

Sequencing of FES, vWA and SE33 STRs in mother–child incompatibilities

M.J. Anjos^{a,*}, M. Carvalho^a, F. Balsa^a, L. Andrade^a,
V. Lopes^a, A. Serra^a, J. Gamero^b, F. Corte-Real^c,
D.N. Vieira^c, M. Vide^a

^a*Service of Forensic Genetics, Delegation of Coimbra, National Institute of Legal Medicine, Largo da Sé Nova, Coimbra, 3000-213, Portugal*

^b*Department of Legal Medicine, Faculty of Medicine, University of Cádiz, Spain*

^c*National Institute of Legal Medicine, Largo da Sé Nova, Coimbra, 3000-213, Portugal*

Abstract. Our casuistic in paternity testing showed several isolated incompatibilities present in different STRs like FES, SE33, vWA, among others, include homozygote and heterozygote situations, and appear both in father–child and mother–child pairs. As we assume, in paternity testing, that the mother is the real one, most of incompatibility situations are assigned to a mutation in putative father. In fact, only sequencing allows the real explanation for the event. © 2003 Elsevier B.V. All rights reserved.

Keywords: STR; Mutation; Sequencing

1. Introduction

In this work, we present some cases of mother–child incompatibilities, one of them (3) that seems to be in a first analysis due to a mutation in the father–child pair.

2. Material and methods

DNA from four families was extracted using Chelex 100 [1]. PCR singleplex reaction of each STR was prepared with primers and amplification conditions according to the authors (FES [2], SE33 [3] and vWA [2]) and carried out in a thermocycler GeneAmp[®] PCR System 9600 (Perkin Elmer). Allele designation was made using sequenced allelic ladders.

PCR product was purified with MicroSpin Sephadex G-50. Manual electrophoresis was done to separate the alleles in cases 2 to 4. Re-amplification of each one was carried out using the same primers followed by other purification.

* Corresponding author. Tel.: +351-239-854-230; fax: +351-239-820-549.

E-mail address: geneforense@dcinml.mj.pt (M.J. Anjos).

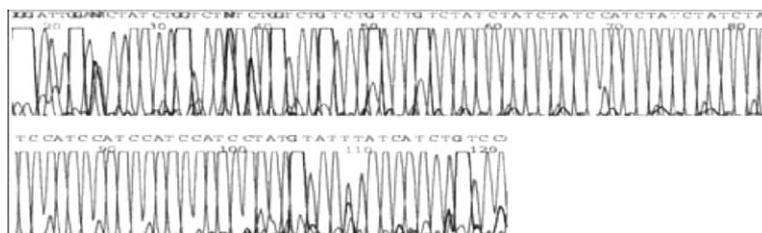


Fig. 3. vWA: electropherogram of child's allele 17.

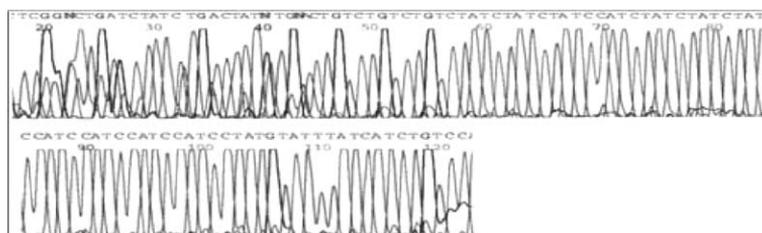


Fig. 4. vWA: electropherogram of child's 2 allele 17.

Case 4—Mothers allele 14 showed the structure already described. The child's 2 allele 17 (Fig. 4) revealed an overlapping of sequences where we can distinguish one structure identical to allele 14. It could be a false homozygote with alleles 14 and 17.

Several phenomena for human diversification are described like meiotic recombination and errors during replication [7]. Possible mechanisms like the unequal crossover between alleles or sister chromatids and point mutations could be a possible explanation for incompatibilities (detected in cases 4 and 2, respectively).

When a mutation is present in the primer binding region for any particular allele there are several possible results for PCR: no effect on amplification; weak amplification of the mutant sequence, resulting in peak signal imbalance in a heterozygote; non-amplification of the mutant allele, resulting a homozygous genotype in a true heterozygote [8]. Probably, cases that showed homozygote PCR products (cases 1 and 4) could have such a kind of mutation. However, we cannot explain what happened in case 3: sequencing of child's allele 17 (150.92 bp) revealed an identical structure to mother's allele 14 (138.76 bp).

In conclusion, we think that sequencing alleles in incompatibility cases should always be done for confirmation, especially in the father–child ones.

References

- [1] P.S. Walsh, et al., *BioTechniques* 10 (4) (1991) 506–513.
- [2] C. Kimpton, et al., *Forensic Sci. Int.* 71 (1995) 137–152.
- [3] P. Wiegand, et al., *Int. J. Leg. Med.* 105 (1993) 315–320.
- [4] A. Urquhart, et al., *Int. J. Leg. Med.* 107 (1994) 13–20.
- [5] A. Möller, et al., *Int. J. Leg. Med.* 106 (1994) 319–323.
- [6] A. Möller, et al., *Int. J. Leg. Med.* 106 (1994) 262–267.
- [7] A.J. Jeffreys, et al., *EMBO J.* 17 (14) (1998) 4147–4157.
- [8] S. Walsh, *J. Forensic Sci.* 43 (5) (1998) 1103–1104.