

## Polyglutamine Diseases after Genetic Analysis in Patients Clinically Diagnosed as Parkinson's Disease in Bangladesh

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### Abstract

**Introduction:** Polyglutamine (polyQ) diseases are Huntington's Disease (HD), Dentatorubropallidoluysian Atrophy (DRPLA), Spinobulbar Muscle Atrophy (SBMA) and the Spinocerebellar Ataxias (SCA) type 1, 2, 3, 6, 7 and 17. These diseases are characterized by an expansion of the CAG-trinucleotide repeat region in the respective disease-related genes.

**Objective:** To find out Polyglutamine (polyQ) diseases by genetic analysis from those patients presenting with Parkinsonism in the Neurology department of Mymensingh Medical College hospital.

**Materials and Methods:** This study was conducted on 7 healthy people and 9 patients of Neurology Department, Mymensingh Medical College Hospital in 2010. 5ml blood was collected from each individual by venipuncture. The complaints of patients along with physical and/or psychological findings, family history including demographic data were recorded with a questionnaire by the neurologists of Hospital. Informed consents from the patients were taken and ethical clearance sought as well. Extraction of genomic DNA from the venous blood using FlexiGene DNA kit (Qiagen, Japan) was performed in Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh. The extracted DNA was stored, accumulated and 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 up to amplification.

**Results:** HD PCR products reveal the DNA product of about 110bp (no. of CAG repeats=21) to 150bp (no. of CAG repeats=34) in both healthy individual and patient repeats=36). SCA2 PCR products reveal the DNA products of about 150 bp (no. of CAG repeats=23) except one patient

and it was 175bp (no. of CAG repeats=30). SCA3 PCR product size of both healthy individual and patient DNA was within about 250 (no. of CAG=11) to 300 bp (no. of CAG repeats=28) except one patient which was about 320bp and its CAG repeats was about 34. SCA6 PCR product size of both healthy individual and patient DNA was about 150bp (no. of CAG=16). DRPLA PCR product size of healthy individual and patients DNA was about 142 (no. of CAG repeats=19) to 165bp (no. of CAG repeats=27) except one healthy individual DNA which was about 205 bp and its CAG repeat was 40.

**Conclusion:** Genetic analysis and PCR has been an important tool to visualize the root cause of the diseases-the CAG repeat can facilitate a definitive clue to address Poly Q diseases. This is so far first time study in Bangladesh on the range of CAG repeats in patients as well as healthy individual.

**Key-words:** Polyglutamine(PolyQ), CAG repeat, Huntington's Disease, Spinocerebellar Ataxias(SPA).

### Introduction

Polyglutamine (polyQ) diseases are Huntington's Disease (HD), Dentatorubropallidoluysian Atrophy (DRPLA), Spinobulbar Muscle Atrophy (SBMA) and the Spinocerebellar Ataxias (SCA) type 1, 2, 3, 6, 7 and 17. These diseases are caused by an expansion of the CAG-trinucleotide repeat region in the respective disease-related genes. Although the different polyQ proteins are widely expressed in cells throughout the brain, there is a high variability in the cell type loss in different brain areas. In most polyQ disorders the disease becomes manifested when the polyQ expansion exceeds 36–40 glutamines. The length of the polyQ expansion is inversely correlated with the age of onset of the disease. Dominantly inherited disease caused by a toxic gain of function of the polyQ-expanded protein<sup>1</sup>.

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Huntington's disease prevalence in white population is 5-7 per 100,000 and in Japanese<sup>2</sup> is 0.5 per 100,000. Higher incidence of HD has been observed in white populations than African or Asian<sup>3</sup>. Wide global variations in relative prevalence of SCA patients have been observed. SCA1 has been reported to be far more common in Russia than any other SCA subtypes<sup>4</sup>. Takano et al reported that the general prevalence of SCA1 and SCA2 is significantly higher among white SCA pedigrees (15% and 14% respectively) than in the Japanese<sup>2</sup>. SCA2 is also the most common (12.6%) among Korean patients followed by SCA6 (6.9%) and SCA3 (4.6%)<sup>5</sup>. SCA3 is quite frequent in many countries with different ethnic backgrounds, such as Portugal (80%), Germany (40%), Japan (40%), and France (30%). SCA6 and DRPLA are less frequent in white population (5% and 0% respectively) than in Japanese populations (11% and 20% respectively).

