Int J Legal Med (1999) 112 : 192–194

ORIGINAL ARTICLE

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Mutation of the repeat number of the HPRTB locus and structure of rare intermediate alleles

Received: 22 December 1997 / Received in revised form: 27 April 1998

Abstract During routine paternity testing a mutation of a paternal allele at the HPRTB locus was observed. The opportunity was taken to analyse this mutation at a molecular level. The repeat sequence is flanked by an imperfect repeat sequence and this region could be involved in the mutation mechanism. For this reason, we also examined the structure of "intermediate" alleles. Sequencing confirmed the insertion of a perfect repeat motif and revealed a deletion of a dinucleotide some 50 nucleotides downstream from the repeat sequence for the intermediate alleles. It is likely that these intermediate alleles are rare biallelic deletion polymorphisms and are probably not involved in the mutation or variation mechanism of this locus.

Key words Short tandem repeat (STR) · Mutation · DNA analysis · Alleles · Paternity testing

Introduction

The triple triplex STR system described by Alford et al. (1994) was validated for forensic testing in the Flemish population by Mertens et al. (1997). During routine paternity analysis using these nine STR loci, a mutation was observed at the HPRTB locus. As new mutations could contain clues to the understanding of the mutation or variation mechanism, we undertook molecular analysis of this locus.

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Materials and methods

Primer sequences and PCR amplification conditions of HPRTB alleles were as described before (Alford et al. 1994) and PCR products were visualised by silver staining. Products cloned in pGEM-T (Promega Corp. Madison, Wis.) were size selected by electrophoresis on an agarose gel and sequenced using the ABI Prism dye terminator cycle-sequencing ready reaction kit and an ABI Prism model A 310 apparatus (Perkin Elmer Applied Biosystems, Foster City, Calif.). Typing for the HLA DRB1, DQB1 and DPB1 loci was performed using a reverse dot blot system (INNO-LiPA, Innogenetics, Zwijndrecht, Belgium). An AMP-FLP kit (Lifecodes Co, Stamford, Conn.) was used to type the VNTR loci D1S80 and D17S5.

Results and discussion

In order to legally confirm biological paternity in a case of a natural child, alleles for nine loci (Alford et al. 1994) from both parents and their daughter were genotyped. Of these, eight loci gave concordant results except for the X-linked HPRTB locus where the mother was homozygous for allele 12, the father had a 13 repeat allele and the daughter inherited a 12 and 14 repeat allele. When the results of the HPRTB locus were not taken into account, the probability of inclusion was calculated to be W = 99.951%. Further evidence for inclusion was obtained from typing of HLA DRB1, DPB1 and DQB1, and of AMPFLPs D17S5 and D1S80. The total probability of inclusion at this point was W = 99.997%.

Inserts of cloned alleles were obtained by digestion with restriction enzymes PstI and ApaI followed by separation on 1.5% agarose gels by electrophoresis. This method enabled distinction between the longer and the shorter alleles of the daughter. Sequencing of the HPRTB alleles 12, 13 and 14 was undertaken and the sequences were compared with the compiled sequence (Genbank #M26434; Fig. 1a). The presence of 12, 13 and 14 repetitions of the repeat motif ATCT was confirmed and no differences in the flanking sequences were observed. The HPRTB STR contains, on the 5′ side of the repeat, a number of imperfect repeats (Fig. 1b) where three repeat mo-

a)

22798-**ctctccagaatagttagatgtaggt**ataccactttgatgttgacactagttta

cctagaacttatcttctgtaaatctgtctctatttccatctctgtctccatctttGTCTC

TATCTCTATCTGTCTATCTCT**ATCTATCTATCTATCTATC**

TATCTATCTATCTATCTATCTATCTATCTaaagcaaattcatgcccttct

cctatttattgaatcgagaccatagac***AG***gggtgagagaaagaatttggc

aggaat**gggg atgtgtatta tctgtggcat**-23080

b) ---GTCTCTATCTCTATCTGTCTATCTCT[ATCT]$_{12}$---

c) ---[NT]$_{13}$[ATCT]$_{12}$--- with N = G, A, or C

Fig. 1 Sequence organization at the HPRTB locus. Primer sequences are indicated in small characters in bold (**a**). The position of the sequence corresponds to the numbering in Genbank, access number M26434. The perfect repeat, indicated in bold capitals and containig 12 repetitions ATCT, is preceded by a region (in capitals) containing the repeat motif (underscored) but interrupted. The deletion of the dinucleotide AG downstream from the repeat and found in alleles 12.-2 and 13.-2 is indicated in bold italic capitals. Each second position of the imperfect repeat (and of the perfect repeat) is occupied by a T-residue (**b**, **c**)

tifs (underscored) are interrupted by a CT or by a GTCT motif. Taken together, each second position in the perfect and imperfect repeat is occupied by a T-residue (Fig. 1c). So-called intermediate alleles have been found (Hammond et al. 1994; Mertens et al. 1997) to differ by two nucleotides in length as estimated on a gel. In order to ascertain whether these intermediate alleles are due to changes in the repeat region, an 11A and a 12A allele were sequenced. A deletion of a dinucleotide AG, 50 nucleotides downstream from the repeat region, was found in both cases (Fig. 1a).

