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CASE REPORT

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Recent observations in human DNA-minisatellite mutations

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Abstract We report maternal and paternal mutation rates at loci D1S7 (MS1), D7S21 (MS31), D12S11 (MS43A), and D7S22 (G3). The respective mutation rates were as follows:

System	Maternal meioses	Paternal meioses
D1S7	4.3%	4.2%
D7S21	0.2%	1.2%
D12S11	0.05%	0.4%
D7S22	0.1%	0.8%

At loci D7S21, D12S11, and D7S22 statistically significant differences in mutation rates exist between the sexes. No such difference was observed at locus D1S7. However inspection of the latter data reveals that by mutation at spermiogenesis approximately two-thirds of the fragments showed an addition of repetitive units, while a 50:50 ratio was encountered in the series of maternal mutations. We also report the observation of naturally occurring 3-fragment patterns.

Key words DNA minisatellite loci · Mutations · Sex differences

Zusammenfassung Die Studie berichtet von den neuesten Daten zu Mutationsraten an den DNA-Minisatellit-Loci D1S7 (MS1), D7S21 (MS31), D12S11 (MS43A) und D7S22 (G3).

Folgende Beobachtungen wurden gemacht:

System	Maternelle Meiosen	Paternelle Meiosen
D1S7	4.3%	4.2%
D7S21	0.2%	1.2%
D12S11	0.05%	0.4%
D7S22	0.1%	0.8%

Der Vergleich der mütterlichen und väterlichen Mutationsraten zeigt, daß signifikante Unterschiede an den Loci D7S21, D12S11 und D7S22 vorliegen. Am Locus D1S7 konnte ein derartiger Unterschied nicht beobachtet werden, doch zeigte sich hier, daß (fast) 40 mutierte väterliche Fragmente größer geworden waren, während 20 Fragmente durch die Mutation verkleinert wurden.

Neben diesen Zahlen werden 2 Familien vorgestellt, in denen natürliche 3-Fragment-Muster beobachtet wurden.

Schlüsselwörter DNA-Minisatellitloci · Mutationen · Geschlechtsunterschiede

Introduction

Germline length mutations at human DNA loci have been encountered in human pedigrees [11, 17, 23, 27]. Various authors have shown that at some loci, paternal and maternal mutations arise with similar frequency, whereas at others, a strong male bias exists [11, 19, 21, 23, 29]. This has to be taken into consideration, especially in parentage testing. In previous papers we reported mutation rates at VNTR loci [10, 11]. Due to the increased number of observations, we are now able to update and to review earlier data and to describe the expansion of repeats in mutant paternal alleles at 2 loci. We further describe the natural occurrence of 3-band patterns.

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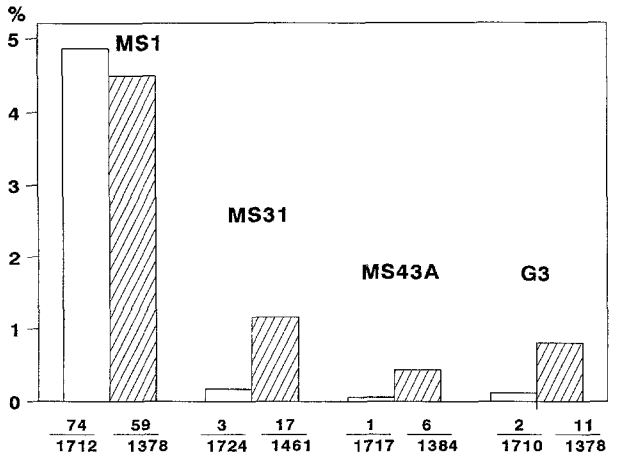


Fig. 1 Comparison of mutation rates in maternal (□) and paternal (▨) meioses. The total number of investigated meioses along with the observed mutation rates is illustrated

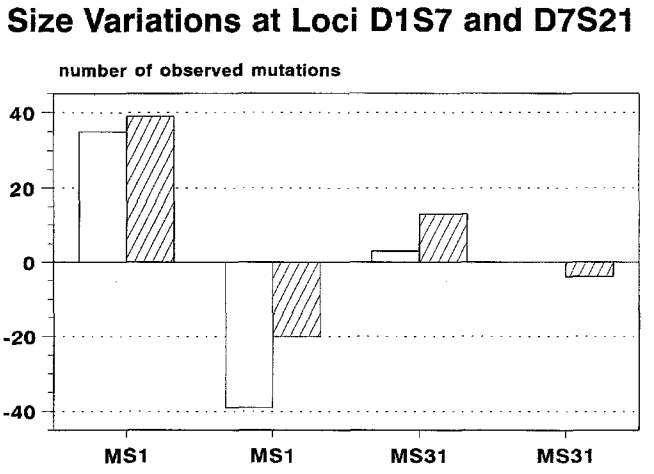


Fig. 2 Illustration of observed gains and losses in mutant alleles (□ = maternal meioses; ▨ = paternal meioses)

