



Case series

CTA/CTG expansions at the SCA 8 locus in multiple system atrophy

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ARTICLE INFO

Article history:

Received 2 April 2008

Received in revised form 29 August 2008

Accepted 5 September 2008

Keywords:

SCA 8

Multiple system atrophy

Ataxia

KLHL1

ABSTRACT

Objective: Spinocerebellar ataxia type 8 (SCA 8) is an autosomal dominant disorder characterized by cerebellar ataxia with additional features, such as upper motor neuron signs, urinary incontinence and dysphagia. From a clinical standpoint, SCA 8 and the cerebellar form of multiple system atrophy (MSA-C) share several common features.

Methods: We studied the presence of expanded SCA 8 alleles in 10 sporadic patients with probable MSA-C. **Results:** We found 1 patient with a heterozygous CTA/CTG repeat expansion in the pathological range. Clinically this subject presented no features that differed from the other subjects carrying smaller repeat sizes.

Conclusions: We believe that the association of SCA 8 repeat expansions with sporadic, atypical and heterogeneous phenotypes is debatable and should be interpreted with caution. Our personal conclusion is that testing in such patients may become a source of diagnostic confusion.

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1. Introduction

Spinocerebellar ataxia type 8 (SCA 8) is an autosomal dominant (AD) neurodegenerative disorder characterized clinically by cerebellar ataxia of varying severity occurring alone or associated with additional features, such as upper motor neuron signs, urinary incontinence, dysphagia, and behavioral symptoms [1]. SCA 8 is a trinucleotide repeat disorder but unlike the previously described spinocerebellar ataxias (SCAs), the expansion lies in a transcribed but untranslated CTG repeat on chromosome 13q21 with a short polymorphic adjacent CTA repeat (CTA/CTG) in the antisense RNA of the Kelch-like 1 gene (KLHL1) [2]. The CTA/CTG repeat is unstable with a tendency to expand on maternal and to contract on paternal transmission. There is also some evidence for somatic mosaicism of repeat length [3].

The shortest number of combined CTA/CTG repeats that is considered to be pathogenic is a matter of debate. Previous studies demonstrated that more than 99% of healthy controls have 16–37 CTA/CTG repeats [3] with potentially pathogenic repeats ranging from 71 to more than 1300 [4]. Therefore, Izumi et al. [5] tentatively classified the pathogenic expanded CTA/CTG SCA 8 alleles into three classes: intermediate-sized alleles (50–84 CTA/CTG repeats),

large expanded alleles (85–400 CTA/CTG repeats), and very large expanded alleles (>400 CTA/CTG repeats). In addition to this uncertainty, the SCA 8 expansion has been described not only among autosomal dominant familial cases but also in autosomal recessive and sporadic ataxia patients, in non-ataxic healthy subjects, psychiatric patients, and in patients with various other neurological diseases with a known etiological cause [5,6]. Additionally, the mutation has an exceptionally low penetrance, raising doubts about its pathogenic role, leading to speculation of plausible mechanisms, such as linkage disequilibrium of the CTG expansion with another as yet unidentified causal mutation [5–7].

Owing to the clinical similarities of SCA 8 and the cerebellar form of multiple system atrophy (MSA-C) [1,8], we studied the presence of SCA 8 expansions in sporadic patients with probable MSA-C.

2. Materials and methods

Ten unrelated patients with probable MSA-C, according to the Consensus Committee [8] [late onset sporadic ataxia with early dysautonomia (orthostatic hypotension, impotence and/or urinary incontinence or retention not explained by medications or other conditions)] followed for at least 5 years were selected. Blood samples were obtained and genomic DNA was extracted by a standard method [9] after informed consent was obtained. A combination of PCR and Southern blotting was used as described previously in order to reach better sensitivity for the detection of expansions. Although the primary objective of our study was to test the occurrence of SCA 8 mutations on MSA-C patients, we also tested all our

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patients for mutations that are part of the SCA panel routinely used at our laboratory (SCA 1, 2, 3, 6, 7 and DRPLA loci).

