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Observation of null alleles apparently due to deletions

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Abstract After examining 2 paternity cases in 17 classical, 4 RFLP and 5 PCR-VNTR systems, isolated pseudo-exclusions were observed in the polymorphism D2S44 (YNH24). In both cases the "exclusions" were due to apparent opposite homozygosity. The application of different restriction enzymes, PCR amplification and varying electrophoretic conditions each led to an equivalent result of a 1-band-pattern with a mismatch between both father/child pairs. From these results the authors conclude that a complete or almost complete loss of the alleles is the most probable explanation.

Key words D2S44 (YNH24) · RFLP · PCR · 'Opposite homozygosity' · Deletion

Introduction

The examination of restriction fragment length polymorphisms (RFLP) with single locus probes has been established for several years (Balazs et al. 1989; Brinkmann et al. 1991; Bär and Kratzer 1992). The mutation rates are below 1% with the exception of D1S7 (MS1) with approximately 5% (Jeffreys et al. 1988; Henke et al. 1993). The existence of null alleles has also been demonstrated from an excess of homozygotes (Steinberger et al. 1993; Chakraborty et al. 1994). In this study, 2 paternity cases are demonstrated where isolated pseudo-exclusions were found in the polymorphism D2S44 due to deletions.

Materials and methods

RFLP fragments were visualized applying a standard protocol for extraction, digestion, separation and blotting (Brinkmann et al. 1991) in combination with chemiluminescence detection (Cellmark Diagnostics, UK). In the 2 families concerned a variety of modifications was applied to D2S44:

- PCR amplification according to a previous report (Möller et al. 1994)
- digestion with the following additional enzymes: PstI, HaeIII, AluI, StuI
- extremely short electrophoretic runs allowing the visualization of fragments down to 500 bp for RFLP detection and down to 140 bp for PCR product detection (Chakraborty et al. 1994)
- increase of hybridization sensitivity by reducing the temperature and increasing the salt concentration.

Biostatistics

The Essen-Möller approach was applied for all systems. The allele frequencies were determined using a ± 3 SD window (Wiegand et al. 1991) for D2S44. The probability of paternity was calculated as described by Henke et al. (1993) considering the frequency of new mutations.

Other PCR based systems (Table 1) were demonstrated applying published protocols (Rand et al. 1992; Wiegand et al. 1993).

Results

So far, we have investigated 408 paternity cases using the probe YNH24. Among the exclusions obtained all but 2 were in combination with at least 2 other systems. These 2 cases showed an obvious opposite homozygosity in the polymorphism D2S44 after HinfI digestion. All other systems showed matching trios (Table 1). Phenotypically the father and child in each family showed 1 allele only, but with different fragment sizes (Fig. 1).

Case 1: The classical approach (17 systems) and 9 DNA systems led to a combined probability of paternity of W = 99.99999% considering the isolated D2S44 pseudo-exclusion and assuming a mutation rate of 0.86% (Henke et al. 1993). The probability values were 99.98% (classical systems), 99.95% (RFLPs), 99.95% (PCR-VNTRs).

Case 2: The classical approach (17 systems) and 9 DNA systems (Table 1) led to a combined probability

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Table 1 Summary of the results in 2 paternity cases. EM-value = 10 + log(1-W)/W (probability of paternity), m = mother; ch = child; pf = putative father

	Single locus polymorphism		PCR-VNTR	Number of class. systems	EM comb.
	Compatible	Incompatible (kbp)			
Case 1:	MS8 MS43a MS31	YNH24	YNZ22, MCT118, COL2A1 ACTPB2, TH01	17	
		m = 4.737 ± 0.142 3.118 ± 0.094 ch = 4.736 ± 0.142 pf = 2.270 ± 0.068			
	EM (SLS) = 10.1563		EM (PCR) = 6.5754	EM (class.) = 6.3712	3
Case 2:	MS8 MS43a MS31	YNH24	ApoB, MCT118, VWA, ACTBP2, TH01	17	
		m = 2.294 ± 0.069 2.717 ± 0.082 ch = 2.294 ± 0.069 pf = 2.700 ± 0.081			
	EM (SLS) = 10.3986		EM (PCR) = 7.9187	EM (class.) = 8.8463	7.1635