Methods

Parent/child pairs from consecutive paternity cases constituted the basis for this study. The kinship status was established by conventional hemogenetic tests (W > 99,8%, at a given a priori of 0.5). Non-paternity was concluded on the basis of at least 2 exclusions. Mutations were studied at loci D1S7, D7S21, D12S11, and D7S22. The numbers of maternal meioses ranged between 1710 and 1724, whereas at least 1378 paternal meioses could be investigated (Fig. 1).

Techniques for DNA isolation, endonuclease restriction, electrophoresis and hybridizations have been described elsewhere [10]. Membranes were hybridized consecutively with probes MS1, MS31, MS43A, G3, YNH24 and MS205 [22, 26, 32]. The size of fragments was measured independently on autoradiographs or lumigraphs by 2 staff members using a digitising tablet [9]. Fragments differing in mobility by 0.5 mm could be resolved. The "local reciprocal method" was used to estimate the fragment sizes.

Definition of a mutation at a DNA locus

If in serologically established families one single restriction fragment of a child could not be exactly attributed to either parent by visual comparison in side-by-side runs we declared this a mutation, regardless of whether the respective difference of size measurement was within the range of our standard deviation of 1.8% or not [9, 11]. The standard deviation is only used to calculate the "allele" frequency.

Results

Mutation rates in maternal and paternal meioses

Figure 1 shows the number of meioses investigated and respective mutation rates in both sexes. Since mutations involving changes of less than 100 bp cannot always be reliably resolved in electrophoresis systems, the true mutation rate, especially at locus D1S7, is presumably higher than reported here. At locus D1S7 mutations arise in both sexes with equal frequency. This result does not confirm the observation of Olaisen et al. [23], who reported different mutation rates for MS1 with a ratio exceeding 2:1 between the sexes. For the other loci (D7S21, D12S11 and D7S22) the inspection of data reveals a statistically significant difference in mutation rates between the sexes. The difference at locus D7S21 is highly significant at the 0.1% level, while the differences at loci D12S11 and D7S22 are significant at the 5%, and at the 1% level, respectively. Our findings at locus D7S21 compare favorably with those of Olaisen et al. [23] (4 mutations in paternal meioses versus 0 mutations in maternal ones).

Size of mutated fragments

We have also analysed the sizes of the mutated MS1 alleles with respect to the sizes of their progenitors.

By mutation at spermiogenesis, approximately two-thirds of the D1S7 fragments showed an addition of repetitive units, while approximately a 50:50 ratio was encountered in the series of maternal mutations. This sex difference is statistically significant at the 5% level. It should be mentioned that at locus D7S21 there is also a clear tendency towards a size gain in paternal mutations (Fig. 2).

Natural occurrence of 3-fragment patterns

Using probes MS1, MS31 and G3, individuals could be observed showing 3 fragments instead of either 2 or 1.

Two of these case studies are presented here:

a.) 3 fragments in mother and child at locus D7S21 (Fig. 3).
Using probe MS31 in combination with the enzyme Hinf I, a 3-fragment pattern was observed in a mother and her child, while other probe/enzyme combinations detected only the expected 2 fragments (data not shown).

b.) A child with 3 fragments at locus D1S7 (Fig. 4a, b)
Using probe MS1 and the enzyme Hinf I a 3-fragment

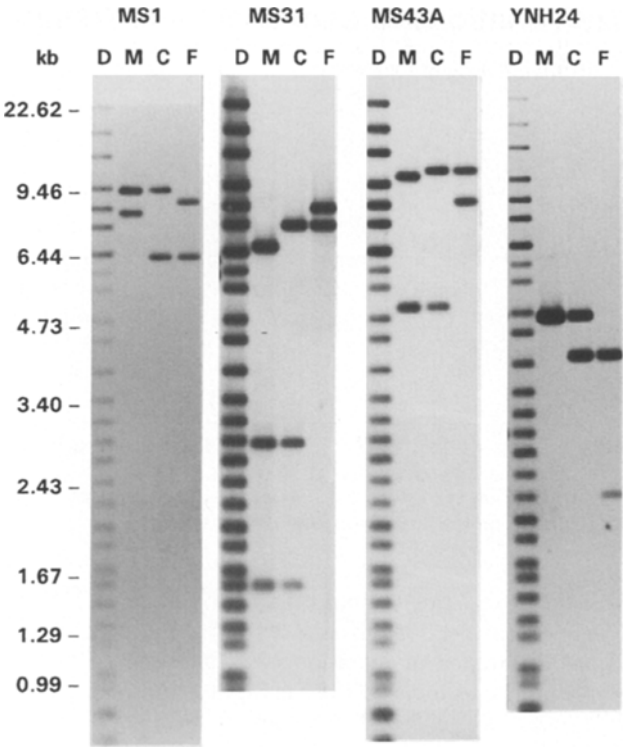


Fig. 3 Mother (M) and child (C) revealing a 3-band pattern in the Hinf I/MS31 combination. F = Father

