VWA STR genotyping: further inconsistencies between Perkin-Elmer and Promega kits

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SHORT COMMUNICATION

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VWA STR genotyping: further inconsistencies between Perkin-Elmer and Promega kits

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Abstract We report a simultaneous study of the VWA STR locus by the Perkin-Elmer Profiler Plus kit and the Promega GenePrint CTTv kit in a population sample from North Portugal and in 418 meiosis from family material and paternity cases. PCR amplification and genotyping were performed according to the manufacturer's instructions using ABI 377 or ABI 310 automatic sequencers. Biological kinship in family material and paternity cases was validated by the use of the STR loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11, FGA, CSF1PO, TH01 and TPOX. Out of 434 unrelated individuals we found 4 inconsistencies between the genotypes obtained using each kit. No exclusions were found in the meiotic analyses. In all cases, these inconsistencies were due to an annealing failure of the Perkin-Elmer forward primer resulting in false homozygotes. Sequencing analysis revealed an A-to-T substitution at position 1631 (GenBank sequence M25858), 52 bases upstream of the first TCTA motif of the repeat region. An estimate of the null allele frequency (*s*) in this study is thus obtainable from the expression $s = 4/(2 \times 434) = 0.46\%$. The relatively high frequency of these discrepancies in our population demonstrates the need for caution when comparing genotype or gene frequency estimates made from amplicons produced by different primers, when evaluating apparent exclusions in paternity testing and when searching for a match between individual genetic profiles in forensic databases. Our findings are also compared with those previously reported.

Keywords STR · VWA · PCR · Kits · Null alleles

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Introduction

Since it was first described (Kimpton et al. 1992), the polymorphic STR at intron 40 of the VWA locus (von Willebrand coagulation factor) has been widely used in population (e.g. Möller et al. 1994; Prata et al. 1996; Gusmão et al. 1997; Hantschel et al. 1999; Perez-Lezaun et al. 2000) and forensic studies (e.g. Pestoni et al. 1995; Sparkes et al. 1996; Neuhuber et al. 1999). More recently, commercial kits from different manufacturers have become available for its typing in multiplex systems.

The use of different primers for VWA genotyping has however introduced the risk of inconsistency between results from defined protocols due to polymorphisms in the different primer annealing regions.

In this work we report the results of the simultaneous study of the VWA STR locus by the Perkin-Elmer (PE, Foster City Calif.) Profiler Plus kit and the Promega GenePrint CTTv kit (Promega, Madison Wis.) in a population sample from Northern Portugal, in family studies and in paternity cases. Furthermore, the reason for the inconsistencies found in this work was evaluated by sequencing analysis using a new set of primers.

A comparison of our findings with those obtained in recent publications (Kline et al. 1998; Walsh 1998; Budowle et al. 1999; Budowle 2000; T. Ribeiro, personal communication; M. Anjos, personal communication, see acknowledgements) is also reported.

Material and methods

Samples and DNA extraction

Samples were obtained from 434 unrelated volunteer donors from North Portugal and 418 meioses from family material and paternity cases were also examined. Samples were obtained by venipuncture or buccal swabs and DNA was extracted according to Singer-Sam et al. (1989).

PCR amplification and fragment analysis

PCR amplification and genotyping were performed according to the manufacturers instructions (PE Profiler Plus kit and the Promega GenePrint CTTv kit) using the PE ABI 377 or ABI 310 sequencers and the GeneScan software.

Kinship validation studies

Biological kinship in family material and paternity cases was validated ($W \geq 99.99\%$) by the use of the STR loci D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11, FGA, CSF1PO, TH01 and TPOX.

Sequencing

The primers used for sequencing in the VWA system were:

- P1: 5' GTG AAC TCC TCA GAC TGA TCC TAT AAG G (this work)
- P2: 5'GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG (Kimpton et al. 1992).

The choice of this primer pair was the result of an experiment to try to find out whether one of the primers used by Kimpton et al. (1992) was common to one of the PE Profiler Plus VWA primers. Previously amplified samples with the Kimpton et al. VWA primers were re-amplified in two separate PCR reactions: one contained the Kimpton et al. forward primer and PE Profiler Plus primers, whilst the other was set up with the reverse primer of Kimpton et al. together with PE Profiler Plus primers. PCR products were only obtained when using the forward primer of Kimpton et al. (amplicons with similar size range as with the Kimpton et al. primer pair), concluding that one of the PE primers overlaps the reverse primer of Kimpton et al.. In conclusion, the mutation responsible for the VWA null alleles when using the PE kit must be in the annealing region of the PE forward primer. Given the known size of VWA amplicons with the PE kit, a new primer (P1) was designed that would clearly anneal outside the PE forward primer (5' end anneals 141 bp upstream of the repeat region).

