MUTATIONS OF D2S44 AND D4S139 ALLELES AND PRESENCE OF TWO-FRAGMENT ALLELES FOR D4S139

A. Vandenberghe, N. Mommers, I. Peeters, M. Vandenbroeck and L. Muylle.

Department of Biochemistry, University of Antwerp, B-2610 Wilrijk, Belgium, Blood Transfusion Center, B-2650 Edegem, Belgium, and Faculty of Pharmacy, University Claude Bernard, F-69373 Lyon CEDEX 08.

INTRODUCTION

Short DNA sequences with a variable number of tandem repeats (VNTR) are highly polymorphic and informative and are frequently applied to solve a number of genetic problems. A major condition for using these probes in paternity testing is the absence of mutations upon transmission which could be interpreted as false negatives. We studied the transmission of alleles from three VNTR probes frequently applied in paternity testing: YNH24 (D2S44), pH30 (D4S139) and MS43A (D12S11). Mutations, albeit at low frequency, were found with the first two. In some cases, interpretation of the D4S139 locus becomes more complex due to the presence of an additional RFLP and of methylation events.

MATERIALS AND METHODS

DNA was digested with the restriction enzyme *Hinf*I following the instructions and using the solutions of the manufacturer (Boehringer, Mannheim, Germany). Separation, transfer onto nylon membranes and hybridization with radiolabelled DNA probes was done according to routinely applied laboratory procedures. Manually measured migration distances were plotted on a standard curve obtained with Analytical Marker DNA Wide Range (Promega corp., Madison, WI). Values were rounded off to the closest 100pb, 50pb was rounded up to the higher value.

RESULTS AND DISCUSSION

Mutations

No mutations for MS43A were found. Smith et al.(1990) described 2 mutations during the transmission of 298 alleles while others did not find mutations for this probe when analyzing 688 gametes (Jeffreys et al., 1988). We observed 2 mutations for YNH24 and 4 for pH30 (Figures 1a, 1b and Table 1). No allelic association of the mutation was observed (Table 2) and the sex of the receiving offspring seems not to be involved (3 boys and 3 girls). The age of the parent does not seem to play any role: the mean age of the fathers of the mutated children at birth was 29.6 year, while for 100 controls this was 31.3 years. Only expansions were observed. Expansions are also seen in a growing number of pathologies (reviewed by Wieringa, 1994). These mutations involve the expansion of the number of repeats of a triplet sequence. Although VNTR core sequences are longer (31 for YNH24, 32 for pH30, 45 for MS43A), an analogous mutation mechanism cannot be excluded. Recently the involvement of a certain genetic environment predisposing to the instability of

triplet repeats (Neville et al.1994) or VNTRs (Jeffreys et al. 1994) was suggested. Weber and Wong (1993) reported mutations of short tandem repeats (STRs) and observed a preference for increasing over decreasing (31:9). Similar results were reported by Vergnaud et al. (1991) (30:12) for a new VNTR locus. Although STRs have shorter repeat sequences than VNTRs, the majority of studies conclude to absence of unequal recombination between homologous chromosomes but favours rather polymerase or strand slippage (Jeffreys et al. 1990;) or even more complex reactions (Jeffreys et al. 1994) as the mechanism of choice.

One of the mutations is from maternal origin while the others all are from paternal origin. The parental origin of the mutation was derived considering the parental allele most similar in size to the mutant allele. Another maternal mutation for YNH24 was reported by Endean (1989) but a superior mutation rate for paternal VNTR alleles was reported by Jeffreys et al. (1990) (15:5), Vergnaud et al. (1991) (51:1) and Nürnberg et al.(1989) (7:1) and for STR alleles by Weber and Wong (1993) (15:4). It remains to be proven wether the higher male bias in the generation of new alleles is simply the reflection of a larger number of cell divisions during spermatogenesis.

