Genetic testing for Spinocerebellar Ataxias (SCA) in Parkinsonism

MD SIDDIQUR RAHMAN1, YOSHITAKA NAGAI2, HAKIKO POPIEL3, MUZAHED UDDIN AHMED4, MD JALAL UDDIN5, TALSUSHI TODA6

Abstract:
Objective: The study was conducted to find out Spinocerebellar Ataxias (SCA) by genetic analysis from those presenting with parkinsonism in the Neurology department of Mymensingh Medical College. Materials and methods: A sample of about 5ml blood was collected by venipuncture in EDTA tube with informed consent from the patients following institutional ethics committee approval by genetic study from 7 healthy people and 9 patients. The neurological disorder along with a complete physical and/or psychological, as well as family history and demographic data was recorded with a prescribed questionnaire by the neurologists of Mymensingh Medical College. Extraction of genomic DNA from the venous blood using FlexiGene DNA kit (Qiagen, Japan) was performed in Department of Medicine, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh. The extracted DNA was stored and accumulated and then these DNA were sent to Division of Clinical Genetics, Department of Medical Genetics, Osaka University Medical School, Suita, Osaka 565 0871, Japan for PCR and further analysis. PCR amplification of the CAG repeat was performed for the SCA1, SCA2, SCA3, SCA6 loci using primers SCA1N-F1 and SCA1N-R1, SCA2-F1 and SCA2-R1, MJDF1 and MJDR1, SCA6-F1 and SCA6-R1, respectively. Results: SCA1 PCR of both healthy individual and suspected PD patients DNA is about 250 bp (no. of CAG repeats=36). SCA2 PCR products reveal the DNA products of about 150 bp (no. of CAG repeats=23) except one patient that we suspected and it was sequenced and revealed 175bp (no. of CAG repeats=30). SCA3 PCR products reveal the DNA products of about 250 bp (no. of CAG repeats=23) except one patient that we suspected and it was sequenced and revealed 175bp (no. of CAG repeats=30). SCA3 PCR product size of both healthy individual and patient DNA is within about 250 (no. of CAG=11) to 300 bp (no. of CAG repeats=28) except one patient which is about 320bp and its CAG repeats is about 34. SCA6 PCR product size of both healthy individual and patient DNA is about 150bp (no. of CAG=16). Conclusion: This is the first time from Bangladesh regarding the range of CAG repeats in patients as well as healthy individual. Key words: Spinocerebellar ataxias (SCA), parkinsonism, genetic testing

Introduction:
The autosomal dominant spinocerebellar ataxias (SCAs) are a complex group of neurodegenerative disorders characterized by progressive cerebellar ataxia of gait and limbs variably associated with ophthalmoplegia, pyramidal and extrapyramidal signs, dementia, pigmentary retinopathy and peripheral neuropathy1. There are several subtypes of SCAs: SCA1, SCA2, Machado-Joseph disease (MJD)/SCA3, SCA6, SCA7, SCA17 (Table 2). In all cases, expansion of CAG/ repeats in the respective genes have been implicated in the pathogenesis of the disease2-10. For example, the number of CAG repeats at the SCA1 locus varies

1. Professor, Department of Medicine & Director, Veterinary Clinic, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh and former JSPS Postdoctoral fellow, Division of Clinical Genetics, Department of Medical Genetics, Osaka University Medical School, 2-2-B9 Yamadaoka, Suita, Osaka 565-0871, Japan.
2. Associate Professor, Division of Clinical Genetics, Department of Medical Genetics, Osaka University Medical School, 2-2-B9 Yamadaoka, Suita, Osaka 565-0871, Japan.
3. Postdoctoral fellow, Division of Clinical Genetics, Department of Medical Genetics, Osaka University Medical School, 2-2-B9 Yamadaoka, Suita, Osaka 565-0871, Japan.
4. Ex Professor, Department of Medicine, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.
5. Assistant Professor, Department of Neurology, Mymensingh Medical College, Mymensingh 2200, Bangladesh
6. Professor and Chairman, Department of Medical Genetics, Osaka University, School of Medicine, Japan.
from 25 to 36 in normal individuals, while among affected individuals the range is 40 to 81\(^3\). Similarly, polymorphic CAG repeats in the ataxin2 gene (SCA2 locus) varies from 15 to 29 repeats among normal individuals and from 35 to 59 among affected individuals\(^3,9\).