Fig. 4 **a** Child (C) reveals a 3-band pattern in the Hinf I/MS1 combination. The fragment at approx. 7.3 kb cannot be attributed to either parent. **b** Digestion experiments using the DNA of the child (C in **a**) a = HinfI, b = MboI, c = AluI, d = HaeIII, e = DdeI All MS1 probed samples reveal a 3-band pattern. The combination Hae III/MS31 also shows a 3-band pattern. No fragments are detected in the Hae III/MS43A combination

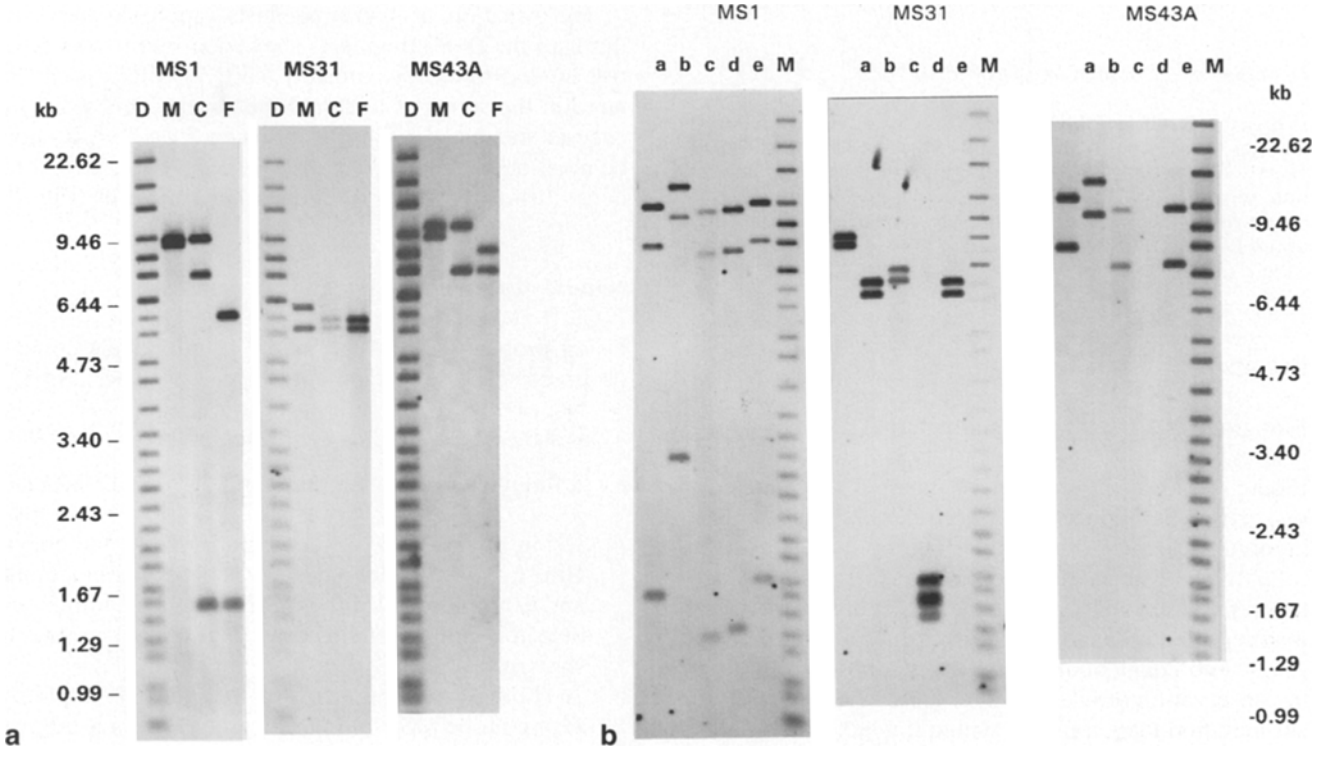
pattern could be observed in a child. One of these fragments could not be attributed to either parent. DNA from this child was additionally digested with enzymes MboI, AluI, HaeIII, and DdeI. All digested samples showed a 3-fragment pattern with probe MS1.

