

Spinocerebellar ataxia 1: case and cohort-based studies in India

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Spinocerebellar ataxia 1 (SCA1) is a late-onset neurodegenerative disease caused by CAG (coding for glutamine) repeat expansions or polyglutamine (polyQ) aggregates in the *ATXN1* gene. Apart from the causative CAG repeat expansions in the *ATXN1* locus, environment and ethnicity have been hypothesized to affect clinical variability. This review brings together studies on SCA1 conducted in India and explores disease heterogeneity within the Indian population in general and within a cohort of SCA1 individuals in a South Indian village. The studies support existence of genetic factors in addition to CAG repeat sizes that are likely to influence SCA1 progression.

Keywords: Cohort studies, clinical variability, heterogeneity, genetic factors, neurodegenerative disease.

SPINOCEREBELLAR ataxias (SCAs) comprise a heterogeneous group of autosomal dominantly inherited ataxias. To date, more than 30 SCA genes or loci have been identified¹. They show, as common features, progressive neurodegeneration of neuronal subsets in distinct brain areas. Polyglutamine (PolyQ) aggregates are observed in SCAs caused by expansion of CAG (coding for glutamine) repeat sequences in certain genes².

Amongst the SCAs, SCA1 was the first to be genetically characterized with an expansion of CAG trinucleotide repeats located within exon 8 of the *ATXN1* gene³. In normal individuals, the CAG repeat number varies between 4 and 39 repeats, which are occasionally interrupted by 1–3 CAT triplets. CAT (coding for histamine) interruptions of the polyCAG tracts within the *ATXN1* gene render the allele non-pathogenic⁴, possibly by stabilizing the polyQ containing protein⁵. In contrast, in SCA1 patients, the repeats are expanded beyond 39 and do not contain any CAT interruptions⁶. Typically SCA1 is characterized by progressive cerebellar ataxia of gait and limbs, variably associated with ophthalmoplegia, pyramidal and extrapyramidal signs, pigmentary retinopathy and peripheral neuropathy⁷. Age of onset of symptoms is typically between 20 and 50 years, and the disease culminates with the death of the individual within 10–20 years after its onset⁸.

Currently, no treatment is available either for curing SCA1 or delaying the onset of the disease⁹. Majority of

SCA1 studies in India are hospital-based^{10–15} and hence cross-sectional; thus they fail to reveal characteristic clinical features of disease progression. Specific cohort-based studies on the natural history and clinical characteristics of SCAs are gaining importance because such studies could help in understanding the preclinical stages of SCA, where therapy is likely to be most effective. Such cohort-based studies exploring the clinical and biological characteristics of at-risk cases of SCA1 have so far been conducted in the US, Europe, South Africa^{16–18} and more recently in India¹⁹.

Functions of Ataxin1 protein in the cellular context

ATXN1 encodes a polyQ containing protein (Ataxin1 or ATXN1) which localizes to both the cytoplasm and nucleus²⁰. Although the exact function of ATXN1 in the context of neurodegeneration remains to be understood completely, it is known to play a role in regulation of transcription and RNA processing/metabolism^{21–23}. In addition to its interaction with transcriptional repressor (*capicua*, CIC) and mRNA splicing factor (RBM17), ATXN1 transiently interacts with a number of other transcription regulators *in vivo*, such as RORa, Tip60, SMRT, LANP and PQBP-1 (refs 24–26). ATXN1 also binds RNA in a manner inversely proportional to its polyQ length²⁷. Additionally, ATXN1 undergoes several post-translational modifications, including phosphorylation, ubiquitination, sumoylation and transglutamination²⁸. Such modifications can alter the stability of ATXN1 or its activity in the regulation of target gene expression and, therefore contribute to SCA1 toxicity²⁸. Studies in the cerebella of SCA1 mice and patients have shown transcriptional dysregulation of several intracellular calcium signalling genes prior to the onset of disease phenotypes, indicating that cellular and transcriptional changes indeed antedate neuronal death^{29–31}.

Importance of cohort studies for SCA1

Although studies in mice models have helped understand the cellular functions of ATXN1, its role in the context of pathogenesis/disease progression in humans still needs to be elucidated. Studies conducted in patient cohorts, where

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progression of the disease can be monitored over a period, will help in better understanding of the varied effects of ATXN1 during SCA1 pathogenesis. Such studies when conducted in patients facing similar environmental influences could also help in delineating additional genetic factors that affect disease progression. In fact, such cohort-based studies have indicated the influence of factors other than polyQ size to affect disease onset in SCA1^{18,32}. Another cohort-based study has shown the lack of correlation between CAG repeat size and disease progression, indicating the influence of other confounding factors after disease onset¹⁷. Thus, cohort-based studies are necessary for understanding all factors that lead to disease characteristics and progression in SCA1 individuals.