The four samples presenting null alleles in this study together with control samples with amplifiable alleles, were amplified with the new set of primers using the following conditions: 5 ng genomic DNA in 25 µl total reaction volume containing 200 µM dNTPs (PE), 0.25 µM each primer (PE) and 0.5 U Taq DNA polymerase (MBI Fermentas, Vilnius, Lithuania) in 1 × PCR buffer (MBI Fermentas). The reaction was carried out in 35 cycles of 95 C for 30 s, 54 C for 1 min and 72 C for 1 min, with a final extension step of 60 C for 30 min.

The PCR products were separated in native polyacrylamide gel electrophoresis (T 9%, C 5%) using a discontinuous buffer system (Luis and Caeiro 1995). The DNA fragments were visualised by silver staining according to Budowle et al. (1991).

Individual alleles were excised after electrophoresis and extracted from the gel with TE buffer by freezing for 30 min and heating at 65 C for 15 min, 3 times. The extracted alleles were then re-amplified using the same conditions as described before. The fragments were purified using Microspin S-300 HR columns (Pharmacia, Upsala, Sweden), according to the manufacturers' instructions.

The sequencing reaction was done with the BigDye Terminator Cycle Sequencing Ready Reaction kit (PE), in a total reaction volume of 5 µl. The samples were additionally purified with an $MgCl_2$/ethanol based protocol.

Electrophoresis was carried out on a PE ABI 377 DNA sequencer and the results analysed using the ABI PRISM 377-18 data collection software.

Results

The meiotic analysis provided no evidence of mendelian incompatibilities or segregation distortions.

In 434 unrelated individuals we found the following inconsistencies:

- Two individuals typed 16 with PE profiler Plus and 16/18 with Promega GenePrint CTTv
- One individual typed 16 with PE profiler Plus and 16/19 with Promega GenePrint CTTv
- One individual typed 18 with PE profiler Plus and 17/18 with Promega GenePrint CTTv.

In order to assess if changes in the PE PCR protocol would amplify the null alleles, the annealing temperature recommended (59 C) was reduced to as low as 48 C and still no amplification occurred.

Sequencing analysis with the new set of primers used in this work revealed an A-to-T substitution at position 1631 (GenBank sequence M25858), 52 bases upstream the first TCTA motif of the repeat region.

Discussion

The genotyping inconsistencies here described are due to an annealing failure of the Perkin-Elmer forward primer caused by a sequence variation in the binding region and resulting in false homozygotes. The only variation detected in this work outside the repeat region was an A-to-T substitution which must lie somewhere in the PE forward primer annealing region. This mutation is most likely the same as the one detected by Walsh (1998) at the second position from the 3' end of the PE forward primer binding region.

This type of inconsistency has been detected by various groups (Kline et al. 1998; Walsh 1998; Budowle et al. 1999; Budowle 2000) and not only for the VWA locus (e.g. Gusmão et al. 1996; Walsh 1998; Budowle 2000). However, as this is the most frequently represented locus in the various multiplex kits available from different man-

Table 1 Comparison of our findings for the VWA locus with those obtained in previous publications (*OCME* Office of Chief Medical Examiner, New York City)

References	Sample size	Inconsistencies (PE vs Promega kits)		
		vWA	Null alleles	Origin
1. Kline et al. (1998)	600	1	19	African-American
2. OCME (Walsh 1998)	200	1	17	US Hispanic
3. Budowle et al. (1999)	490	0	–	–
4. Budowle (2000)	750	0	–	–
5. This work	434	4	17, 18 (2), 19	Portuguese (North)

ufacturers, it allows a direct genotype comparison of one sample with distinct primer pairs in the same laboratory.

An estimate of the null allele frequency(*s*) using the PE kit simultaneously with the Promega kit is thus obtainable from the expression $s = 4/(2 \times 434) = 0.46\%$.

The mutations responsible for these discrepancies do not seem to be characteristic of a specific population group nor is its origin rooted to a specific allele (it is present in distinct allelic backgrounds, Table 1). Although in these works the null alleles detected were associated with 17, 18 and 19 repeats, the association with allele 16 in 5 individuals has also been described (T. Ribeiro, personal communication). Furthermore, it has also been detected (M. Anjos, personal communication) in tandem with allele 18 (in three unrelated individuals) and with allele 17 (in one individual).

An estimate of the mutation frequency at the VWA STR locus, combining the available data (Table 1) is 0.12%, but varies between 0 and 0.46% according to the population sample. The relatively high frequency of these discrepancies, at least in some populations, demonstrates the need for caution when comparing genotype or gene frequency estimates made in amplicons produced by different primers, when evaluating apparent exclusions in paternity testing and when searching for a match between individual genetic profiles in forensic databases.

These precautions are not only required by the type of discrepancy described here. Indeed, since the primers mentioned amplify quite different genomic lengths (157–197 bp for Perkin-Elmer and 127–167 bp for Promega) other length or sequence variations outside the overlapping region can occur. Databases and gene frequency estimates as well as forensic case work results are therefore strictly comparable only when using the same set of primers.

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