vWA STR LOCUS - STRUCTURE AND VARIABILITY

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INTRODUCTION

Biological individualization of samples is studied through DNA STR loci analysis and is the principal goal of Forensic Genetics. STR loci study was initiated in the 90s on the majority of Forensic Laboratories. vWA is one of the most studied STR loci all over the world. It is included in any STR set proposed by Forensic Groups as the 13 core loci of the CODIS System used in routine cases by most Forensic Genetic Laboratories.

When performing paternity investigation and identification cases with STR loci, once in a while we came through rare or new alleles. Sequencing is essential to confirm the structure of these alleles. Also in mutation cases, specially in primer binding mutation cases, allele sequence should be performed. The high primer binding mutation observed in this locus and the interest in structure analysis of rare alleles observed in certain population groups were the main reasons for performing vWA sequence. More than one hundred vWA samples have been sequenced with DNA Sequencing Kit Big Dye™ Terminator Cycle Sequencing with forward and reverse primers (Kimpton et al, 1992 GenBank M25858). Sequencing were performed in an ABIPRISM 377 DNA Sequencer and analysed by Sequencing Analysis.

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 population and an African population mainly from Cabo Verde Islands and Angola.

RESULTS AND DISCUSSION

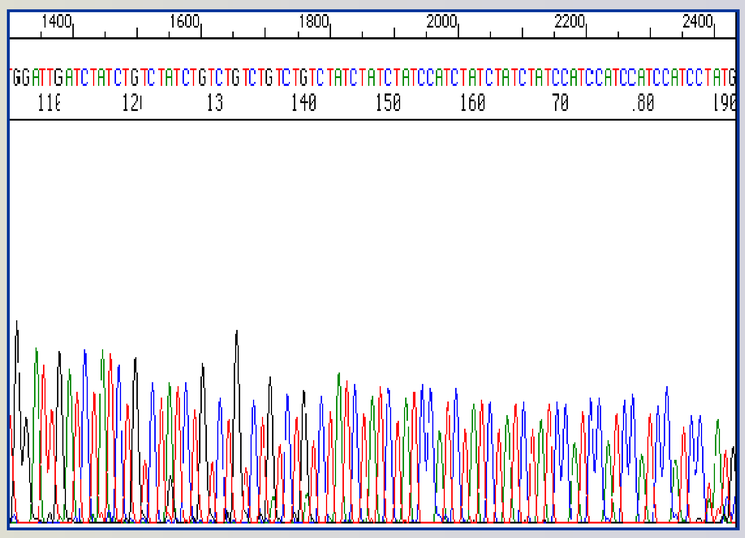
The **consensus vWA repeat region structure** is **TCTA (TCTG)3-6 (TCTA)n** followed by a **TCRA TCRA TCCA TCCA structure where R represents C or T**. Table 1 shows allele nomenclature, number of alleles sequenced and sequence structure at the vWA locus in two populations – a Portuguese population and an African population.

Table1. Allele nomenclature, number of alleles sequenced and sequence structure at the vWA locus in Portuguese Caucasian and African populations

	Structure of the repeat region (in bold)				
Alleles	**Portuguese Population**	**n**	**Alleles**	**African Population**	**n**
11	-		**11**	**TCTA (TCTG)3 (TCTA)7** TCCA TCTA TCCA TCCA	***3***
12	-		12	-	
13	**TCTA (TCTG)3 (TCTA)9** TCCA TCTA TCCA TCCA	**1**	13	-	
	TCTA (TCTG)4 (TCTA)8 TCCA TCTA TCCA TCCA	**2**		**TCTA (TCTG)4 (TCTA)8** TCTA TCTA TCCA TCCA	**4**
14	**TCTA (TCTG)3 (TCTA)10** TCCA TCTA TCCA TCCA	**2**	14	-	
	TCTA (TCTG)4 (TCTA)9 TCCA TCTA TCCA TCCA	**1**		**TCTA (TCTG)4 (TCTA)9 TCRA** TCTA TCCA TCCA	2
	Non-consensus structure	**5**			**4**
	TCTA TCTG TCTA (TCTG)4 (TCTA)3 TCCA (TCTA)3 TCCA TCCA TCCA TCCA				
15	**TCTA (TCTG)4 (TCTA)10** TCCA TCCA TCCA TCCA	1	15	**TCTA (TCTG)4 (TCTA)10** TCCA TCTA TCCA TCCA	1
16	-		16	**TCTA (TCTG)3 (TCTA)12** TCCA TCTA TCCA TCCA	3
	TCTA (TCTG)4 (TCTA)11 TCCA **TCRA** TCCA TCCA	**10**		**TCTA (TCTG)4 (TCTA)11** TCCA TCTA TCCA TCCA	2
17	**TCTA (TCTG)4 (TCTA)12** TCCA **TCRA** TCCA TCCA	**12**	17	**TCTA (TCTG)4 (TCTA)12** TCCA TCTA TCCA TCCA	**8**
18	**TCTA (TCTG)4 (TCTA)13** TCCA **TCRA** TCCA TCCA	11	18	**TCTA (TCTG)4 (TCTA)13** TCCA TCTA TCCA TCCA	**4**
	TCTA (TCTG)5 (TCTA)12 TCCA TCTA TCCA TCCA	1		-	
19	**TCTA (TCTG)4 (TCTA)14** TCCA **TCRA** TCCA TCCA	8	19	**TCTA (TCTG)4 (TCTA)14** TCCA TCTA TCCA TCCA	3
	-			**TCTA (TCTG)5 (TCTA)13** TCCA TCTA TCCA TCCA	2
20	**TCTA (TCTG)3 (TCTA)16** TCCA TCTA TCCA TCCA	1	20	-	
	TCTA (TCTG)4 (TCTA)15 TCCA **TCRA** TCCA TCCA	2		-	
21	**TCTA (TCTG)4 (TCTA)16** TCCA TCTA TCCA TCCA	2	21	-	
	TCTA (TCTG)5 (TCTA)15 TCCA TCTA TCCA TCCA	3		-	
	TCTA (TCTG)6 (TCTA)14 TCCA TCTA TCCA TCCA	1		**TCTA (TCTG)6 (TCTA)14** TCCA **TCRA** TCCA TCCA	2
22	**TCTA (TCTG)5 (TCTA)16** TCCA **TCRA** TCCA TCCA	2	22	-	