There are some reports of HD, based on family history and clinical findings from India<sup>6</sup> but none from Bangladesh. Recently and for the first time from India<sup>7</sup>, analyzed the CAG repeats in the Huntingtin gene of HD patients where the range of expanded CAG repeat number was 41-56 and the maximum number of alleles (12) was found in Bengali population of that country but there are no reports yet from Bengali population in Bangladesh.

First genetically responsible family with Machado–Joseph disease (MJD) (SCA3) in India was reported by Chakravarty et al<sup>8</sup> in 1996 among Bengali family. Subsequently, genetic studies in families with cerebellar ataxias have been made by Wadia et al<sup>9</sup> and Peng et al<sup>10</sup> in Mumbai and Sinha at Ranchi<sup>11</sup> and updated reviews on the subject have been written by Sinha<sup>12</sup>, Banerjee and Chakravarty<sup>13</sup> and Wadia<sup>14</sup>. More recently two multi authored communications highlighted on the molecular genetic aspects of autosomal dominant hereditary ataxias in India including Bengali family<sup>15,16</sup>. Basu et al in their study<sup>16</sup> 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 and they have not detected any patient with expansion in the DRPLA loci. Ghosh et al reported five ethnic Bengali subjects with positive family history and found 3 cases of SCA1, 2 with SCA3 mutation and none with SCA2<sup>17</sup>. Chakravarty and Mukherjee 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 and West Bengal, a state of India are known as Bengali)<sup>18</sup>. The people of Bangladesh and India seem to be alike but are not necessarily genetically akin. The study was carried out to know the status of HD, DRPLA, SCA 1, 2, 3 and 6 in Bangladesh by genetic testing who were suffering from Parkinson Disease (PD).

## Materials and Methods

This study was carried out in the department of Neurology, Mymensingh Medical College Hospital from April 2010 to December 2010. Informed consents were taken from each of study patients and healthy persons. A sample of about 5ml blood was collected by venipuncture in EDTA tube. The complaint of patients along with physical and/or psychological findings, family history and demographic data (sex, age at onset, age at referral, residence) as well was recorded with a prescribed questionnaire by the neurologists of Hospital. Ethical clearance was taken from ethical review committee of the medical college. Extraction of genomic DNA from the venous blood using FlexiGene DNA kit (Qiagen, Japan) was performed in Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, 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° 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° C in a heating block or water bath and stored at -70° 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 in the 1T15 gene was performed with primers HD1 and HD319 and SCA1, SCA2, SCA3, SCA6 and DRPLA loci were amplified using primers SCA1N-F1 and SCA1N-R1, SCA2-F1 and SCA2-R1, MJDF1 and MJDR1, SCA6-F1 and SCA6-R1, CTG-B37-F and CTG-B37-R, respectively (Table-I) and the conditions for PCR and interpretation of the results were performed as described earlier<sup>20-22</sup>. 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 of Huntingtin (1T15) gene and SCA1, SCA2, SCA3, SCA6 and DRPLA loci

Primer	Sequences
HD 1	ATG AAG GCC TTC GAG TCC CTC AAG TCC TTC
HD3	GGC GGT GGC GGC TGT TGC TGC TGC TGC
SCA1N-F1	CTG GCC AAC ATG GGC AGT CTG AG
SCA1N-R1	GGA GAA CTG GAA ATG TGG ACG TA
SCA2-F1	CCC TCA CCA TGT CGC TGA AGC
SCA2-R1	CGA CGC TAG AAG GCC GCT G
MJD-F1	CCA GTG ACT ACT TTG ATT CG
MJD-R1	CTT ACC TAG ATC ACT CCC AA
SCA6-F1	CAC GTG TCC TAT TCC CCT GTG ATC C
SCA6-R1	TGG GTA CCT CCG AGG GCC GCT GGT G
CTG-B37-F	CAC CAG TCT CAA CAC ATC ACC ATC
CTG-B37-R	CCC TCC AGT GGG TGG GGA AAT GCT C

