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

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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.

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Cycle sequencing of the re-amplified product was made using the ABI Prism Big Dye V3.0 Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS (Applied Biosystems). After sequencing, samples were purified by MgCl₂/ethanol precipitation. Detection was carried out using an ABI PRISM[®] 377 DNA Sequencer.

3. Results

Case 1 (FES) P. father: 10, 12 mother: 12 child: 10, 13. Case 2 (SE33) P. father: 18, 29.2 mother: 17, 31.2 child: 29.2, 32.2. Case 3 (vWA) P. father: 18, mother: 14, 18 child: 17, 18. Case 4 (vWA) P. father: 15, 17 mother: 14, 18 child 1: 15, 18 child 2: 17.

4. Discussion and conclusions

Case 1—Mother's allele 12 revealed a consensus structure [4,5] in 12 repeats with an overlapping of bases after that—seems that there are two different sequences: one of allele 12 (with flanking region) and the other of allele 13. The child's sequence revealed an overlapping of two sequences that could correspond to alleles 10 and 13 (Fig. 1).

Case 2—Mother–child alleles (31.2 and 32.2) revealed a type II structure [6]. In Fig. 2, we can observe an "N" in the last repeat before the 3'-flanking region: the G to A transition (a possible deamination) caused an increment of one repeat unit.

Case 3—Analysis of mothers allele 14 revealed the structure described for this allele [4,5]. In child's sequence (Fig. 3), we can see an identical structure to mother's allele 14 (flanking region included) instead of the expected structure for allele 17.



Fig. 1. FES: electropherogram of mother's allele 12.



Fig. 2. SE33: electropherogram of child's allele 32.2.



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.

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