The pH30 complex polymorphism

In 10 unrelated persons from the 782 examined, a third fragment was observed with probe pH30 (Figure 1c) including one case with 4 fragments (see use of *Hinf*I). The other VNTR probes did not detect such complex patterns. From 8 persons this third fragment was transmitted to their offspring. The occurrence of an additional fragment for pH30 has been described by Waye and Fourney (1990) for genomic DNA digested with the restriction enzyme *Hae*III in about 1% of 547 individuals. They conclude to the occurrence of a VNTR polymorphism on both chromosomes with, in addition, a bi-allelic RFLP on one of the two chromosomes. We here conclude to the presence of an internal additional *Hinf*I site. This site should be situated in the repeat sequence since no unique fragment length was observed for persons with three fragments (see Table 3).

By observing the transmission, the chromosome carrying the VNTR in combination with the biallelic polymorphism could be identified. Since all fragments show distinct fragment sizes, we can reasonably assume the presence of linkage equilibrium between the VNTR and the RFLP. However the RFLP is clearly associated with longer VNTR fragment lengths (allele frequencies of D4S139 show a maximal value around 6 kb for DNA hydrolyzed with *Hinf*I).

Use of the restriction enzyme *Hinf*I

An unusual observation was made with a 2 fragment allele of D4S139 (pH30) (Fig. 1d), where a weak fourth fragment is present in the mother and in the child profile. The *Hinf*I recognition site contains a cytosine residue in the 3'position that can be followed by a guanine residue. Methylation can reduce the activity of the enzyme (Nelson and McClelland, 1987) and produce partial digestion patterns. An almost identical observation was made earlier by Budowle et al. (1990). Since

the additional fragment is also transmitted to the child, we suppose that one of the *HinfI* sites present in this family contains a methylable CpG sequence and that partial digestion occurred. Since the length of the third fragment (12.5 kb) is not the sum of the two other fragments (11.3 and 6.6 kb), the methylable CpG should not be part of the additional internal restriction site but should belong to one of the sites at the extremities of the detected region. Although not really bothering the interpretation for paternity testing, the phenomenon of methylation and partial digestion is disavantageous for the comparison of forensic samples.

REFERENCES

Budowle B, Waye JS, Shutler GG, Baechtel FS (1990) *Hae*III -a suitable restriction endonuclease for restriction fragment length polymorphism analysis of biological evidence samples. J Forens Sci 35:530-536.

Endean DJ (1989) RFLP analysis for paternity testing: observations and caveats. In: Proceedings of the International Symposium on Human Identification, Promega Corp., Madison, pp. 55-69.

Jeffreys AJ, Royle NJ, Wilson V, Wong Z (1988) Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature 332:278-281.

Jeffreys AJ, Neumann R, Wilson V (1990) Repeat unit sequence variation in minisatellites: a novel source of DNA polymorphism for studying variation and mutation by single molecule analysis. Cell 60:473-485.

Jeffreys AJ, Tamaki K, MacLeod A, Monckton DG, Neil DL, Armour JAL (1994) Complex gene conversions events in germline mutation at human minisatellites. Nature Genet 6:136-145.

Nelson M, McClelland M (1987) The effect of site-specific methylation on restriction modification enzymes. Nucl Acids Res 15:r219-r230.

Neville CE, Mahadevan MS, Barceló MS, Korneluk RG (1994) High resolution genetic analysis suggests one ancestral predisposing haplotype for the origin of the myotonic dystrophy mutation. Hum Mol Genet 3:45-51.

Nürnberg P, Roewer L, Neitzel H, Pöpperl A, Hundrieser J, Pöche H, Epplen C, Zischler H, Epplen JT (1989) DNA fingerprinting with oligonucleotide probe $(CAC)_5/(CTG)_5$: somatic stability and germline mutations. Hum Genet 84:75-78.

Smith JC, Anwar R, Riley J, Jenner D, Markham AF, Jeffreys AJ (1990) Highly polymorphic minisatellite sequences: allele frequencies and mutation rates for five locus-specific probes in a Caucasian population. J Forens Sci Soc 30:3-18.