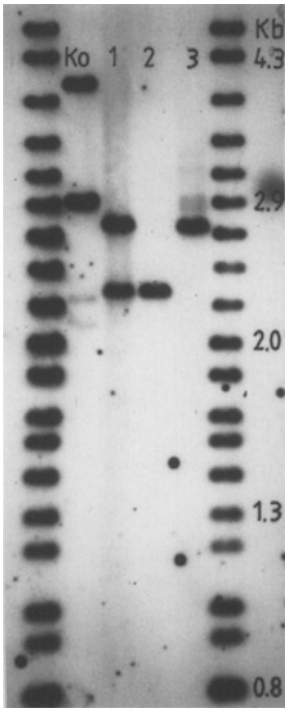

Fig. 1 Hybridization results in one of the paternity cases with the single locus probe YNH24 after shortening of the electrophoresis step. 1 = mother, 2 = child, 3 = putative father (pf), Ko = control DNA (mixture of a laboratory DNA and the cell line K562). The putative father and the child each show a homozygote band pattern with a pseudo-exclusion

of paternity of W = 99.8545% considering the isolated D2S44 pseudo-exclusion and assuming a mutation rate of 0.86% (Henke et al. 1993).

PCR amplification (D2S44) results in mismatching 1-band patterns in both father-child pairs. The application of 4 additional restriction enzymes resulted in mismatching 1-band patterns only in both father-child pairs. Depending on the restriction sites the fragments obtained varied between approximately 12.5 kb (PstI) and 1.2 kb (HaeIII).

Shorter electrophoretic runs for RFLP and PCR-based analysis also resulted in the detection of only 1-band patterns. No additional band could be observed after variation of the hybridization conditions.

Discussion

The pseudo-exclusions observed in 2 families in the polymorphism D2S44 (YNH24) can be due to 4 reasons: I. failure to detect a rare variant, II. mutations, III. new mutation and IV. deletion.

Loss of existing variants

Two major reasons can exist:
1. loss by fast migration, i.e. extremely small fragments could not be detected after standard electrophoresis. Non-detectable alleles (so-called null alleles) are known to occur within the polymorphism D2S44 (Steinberger et al. 1993; Chakraborty et al. 1994). Both flanking regions of the HaeIII-digested YNH24 probe have together a length of 430 kp (Möller et al. 1994). Comparison of the HaeIII profiles with HinfI profiles indicated that the HinfI fragments are approximately 1 kb longer. As a consequence we would not expect fragments shorter than 1000 bp after HinfI digestion. The other enzymes resulted in either longer (PstI) or shorter (HaeIII, AluI, StuI) fragments compared to HinfI digestion. Our PCR approach resulted in fragments of shorter length as after HaeIII digestion, because the primer sites are located next to the repeat re-

gion. Defined electrophoretic conditions were chosen to visualize fragments between 0.5 and 0.1 kb. No faint band could be visualized not even in highly improbable regions.

2. Loss due to poor sensitivity: in addition and partly in combination with the different electrophoretic conditions we have tested a variety of modifications known to be associated with increasing sensitivity. In particular the parameters of hybridization and exposure were varied but none resulted in any faint signal, although the band visualized was quiet intensive (Fig. 1). In addition the southern blotting PCR amplification was performed which had been previously optimized (Möller et al. 1994). This approach is known to be more sensitive than RFLP technology which could be shown in the previous study. More importantly one would expect here a loss of the longer fragment (preferential amplification) but not of the shorter one. From these experiments and observations we would therefore exclude the possibility to lose existing variants.

Mutations

Mutations involving single or very few bases (exchanges, deletions, insertions) can either result in a loss of restriction sites, the creation of new restriction site(s) or the loss of primer annealing sites. Since a variety of different sites of the locus were involved (4 enzyme sites and 2 primer annealing sites), it is impossible that all sites were simultaneously mutated thus leading to equivalent patterns in all methods. Even if one would assume a shorter insertion preventing probe annealing one would not expect a negative PCR result.

New mutation

The hypothesis could be: the putative father is homozygote in one of his gametes which has mutated to length conformity with the maternal allele of the child. As a consequence, a homozygote type 1 (putative father) is converted into a homozygote type 2 (child). This possibility is statistically extremely improbable. The other possibility of new mutation leading to a mismatched heterozygote is of course statistically much more frequent. Since we have never observed the latter occurrence we would exclude this new mutation hypothesis for our observations.

Deletion

All results can be explained with this hypothesis. Deletion will result in a simultaneous failure of a variety of technologies and modifications. In particular the failure of different enzymes, of highly sensitive approaches such as PCR and electrophoretic and hybridization variations can only be explained by this assumption.

Since deletion has to be concluded in 2 families out of 400 investigated so far, it could be considered to play a major role in the occurrence of an excess of homozygotes (Valverde et al. 1993; Chakraborty et al. 1994).

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