3. Results

Clinical characteristics of each patient are found in Table 1. Mean age for the 10 patients with a diagnosis of MSA-C was 60.7 (52–68) years with 6 (60%) male patients. Mean age of onset was 55.5 (47–63) years. By definition, all subjects presented with cerebellar ataxia and dysautonomia. Namely, subjects 1, 2, 4, 5, 8 and 9 had urinary incontinence; subjects 2, 3, 6, 7, 9 and 10 had erectile dysfunction; subjects 1, 2, 3, 4, 6, 8 and 10 had orthostatic hypotension. Gait ataxia was the presenting symptom for all patients. Dysarthria was reported as an initial symptom in 4. Four patients had pyramidal tract signs and two had rigid akinetic symmetric Parkinsonism. A history suggestive of REM sleep behavior disorder was evidenced in 7 patients. Three patients complained of mild memory deficits that were not significant to interfere with daily living activities. Screening using the MMSE did not reveal evidence of dementia in any of the patients.

None of the patients had a family history of neurological disease resembling ataxia or Parkinsonism. Resting tremor was not observed in any of our patients. Mild to moderate action tremor was observed in subjects 2, 4, 5, 6 and 9. Sensation was normal for all modalities in all subjects. Neuroimaging studies were performed in all subjects. All were initially assessed with brain CT scan showing signs of cerebellar and pontine atrophy; subjects 1, 2 and 8 were additionally investigated with brain MRI scans revealing pontocerebellar atrophy with typical signal changes in the pons (“cross sign”).

Combined CTA/CTG repeats were within normal range for both alleles in 9 MSA-C patients, ranging from 20 to 28 repeats (Table 1). Patient 1 presented 22/76 repeats. From a clinical standpoint this subject presented no distinctive features that differed from the other subjects carrying smaller repeat sizes. The remaining tests that are part of our SCA panel showed repeat lengths with normal range.

4. Discussion

Recently molecular and pathologic investigations provided objective evidence of the pathogenic role CTA/CTG repeat expansions in neuronal degeneration. He et al. [10] developed a knockout mouse model for the KLHL1 gene and showed that mice either homo or heterozygous for the KLHL1 gene deletion presented significant gait abnormalities and incoordination at an early age. These motor signs correlated with significant Purkinje cell dendritic deficits and were more pronounced in the homozygous mice, confirming the hypothesis that loss of KLHL1 function leads to degeneration and is likely to play a significant role in the under-

lying pathophysiology of SCA 8. Finally, Ito et al. [11] confirmed similar findings in a pathological study of one patient from a SCA 8 family. This case showed degeneration of Purkinje cells, inferior olive, substantia nigral and periaqueductal area with fibrillary intracytoplasmic 1C2-positive inclusions.

On the other hand, “potentially pathogenic” SCA 8 CTA/CTG repeats ranging from 68 to 259 have been reported in normal subjects as well as in patients with Lafora disease, levodopa responsive Parkinson’s disease, corticobasal degeneration, Alzheimer’s disease, essential tremor, patients with non-ataxic/non-specified neurological conditions, in addition to genetically proven SCA 1, 2, 6 and Friedreich’s ataxia subjects [5,6,12–14]. Additionally, the most striking “false positive” case of SCA 8 was published by Factor et al. [15] in a pathologically confirmed MSA-C case who carried 145 CTA/CTG repeats in the SCA 8 allele. To our knowledge, two previous studies analyzed the SCA 8 allele in patients with MSA-C. Sobrido et al. [6] analyzed 153 patients with familiar and sporadic forms of ataxia, including 13 with MSA-C, while Schöls et al. [16] studied 124 patients with idiopathic sporadic cerebellar ataxia, including 20 that fulfilled criteria for probable MSA-C. Neither study found molecular abnormalities in their respective MSA-C subgroup. Therefore, our case with MSA-C and an expansion in the SCA 8 gene is the second in the literature, with the caveat that pathological confirmation is pending.