Wide global variation in relative prevalence of SCA subtypes among autosomal dominant cerebellar ataxia (ADCA) patients has been observed. SCA1 has been reported to be far more common in Russia\(^11\) than any other SCA subtypes. Recently, Takano et al\(^12\) have reported that the general prevalence of SCA1 and SCA2 is significantly higher among white SCA pedigrees (15% and 14%, respectively) than in the Japanese (3% and 5% respectively), whereas relative prevalence of SCA3 is higher in the Japanese pedigrees (43%) than in whites (30%). Also, SCA6 appear to be less frequent in white populations (5%) than in Japanese populations (11%) respectively\(^12\). It has been reported that SCA2 is exclusively responsible for all ataxia cases in the Indian population\(^13\). In a study of six Indian SCA2 pedigrees, Wadia et al\(^13\) observed CAG repeat expansion in 14 affected family members at the SCA2 locus. All of these patients showed slow saccades and peripheral neuropathy. An inverse correlation between repeat size and age at onset was observed with repeat numbers varying from 36 to 45 repeats\(^13\). Similar observation has been reported by Saleem et al\(^14\) in an independent set of 39 SCA pedigrees principally from northern India. SCA2 is also the most common form of hereditary ataxias among Korean patients\(^15\), constituting 12.6% of all SCA patients, followed by SCA6 (6.9%) and SCA3 (4.6%).

SCA3 is more common in Germany\(^16\), Brazil\(^17\), United States\(^18,19\), Portugal\(^20\) and Japan\(^21\). In Portugal, expansion at SCA3/MJD locus was observed in 74% of ADCA patients, followed by expansion at SCA2 locus in 4% of patients. The investigators did not find any SCA1, SCA6 mutation\(^20\). Similarly, in the Japanese population, Sasaki and Tashiro\(^21\) have observed that 24.6% of patients possessed CAG expansion at the SCA3 locus, followed by expansions at SCA6 (11.8%), SCA1 (10.5%) and SCA2 (4.4%) loci. SCA6 is the most common (5%) expansion mutation in SCA patients in the United Kingdom, followed by SCA2\(^22\).

Expansion at the SCA6 locus is also very frequent (13%) in ADCA families of Germany\(^23\). Spinocerebellar ataxia type 3 (SCA3), can present with parkinsonism. However, classically, atypical features, including pyramidal and cerebellar signs, peripheral neuropathy, and/or anterior horn cell dysfunction, are also seen\(^24\). To our knowledge, there are no previous reports of regarding the CAG repeat in patients as well as healthy individuals in Bangladesh. In this study we present the results of DNA analysis of CAG repeats in the healthy individual and as well as patients provisionally diagnosed as Parkinson Disease (PD) in Department of Neurology, Mymensingh Medical College, Mymensingh-2200, Bangladesh.