## Results

HD PCR products reveal the DNA product of about 110bp (no. of CAG repeats=21) to 150bp (no. of CAG repeats=34) in both healthy individual and patient DNA (Figure-1). SCA1 PCR of both healthy individual and patients DNA was about 250 bp (no. of CAG repeats=36) (Figure-2). SCA2 PCR products reveal the DNA products of about 150 bp (no. of CAG repeats=23) except one patient and it was 175bp (no. of CAG repeats=30) (Figure-3). SCA3 PCR product size of both healthy individual and patient DNA was within about 250 (no. of CAG=11) to 300 bp (no. of CAG repeats=28) except in one patient which was about 320bp and its CAG repeats was about 34 (Figure-4). SCA6 PCR product size of both healthy individual and patient DNA was about 150bp (no. of CAG=16) (Figure-5). DRPLA PCR product size of healthy individual and patients DNA was about 142 (no. of CAG repeats=19) to 165bp (no. of CAG repeats=27) except one healthy individual DNA which was about 205 bp and its CAG repeat was 40 (Figure-6).

## Discussion

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 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<sup>23</sup>. Among the polyglutamine diseases, we checked only HD, SCA1, SCA2, SCA3, SCA6 and DRPLA.

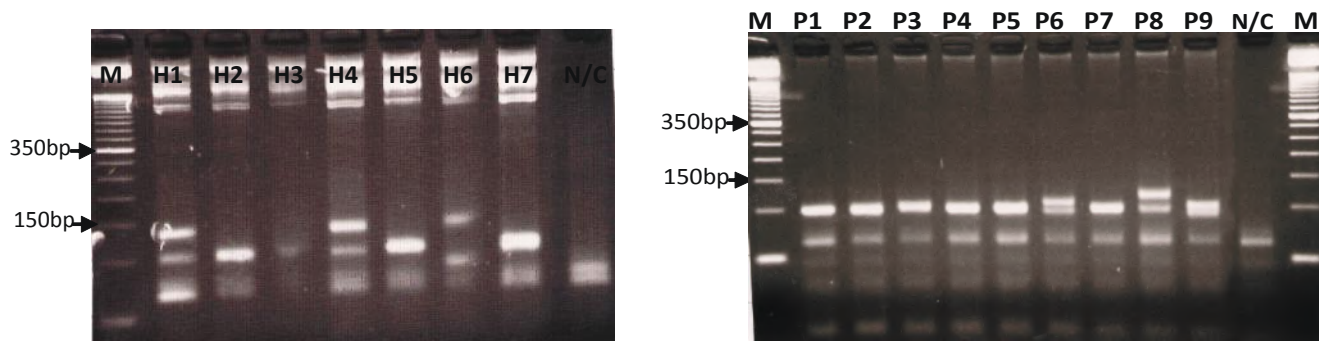
The PCR product size of healthy individual and patient DNA was within about 110 (no. of CAG repeats=21) to 150bp (no. of CAG repeats=34) and this range is within the normal limit in India<sup>24</sup> and therefore revealed no difference between the healthy individual and patient DNA. The SCA1 PCR product size of both healthy individual and patient DNA was about 250bp (no. of CAG repeats=36) and therefore revealed no difference between the healthy individual and patient DNA. The SCA2PCR product size of both healthy individual and patient DNA was about 150bp (no. of CAG repeats=23) except no. 8 of patient sample which was about 175bp and this product was sequenced and it revealed 30 CAG repeats that is within the normal range (according to published report of India). The SCA3 PCR product size of both healthy individual and patient DNA was within about 250 (no. of CAG repeats=11) to 300bp (no. of CAG repeats=28) except no. 9 of patient which was about 320bp and its CAG repeats no. was about 34 that is not pathogenic (pathogenic range is 62-79 in India). The SCA6 PCR product size of both healthy individual and patient DNA was about 150bp (no. of CAG repeats=16) and therefore revealed no difference between the healthy individual and patient DNA. The DRPLA PCR product size of both healthy individual and patient DNA was within about 142 (no. of CAG repeats=19) to 165bp (no. of CAG repeats=27) except the no. 7 of healthy individual which was 205bp and its CAG repeat was 40. It seems very exceptional because the normal range of CAG repeat is detected as 8-25 in India<sup>15</sup>.

