SCA 1, SCA 2 & SCA 3/MJD mutations in ataxia syndromes in southern India


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Background & objectives: Spinocerebellar ataxias (SCAs) are often caused by expansions of CTG/CAG trinucleotide repeat in the genome. Expansions at the SCA1, 2 and 3 loci are the most frequent, but differences in their relative proportion in regions occur across the world. We carried out this study to assess the occurrence of SCA1, 2 and 3, at a tertiary neuro-psychiatric center in Bangalore, Karnataka.

Methods: Probands (N=318) who were diagnosed to have an ataxia syndrome (progressive degenerative ataxia of unknown cause) attending the clinical services of the National Institute of Mental Health and Neuro Sciences (NIMHANS), Bangalore, were evaluated over a period of three years. Standard protocols were used for both clinical and molecular diagnosis.

Results: Genotyping established that SCA1, 2 and 3 accounted for more than one third of the ataxia cases seen in the clinic. In the cases with established family history and autosomal dominant inheritance SCA1 was most prevalent followed by SCA2 and SCA3.

Interpretation & conclusions: Our findings suggested SCA1 rather than SCA2 to be the more common mutation in southern India. Large numbers of SCA3 probands were also identified. Differences in prevalence of these syndromes within India need to be explored further for founder effects, correlations with phenotype, and patterns of outcome. Family history was not apparent in almost a fifth of those tested positive, highlighting the value of testing even in the absence of family history. Molecular testing should be extended to cover the other forms of ataxia, of which a large number are now known. Combined efforts to confirm the presence of these less common forms, as well as family studies to detect novel mutations, are necessary in this context in India.

Key words Genetic markers - prevalence - spinocerebellar ataxia

Spinocerebellar ataxias (SCAs) are a group of neurological disorders, many caused by an expansion of unstable (CAG) triplet repeats in the genome. Evidence of anticipation, expansions of repeats in
subsequent generations and repeat instability has been demonstrated in many of the syndromes\(^1\)\(^-\)\(^3\). Traditional classification of ataxias has been based on the neuropathological and clinical criteria\(^1\)\(^-\)\(^5\). The clinical phenotypes in SCA are variable and often appear to overlap with one another, making accurate classification difficult without the genotype. Molecular dissection has aided in better understanding of the SCAs. Currently the classification of SCA is based on molecular genetics and there have been several genetic loci identified\(^1\). In spite of this, the precise biology of the genotype to phenotype conversion, is still incompletely understood.

Though a common diagnosis in referral clinics, no primary epidemiological data are available. One to five per 100,000 is the estimated worldwide prevalence rates for ataxias as a group\(^6\). At present, treatment is symptomatic, and it is hoped that a more detailed evaluation of genetic variations and clinical phenotype will help us better understand the biological basis of the clinical syndrome, and optimize or evolve improved management strategies.

Expansions at the SCA1, 2 and 3 loci are the most frequent, but there exist differences in their relative preponderance, worldwide\(^7\)\(^-\)\(^1\(^1\). Moreover, instances of high rates of SCA6, 7, and 8 have also been reported in certain samples from the American continent, Europe, Australia, Asia and South Africa. High frequency of SCA6 is reported from Japan, Germany and North American countries\(^8\). Similarly, SCA7 is reported as a common SCA type from South African families of Black ethnic origin\(^7\); European\(^8\) and South American countries\(^8\). SCA8 is relatively rare with only a handful of reports from Europe and North America\(^6\).

Observations regarding the ataxias have been reported from India for many years. The clinical syndromes, imaging studies, and familial basis have been well documented\(^1\(^2\)\(^-\)\(^1\(^5\). Several molecular studies have been reported over the past few years (Table I). SCA2 is reported as the most common diagnosis in a sample from Delhi\(^1\(^6\). In Kolkata, two studies, reported SCA2 as the most common mutation\(^1\(^4\),\(^1\(^7\), while another study done in the same region on 14 families from Bengal reported SCA3 as the most frequent mutation\(^1\(^8\). In our preliminary analysis of 60 families we have reported the prevalence of SCA1 as the most common mutation\(^1\(^9\). There seems to be a significant variation in the pattern of distribution of the ataxia syndromes within India.

As part of a larger effort exploring genetic basis of ataxia, we carried out an evaluation on probands attending the neurological clinics of the National Institute of Mental Health and Neuro Sciences (NIMHANS), a neuro-psychiatry medical facility in Bangalore, India from June 2003 to June 2006.

