What is expanded in progressive myoclonus epilepsy?

It has been discovered that the gene for cystatin B (*CSTB*) on chromosome 21q22.3 is responsible for progressive myoclonus epilepsy of Unverricht-Lundborg type (EPM1; ref. 1). Comprehensive mutation analyses failed to identify most of the mutations in unrelated patients[2,3], and it was suggested that a common mutation might be an unstable sequence in the 5′ region of the gene[3]. Recently, it was shown that the common mutation mechanism in patients from various geographic and ethnic origins appears to be an expansion of a repeated DNA sequence in the 5′ flanking region of *CSTB*[4,5]. However, the exact nature of the repeated sequence is controversial; it is either an expansion of novel 18- and 15-mer minisatellites[4] or an expansion of a polymorphic dodecamer repeat CCCCGCCCCGCG[5]. The resolution of the nature of the repeat has important implications for the mechanism of this expansion in EPM1 (ref. 6).

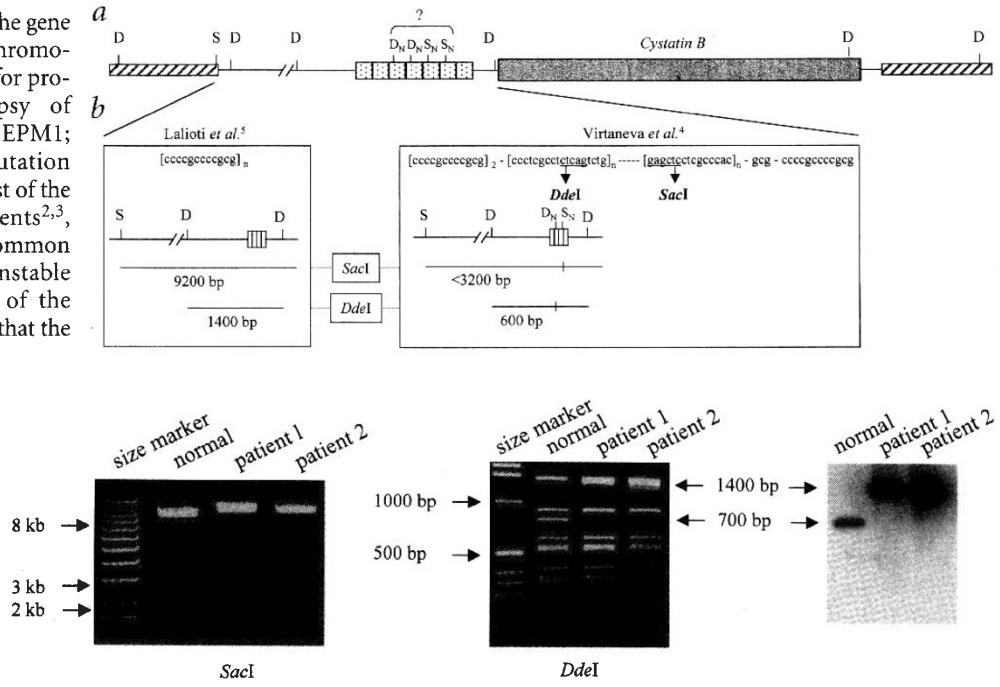

Fig. 1 ***a***, A schematic representation of the clones containing the expanded *CSTB* alleles (not to scale). The vector-derived sequences are cross-hatched, *CSTB* is grey, and the repeats are represented as boxes. *Sac*I (S) and *Dde*I (D) restriction sites are shown above. D_N and S_N represent potential restriction sites that would be present in the *de novo* 15- and 18-mer minisatellite sequences of Virtaneva *et al.*[4] ***b***, Expected fragments produced by digestion of two different mutant clones, both contain expanded repeats. If the 15-mer were present, it would introduce multiple *Sac*I sites into the insert—producing two fragments, one smaller than 3.2 kb and the other approximately 6 kb. If only the dodecamer were present, *Sac*I would cut only once in the vector linearizing the plasmid as shown in ***c***. If the 18-mer were present, *Dde*I digestion would produce a diagnostic fragment of 600 bp in addition to fragments from the insert and vector; if it were not present, a larger band of 1,400 bp would be expected as detected in *c*. *Sac*I and *Dde*I digestion of normal and mutant clones. Note the absence of the 3.2-kb (for *Sac*I) and 600-bp (for *Dde*I) fragments, which would be diagnostic for the minisatellites of Virtaneva *et al.*[4]. For the *Dde*I digestion, Southern blotting and hybridization with an oligonucleotide containing two dodecamer repeats verified that the 700-bp normal and 1,400-bp mutant bands (indicated by the arrows) contained the repeats.

If the expanded sequence consisted of the reported 15- and 18-mers, restriction enzymes *Sac*I and *Dde*I should cleave the expanded DNA. Thus, we have digested two patient-derived CSTB genomic clones containing the larger mutant EPM1 alleles. This experiment clearly shows that the proposed minisatellite sequences reported by Virtaneva and colleagues[4] are not present in our clones (Fig. 1), as these two enzymes failed to cleave the 'expanded' DNA. The conclusions of Virtaneva *et al.* may have been drawn from the sequencing gels presented in their article (and possibly others), and we propose that they have interpreted cross-bands as real nucleotides (Fig. 2).

18-mer	T T T A T T
12-mer	CxCCxCGCCxCxCxGxCxG
15-mer	T T A A

Fig. 2 A comparison of the dodecamer repeat expanded in EPM1 with the microsatellite repeats reported by Virtaneva *et al.*[4]. An 'x' has been inserted into the sequence of the dodecamer to allow for the alignment of the sequences. The additional bases in the reported 18-mer and 15-mer, which result from cross-banding, are shown above and below, respectively.

We conclude that the common mutation mechanism in EPM1 is the expansion of the dodecamer repeat, not the expansion of *de novo* 15- or 18-mer minisatelites. This implies that the EPM1 expansion alleles were probably a result of replication errors, as hypothesized for the trinucleotide repeat disorders[6], and not produced by recombination, as for minisatellites[7].

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