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Trinucleotide repeat spinocerebellar ataxias: experience of a tertiary care centre in Western India with review of Indian literature

[1]Satish V Khadilkar *MD DM*, [2]Rajesh Dabi *MD DM*, [1]Pramod Dhonde *MD DM*, [3]Nilesh Nadkarni *MD DM*, [4]Smita Kulkarni, [4]Dhananjaya Sarnath *MD*

[1]Department of Neurology, Grant Medical College and Sir J.J. Group of Hospitals, Mumbai; [2]Neurocare clinic, Jalgaon; [3]Apollo Victor Hospital, Goa; [4]Reliance Life Sciences, Rabale, Navi, Mumbai; India

Abstract

Consecutive index cases with trinucleotide repeat spinocerebellar ataxia (SCA) presenting from August 2006 to August 2008 to a tertiary care university department were studied clinically, radiologically and genetically (repeat expansions at SCA 1, 2, 3, 6, 7, 10 and 12). CAG repeat expansions were seen in 14 patients. Inverse relationship between CAG repeats and age of presentation was conspicuous. SCA 2 (10/14) was the commonest followed by SCA 6 (2/14), SCA 3 (1/14) and SCA 1(1/14). In one patient of SCA 6, 'hot cross bun' sign was seen on MRI Brain. Sixteen cases were negative for the genetic tests.

INTROUDCTION

Spinocerebellar ataxias (SCAs) are autosomal dominant inherited ataxias with 31 specific nucleotide repeat mutations identified.[1] These ataxias are phenotypically heterogeneous and the prevalence of genotypes varies amongst studied populations. Recent data suggest SCA 3 to be the commonest subtype in the world; and other trinucleotide repeat SCAs occur with lesser frequencies.[1] In the Indian context, there are regional differences in prevalence of genotypes of trinucleotide repeat SCAs as documented by studies from the north, south and east of India.[2-8] As yet, there is no prevalence study of trinucleotide repeat SCAs from the western region. In the current study, we present our findings on frequency of SCA mutations in a tertiary care centre in Western India, review the available Indian literature on trinucleotide repeat SCAs and propose an algorithm for genetic evaluation of trinucleotide repeat SCAs in India.

METHODS

The study was carried out in our tertiary care Neurosciences centre from August 2006 to August 2008. Consecutive patients with chronic progressive cerebellar ataxia were included in the study. Patients with known non genetic aetiology were excluded. Patients were clinically evaluated. Investigations included complete blood count, renal/ liver / thyroid function tests, HIV test, MRI Brain, vitamin B12 and nerve conduction study. Molecular diagnostic tests for SCA with specific nucleotide repeat expansions for SCA 1, 2, 3, 6, 7, 10 and 12 were based on by Polymerase Chain reaction to investigate SCA gene amplification.

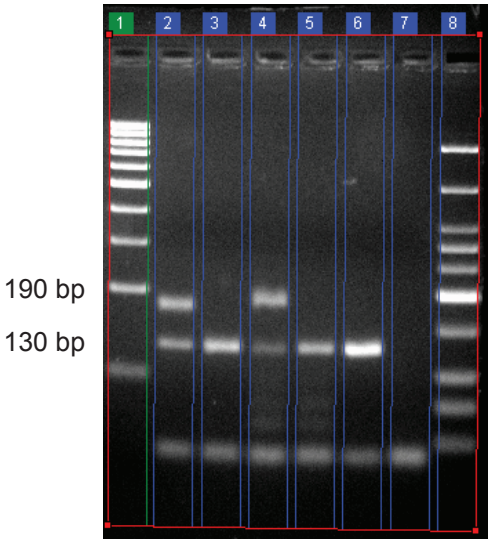
Polymerase chain reaction and agarose gel electrophoresis

Genomic DNA was extracted from peripheral blood using Qiagen DNA extraction kit (Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) amplification of the genes SCA 1, SCA 2, SCA 3, SCA 6, SCA 7 and SCA 12, harbouring the CAG trinucleotide repeat sequences, and SCA 10 with pentanucleotide ATTCT-repeat region, was performed in independent reactions as described by Kim *et al*.[9]

RESULTS

Thirty patients fulfilled the inclusion criteria. All were index cases. Family members were not included in the study. There were 24 males and 6 females. The age of presentation varied from 12-65 years. The average age of presentation was 33.5 ± 1.41 years. Autosomal dominant history was present in 8 patients; whereas family history of remaining cases was not sufficient to classify

Address correspondence to: Prof S V Khadilkar, 110, New Wing, First Floor, Bombay Hospital, 12, New Marine Lines, Mumbai, India 400020. Mobile: 919820024689, e-mail: khadilkarsatish@gmail.com

