The effect of CAT trinucleotide interruptions on the age at onset of spinocerebellar ataxia type 1 (SCA1)

Zenjiro Matsuyama, Yuishin Izumi, Masakuni Kameyama, Hideshi Kawakami, Shigenobu Nakamura

Abstract
The effect of CAT trinucleotide interruptions in the CAG trinucleotide repeats of the SCA1 gene on the age at onset of spinocerebellar ataxia type 1 (SCA1) was investigated. The number of CAG repeats in SCA1 was determined by polymerase chain reaction (PCR) analysis, and the presence of CAT interruptions was assessed on the basis of the sensitivity of the PCR products to the restriction endonuclease \( Sfa\)NI, which recognise CAT trinucleotides. Only one in 17 expanded SCA1 alleles from 17 SCA1 patients was interrupted by CAT. The \( Sfa\)NI sensitive SCA1 allele from this single patient contained 58 CAG repeats, which would predict an age at onset of SCA1 of 22.0 years, in contrast to the actual 50 years. In addition, the brain stem atrophy of this patient was mild compared with that of a patient with 52 uninterrupted CAG repeats. A sequence analysis showed that the repeat portion of the patient contained \((CAG)_{45}CATCAG\) CAT\((CAG)_{10}\). From these results, we suggest that the age at onset of SCA1 is not determined by the total number of CAG repeats (58) but by the number of uninterrupted CAG repeats.

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Spinocerebellar ataxia type 1 (SCA1), an autosomal dominant neurodegenerative disease characterised by the progressive loss of neurons in the cerebellum, brain stem, and spinocerebellar tract, is caused by the expansion of a polymorphic CAG repeat in the coding region of the SCA1 gene.\(^1\) Whereas the CAG repeat of expanded SCA1 alleles appears to be uninterrupted, most unexpanded alleles (98%) are interrupted by CAT' trinucleotides.\(^2\) The interruption consists of a single region with one to three CAT trinucleotides\(^3\) or two regions each with two CAT trinucleotides.\(^4\)

The purpose of this study was to determine whether CAT interruptions influence the age at onset of SCA1. We determined the number of CAG repeats in the SCA1 alleles of SCA1 patients, and examined the polymerase chain reaction (PCR) products containing the CAG repeats of SCA1 for susceptibility to the restriction endonuclease \( Sfa\)NI, which recognises CAT interruptions. We also describe an unusual SCA1 patient with an expanded SCA1 allele containing 58 CAG repeats with an age of onset (50 years) much older than that calculated from the total number of repeats.

Figure 1 (A) MRI sagittal image of the brain (1.5T, TR 400, TE 19) of the SCA1 patient with the continuous 52 CAG repeats. There was severe atrophy in the brain stem, but the cerebellar atrophy was mild in the 42 year old patient with 8 years duration of disease. (B) MRI sagittal image of the brain (1.5T, SE 256, TR 150, TE 20) of the unusual SCA1 patient with the sequence of \((CAG)_{45}CATCAG\) CAT\((CAG)_{10}\). There was mild or moderate cerebellar atrophy. The brain stem was well preserved in the 69 year old patient with 19 years duration of disease.
Case report

The patient was evaluated at the age of 69 for dysarthria and gait disturbance. He had noticed these symptoms at the age of 50 and his mother and sister had the same symptoms. He was diagnosed as having spinocerebellar ataxia type 1 by gene analysis. At the age of 69, he was 165 cm tall, weighed 64 kg, and had muscle wasting of his lower extremities bilaterally. There was generalised hyporeflexia, decreased muscle tone of his lower extremities bilaterally, bilateral extensor plantar responses, cerebellar ataxia, and dysarthria. Brain MRI showed cortical and vermal cerebellar atrophy, but the brain stem was well preserved (fig 1B).

Methods

To determine the number of CAG repeats in the SCA1 gene of 17 SCA1 patients, high molecular weight DNA was extracted from their leucocytes as described by Sambrook et al. We obtained informed consent from all SCA1 patients. PCR was performed for 30 cycles (denaturation for one minute at 95°C, annealing and extension for two minutes at 72°C) with primers S (5'-GAGCCAGACGCAGGGACAA -3') and r-S (5'-ACGGGGATGGCCGGAGGAGAG-3'). The 5' end of primer S was labelled by incubation with bacteriophage T4 polynucleotide kinase (Takara, Kyoto, Japan) and [32P]ATP at 37°C. Gel electrophoresis was performed on 8% HydroLink Long Ranger gels (AT Biochem, PA, USA) containing 7 mol/l urea and 42% formamide. The CAG repeat length was determined by comparison with a standard M13 sequencing ladder.