Bangladesh and India are in the same geographical region but the people are not necessarily genetically akin. In this study, 7 healthy individual of Mymensingh and 9 patients who

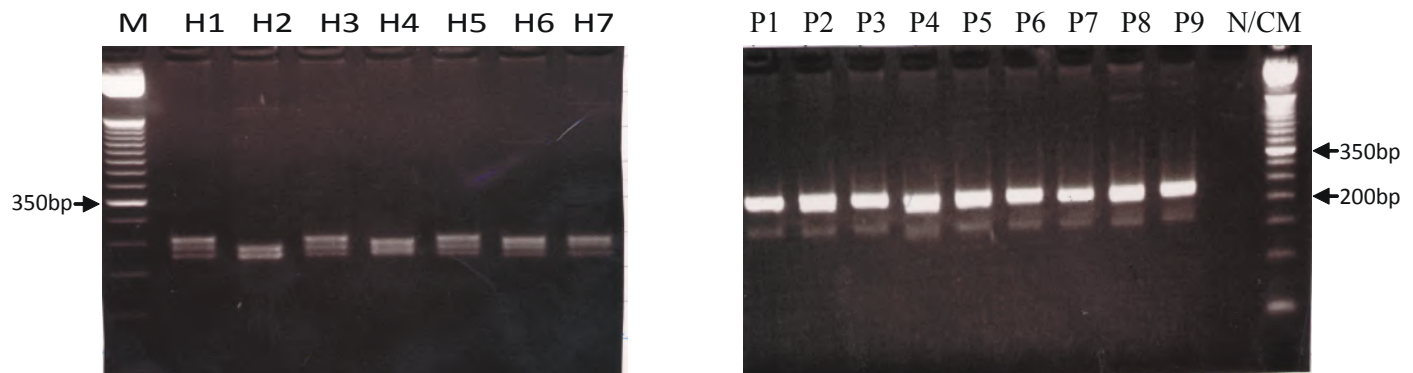


who reported to the neurologists of Mymensingh Medical College Hospital with a complaint of Parkinson Disease had been examined. The age groups of all the patients were 57-80 years and all the patients had tremor and most of the patients had the tendency to fall down and memory impairment. People with neurological diseases, such as Parkinson's disease and Huntington's disease, may also experience cognitive and emotional symptoms, including anxiety, depression and memory deficits. Parkinson's (PD) and Huntington's disease (HD) are chronic neurodegenerative conditions of the brain with a variety of clinical presentations including a disorder of movement and a range of nonmotor deficits. HD is genetic in origin and the causative gene and protein known, namely mutant Huntingtin, which leads to widespread early neuronal dysfunction and death throughout the brain. In contrast, the etiology of sporadic PD is unknown, and the pathology targets the nigrostriatal dopaminergic neurons with the formation of  $\alpha$ -synuclein positive Lewy bodies. In both diseases, the ability to accurately diagnose the disease in the early stages and monitor progression over time remains a major challenge given the majority of the pathology is sited deep within the CNS<sup>24</sup>.