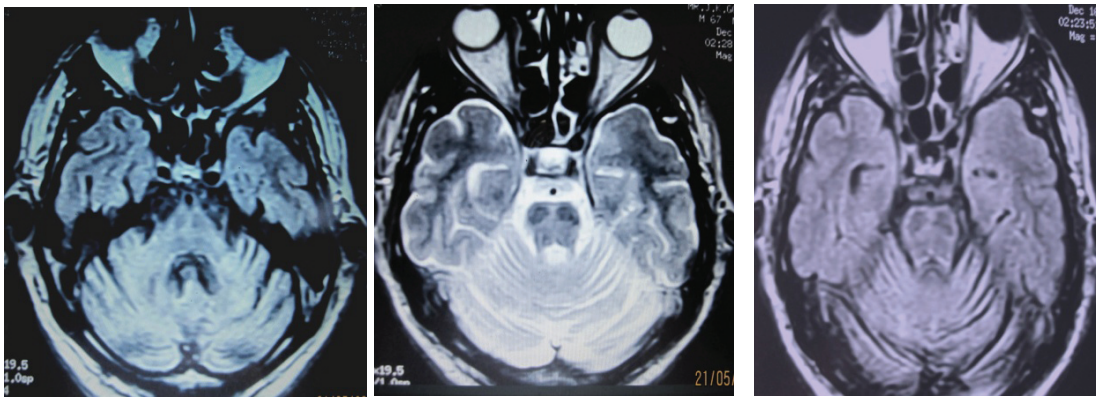


1	100 bp Molecular Wt. Marker	Amplicon sizes (bp)
2	SCA 2 Positive Control	190 bp ; 130 bp
3	Normal Control	130 bp
4	**Sample 1**	**200 bp ; 130bp**
5	Sample 2	130 bp
6	Sample 3	130 bp
7	Reagent Control	
8	Low Mol. Wt. Marker	

Figure 1: SCA 2 PCR Amplification

them as sporadic or late onset cerebellar ataxias with positive family history. Representative PCR amplifications of the nucleotide repeats in normal controls and patients with expanded repeats in SCA 2 patients is shown in Figure 1.

The most common SCA in our study was SCA 2 (10 patients). Two patients with SCA 6 and one each of SCA 1 and SCA 3 were detected. However, 16 of 30 patients did not show presence of the investigated SCA specific repeat expansions. Clinical and investigative parameters are tabulated in Table 1. Mean age of onset was 26 years in SCA 2 patients. Saccadic eye movement abnormalities and ataxia were consistently seen in SCA 2. Spearman correlation coefficient between age of onset and CAG repeats was -0.67 supporting the inverse correlation in SCA 2 patients. The patients with SCA 6 were in sixth and fifth decade at time of presentation. SCA 3 and SCA 1 presented during fifth and third decade respectively. MRIs showed atrophy of the cerebellum, and in some cases, brainstem. One patient with SCA 6 had the 'hot cross bun sign' on his MRI (Figure 2). This Patient was 45 years old and had severe cerebellar ataxia, without involvement of any other neurological structures. The clinical and laboratory examination did not detect any autonomic dysfunction. Thus, the diagnosis of MSA was very unlikely and the genetic tests confirmed SCA 6 mutation. Electrophysiology was done in 28 patients. Amongst them 4 patients of SCA 2 showed axonal neuropathy while others with known genetic abnormalities had normal nerve conduction study (Table 1).



A. T1 W image B. T2 W image C. FLAIR image

Figure 2: Hot cross bun sign in patient of SCA6 on MRI on T1, T2 and FLAIR axial images.

DISCUSSION

Patients with SCA are encountered all over India. Hospital based studies have analysed relative frequency of SCA genotypes in various regions of India (Table 2).[2-8] These studies have been carried out on the Eastern, Northern and Southern parts of population. From the western part of India, a detailed evaluation of SCA 2 families is available.[8] However, there is no prevalence data from western region of India. This hospital based study was done to obtain this information.

In the present study, in keeping with information from the Northern and Eastern India, SCA 2 was found to be more common. This is in contrast with SCA 3 being the commonest SCA worldwide.[1] The SCA 2 patients, as a group, tended to have uniform clinical manifestations dominated by slow saccades and limb ataxia. It is noteworthy that, cerebellar tremors were much uncommon as compared to dysdiadochokinesia, probably a manifestation of pattern of degeneration. The number of repeats correlated inversely with the age at onset, a reflection of phenomenon of anticipation. Other subtypes i.e. SCA 6, 3 and 1 were infrequent. As Table 2 shows, there is a regional variation in the prevalence of subtypes of trinucleotide repeat SCAs in India. Available studies from the Southern region show the prevalence of SCA1 to be the most common, while the Eastern and Northern studies show SCA 2, 1, 3 and 12 in various comparable proportions. Overall, in India, SCA 2 seems to have higher prevalence as compared to other regions in the world.

We also found that in one patient of SCA 6, 'hot cross bun' sign was seen on MRI Brain. (Figure 2). The sign, initially described in multiple system atrophy, has been seen inconsistently in some SCAs as well. In a large study from Taiwan consisting of 138 patients of trinucleotide repeat SCAs, the sign was noticed in 25% of SCA 2, 1.3% of SCA 3 and 1 patient each of SCA 7 and SCA 8.[10] To the best of our reading, it has not been documented in SCA 6 patients.

Taking into account the available information on SCAs in a resource limited developing country

Table 1: Clinical and investigative parameters of the study patients with SCA.