The same samples were also probed with MS31. Here, only 2 fragments were detected, except in the combination HaeIII/MS31, where a 3-fragment pattern also appeared. Using probe MS43A, only 2 fragments could be detected. As expected, no fragments could be seen in the HaeIII digested sample, which is obviously due to the very small size of the fragments.

Discussion

Different mutation rates in maternal and paternal meioses.

It is well known [10, 11, 23, 27] that probes MS31, G3 and MS43A detect low-mutant loci, while the MS1 mutation rate is substantially higher. The observed minisatellite mutation frequencies are in accordance with published findings [23, 27]. Our data however, clearly show higher mutation rates in paternal meioses in comparison with maternal ones at loci D7S21, D12S11, and D7S22. While our observations at locus D7S21 are fairly consistent with those of Olaisen and co-workers, our findings at locus D1S7 clearly differ from the results of the Norwegian study. The reason for this particular difference is unclear.



Size of mutated fragments

Olaisen and co-workers [23] were able to demonstrate a tendency towards fragment size increase at mutation. Jeffreys and co-workers [19] reported a major bias (75%) towards gains in repeats rather than losses, when they investigated sperm mutations at locus D1S8. Our observations at locus D1S7 show that the number of mutant alleles with increased repeat sizes is almost twice as high (39) as the number of alleles with size losses (20). This, however is only true for the paternal meioses, and not for the maternal ones, where such differences were not seen (35:39). At locus D7S21 there might be a simular bias. It is now clear that the bias renders previous computer modelling of minisatellite evolution invalid [7, 8], based on the assumption that repeats are gained or lost with equal frequency.

Individuals with naturally occurring 3 fragment patterns

Our interpretation of the findings in the first case is that the mother has transmitted 2 fragments to her child, indicating that the respective chromosome has an internal Hinf I restriction site at locus D7S21.

The case of the second family is not that simple. At present one can only speculate on the genetic background of these complex observations although there are some similarities with earlier findings [23, 31]. Further studies by means of single molecule analysis [18] should be carried out.

Instability of minisatellite repeats

Mutations are obviously due to a number of different mechanisms. However, the issue which major factor is involved in de novo length changes is still incompletely understood [4, 5, 14, 16, 28, 31]. Minisatellite mutations are not restricted to the germline, but may also occur somatically [1, 8, 31], indicating that any tissue will contain a majority of cells with 2 progenitor minisatellite alleles, plus a certain amount of cells containing mutated length alleles. Because the proportion of cells possessing mutated alleles is significant, the heterogeneity in new alleles may prevent their detection by Southern blot analysis [5]. This however, is not true if a mutation occurs in a very early stem cell lineage, which will create a tissue either mosaic for original non-mutant cells plus cells descended from the same mutant progenitor cell (leading to a tissue with 3 alleles) or tissue homogeneously composed of mutant cells. Such a process could result in the divergence of single locus profiles between different tissues from the same individual. To our knowledge only one example of an early stem cell mutation in man has been described (Jeffreys, Patel and L. Henke, unpublished data, quoted in 19), although very low level mosaicism can be routinely detected in blood and sperm DNA [8]. Hundrieser et al. [12] observed an apparent somatic recombination in a monozygotic twin. This report however, was revoked later when it turned out that one maternal allele was exclusively inherited by part of the peripheral blood cells of one twin.

It now appears likely that repeat sequence mutation is a common cause of human disease [3, 6, 13, 24, 33] and the following questions should be addressed. Which mechanisms cause mutations of repeat sequences? Why are some repeat loci unstable while others are not? Jeffreys et al. [19] have demonstrated that the mechanics of change in repeat copy number is not a simple process and may differ from one repeat to another. Sperm mutations at locus D1S8 (MS32) show a consistent bias towards gains of repeats [19]. There is evidence for a similar bias at other loci. These observations deserve further attention, because it is obvious that mutations provide a basis for evolutionary expansion of VNTR fragments [14, 19, 20]. The range of structures described in mutant minisatellite alleles is surprisingly complex [19, 25]. Analyses of polymorphic sites flanking the minisatellite repeat array have failed to reveal exchange of flanking markers predicted for the products of unequal exchange between alleles [29–31], suggesting that processes such as replication slippage and unequal exchange may be the major reasons for mutations [7]. It is now clear, that the majority of mutants have not arisen by simple (and non-polar) intra-allelic reduplication/deletion events [2, 19].

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Note added in proof Most recently Holmlund et al. (Forens Sci Intl 66:105–109; 1994) published an article, that reports the observation of an MS43A allele with an internal *Hinf*I restriction site. It appears worthwhile to mention, that we have also seen such kind of event while this article was in press.

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