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**Table I. Distribution of confirmed SCA mutations in India**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Site</th>
<th>N</th>
<th>SCA1</th>
<th>SCA2</th>
<th>SCA3</th>
<th>U-SCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saleem et al(^1(^6)</td>
<td>Delhi</td>
<td>40</td>
<td>3</td>
<td>10</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>Basu et al(^1(^7)(^8)</td>
<td>Calcutta</td>
<td>26</td>
<td>5(19.2)</td>
<td>7(26.9)</td>
<td>3(11.5)</td>
<td>10(38.5)</td>
</tr>
<tr>
<td></td>
<td>(Kolkata)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Srivastava et al(^1(^9)</td>
<td>Delhi</td>
<td>77</td>
<td>12(15.6)</td>
<td>19(24.7)</td>
<td>2(2.6)</td>
<td>37(48)</td>
</tr>
<tr>
<td>Chakraverty &amp; Mukherjee(^1(^1)</td>
<td>Calcutta</td>
<td>14</td>
<td>2(14.3)</td>
<td>4(28.6)</td>
<td>5(35.7)</td>
<td>3(21.4)</td>
</tr>
<tr>
<td></td>
<td>(Kolkata)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinha et al(^1(^4)</td>
<td>Calcutta</td>
<td>28</td>
<td>4(14.3)</td>
<td>16(57.1)</td>
<td>0(0)</td>
<td>8(28.6)</td>
</tr>
<tr>
<td></td>
<td>(Kolkata)</td>
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<td></td>
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<tr>
<td>Rengaraj et al(^1(^7)(^8)</td>
<td>Vellore</td>
<td>17</td>
<td>17</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Mittal et al(^1(^8)</td>
<td>Delhi(^/)</td>
<td>167</td>
<td>37</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Bombay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Wadia et al(^1(^2)(^9)</td>
<td>Bangalore</td>
<td>51</td>
<td>*</td>
<td>14</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>(Mumbai)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Present study</td>
<td>Bangalore</td>
<td>284</td>
<td>34(32.4)</td>
<td>24(22.9)</td>
<td>15(14.3)</td>
<td>32(30.4)</td>
</tr>
</tbody>
</table>

N=Total number of index/families.

Figures in the parentheses are percentages.

U-SCA Negative for the SCA types tested.

* Details of analysis other than SCA1 were not mentioned.

# These studies were not comparable as families had history of ataxia but were not ADCA.

\(^\$\) 1 case of SCA6 (3.85%) was reported of the 26 families.

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Material & Methods

The patients were invited to participate in the study, after informed consent, and the ethics committee of the Institute had approved the study design. The study was carried out on 318 index patients from 284 families who agreed to take part in this study. The four large States of southern India contributed to 97 per cent of the sample Karnataka (n=205), Tamil Nadu (n=50), Andhra Pradesh (n=41) and Kerala (n=12) along with a small proportion from other States of India and the sub-continent. Ataxia was diagnosed based on the detailed evaluation of the clinical and family history by a neurologist; and was rated using the International Co-operative Ataxia Rating Scale (World Federation of Neurology)\(^2\). A well established positive family history was available in 127 (44.7%) families, and an autosomal dominant pattern of inheritance could be reliably confirmed in 105 families. During clinical evaluation 10 ml of venous blood was drawn for molecular testing after informed consent was obtained. DNA was isolated from peripheral blood leukocytes using a modification of the salting-out procedure\(^2\). Repeat sizes were estimated at the SCA1, SCA2, and SCA3/MJD loci by PCR as reported earlier\(^2, 22, 23\) in which one of the primers was fluorescently labeled to enable detection. The labeled PCR products were electrophoresed on a 5 per cent denaturing gel and allele sizes scored using Gene Scan analysis on an ABI Prism 377 automated DNA sequencer (Perkin Elmer, Foster City, USA). To assay for CAT interruptions, PCR products were digested with Sf\(\alpha\)NI prior to electrophoresis\(^24\).

Statistical analysis: The Pearson's correlation coefficient was used to test linear association between repeat length and age of onset, unless specified otherwise. The Student's t test was used to test mean difference between groups, which were considered at probability level less than or equal to 0.05. The Bonferroni method was used for multiple comparison. SPSS ver 10 software package was used to do the statistical analysis.