Very little is known about mutation mechanisms, but mutational behaviour is probably locus dependent. As pointed out before (Edwards et al. 1991) the sequence of the repeat unit does not seem to be the primary factor of the polymorphism and thus of the mutation mechanism. The majority of studies exclude recombination between homologous chromosomes, favouring polymerase or strand slippage (Jeffreys et al. 1990; Kayser et al. 1997) or even more complex mechanisms (Jeffreys et al. 1994).

As observed in RFLP (Henke and Henke 1995) and other STR systems (Brinkmann et al. 1995; Klintschar and Neuhuber 1998), repeat mutations are often of paternal origin, correlating with the fact that at least 10 times more cell divisions occur between the zygote and sperm than between the zygote and ovum (Vogel and Rathenberg 1975; Crow 1993). This case also illustrates that mutations tend to generate larger alleles (Weber and Wong 1993). However, mutations of maternal origin and reductions in length have also been reported (Jeffreys et al. 1988). It is important to note that mutation mechanisms can be sex-dependent as was recently observed during the formation of disease-related deletions and duplications (Lopes et al. 1997).

Furthermore, mutational behaviour is also locus-dependent and correlates with a genetic environment predisposing to the instability of triplet repeats (Neville et al. 1994) or VNTRs (Jeffreys et al. 1994). It has also been reported (Edwards et al. 1991), that the sequence of the repeat unit does not seem to be the primary factor of the polymorphism and thus of the mutation mechanism. Tandem reiteration, regardless of the repeat sequence, probably induces variation but is not the exclusive factor. Another factor could be the sequence surrounding the repeat. For HPRTB, the repeat is flanked on the 5′-side by an apparently related region containing imperfect repeats. Some loci also possess such a region, while others do not. For instance, F13A01 (Genbank #M21986, J03834) has a repeat structure aaagatagaaagat[aaag]$_n$ and LIPOL (Genbank #D83550) has a structure attatttt[attt]$_n$attttt. It remains to be proven if this difference in flanking sequence structure is associated with different mutation mechanisms. The occurrence of a de novo mutation gave us the opportunity to screen the involved paternal allele for sequence differences that might be involved in the mutation mechanism.

Comparison of the daughter and father sequences showed the addition of a perfect repeat unit to the tandem repeat sequences. No differences in adjacent sequences were observed when compared with other alleles or with the compiled sequence in Genbank. The sequence organization of the STR HPRTB locus is noteworthy: a region containing some imperfect repeats immediately flanks the perfect repeat sequence. This imperfect repetition sequence has a T-nucleotide at every second position and could be suggestive of the involvement of a dinucleotide of this sequence in the mutation mechanism. We earlier described two “intermediate” alleles for HPRTB indicated as 11A and 12A (Mertens et al. 1997). Estimation of the length by comparing electrophoretic migration profiles, suggested a difference of 2 nucleotides with the frequent alleles. At this point, we raised the question whether the generation of intermediate alleles involved a dinucleotide in the imperfect repeat region reflecting instability of this region. Sequencing revealed that intermediate alleles were due to a deletion some 50 nucleotides away from the repeat sequence. The 11A allele has the structure of a 12-allele with a deletion of a dinucleotide AG and 12A is a 13-allele with the same deletion in the same position. Consequently, their correct nomenclature should be 12.-2 and 13.-2 (Bär et al. 1997). It is tentative to conclude that intermediate alleles for both loci are not involved in the mutation mechanism but are rather rare biallelic deletion polymorphisms. Alleles 12.-2 and 13.-2 are associated with the two most frequent alleles of this STR i.e. alleles 12 and 13 (Mertens et al. 1997) and it is probable that intermediate alleles associated with other alleles will be found when more chromosomes are typed. The recent finding in a Hungarian population study (Füredi et al. 1996) of two such alleles, designated 10M and 11M, supports our hypothesis.

Acknowledgements We thank Susan Gamon for helping with the preparation of the manuscript and Sandrine Radawieck for technical support.

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