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ORIGINAL ARTICLE

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Structure of new mutations in 2 STR systems

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Abstract Isolated father/child mismatches in cases with a high probability of paternity (W > 99.9%) have been investigated using short tandem repeat (STR) systems. According to the high probability of paternity new mutations could be assumed in these cases. A new mutation could be observed in 3 cases using the STR system HumACTBP2. Two of these cases showed a deletion and 1 case an insertion of 1 repeat (AAAG-motif) which could be verified by sequencing. In another paternity case a new mutation – 1-repeat insertion (TCTA-motif) – in the HumVWA system was detected and verified by sequencing. These findings led to a new mutation rate of 0.7% (n = 453 meioses) for HumACTBP2 and 0.2% for HumVWA (n = 484 meioses).

Key words Short tandem repeat (STR) systems · HumACTBP2 · HumVWA · New mutations · Sequencing

Zusammenfassung Untersucht wurden isolierte Vater/Kind-Ausschlüsse mit Short Tandem Repeat (STR)-Systemen in Paternitätsfällen mit sehr hoher Vaterschaftswahrscheinlichkeit (W > 99.9%). Aufgrund der hohen Vaterschaftswahrscheinlichkeit war von Neumutationen auszugehen. Mit dem STR-System HumACTBP2 wurde eine Neumutation in 3 Paternitätsfällen beobachtet. In 2 Fällen wurde eine Deletion und in einem Fall eine Insertion um jeweils 1 Repeat (AAAG-Motiv) durch Sequenzierung nachgewiesen. Im HumVWA-System konnte in einem weiteren Paternitätsfall eine 1-Repeat-Insertion (TCTA-Motiv) durch Sequenzierung bestätigt werden. Die 3 beobachteten Neumutationsfälle ergaben für HumACTBP2 eine Mutationsrate von 0.7% (n = 453 Meiosen). Für HumVWA lag die Mutationsrate bei 0.2% (n = 484 Meiosen).

Schlüsselwörter Short Tandem Repeat (STR) Systeme · HumACTBP2 · HumVWA · Neumutationen · Sequenzierung

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Introduction

Genetic new mutations in VNTR polymorphisms are more common than in coding regions (Futuyma 1990). Frequencies published so far can approach the 1% level (Henke et al. 1993) and, in selected systems like MS1 even reach the 5% level (Jeffreys et al. 1988). The available publications have been dealing with new mutations in RFLPs. This paper will deal with microsatellite new mutations and their molecular structures.

Materials and methods

The new mutations have been observed in paternity analysis. Paternity analysis performed in this laboratory usually applies a package of 16–18 classical marker systems in a first approach (Table 1). A supplementary DNA analysis is recommended if there is only an isolated or questionable exclusion in the first approach or if the probability values of paternity are too small (below 99.9%). – Usually 4–6 DNA microsatellite systems are applied, but their number can be increased if necessary. The PCR protocols and the conditions for electrophoretic separation have been published elsewhere (D1S80, ApoB, YNZ22 according to Rand et al. 1992; HumACTBP2, HumTHO1 according to Wiegand et al. 1993; HumFABP, HumVWA, HumMBP according to Möller et al. 1994a; HumFES/FPS, HumD21S11 according to Möller et al. 1994b; HumF13A1 according to Puers et al. (1994). Sequence data were obtained as described by Möller and Brinkmann (1994) for HumACTBP2 and Möller et al. (1994b) for HumVWA.

Results

Case 1

The classical approach (16 systems) showed no exclusion but the combined W-value was only 84.2%. Inclusion of 6 additional PCR-VNTRs showed no exclusion with the exception of ACTBP2 (Table 1), which showed a mismatch between pf (putative father) and ch (child). Assuming genetic new mutation the child showed a 1 repeat deletion

Table 1 Summary of the main results in 4 paternity cases. (EM = Essen-Möller, m = mother, ch = child, pf = putative father) The repeat structure of the incompatible STR systems ACTBP2 and VWA was determined by Taq Cycle Sequencing as described by Möller and Brinkmann (1994) and Möller et al. (1994b). For ACTBP2 the allele designation is arbitrary; the number of repeats is given in brackets (Möller and Brinkmann 1994). The allele designation for VWA is according to the repeat number. Incompatible alleles are indicated with an asterisk. Classical systems used: ABO, MNSs, Rhesus, Kell, Fy, Jk, Gm, Km, Hp, Gc-sub, C3, Tf, Pi, EAP, PGM-sub, GPT, EsD,GLO.

Case	1	2	3	4
Type of paternity	Triplet	Triplet	Duo	Triplet
No. of class. system	16	18	16	17
EM	9.272	5.0792	8.2507	4.9542
DNA (compatible syst.)	D1S80, ApoB, YNZ22, TH01, VWA	D1S80, ApoB, YNZ22, TH01, VWA, FABP, F13A1, D21S11, FES	D1S80, ApoB, TH01, VWA	D1S80, ApoB, YNZ22, TH01, ACTBP2, MBP, FABP, F13A1, FES, D21S11
DNA (incompatible syst.)	ACTBP2: m = 15, 19 ch = 15, 6 (18*) pf = 18, 7 (19*)	ACTBP2: m = 3, 23 ch = 3, 9 (21*) pf = 15, 8 (20*)	ACTBP2: m = (not available) ch = 17, 4 (16*) pf = 26, 5 (17*)	VWA: m = 18, 19 ch = 18, 20* pf = 14, 19*
total EM	7.3612	1.0001	6.7095	4.6021

relative to the putative father. If ACTBP2 was omitted the combination of all other systems led to a combined probability of paternity of W = 99.997%. The inclusion of the ACTBP2 phenotypes assuming a new mutation rate of 1% and r = 0.1 (Henke et al. 1993) led to a combined probability of paternity of W = 99.6158%.