Another caveat of the case presented here, with a 76 CTA/CTG repeat expansion in one SCA 8 allele, is the fact that the upper limit of normal for such expansions remains imprecise. In the initial description from 1999, Koob et al. [17] reported that their control samples showed combined CTA/CTG repeats ranging from 16 to 92, although more than 99% of the normal alleles had 16–37 repeats. Among the ataxic patients belonging to this initial SCA 8 kindred, alleles ranged from 110 to 130 CTA/CTG repeats. In the following year, the same group published data on the repeat size range found in affected members of 11 different SCA 8 families, showing a broader range from 71 to 800 CTG repeats. They acknowledge that alleles with <100 and >250 CTA/CTG repeats are less pathogenic [4]. In another series that used 91 CTA/CTG repeats as the cut off value, no ataxic patient presented repeats between 40 and 90, and 98% of their controls had SCA 8 CTA/CTG repeat sizes below 40. In this study, the authors present a thorough literature review, showing that by 2004 only four ataxic patients had been reported with expansions below their cut-off value [18]. Cellini et al. [1] analyzed 167 patients with ataxia, 161 healthy controls and 125 psychiatric patients finding five ataxic patients with SCA 8 CTA/CTG expansions between 90 and 320. One of the psychiatric patients carried what they considered as a large normal allele of 75 repeats, using the repeat size distribution described by Silveira et al. [3]. The case presented here lies immediately above this limit and within the range considered as “potentially pathogenic” in the Moseley et al. [4] study mentioned above. Previous studies

Table 1
Clinical and genetic profile of the subjects with probable MSA-C.

Case	Age (y.o)	Onset (y.o)	Gender	Cerebellar ataxia	Autonomic disorder	Pyramidal signs	PK	Neuroimaging (CT/MRI)	SCA 8 expansion	RSBD
1	52	47	F	+	+	–	–	CA	22/76	+
2	55	50	M	+	+	–	–	CA	23/28	+
3	68	62	M	+	+	–	–	CA	23/25	+
4	58	53	F	+	+	+	–	CA	20/25	+
5	64	59	F	+	+	+	–	CA	22/26	+
6	53	47	M	+	+	–	–	CA	23/25	–
7	67	62	M	+	+	–	+	CA	27/24	–
8	64	59	F	+	+	+	–	CA	25/22	–
9	68	63	M	+	+	+	–	CA	23/22	+
10	58	53	M	+	+	–	+	CA	25/20	+

PK: Parkinsonism; RSBD: REM sleep behavior disorder; CA: cerebellar atrophy.

have shown that both CTA and CTG tracts are polymorphic implying that CTA/CTG expansions at the SCA 8 locus may be rare polymorphisms with a clinical significance that remains to be determined [12]. In fact previous studies indicated that SCA 8 expansions may imply in a risk for the development of ataxia by only 2-fold to 5-fold, compared with a >1000-fold genetic risk associated with other SCAs [9]. Finally, in a study of 1.262 German ataxia patients, Schöls et al. found intermediate and expanded CTG repeats with similar frequencies in patients with and without established genetic diseases, again, questioning the pathogenicity of such mutations [19].

5. Conclusions

In the light of these and other observations, the association of SCA 8 repeat expansions with sporadic, atypical and heterogeneous phenotypes has become debatable and should be interpreted with caution [5–7,14,15]. On the other hand, the importance of screening for SCA 8 in patients with a compatible phenotype, namely those with autosomal dominant ataxia, should not be underestimated. The series by Zeman et al. [18] found that although CTA/CTG repeat expansions over 91 are relatively common among unaffected controls (0.5%), these mutations are significantly more common among patients with hereditary ataxia (3.5%), confirming not only the association between the SCA 8 expansion and a cerebellar clinical syndrome but also the importance of this genetic test. In their review of the published series, the authors found support to their results confirming that CTA/CTG repeat expansions above the same cut off occurred in 3.4% of ataxia patients and among 0.7% of all controls. Juvonen et al. [9] calculated the predictive values of a positive SCA 8 repeat expansion (cut off of 80) to be of 47.4% in sporadic patients, rising to 86.3% in cases with dominant inheritance, indicating the importance the test when performed for the specific phenotype. Finally, Izumi et al. [5] reported a frequency of pathogenic CTA/CTG expansions of 1.9% in patients with dominant ataxias, while patients with other neurodegenerative disorders or normal controls had much lower rates (0.3–0.5%).

Our personal conclusion is that testing in such patients may become a source of diagnostic confusion. Clinicopathologic correlation studies as well as objective demonstration of a pathogenic role for SCA 8 expansions in these atypical cases should be performed before testing for SCA 8 is recommended in clinical settings.

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