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An isolated exclusion in the FGA system

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Abstract. In a paternity test with 35 short tandem repeats (STRs), an isolated exclusion for the FGA system was observed (probability of paternity >99.999%). Different mechanisms suitable to explain this finding like polymerase slippage, primer mismatch, a deletion of the FGA locus or gene conversion after formation of the zygote are discussed.

Keywords: FGA; Deletion; Mutation; Gene conversion

1. Introduction

Short tandem repeat (STR) polymorphisms are prone to germline mutations. The gametic mutability of STR loci routinely used for paternity testing has been closely examined previously [1,2] and single step mutations due to polymerase slippage were identified as most common mutation events. In a paternity test with 35 STRs, an isolated exclusion for the FGA system with a contraction for at least three repeats was observed (Fig. 1). This mutation event cannot easily be explained by simple slippage.

2. Materials and methods

DNA was extracted from Blood and saliva samples twice following standard protocols from Qiagen and amplified for 35 STRs.

3. Results and discussion

In the FGA system, child and mother were homozygous for 21, whereas the alleles of the putative father were 24 and 25 (Fig. 1). Sequencing revealed consensus alleles for FGA. Typing 27 autosomal STRs and eight Y-chromosomal STRs lead to a probability of paternity of >99.999%. There is no evidence of relatives of the putative father as genitors.

The most obvious explanation for a mutation in an STR locus would be a contraction of the repeat stretch due to polymerase slippage. However, these mutations are almost invariably confined to a single repeat [1,2]. Larger contractions or expansions (as observed in disease related triplet sequences) are considered extremely rare, and to be the consequence of recombination rather than slippage. Moreover, the fact that the child is homozygous (the paternally inherited allele has mutated into the same allele as the maternally inherited!)

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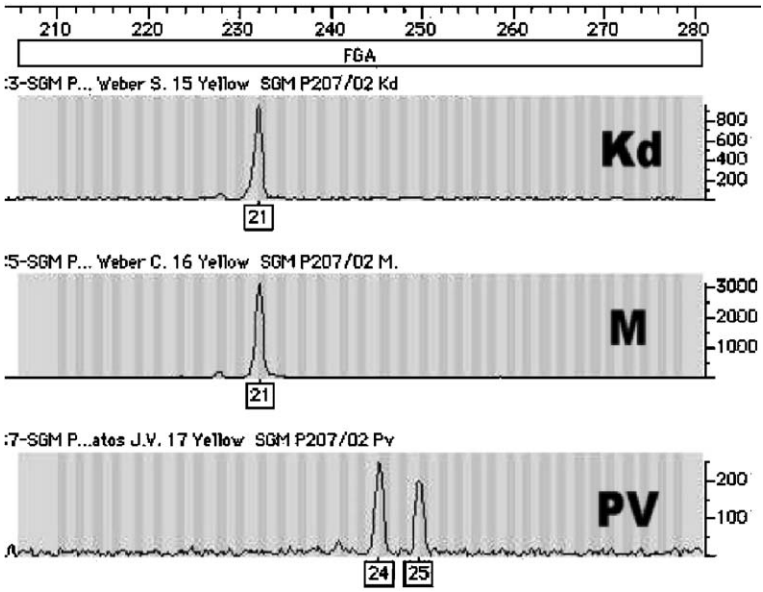

Fig. 1. FGA results with SGM kit.

merits attention: Only by incidence slippage would result in homozygote offspring. Bearing this in mind, we considered alternative mutational mechanisms. One possibility would be a loss of the paternal allele during meiosis, either by introduction of a primer binding mismatch mutation or by deletion of the FGA locus. A primer binding site mutation could be ruled out by amplification with three different primer sets.

Recently, a 11-kb deletion of the fibrinogen alpha-chain gene comprising the FGA polymorphism associated with afibrinogenemia was reported [3]. We succeeded to eliminate this possibility by performing a PCR specific for the 11-kb deletion (Fig. 2). To identify for larger, unknown deletions, we analyzed the chromosomal region surrounding the FGA system. Close polymorphisms of forensic interest are MNSs and FABP. We typed these

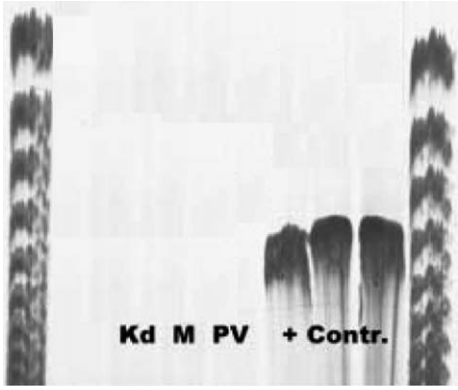

Fig. 2. PCR results from FGA fibrinogen alpha-chain gene comprising the FGA locus.

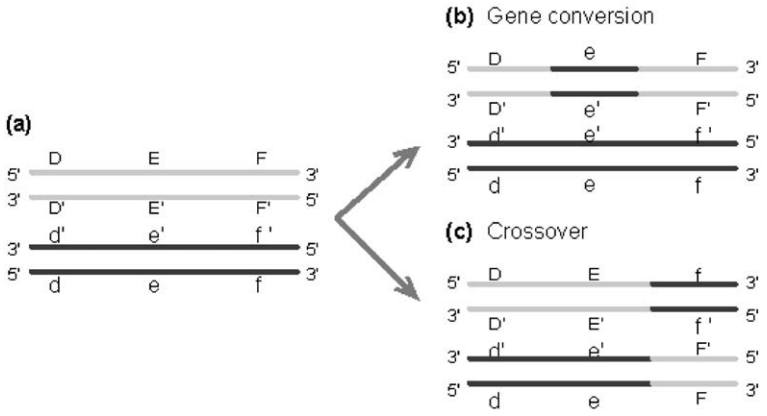

Fig. 3. Comparison between gene conversion and DNA crossover. (a) Two DNA molecules. (b) Gene conversion—the black DNA *donates* part of its genetic information (e–e′ region) to the grey DNA. (c) DNA crossover—the two DNAs *exchange* part of their genetic information (f–f′ and F–F′).

polymorphisms and four further close STRs (D4S1625, D4S1629, D4S2999, and D4S3021) without finding a further exclusion. Moreover, in four of the loci, the child was heterozygous. Finally, we used the relative signal intensities in a multiplex PCR to rule out a deletion of one FGA allele: In the case of a deletion in the child (= only one copy of the locus), the signal of THO1 (relative to the other loci in the multiplex) should be half compared to that for the mother (= two copies). However, in the present case, the mean relative amplification for TH01 in five reactions did not differ between child and mother, allowing us to eliminate a deletion (Fig. 2).

However, gene conversion might be a mechanism that could satisfyingly explain this unusual mutation. Although little is known about gene conversion in mammals, it is well-described in fungi. Basically, it is a nonreciprocal genetic exchange in which the sequence of one DNA-strand (acceptor) is altered to become identical to the sequence of another DNA strand (donor, Fig. 3b) following damage to the acceptor strand. It is thus considered to be part of a mismatch repair mechanism. Gene conversion is usually observed during meiosis, but it is considered to occur during mitosis as well. We thus favor gene conversion after formation of the zygote as the most likely mutational mechanism in this case as (having excluded a deletion) it is the only mechanism explaining the homozygosity of the child which is capable of introducing a three or four repeat contractions. This is the first mutation of that kind reported for FGA.

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