Case 2

The classical approach (18 systems) showed no exclusion and the combined W-value (= probability of paternity) was W = 99.9988%. The court nevertheless asked for an extended investigation and we applied 10 DNA systems (Table 1):

The phenotypes of the triplet matched with only 1 exception i.e. HumACTBP2 (Table 1, Fig. 1a). Assuming genetic new mutation, the child showed a 1-repeat (= 4 bp) insertion relative to the putative father. Inclusion of all systems without HumACTBP2 led to a combined W = 99.999999999% and the inclusion of ACTBP2 assuming a new mutation frequency of $\mu = 0.007$; (r = 0.1) (Henke et al. 1993) led to a combined value of 99.9999999%.

Case 3

This was a deficiency case with only the putative father and the child. The classical approach (16 systems) showed no exclusion. Again, we were asked to apply DNA systems and 8 additional systems gave matches (while ACTBP2 indicated possible incompatibility). Sequencing data revealed (under the assumption of new mutation) a deletion of 1 repeat in 1 allele of the child. – The statistical values were: W = 99.9994% without ACTBP2, W = 99.9229% with the inclusion of ACTBP2.

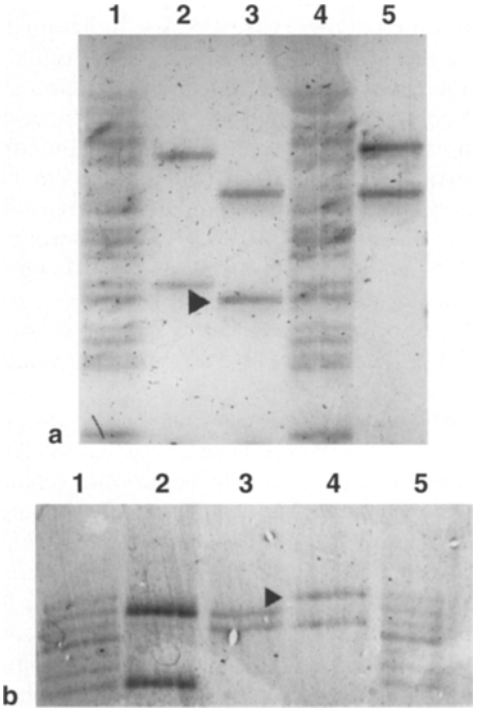

Fig. 1 a, b Example of a new mutation in the **a** HumACTBP2 system: 1,4 = allelic ladder (Wiegand et al. 1993); 2 = putative father; 3 = child (1-repeat deletion is indicated with an arrow); 5 = mother and **b** HumVWA system: 1,5 = allelic ladder (nomenclature according to the number of repeats – alleles 14–20; Möller et al. 1994b); 2 = putative father, 3 = mother, 4 = child (1-repeat insertion is indicated with an arrow)

Case 4

The classical approach (17 systems) showed no exclusion and the combined value was W = 99.999%. Again, we had to apply STR analysis with 8 STR systems which showed compatible phenotypes with the exception of HumVWA

(HumVWA – Fig. 1b). Sequencing data showed (under the assumption of new mutation) an insertion of 1 repeat in the child relative to the putative father. The combined W-value (considering VWA) was 99.9993% ($\mu = 0.002$, $r = 0.34$).

Discussion

So far, we have investigated 453 meioses in ACTBP2 and 484 meioses in VWA. Assuming new mutations in the above mentioned cases the incidence would be 0.7% (ACTBP2) and 0.2% (VWA). Together with another new mutation case (MBP, Möller et al. 1994a), the overall incidence seems to be quite low. A total of 2193 meioses in 7 STR systems have been investigated and no further deviations from Mendelian expectations were observed. Comparison of these results with those obtained from RFLP analysis indicates that RFLPs are much more associated with new mutations than the microsatellites investigated so far (Henke et al. 1993). This is in accordance with previously published hypotheses that had predicted an association between the number of repeats and the incidence of new mutations (Jeffreys et al. 1988). Even within the 2 systems investigated in this paper, ACTBP2 with more than 30 alleles shows a 3-fold higher (0.7%) rate of new mutation than VWA with 10 alleles (0.2%). Furthermore, we have sequenced a multiplicity of alleles in different STRs and could subdivide these into different categories relative to the extent of their sequence micro-heterogeneity within the repeat region. There were 2 systems with extensive structural variation, i.e. ACTBP2 and D21S11, systems with intermediate variation such as VWA and systems with very little microheterogeneity e.g. THO1. Consequently, the new mutations observed here are associated with a high level or at least an intermediate level of micro-variation. The number of observations is too small to draw any further conclusions, but 3 further points might be interesting in the future: (1) genetic new mutations observed so far are either insertions or deletions of 1 repeat, (2) deletions and insertions are so far in balance, (3) so far, no maternal new mutations have been observed which could again lead to an excess of paternal new mutations, which have also been observed in RFLP analysis (Henke et al. 1993).

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