Clinical features	SCA 2 (n=10)	SCA 6 (n=2)	SCA 1 (n=1)	SCA 3 (n=1)	Unknown (n=16)
Slow saccades	10	0	1	0	2
Dysdiadochokinesia	9	2	1	1	14
Dysmetria	9	2	1	1	16
Cerebellar tremor	2	0	0	0	4
Dysarthria	9	2	1	1	16
Impaired heel knee heel test	10	2	1	1	16
Parkinsonian features	0	1	0	0	0
Cognitive impairment	1	0	0	0	0
Generalised areflexia	3	0	0	0	2
MRI Features	Cerebellar atrophy(3) pontocrebellar atrophy(3) Cerebral and cerebellar atrophy(1)	Hot cross bun (1) Cerebellar atrophy (1)	Cerebellar atrophy	Cerebellar atrophy	Cerebellar atrophy(5) Remaining (NA)
NCS	Sensory axonal neuropathy (4)	Normal	NA	Normal	Sensory axonal neuropathy(2)
CAG repeats	41-60	20, 22	42	80	0

NA= not available

Table 2: Relative frequencies of different SCAs in India

	Number of subjects/families	SCA1 (%)	SCA1 (%)	SCA3 (%)	SCA6 (%)	SCA7 (%)	SCA 10 (%)	SCA12 (%)	Unknown SCA (%)
Saleem *et al*[2] (North)	42 (families)	3	10	2	0	0	NA	NA	24
Shrivastava *et al*[3] (North)	77 (families)	NA	NA	NA	NA	NA	NA	5	NA
Basu *et al*[4] (East)	57 (subjects)	6 (10.5)	10 (17.5)	4 (7.0)	1 (1.8)	0 (0)	NA	NA	36 (63.2)
Chakravarty *et al*[5] (East)	14 (families)	2 (14.3)	4 (28.6)	5 (35.7)	NA	0	NA	NA	3 (21.4)
Sinha *et al*[6] (East)	28 (families)	4 (14.3)	16 (57.1)	0	0	0	NA	0	8 (28.6)
Krishna *et al*[7] (South)	105 (families)	34 (32.4)	24 (22.9)	15 (14.3)	NA	NA	NA	NA	32 (30.4)
Wadia *et al*[8] (West)	51 (subjects)	NA	14	NA	NA	NA	NA	NA	NA
Present study (West)	30 (subjects)	1 (3.3)	10 (33.6)	1 (3.3)	2 (6.7)	0	0	0	16 (53.3)

NA= not available

that India is, we propose an algorithm for testing of degenerative cerebellar ataxias (Figure 3), based on reported data and review of Indian literature from various regions.

In conclusion, in India, SCA 2 appears to be the commonest trinucleotide repeat SCA. Trinucleotide repeat SCAs like 1, 2, 3, 6 and 12 are also seen. In South India, SCA 1 appears more commonly. Based on this information, an algorithm has been proposed. The present study also provides relative frequencies of trinucleotide repeat SCAs seen in a tertiary clinic in western part of India. The available data is limited in the context of total population of India and further multicenter analysis of SCAs in larger numbers is necessary to validate the proposed algorithm.

DISCLOSURE

Conflict of interest: None

Source of support in the form of grants or others: None

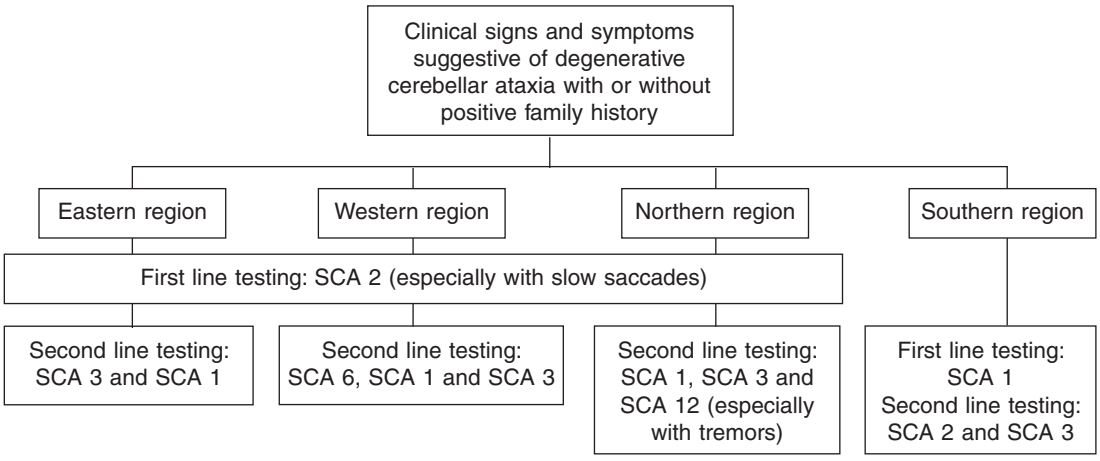


Figure 3. Proposed algorithm for genetic testing for SCA in India.

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