Materials and Methods:
A sample of about 5ml blood was collected by venipuncture in EDTA tube with informed consent from the patients following approval by institutional ethics committee for genetic study from 7 healthy people and 9 patients who came to Department of Neurology, Mymensingh Medical College, Mymensingh 2200, Bangladesh. The neurological disorder along with a complete physical and/or psychological, as well as family history and demographic data (sex, age at onset, age at referral, residence) was recorded with a prescribed questionnaire by the neurologists of Mymensingh Medical College. Extraction of genomic DNA from the venous blood using FlexiGene DNA kit (Qiagen, Japan) was performed in Department of Medicine, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh. Briefly, pipetting of 7.5 ml Buffer FG1 into a 15 ml centrifuge tube and then adding of 3 ml whole blood and mixing by inverting the tube 5 times. Centrifuging for 5 min at 2000 x g in a swing-out rotor and discarding the supernatant and leaving the tube inverted on a clean piece of absorbent paper for 2 min, taking care that the pellet remains in the tube. Adding of 1.5 ml Buffer FG2, closing the tube, and vortexing immediately until the pellet is completely homogenized. Inverting the tube 3 times, placing it in a heating block or water bath, and incubate at 65 degree C for 10 min. Adding of 1.5 ml isopropanol (100%) and
mixing thoroughly by inversion until the DNA precipitate becomes visible as thread or a clump and centrifuging for 3 min at 2000 x g. Discarding the supernatant and briefly invert the tube onto a clean piece of absorbent paper, taking care that the pellet remains in the tube. Adding of 1.5 ml 70% ethanol and vortex for 5 second and centrifuging for 3 min at 2000 x g. Discarding of the supernatant and leaving the tube inverted on a clean piece of absorbent paper for at least 5 min, taking care that the pellet remains in the tube. Air-drying the DNA pellet until all the liquid has evaporated and adding 300 microliter FG3, vortexing for 5 second at low speed, and dissolve the DNA by incubating for 1 hour at 65 degree C in a heating block or water bath and stored at -70 degree C until used.

The extracted DNA was stored and accumulated and then these DNA were sent to Division of Clinical Genetics, Department of Medical Genetics, Osaka University Medical School, Suita, Osaka 565 0871, Japan for PCR and further analysis. PCR amplification of the CAG repeat was performed for the SCA1, SCA2, SCA3, SCA6 loci using primers SCA1N-F1 and SCA1N-R1, SCA2-F1 and SCA2-R1, MJDF1 and MJDR1, SCA6-F1 and SCA6-R1, respectively (Table 1) and the condition for amplification were essentially same as described earlier. PCR products were checked in 3% agarose gel and the sequencing of the suspected PCR products were performed using Genescan system (version 2.02) in an ABI-377 automated DNA sequencer.

**Table-I**

*PCR Primer Sequences used in this study for detection of SCA1, SCA2, SCA3, SCA6 loci.*

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCA1N-F1</td>
<td>CTG GCC AAC ATG GGC AGT CTG AG</td>
</tr>
<tr>
<td>SCA1N-R1</td>
<td>GGA GAA CTG GAA ATG TGG ACG TA</td>
</tr>
<tr>
<td>SCA2-F1</td>
<td>CCC TCA CCA TGT CGC TGAAGC</td>
</tr>
<tr>
<td>SCA2-R1</td>
<td>CGA CGC TAG AAG GCC GCT G</td>
</tr>
<tr>
<td>MJDF1</td>
<td>CCA GTG ACT ACT TTG ATT CG</td>
</tr>
<tr>
<td>MJDR1</td>
<td>CTT ACC TAG ACT TTC ATT CG</td>
</tr>
<tr>
<td>SCA6-F1</td>
<td>CAC GTG TCC TAT TCC CTT GTG ATC C</td>
</tr>
<tr>
<td>SCA6-R1</td>
<td>TGG GTA CCT CCG AGG GCC GCT GGT G</td>
</tr>
</tbody>
</table>