Allele 14 non-consensus structure

(TCTA) (TCTG)(TCTA)(TCTG)4(TCTA)3(TCCA)(TCTA)3



In both populations the same non-consensus structure of allele 14 has a prevalence of 60%.

Table2. vWA mutations in paternity casework

More than 3300 meiosis in vWA have been studied and a total of **8 mutations** were detected – **average mutation rate 0.0024**

Maternal mutations- 1 Mutation rate 0.0003 | **Paternal mutations- 5** Mutation rate 0.0015 | **Undetermined- 2** Mutation rate 0.0006

	W without mut	A Father	Mother	Child	W with mutation
Case1	W=99,99 998%	**16***	**17-18**	**17***	W=99,993%
Case2	W=99,99 8%	**14-18**	**17-18-19**	**14-17**	**-**
Case3	W= 99, 99 995%	**18-19**	**16-19**	**19-20**	-
Case4	W=99,99999998%	**17-17**	**15-17**	**15-16**	W=99,999997%
Case5	W=99,99993%	**17-18**	**18-20**	**18-19**	W=99,98%
	W=99,999997%	**17-18**	**18-20**	**18-20**	**(Brother)**
Case6	W=99,999 998%	**17-19**	**15-16**	**15-18**	W=99,9996%
Case7	W=99,99996%	**16-20**	**17-19**	**19-21**	W=99,994%
Case8	W=99,999995%	**18-20**	**14-17**	**17-19**	W=99,9996%

*Null allele either with SGM Plus or Powerplex

Table3. Mutations at the binding primer site

Analysis were peformed with **Profiler Plus/SGM Plus and Powerplex1.2 /Powerplex16 - 6 mutations at the binding primer site** have been detected – **mutation rate 0.0018**

	W without mut	A Father	Mother	Child	W with mutation
Case1	W=99,9991%	15-18	16-16	**18-18**	**Profiler Plus**
		15-18	16-**16**	**16-18**	**Powerplex 1.2**
Case2	W=99,9991%	16-18	**17-17**	16-17	**SGM Plus**
		16-18	**16-17**	16-17	**Powerplex 1.2**
Case3	W=99,9991%	**16-16**	14-16	14-16	**SGM Plus**
		16-17	14-16	14-16	**Powerplex 1.2**
Case4	W=99,999999997%	16-19	**17-17[1]**	17-19	**SGM Plus**
		16-19	**16-17[1]**	17-19	**Powerplex 1.2**
Case5	W=99,9999992%	17-18	**17-17**	**18-18**	**SGM Plus**
		17-18	**16-17**	**16-18**	**Powerplex 1.2**
Case6	W=99,999998%	**17-17**	16-16	**16-16**	**SGM Plus**
		17-18	16-16	**16-18**	**Powerplex 1.2**

[1]Angola

CONCLUSIONS

1. Internal variability was detected at allelic level in vWA locus. In Portuguese Caucasian Population, the **most common consensus structure** seems to be **TCTA(TCTG)4(TCTA)n**, with a prevalence of TCTA(TCTG)3(TCTA)n structure in small alleles as **alleles 11 and 13** and the prevalence of **TCTA(TCTG)5-6(TCTA)n** in bigger alleles as **alleles 21 and 22.**

In African Population, the most common structure is also **TCTA(TCTG)4(TCTA)n**, which seems to be more frequent in all alleles when comparing to the Portuguese Population. **Allele 11** only detected in the African Population has a TCTA(TCTG)3(TCTA)n structure while in **allele 21** a **TCTA(TCTG)6(TCTA)n** structure was encountered in this population.

2. Considering allele structure, the **most stable alleles** in the **Caucasian Population** seems to be **alleles 16 and 17** and the most stable in the **African Population** are **alleles 17 and 18**. Although twenty **allele 17** samples have been analysed from both populations, the structure observed was always the same in both populations – **TCTA(TCTG)4(TCTA)12.**

3. When performing more than **3000 paternity investigation cases** with multiplex systems, **several types of vWA mutations** have also been encountered – null allele, three allele genotype and undetermined, paternal and maternal mutations. **Mutations at the binding primer site** when performing Profiler Plus and SGM Plus have also been detected, which means that any vWA homozygous genetic incompatibility needs to be confirmed with another set of primers.