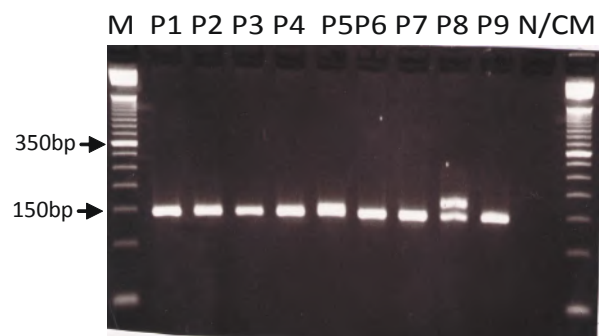
Analysis of the CAG repeat locus among 28 unrelated clinically diagnosed HD patients from the eastern part of India showed that the range of expansion of CAG repeats was between 41 and 56 in the huntingtin gene. The smallest size of the CAG repeat which causes HD is not defined precisely. In Western populations and in the Japanese, the lowest number has been reported to be around 37. 28-35 CAG repeats in normal white people<sup>3</sup> while 6-35 in normal and 36-250 CAG repeats in patients has been recorded<sup>25</sup>. 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 MJD/SCA3 mutation is indicated. In our study, the smallest size of the CAG repeat is found to be 23 and 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.



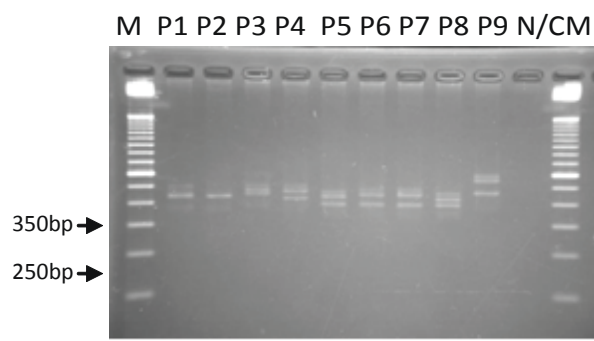
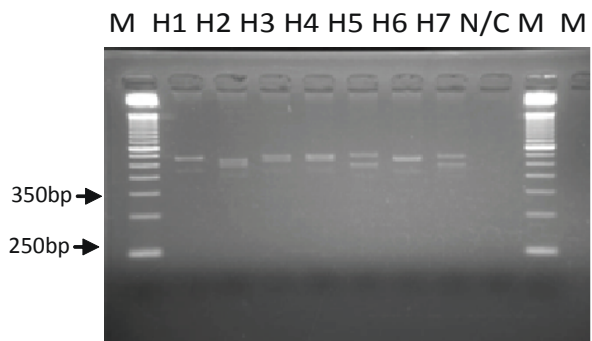
**Fig-1:** HD PCR of 7 healthy individuals DNA (left side) and 9 patients DNA (right side): the PCR product size of both healthy (H1-H7) and patient (P1-P9) DNA is within about 110 (no. of CAG repeats=21) to 150bp (no. of CAG repeats=34) and therefore revealed no difference between the healthy individual and patient DNA, N/C= negative control, M= molecular marker



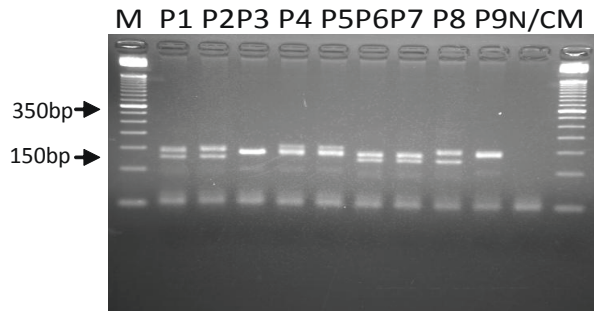
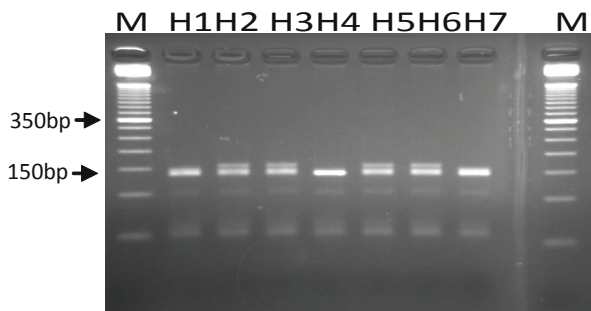
**Fig-2:** SCA1 PCR of 7 healthy individuals DNA (left side) and 9 patients DNA (right side): The PCR product size of both healthy (H1-H7) and patient (P1-P9) DNA is about 250bp (no. of CAG repeats=36) and therefore revealed no difference between the healthy and patient DNA, N/C= negative control, M= molecular marker