**Table-II**

*Different types of SCA with its gene range of CAG repeat and affected regions.*

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Gene</th>
<th>CAG repeat</th>
<th>Affected regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>spinocerebellar ataxia type 1 (SCA1)</td>
<td>ataxin-1</td>
<td>6-44</td>
<td>39-83 Cerebellar Purkinje cells, dentate nucleus, brainstem</td>
</tr>
<tr>
<td>spinocerebellar ataxia type 2 (SCA2)</td>
<td>ataxin-2</td>
<td>15-31</td>
<td>36-63 Cerebellar Purkinje cells, brainstem</td>
</tr>
<tr>
<td>spinocerebellar ataxia type 3 (SCA3)</td>
<td>ataxin-3</td>
<td>12-41</td>
<td>55-84 Dentate nucleus, basal ganglia, brainstem, spinal cord</td>
</tr>
<tr>
<td>Machado-Joseph disease</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>spinocerebellar ataxia type 6 (SCA6)</td>
<td>? 1A calcium channel</td>
<td>4-18</td>
<td>21-33 Cerebellar Purkinje cells</td>
</tr>
<tr>
<td></td>
<td>ataxin-7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>spinocerebellar ataxia type 7 (SCA7)</td>
<td>TATA-binding protein</td>
<td>25-44</td>
<td>46-63 Cerebellar, cerebral cortex, basal ganglia</td>
</tr>
<tr>
<td>spinocerebellar ataxia type 17 (SCA17)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results and Discussion:
SCA1 PCR of both healthy individual and suspected PD patients DNA is about 250 bp (no. of CAG repeats=36) (Figure 1). SCA2 PCR products reveal the DNA products of about 150 bp (no. of CAG repeats=23) (Figure 2) except one patient that we suspected and it was sequenced and revealed 175bp (no. of CAG repeats=30) (Figure 3). SCA3 PCR product size of both healthy individual and patient DNA is within about 250 (no. of CAG=11) to 300 bp (no. of CAG repeats=28) except one patient which is about 320bp and its CAG repeats is about 34 (Figure 4). SCA6 PCR product size of both healthy individual and patient DNA is about 150bp (no. of CAG=16) (Figure 5).

The polyglutamine diseases are a group of inherited neurodegenerative diseases caused by the expansion of a CAG repeat coding for glutamine in each disease-causing gene. Currently there is no effective treatment against the polyQ diseases. In

![Figure 1: SCA1 PCR of 7 healthy individuals DNA (above) and 9 patients DNA (below): the PCR product size of both healthy (H1-H7) and patient DNA (P1-P9) is about 250bp (no. of CAG repeats=36) and therefore revealed no difference between the healthy individual and patient DNA, N/C= negative control, M= molecular marker.](image)

![Figure 2: SCA2 PCR of 7 healthy individuals DNA (above) and 9 patients DNA (below): the PCR product size of both healthy (H1-H7) and patient (P1-P9) DNA is about 150bp (no. of CAG repeats=23) except no. 8 of patient sample which is about 175bp and this product was sequenced and it revealed 30 CAG repeats that is within the normal range, N/C= negative control, M= molecular marker.](image)

the pathogenesis of the polyQ diseases, expansion of the polyQ stretch is thought to cause misfolding of the protein, resulting in pathogenic protein-protein interactions including aggregate formation, leading to neuronal dysfunction and eventual neuronal death. Although extensive research has been performed regarding polyQ-mediated neuronal cell death, recent detailed analyses of brains of polyQ disease patients as well as mouse models have revealed that neuronal phenotypes develop before marked cell death is observed. SCA is one of several polyglutamine diseases.

The first genetically documented family with MJD (SCA3) in India was reported by Chakravarty et al.

19
**Fig.-3:** Sequenced data SCA2 PCR products from one patient (patient no. 8 that was suspected of SCA2 and it revealed 175bp (no. of CAG repeats=30).

**Figure 4:** SCA3 PCR of 7 healthy individuals DNA (above) and 9 patients DNA (below): the PCR product size of both healthy (H1-7) and patient (P1-P9) DNA is within about 250 (no. of CAG repeats=11) to 300bp (no. of CAG repeats=28) except no. 9 of patient which is about 320bp and its CAG repeats no. is about 34 that not pathogenic (pathogenic range is 62-79 in India), N/C= negative control, M= molecular marker.