Insights from SCA1 studies across India reveal the prevalence of heterogeneity

India is a large country with a total population of 1.2 billion³³. Geographically and ethnically the four quadrants (North, South, East and West India) vary in their genetic composition^{34–36}. SCA1 was first reported in ethnic populations of eastern India¹⁰ (Table 1), where DNA from peripheral blood of 57 patients with clinical symptoms of SCA belonging to the region was genetically tested for SCA1, 2, 3, 6 and 7. Among the 57 patients, six individuals tested positive for SCA1 with repeat expansions consisting of 44–52 repeats with varying ages of presentation (20–53 years). Subsequent to this, when the relative frequency of SCAs was analysed in 42 North Indian families by molecular methods, three families tested positive for SCA1 with expanded repeat sizes ranging from 48 to 57. Complete clinical examination along with molecular testing was conducted in another study where 4 families consisting of 7 patients tested positive for SCA1 among the 28 East Indian families with autosomal dominant cerebellar ataxia (ADCAs). The presenting clinical symptom was unsteadiness of gait in all seven SCA1 patients. Expanded repeats were in the range 50–65. An inverse correlation of age of onset with CAG repeat size was observed here as seen in previous studies^{19,38}. These studies were primarily aimed at frequency estimation of different ADCAs and concluded that SCA2 was the most prevalent form in India.

However, in southern India, four independent studies point to a higher incidence of SCA1 over other SCAs^{13,14,19,38}. When the frequencies of SCA1 patients from hospitals were compared amongst the ADCAs, they were twice in southern India (33%, 20/60 families) than in northern India (15%, 17/107 families)¹³.

A community-based study of two ethnic villages, Rajapalayam and Kotamedu in Tamil Nadu, identified SCA1 as the most prevalent type of SCA within this ethnic community³⁸. Clinical analysis revealed unsteadiness of gait to be the first symptom in 80% of the patients. A

single homozygous patient identified did not differ in disease manifestation, severity and progression compared to the heterozygous patients ($n = 17$). This study also revealed 60% of the patients to be asymptomatic, indicating pre-symptomatic carriers who are likely to manifest the disease at a later stage. Additionally, a recent study in an ethnic in-breeding population in South India identified 21 symptomatic (age 17–60 years) and 16 pre-symptomatic SCA1 (age 34–60 years) cases with repeat sizes above 40 CAG from DNA isolated from buccal mouthwashes¹⁹.

To summarize, three groups have conducted exclusive studies on SCA1 in India. One of these groups has addressed the variation observed in the prevalence of SCA1 across India¹³. This group studied the role of various genetic factors such as repeat length, CAT interruption pattern and founder mutation in contributing towards the prevalence of SCA1. They analysed 12 genetic markers spanning a distance of 65 kbp linked to the disease locus, to understand the diversity of founder mutation within the Indian subcontinent as a whole. Three genetic markers, 2 SNPs (rs1476464 and rs2075974) and a microsatellite region (D6S288) are associated with expanded alleles by haplotype analysis¹³. Patients from both southern and northern India showed a haplotype of C-4-C corresponding to the presence of allele C/G at rs1476464, a dinucleotide repeat of 25 at D6S288, and allele C/G at rs2075974, suggesting a founder effect for SCA1 in the Indian population¹³. A recent study reconfirms these data by genotyping for the founder mutation in an isolated ethnic SCA1 cohort¹⁹. This study supports a common genetic founder for SCA1 within the Indian population. Thus, the reasons for marked heterogeneity in the prevalence of SCA1 in India still need to be explored.

