

Are (CTG)_n expansions at the SCA8 locus rare polymorphisms?

Translated (CAG)_n repeat expansions are responsible for five autosomal dominant cerebellar ataxias¹ (ADCA). Recently Koob *et al.*² described an untranslated (CTG)_n repeat expansion in the spinocerebellar ataxia 8 (SCA8) gene in patients with ADCA or recessive cerebellar ataxia. PCR amplifications of the (CTG)_n repeat and an adjacent (CTA)_n repeat showed that normal alleles carry 16–91 combined repeats, whereas those in patients from a large ADCA kindred linked to this locus carry 110–130 combined repeats (107–127 pure CTGs). Potentially pathogenic alleles of 92 to 250 (CTA)_n/(CTG)_n repeats were, however, observed in ataxic patients, thus questioning the established SCA8 pathogenic threshold².

We studied 188 French controls with no family history of neurological disorders and 250 European index patients with different forms of ataxia (Table 1) for SCA8 expansions. Disease specificity was assessed by analysing patients with other central nervous system disorders. We determined (CTA)_n/(CTG)_n repeat sizes by PCR (ref. 2) using primers F3 and HEX-labelled R4, followed by electrophoresis and analysis on an ABI-Prism-377 automated sequencer. Alleles carrying 107 or more (CTA)_n/(CTG)_n repeats were amplified with primers F4 (ref. 2) and SCA8-804 (5′-GATTGC CTTCTGACTCCC-3′) with Pfu polymerase. Agarose-purified PCR products were then both sequenced directly and sub-cloned before sequencing at least two independent clones.

We observed a bimodal distribution of the (CTA)_n/(CTG)_n repeat numbers in ataxic patients: 487 chromosomes con-

tained 2–25 repeats and 13 chromosomes (11 patients, including 2 homozygotes) contained 68–123 repeats. We found expansions of more than 91 (CTA)_n/(CTG)_n repeats in 8 of 148 ADCA families, in an apparently sporadic ataxia patient, in a patient with neuropathologically confirmed Lafora disease and in a patient with familial essential tremor. Similarly, 99% of control alleles (n=373) carried 3–28 (CTA)_n/(CTG)_n repeats, except for 3 alleles with 107, 111 and 123 repeats from patients aged 57, 62 and 64 years, respectively (who were at least 20 years older than the mean age at onset reported for SCA8 patients²). This was unexpected, because Koob *et al.*² found no expansions of more than 91 (CTA)_n/(CTG)_n repeats on 1,200 control chromosomes. Large composite repeats exist, therefore, in a greater proportion of continental Europeans than in the control population used by Koob *et al.*².

We examined segregation in relatives of five of these patients, including four with more than 91 combined repeats. In contrast to alleles with up to 28 composite repeats, those carrying more than or equal to 68 repeats varied in size from –19 to +1 repeats in 8 of 9 transmissions. Disease and expansion did not co-segregate in two kindreds with ADCA (104 CTA/CTGs) and Lafora disease (92–111 CTA/CTGs). In addition, two patients were homozygous for large composite expansions (96/114 and 104/104), although the disease was apparently transmitted by only one parent. In an ADCA kindred (AAD-124, with 108 to 122 CTA/CTGs), expansions co-segregated with the disease in three patients.

We found by sequence analysis that some alleles with more than or equal to

107 (CTA)_n/(CTG)_n repeats presented 1–3 CCG or TTG interruptions in the CTG repeat tract, including family AAD-124, in which it was truncated by 3 independent CCGs. The largest pure CTG repeats were detected in a control individual (CTG₁₀₇), an ADCA patient (CTG₁₀₉) and an apparently sporadic ataxia patient (CTG₁₁₁). We also observed one CTG interruption in the (CTA)_n repeat of some alleles with 1–21 repeats.

We have shown that both CTA and CTG tracts are polymorphic, as already reported², and can also be interrupted. This reveals a further level of complexity and emphasizes the importance of sequencing large alleles. We found pure CTGs of more than or equal to 107 repeats in two patients with sporadic ataxia and ADCA, as well as in a 64-year-old healthy control. It is therefore possible that the disease in the large SCA8 kindred² might be caused by another mutation in, or closely linked to, the same gene. Indeed, large alleles of other polymorphic trinucleotide loci were found in controls of specific origins^{3–9}.

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Table 1 • Chromosomes with CTA/CTG and pure CTG repeats in patients and controls

Groups (number of index patients or controls)	Number of chromosomes with		
	2 to 92	93 to 123 CTA/CTG repeats <107 pure CTG	≥107 pure CTG
ADCA (n=148)	286	9*	1
isolated case with cerebellar ataxia (n=76)	151	0	1
autosomal recessive ataxia (n=26)	52	0	0
SCA1/3/7 (n=26)	52	0	0
other neurological disorders (n=46)	90	2	0
healthy controls (n=188)	373	2	1

ADCA, autosomal dominant cerebellar ataxias not caused by CAG repeats at the SCA1, 2, 3, 6 and 7 loci. *Includes two homozygotes.