For digestion of PCR products with SfαNI, amplification was performed by denaturation at 95°C for five minutes followed by 30 cycles of denaturation (95°C, one minute), annealing (55°C, one minute), and extension (72°C, two minutes) with primers REP-1 (5'-AACTGGAATGTGGACGTAC-3') and REP-2 (5'-CAACATGGGCAGTCTGAG-3'). The PCR product (20 µl) was then digested overnight with 2.5 U SfαNI (New England Biolabs, Beverly, MA, USA) in a final volume of 25 µl at 37°C. The product obtained before and after digestion was then analysed by electrophoresis on 2% agarose gels (fig 2).

For sequencing analysis of the CAG repeat of the unusual SCA1 patient, PCR was performed for 30 cycles (denaturation for one minute at 95°C, annealing and extension for two minutes at 72°C) with primers EcoRI-REP1 and BamHI-REP2, which have EcoRI and BamHI sites at each 5' terminal. The PCR product was double digested with the restriction endonucleases EcoRI and BamHI and subcloned into pBluescript vector (Stratagene, La Jolla, CA, USA). The product was sequenced with Sequenase® version 2.0 (USB, Cleveland, Ohio, USA) and primer KS (5'-TCGAGGTCTCAAGTAGTAT-3') (fig 3).

Results

The expanded allele in all but one of 17 SCA1 patients did not contain CAT interruptions (fig
There was an inverse correlation between the age at disease onset and the number of CAG repeats in 16 expanded SCA1 alleles without CAT interruption. \((r=-0.757, \ p=0.0007)\). An expanded SCA1 allele with CAT interruption contained 58 CAG repeats. The unusual case was without a bivariate normal ellipse \((p=0.999)\). The age at onset in the SCA1 patient with CAT interruption was 50 years, markedly older than expected from the total repeat number (22 years). The sequence of the expanded portion cleaved by SfaNI was \((\text{CAG})_{45} \text{CATCAGCAT}(\text{CAG})_{10}\) (fig 3). The continuous polyglutamine number was 45, which would correspond to an age at onset of 41.8 years. This was within a bivariate normal ellipse \((p=0.50)\).

Fig 1 shows a brain magnetic resonance image (MRI) of (A) the SCA1 patient with 52 continuous CAG repeats (disease duration 8 years) and (B) the unusual patient with 58 CAG repeats interrupted by CAT (disease duration 19 years). The brain stem atrophy of the latter patient was milder than that of the former patient.

Discussion
The CAG repeat in many normal alleles of SCA2 (the gene responsible for spinocerebellar ataxia type 2) has recently been shown to be interrupted, whereas expanded alleles do not contain CAA interruptions.\(^5\)-\(^7\)

The expansion (or contraction) of fewer than four repeats might possibly occur either by simple DNA polymerase slippage or by a DNA hairpin mediated process, while the expansion of more than 10 repeats might occur only by the latter process.\(^8\) The presence of interruptions in CAG repeats of SCA1 is thought to result in the formation of a branched hairpin that may inhibit the expansion. Normal alleles which could serve as a reservoir of future expanded alleles would not contain CAT interruptions and have longer CAG repeats than other normal alleles.

The expanded SCA1 allele of the unusual patient with the CAT interruption contained 58 CAG repeats in total, predicting an onset at 22.0 years of age, although the actual onset was 50 years. The age at onset of SCA1 would reflect the uninterrupted CAG repeat length rather than the total repeat size. In addition, although the total repeat number and duration of illness were greater in this unusual patient than in another SCA1 patient with 52 uninterrupted CAG repeats, the brain stem atrophy of the former patient was milder than that of the latter patient.

Several possible mechanisms have been proposed for the disease. Intraneuronal inclusions (NIIs) have been documented in cerebellar Purkinje cells of transgenic mice overexpressing an expanded CAG repeat of ataxin-1, the gene that causes SCA1, and antibodies to ataxin-1 and ubiquitin detected NIIs in the nucleus pontis centralis, a region prominently affected in SCA1, but failed to detect inclusions in brain regions unaffected by disease.\(^9\)

The presence of NIIs in vulnerable neurons has led to the widely held view that these abnormal depositions are toxic or pathogenic or both. This popular notion was shaken by the report that the formation of nuclear aggregates of ataxin-1 was not required to initiate pathogenesis in transgenic mice.\(^10\) The role of interruption should be further examined to verify these hypothetical mechanisms.

In conclusion, our results provided important clues to the manifestation of the disease, because the interruption could markedly affect the age at onset of SCA1 patients.

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