PolyQ repeat size and SCA1 disease progression in India

SCA1 is associated with a considerable variability of clinical symptoms and anticipation⁷. Correlation studies of CAG repeat size with disease duration and severity are lacking in India. A single hospital-based study showed a correlation coefficient (r^2) of 0.62 between CAG repeat size and age of onset¹⁴. Another study conducted in an ethnic population revealed $r^2 = 0.45$, indicating that CAG repeats explain only about 45% of age of onset¹⁹. Interestingly, this study further shows that maternal inheritance of the disease allele delays onset of the disease¹⁹. This is also the first cohort-based study from an Indian village where genotypic characteristics of families with SCA1 (six families with 37 affected individuals) have been correlated with phenotype and disease progression. Such studies are important in understanding the disease characteristics in individuals with shared environmental influences such as similar lifestyles, food habits and occupation. Pre-symptomatic SCA1 cases beyond the age

Table 1. Distribution of SCA1 in India^{10–15,19,38}

Groups	Regions studied	Number of SCA1 cases identified	Year	Expanded repeat range	Significant conclusions
Saleem <i>et al.</i>	Delhi	3	2000	48–57	<ul style="list-style-type: none"> Hospital-based study of SCA 1, 2, 3, 6, 7, 8 and DRPLA. Comparison of the frequency of large normal alleles (>30 and >31) revealed it to be similar to the Caucasian population.
Basu <i>et al.</i>	West Bengal and Bihar	6	2000	44–52	<ul style="list-style-type: none"> Hospital-based study of SCA 1, 2, 3, 6, 7 and DRPLA in nine ethnic populations. Frequency distribution of large normal alleles was comparable to Caucasians in the population studied.
Sinha <i>et al.</i>	Madhya Pradesh and Bihar	4	2004	50–65	<ul style="list-style-type: none"> Hospital-based study of SCA1, 2, 3, 6, 7, 8, 12 and 17. SCA2 reported as the most common in eastern India.
Rengarai <i>et al.</i>	Tamil Nadu	17	2005	40–48	<ul style="list-style-type: none"> Cohort-based clinical and genetic SCA1 study. First report of pre-symptomatic SCA1-positive individuals.
Mittal <i>et al.</i>	North and South India	37	2005	42–72	<ul style="list-style-type: none"> Hospital-based study of SCA1. Three markers were identified linked to the disease and it has been suggested that prevalence of SCA1 is linked to repeat length and interruption pattern in SCA1.
Krishna <i>et al.</i>	Karnataka, Andhra Pradesh, Tamil Nadu and Kerala	57	2007	42–72	<ul style="list-style-type: none"> Hospital-based study of SCA 1, 2 and 3. SCA1 was found to be common in South India.
Patel <i>et al.</i>	Gujarat	2	2014	49–56	<ul style="list-style-type: none"> Hospital-based study of SCA 1, 2, 3 and 6. SCA2 was found to be the common form.
Sharma <i>et al.</i>	Madhya Pradesh	9	2012	Unknown	<ul style="list-style-type: none"> Single-family study on SCA1. SCA1-positive individuals were identified by PCR and MRI.
Kumaran <i>et al.</i>	Tamil Nadu	37	2014	40–51	<ul style="list-style-type: none"> Cohort-based study. First report of early disease onset in paternally inherited SCA1 cases. First report of old pre-symptomatic SCA1 cases in India.

of 50 years remain undetected for the most part in the general population. The recent identification of individuals aged 50 years or more in a South Indian ethnic population harbouring ATXN1 alleles with polyQ repeat size greater than 40, suggests that such pre-symptomatic cases may be more prevalent among Indians than previously known and such cases could only be discovered through cohort-based studies. Previous cohort study in a Siberian populations has reported similar pre-symptomatic individuals and suggested incomplete penetrance of the mutation as the cause³². The reasons for such incomplete penetrance in India remain unknown. The cohort studies suggest that it may be due to compensatory genetic factors and not the environment.

Conclusion

Although reports point to SCA2 as the most common ataxia in Indian population, studies on small communities in Tamil Nadu, suggest that a larger proportion of people might be affected with SCA1 in South India. Interestingly, founders for SCA1 among North and South Indian

populations are similar. The difference in prevalence of the disease between these two broadly distinct regions thus remains to be understood.

Also, further genetic and molecular analysis on older pre-symptomatic individuals should prove informative and help in the better understanding of genetic factors, other than polyQ size, which contribute to disease onset and severity. Studies involving genome-wide linkage scans^{39,40}, exome sequencing⁴¹ and/or targeted gene sequencing by Next-Generation Sequencing⁴² could be employed for identifying such genetic modifiers of SCA1. Identification of new molecular players that influence pathogenesis/progression of SCA1 in patients should also provide new leads for therapeutic interventions in SCAs. Thus, ethnic studies need to be conducted in India, with deeper emphasis on obtaining clinical phenotypes over a longitudinal period, and these need to be correlated with genetic and molecular data.

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