Results

On genetic testing of the 105 families with confirmed autosomal dominant inheritance SCA1 was found to be the most common mutation (34 families; 32.4%) followed by SCA2 (24 families; 22.9%) and SCA3 (15; 14.3%). There were 157 patients with no apparent family history, of which about 10 per cent (n=16) patients only showed an identifiable mutation. Of these, SCA2 (n=8) was the most prevalent followed by SCA1 (n=5) and SCA3 (n=3). Thus of the 318 individuals tested, 116 showed evidence of expansion of CAG repeats at one of the three loci tested (Table II).

At the SCA1 locus, the allele range in the samples was 21-72 repeats. The prevalence of ataxias is known to be linked to the frequency of large alleles at the locus. It has also been shown earlier that the large normal alleles at the SCA1 locus are conferred stability by CAT interruptions. We thus selected fifteen samples of index patients with intermediate range of expansion 34-36, and tested for the CAT interruption, by Sf\(\alpha\)NI digestion\(^2\). The samples were positive for the CAT interruption, suggesting that they were unlikely to undergo expansions in further transmissions, and were at low risk for becoming ‘pathological’.

At the SCA2 locus the repeat allele range in the normal was from 15-27, while the expanded allele was in the range between 35-66 repeats. There was a very high rate of homozygous 22 allele, as has been reported in other studies\(^2, 16\). However, there were 12 samples with the allele range between 25-30 repeats.

At the SCA3 locus the repeat allele range was from 14-78 repeats. No intermediate size repeats were found.

<table>
<thead>
<tr>
<th>SCA1</th>
<th>SCA2</th>
<th>SCA3</th>
<th>U-SCA</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=318</td>
<td>57</td>
<td>40</td>
<td>19</td>
</tr>
<tr>
<td>M/F</td>
<td>41/16</td>
<td>33/7</td>
<td>13/6</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>35±12</td>
<td>34±14</td>
<td>40±8</td>
</tr>
<tr>
<td>AOO (yr)</td>
<td>30±10</td>
<td>28±14</td>
<td>36±10</td>
</tr>
<tr>
<td>Range (yr)</td>
<td>10-54</td>
<td>3-65</td>
<td>18-53</td>
</tr>
<tr>
<td>Duration of illness (yr)</td>
<td>5±5</td>
<td>5±4</td>
<td>4±3</td>
</tr>
<tr>
<td>Normal allele range</td>
<td>6-44(^{22})</td>
<td>14-31(^2)</td>
<td>13-40(^{23})</td>
</tr>
<tr>
<td>Normal repeat range in tested patients</td>
<td>21-36</td>
<td>15-27</td>
<td>14-38</td>
</tr>
<tr>
<td>Disease range in tested patients</td>
<td>42-72</td>
<td>35-66</td>
<td>69-78</td>
</tr>
</tbody>
</table>

U-SCA: Negative for the SCA types tested
M/F: Male/female
AOO: Age of onset
* Alleles in the range 39-44 repeats are pathogenic only if CAT interruptions are absent.

Superscript numbers denote reference numbers
Values are mean ± SD
in our sample at this locus, and, in addition, no unaffected individuals with expansions were found.

There was a significant difference between the patients and the normal as was expected and a significant linear inverse correlation was observed between repeat size and age at onset for SCA1 ($P<0.001$, $r=-0.803$), SCA2 ($P<0.05$, $r=-0.63$, $P<0.01$), and SCA3 ($P<0.05$, $r=-0.433$, $P<0.05$).

**Discussion**

This study describes the molecular findings in ataxia patients, and the estimated occurrence of ataxias, at a tertiary center in southern India. More than a third of the subjects tested positive for the mutations tested, and SCA1, 2 and 3 were detected in descending order of frequency. The presence of a large number of SCA3 probands suggested that the syndrome is not as rare in India as previously suggested. The four large States of southern India contributed to 97 per cent of the sample (Karnataka, Tamil Nadu, Andhra Pradesh and Kerala) along with a small proportion from other States of India.

Diverse rates of SCAs have been observed since molecular testing became available over the past decade. SCA3 is the most common diagnosis worldwide; accounting for 20-50 per cent of all cases, but is thought to be rare in India. This is followed by SCA2, which accounts for between 10-20 per cent of all diagnoses, and quite common in Europe, USA and the UK. Multiple reports of SCA1 in Italy, South Africa, northern Japan and Russia, reflect the varied prevalence and suggest multiple founder effects. Frequency of SCA1 from South Africa (40%) and Italy (41%) represents the highest frequencies reported from any country.

