W = 99.9996%
Case 7	W = 99.99996%	16–20	17–19	19–21	W = 99.994%
Case 8	W = 99.999995%	18–20	14–17	17–19	W = 99.9996%

Table 3
Six mutations at the primer-binding site obtained with ProfilerPlus/SGMPlus versus Powerplex1.2

	W without mutation	A Father	Mother	Child	Multiplex systems
Case 1	W = 99.9991%	15–18	16	18/16–18	Profiler/Powerplex1.2
Case 2	W = 99.9991%	16–18	17 /16–17	16–17	SGM/Powerplex1.2
Case 3	W = 99.9991%	16/16–17	14–16	14–16	SGM/Powerplex1.2
Case 4	W = 99.999999997%	16–19	17 /16–17	17–19	SGM/Powerplex1.2
Case 5	W = 99.9999992%	17–18	17/16–17	18/16–18	SGM/Powerplex1.2
Case 6	W = 99.999998%	17/17–18	16	16/16–18	SGM/Powerplex1.2

locus. The most common structure seems to be TCTA (TCTG)4 (TCTA)n, with a prevalence of TCTA(TCTG)3(TCTA)n structure in small alleles and the prevalence of TCTA (TCTG)5–6 (TCTA)n in large ones.

The nine observed nonconsensus allele 14 structures were found either in Caucasian or in African samples with a prevalence of 60% in both populations. Although we have analysed 20 allele 17 samples from both populations, the structure observed was always the same. Considering allelic structure, the most stable alleles seem to be alleles 16 and 17 in Caucasian population and alleles 17 and 18 in African population.

Performing more than 3000 paternity investigation cases with multiplex systems, several types of vWA mutations have also been encountered—null alleles, three-allele genotypes and repeat region mutations. A total of eight vWA mutations were detected in paternal investigation cases with a 0.0024 average mutation rate—one maternal mutation, five paternal mutations and two undetermined mutations (Table 2).

Table 3 illustrates six mutations at the primer-binding site obtained when performing ProfilerPlus/SGMPlus versus Powerplex1.2 (mutation rate 0.0018), which means that any vWA homozygous genetic incompatibility observed in paternity investigation needs to be confirmed with two different sets of primers.

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