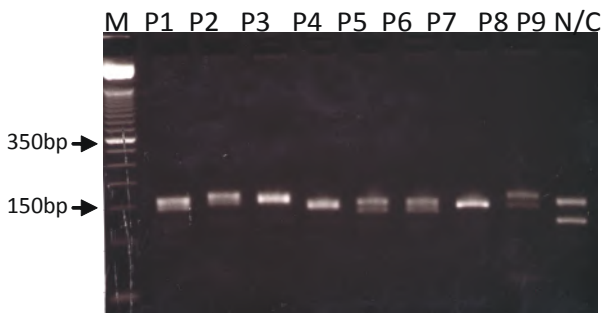
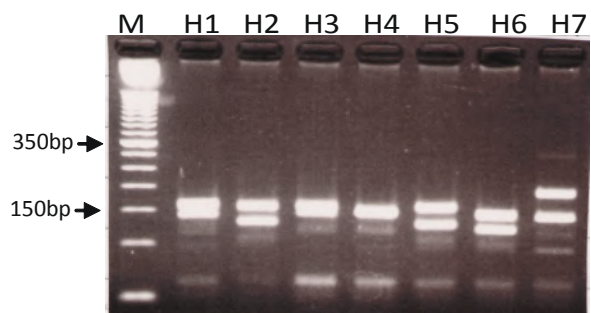
**Fig-3:** SCA2 PCR of 7 healthy individuals DNA (left side) and 9 patients DNA (right side): The PCR product size of both healthy (H1-H7) and patient DNA (P1-P9) is about 150bp (no. of CAG repeats=23) except no. 8 of patient sample which is about 175bp and this product was sequenced and it revealed 30 CAG repeats that is within the normal range (according to published report of India), N/C= negative control, M= molecular marker



**Fig-4:** SCA3 PCR of 7 healthy individuals DNA (left side) and 9 patients DNA (right side): The PCR product size of both healthy 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 that not pathogenic (pathogenic range is 62-79 in India), N/C= negative control, M= molecular marker



**Fig-5:** SCA6 PCR of 7 healthy individuals DNA (left side) and 9 patients DNA (right side): The PCR product size of healthy (H1-H7) and patient DNA (P1-P9) is about 150bp (no. of CAG repeats=16) and therefore revealed no difference between the healthy individual and patient DNA, N/C= negative control, M= molecular marker



**Fig-6:** DRPLA PCR of 7 healthy individuals DNA and 9 patients DNA: The PCR product size of both healthy (H1-H7) and patient (P1-P9) DNA is within about 142 (no. of CAG repeats=19) to 165bp (no. of CAG repeats=27) except the no. 7 of healthy individual which is 205bp and its CAG repeat is 40. It seems very exceptional because the normal range of CAG repeat was detected as 8-25 in India (Saleem Q et al, 2000)<sup>15</sup>

## Conclusion

Polyglutamine diseases are a group of inherited Neurodegenerative disorder caused genetically by the expansion of CAG repeat. Till today, there is no effective treatment against this PolyQ disease. PCR has been an important tool to visualize the root cause of the diseases- the CAG repeat. So this genetic endeavour by seeing the CAG repeats can facilitate to attain a definitive clue to address Poly Q diseases. This study so far the first time in Bangladesh, has found the CAG repeats in patients as well as in healthy individual.

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