In general, SCA 1 and 2 seem to be the most frequent mutation in populations of Caucasian ancestry, while SCA3 and DRPLA are more common in populations of far Eastern ancestry. Within India, as mentioned earlier, SCA2 is the most common form of ataxia in samples from northern, eastern and western India. However, a cluster of SCA3 families has also been reported from eastern India. Multiple cases of SCA1 have been reported in a large isolated community in Tamil Nadu, wherein all the individuals identified were tested positive. Our earlier analysis also suggested that SCA1 is almost twice as common in samples from Bangalore as compared to those from Delhi and the findings of the present study confirmed it. These findings indicate the presence of multiple founder effects in India, as has been suggested.

An association between the prevalence of SCA1 and the frequency of CAT interruptions in the SCA1 gene has been observed. One or more CAT interruptions were found in probands with a stretch of 34-36 CAG repeats at the SCA1 locus. As a general rule, the prevalence of expanded alleles is proportional to the frequency of large alleles with less than two interruptions in that population. Reports of relatively high frequency of unaffected chromosomes harbouring a single interruption in the Indian population, somewhat similar to that seen in the Sakha population, could be predisposing the population to a higher frequency of SCA1. However, the presence of variations - single nucleotide polymorphisms or the frequency of CAT interruptions, may prevent further expansions. These could be viewed as ‘evolutionary’ corrections to the expansion prone CAG repeat structure, for which possible mechanisms have been suggested. The detection of ‘stabilizing’ variations would thus be critical for counselling with respect to future expansions, as well as in estimating the prevalence of at-risk haplotypes in the population. The epidemiology of these ataxias may thus differ within India, influenced by migrations and recent history.

In our sample, majority (> 60%) of the SCA1 probands were from the interior Deccan region (Mysore, Andhra Pradesh, northern parts of Tamil Nadu), while more than half of the SCA3 probands were from northern Karnataka. It is interesting that SCA3 is more frequently diagnosed in samples from northern Karnataka, a region that lies in the hinterland of the erstwhile Portuguese colony of Goa. The worldwide prevalence of SCA3 has been linked to Portuguese maritime trade and colonial expansion. Pang et al. have compared the haplotypes in Indian, Cuban and British patients at the SCA2 locus to conclude that they share a common haplotype and might have a common ancestral mutation. It is generally observed that a specific type of SCA is prevalent in a homogeneous population, as in the two Indian series of Vellore and Bombay, but this is unlikely in a multiethnic community.

Extensive admixture within the Indian population has also been documented, thus stressing the need for further research to investigate the background diversity at each of these loci in different parts of India. Recently admixed populations like the USA have nearly similar rates of all three ataxias, but the clinical syndromes seem to suggest that subjects from different ethnic groups...
may present with varying clinical symptoms. In older admixed populations such as India, it is important to make accurate genotype assessments and explore the relationship between genotype and phenotype variations.

Two additional important issues emerged from our data. In a major proportion (5% of the total index cases) expansions were detected, even when there was no apparent family history. Early death of parents, the varying ages at onset and phenocopies may partly account for this. It has been suggested that sporadic cases could be due to unexplored penetrance, a result of unreliable recording, or due to unknown underlying genetic mutation stressing the need for genotyping even in the absence of clinical symptoms in the family. Secondly, a screen for these three common expansions at SCA1, SCA2 and SCA3 loci provided a confirmatory diagnosis for only 40% of the samples. Thus testing for the other forms of ataxia, of which a large number are now known, assumes significance. Many of these have been reported in single kindreds as yet, while some others are overwhelmingly more common in certain geographical regions (DRPLA/Japan, SCA8/Finland) or communities (SCA12/India).

In conclusion, our results reaffirmed the need for molecular diagnoses in ataxia syndromes and genetic counselling. Available tests need to be extended to the clinical services, for a better understanding of the genetic variations, and to enable efforts to detect novel mutations. This would be critical for developing models to understand the biological and clinical correlates of the mutant alleles, predict outcomes and develop interventions.

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at the SCA1, SCA2 and SCA6 loci in nine ethnic populations of eastern India. *Hum Genet* 2000; 106: 597-604.


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