**Fig.-5:** SCA 6 PCR of 7 healthy individuals DNA (above) and 9 patients DNA (below): the PCR product size of both health (H1-H7) and patient DNA (P1-P9) is about 150bp (no. of CAG repeats=16) and therefore revealed no difference between the control and patient DNA, N/C= negative control, M= molecular marker.
in 1996 among Bengali family. Subsequently, genetic studies in families with cerebellar ataxias have been made by Wadia et al\textsuperscript{13,28} in Mumbai and Sinha at Ranchi\textsuperscript{29} and updated reviews on the subject have been written by Sinha\textsuperscript{30}, Banerjee and Chakravarty\textsuperscript{31} and Wadia\textsuperscript{32}. More recently two multi-authored communications highlighted on the molecular genetic aspects of autosomal dominant hereditary ataxias in India including Bengali family\textsuperscript{14,33}. Basu et al\textsuperscript{33} detected CAG repeat expansion in 6 patients (10.5%) at the SCA1 locus (range of expanded CAG repeat no. 44-52), ten of the 57 patients (17.5%) had CAG repeat expansion at the SCA2 locus (range of expanded CAG repeat no. 39-45), while four (7%) had CAG expansion at the SCA3/MJD locus (range of expanded CAG repeat no. 62-79) and at the SCA6 locus there was a single patient (1.8%) with 21 CAG repeats. Ghosh et al\textsuperscript{34} reported five ethnic Bengali subjects with positive family history and found 3 cases of SCA1, 2 with SCA3 mutation and none with SCA2. Chakravarty and Mukherjee\textsuperscript{35} also reported SCA in Ethnic Bengali; 2 families with SCA1, 4 families with SCA2, 5 families with SCA3 but there are no reports yet from Bengali population in Bangladesh (peoples in Bangladesh & West Bengal, a State of India are known as Bengali).

Bangladesh and India are in the same geographical region and it was the same country until the year 1947 but the people are not necessarily genetically akin. In this study we had examined 7 healthy individual in Mymensingh and 9 patients who came to the neurologists of Mymensingh Medical College and were diagnosed as Parkinson Disease. The age group of all the patients were 57-80 and all the patients has the tremor and most of patients has the tendency to fall down and memory impairment. Spinocerebellar ataxia type 3 (SCA3), can present with parkinsonism. However, classically, atypical features, including pyramidal and cerebellar signs, peripheral neuropathy, and/or anterior horn cell dysfunction, are also seen\textsuperscript{24}. The patients diagnosed as PD were both male and female and they have other complaints but all are diagnosed as PD by the neurologists in Mymensingh Medical College.

The SCA1 PCR product size of both healthy individual and patient DNA is about 250bp (no. of CAG repeats=29) and therefore revealed no difference between the healthy individual and patient DNA. The SCA2 PCR product size of both healthy individual and patient DNA is about 150bp (no. of CAG repeats=23) except no. 8 of patient sample which is about 175bp and this product was sequenced and it revealed 30 CAG repeats that is within the normal range. The SCA3 PCR product size of both healthy individual and patient DNA is within about 250 (no. of CAG repeats=11) to 300bp (no. of CAG repeats=28) except no. 9 of patient which is about 320bp and its CAG repeats no. is about 34 but not pathogenic (pathogenic range is 62-79 in India). The SCA6 PCR product size of both healthy individual and patient DNA is about 150bp (no. of CAG repeats=16) and therefore revealed no difference between the healthy individual and patient DNA.

Spinocerebellar ataxia type 3 (SCA3), can present with parkinsonism but has not been previously reported, to our knowledge from Bangladesh. However, atypical, though also levodopa-responsive, parkinsonism has been previously reported to occur in African American families, suggesting that this phenotype is associated with African ancestry. In this regard, it is perhaps significant that all the individuals with parkinsonism have relatively low numbers of repeats (normal, 16-34; pathologic, 60-84). In families in which linkage analysis is being performed to determine a locus for autosomal dominant parkinsonism suggestive of PD, evaluation for the \textit{MJD/SCA3} mutation is indicated. In our study in Bangladesh we never recorded any CAG repeat within the pathological ranges. This is the first time from Bangladesh regarding the range of CAG repeats in patients as well as healthy individual.

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