Vergnaud G, Mariat D, Apiou F, Aurias A, Lathrop M, Lauthier V (1991) The use of synthetic tandem repeats to isolate new VNTR loci: cloning of a human hypermutable sequence. Genomics 11:135-144.

Waye JS, Fourney RM (1990) Identification of complex DNA polymorphisms based on variable number of tandem repeats (VNTR) and restriction site polymorphism. Hum Genet 84:223-227.

Weber J, Wong C (1993) Mutation of human short tandem repeats. Hum Mol Genet 2:1123-1128.

Wieringa B (1994) Myotonic dystrophy reviewed: back to the future. Hum Mol Genet 3:1-7.

Table 1. Observed mutation frequencies.

probe	# meioses	# mutations	frequency
YNH24	536	2	0.37 %
pH30	536	4	0.75 %
MS43A	517	0	-

Table 2. Origin (p:paternal; m:maternal) and lengths (kb) of mutant alleles.

probe	origin	transmitted alleles	mutant allele
YNH24	p	2.6 & 3.7	2.7
YNH24	m	5.9 & 3.5	3.7
pH30	p	9.7 & 4.8	5.0
pH30	p	10.0 & 5.4	10.1
pH30	p	11.5 & 4.7	11.9
pH30	p	7.1 & 5.1	5.2

Table 3. Length of the D4S139 fragments transmitted as a fragment-pair to the offspring.

lengths of the transmitted fragments (kb)		sum (kb)
14.3	2.8	17.1
8.5	1.8	10.3
10.1	5.1	15.2
12.7	4.6	17.3
10.7	4.2	14.9
11.3	6.6	17.9
4.9	3.5	8.4
4.0	3.3	7.3

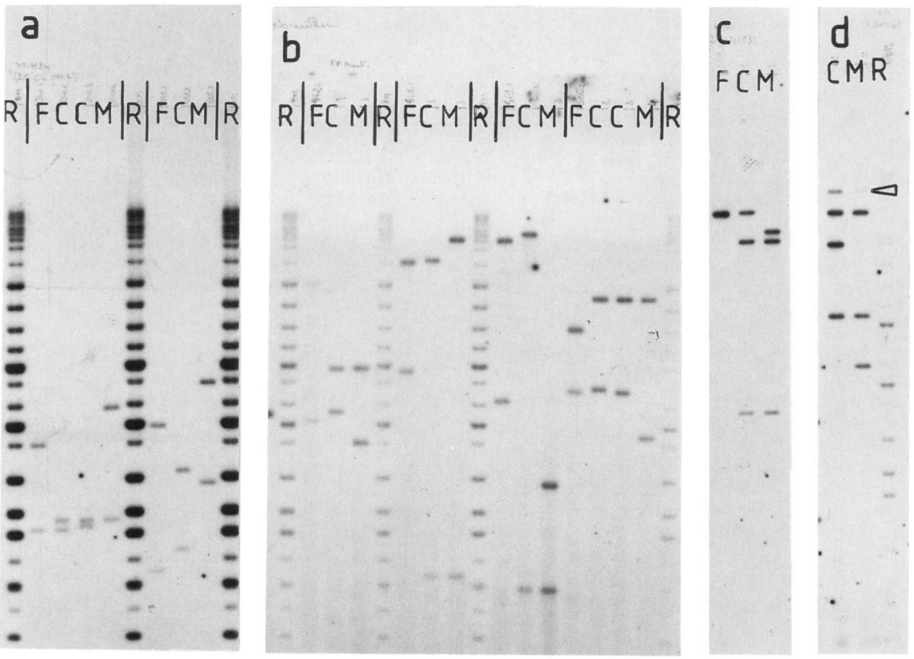

Figure 1.

Mutations found with probe YNH24 are shown in (a) and with pH30 in (b). With probe pH30, sometimes a third fragment is revealed (c) and is transmitted to the offspring. In one case a weak fourth fragment was observed (d, arrowhead) believed to result from methylation of a restriction site sequence. R, reference ladder; F, (alledged) father; C, child; M, mother.