Molecular spectrum and allelic frequency of different subtypes (1, 2, 3, 6 and 7) of Spinocerebellar ataxia in the Indian population

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1. Introduction

Spinocerebellar ataxia (SCA) is a slowly progressive, autosomal dominant disorder that is characterized by a marked intra-familial and inter-familial clinical variability. The global prevalence of SCA is 1 to 5 per 100,000 populations. There are several subtypes of SCA reported; the subtypes prevalent in India include SCA 1, 2, 3, 6, and 7. Studies have reported that SCA subtypes are primarily caused by triplet repeat expansion and in some cases by point mutations, deletions, and missense mutations.

Genes related to SCA subtypes (1, 2, 3, 6, and 7) are ATXN1, ATXN2, ATXN3, CACNA1A, and ATXN7, respectively. SCA is a disorder involving a triplet cytosine-adenine-guanine (CAG) repeat; the normal range of repeats for subtypes 1, 2, 3, 6, and 7 is 6 to 36, 15 to 31, 12 to 40, 4 to 18, and 4 to 19, respectively (according to the American College of Medical Genetics and Genomics, or ACMG). This disorder shows a phenomenon of genetic anticipation in which affected individuals in succeeding generations have an earlier age of onset and more severe clinical features in the next generation, due to the expansion of the repeat number during gametogenesis.

Several studies have reported that the normal and disease range of repeats for different triplet repeat disorders vary considerably between populations (1-3). Screening populations for the polymorphic range of repeats helps to establish the normal range of repeats for a particular geographical region, enabling proper molecular diagnosis. Moreover, repeats that are large but still within the normal range, referred to as large normal alleles, are known to be indicators of disease prevalence (4-7).

Several studies have sought to distinguish the normal range of repeats in different Indian populations (8,9). To explain the frequency of normal and numerous repeats and the prevalence of ataxia in a given population, the current study examined the five most common types of ataxia, namely, SCA1, SCA2, SCA3, SCA6, and SCA7, in samples from 200 healthy controls.
2. Materials and Methods

Blood samples were collected from 200 healthy subjects. Two 2 mL of peripheral venous blood was placed in a EDTA tube for DNA isolation using the standard phenol chloroform method. The quality and quantity of DNA was assured using agarose gel electrophoresis and spectrophotometry, respectively.

Multiplex PCR was performed to amplify SCA genes (Subtypes 1, 2, 3, 6, and 7) using chimeric primers (Table 1). A multiplex PCR reaction of 25 ul consisted of \textit{ATXN1}, \textit{ATXN2}, \textit{ATXN3}, \textit{CACNA1A}, and \textit{ATXN7} gene primers (1.5 pmol each), 2 ul of dNTPs, 1.5 X buffer#1, Taq polymerase (Thermo Scientific Dynazyme (2 U), 7.5ul Q-Solution, and 50-100 ng of genomic DNA purified from peripheral blood as described above.

Reactions were performed on the ABI 9700 thermal cycler for 1 cycle at 98°C for 5 minutes, 35 cycles at 98°C for 30 seconds, 72°C for 1 minute, and a final extension at 72°C for 10 minutes. After PCR, 1.5 uL of the PCR product was added to 4 ul of formamide (HiDyeformamide, Applied Biosystems Inc.) and 0.5 ul of GS500-LIZ. The solution was mix thoroughly and then denatured at 95°C for 5 minutes. Samples were injected into an ABI PRISM 310 genetic analyzer (Applied Biosystems Inc.) and 50-100 ng of genomic DNA purified from peripheral blood as described above.

The size of PCR products was calculated automatically on the basis of a standard curve based on the internal size standard. Each allele represented the number of CAG repeats. While different individuals had the same alleles, these differed slightly in size (bp) from the theoretical values for the ampiclon length of the trinucleotide-repeat region. To reliably define the alleles, those individuals with alleles whose sizes were close to the theoretical values were grouped together.

| Table 1. The primers used in Multiplex PCR for SCA subtypes 1, 2, 3, 6, and 7 |
|------------------|------------------|------------------|------------------|
| Name             | Fluorochrome     | Sequence (5‘-3‘) | Amplicon (bp)    |
| SCA1 F           | FAM              | ggcgtccaaaaaggtcagt AAC TGG AAA TGT GGA CGT AC | 124+CAG          |
| SCA1 R           | PET              | ggtcctaaaaaggtcagtCAA CAT GGG CAG TCT GAG | 59+CAG           |
| SCA2 F           | VIC              | aaaaaggtcagt GGG CCC CTC ACC ATG TCG | 161+CAG          |
| SCA2 R           | VIC              | ccaaaaggtcagtCGG GCT TGC GGA CAT TGG | 102+CAG          |
| SCA3 F           | PET              | ggcgtccaaaaaggtcagt CGG GCT TGC TGT ACT TGT | 75+CAG           |
| SCA3 R           | VIC              | gggtcctaaaaaggtcagt CGG GCT TGC TGT ACT TGT | 75+CAG           |
| SCA6 F           | VIC              | caaaaggtcagt CAG GTG TCC TAT TCC CCT GTG ATC | 96+CAG           |
| SCA6 R           | VIC              | caaaaggtcagt CAG GTG TCC TAT TCC CCT GTG ATC | 96+CAG           |
| SCA7 F           | FAM              | ggcgtccaaaaaggtcagt TGT TAC ATT GTA GGA GCG GAA | 277+CAG          |
| SCA7 R           | FAM              | gtccctaaaaaggtcagt CAC GAG TGT CCC AGC ATC | 277+CAG          |

3. Results and Discussion

This study used multiplex PCR to screen samples from 200 subjects. A representative multiplex PCR result is shown in Figure 1. Samples were used to determine the repeat sizes for the different SCA subtypes (1, 2, 3, 6, and 7) by calculating the ampiclon sizes obtained from multiplex PCR.

The allelic frequency of the SCA subtypes 1, 2, 3, 6, and 7 was determined in the samples. The most prevalent CAG repeat size or allelic frequency was determined for the five SCA subtypes. For SCA1, the frequency was 59% (29 repeats), which means that 59% of the 200 samples had 29 repeats. This repeat size is the most common in the north Indian population.

Those values are used in the program Genemapper to determine the alleles of each loci.

This study revealed the normal range of repeats for different subtypes of SCA in the Indian population. Based on the normal range of repeats in the 200 samples, the normal range of repeats is shifting to higher or lower ranges in some subtypes of SCA. This range varies in comparison to the normal range of repeats according to the ACMG. The range of repeats is shifting in the Indian scenario. Previous studies reported the range of repeats in the Indian population.
and questioned whether those ranges needed to be redefined. Thus, studies with a larger sample size and functional studies need to be conducted to redefine the normal range of repeats in the Indian population.

SCA is an autosomal-dominant, adult-onset genetic disorder. It has multiple subtypes as have been reported, but some subtypes are more prevalent in Indians such as subtypes 1, 2, 3, 6, and 7. This disease is caused by triple repeat expansion where the number of repeats exceeds the normal range.

The principal finding of this paper is a summary of the most common repeat sizes in different subtypes of SCA (1, 2, 3, 6, and 7) in the north Indian population. Different studies have reported different ranges of repeats for particular subtypes of SCA worldwide. In the current study, the most frequent number of repeats for SCA genes \textit{ATXN1}, \textit{ATXN2}, \textit{ATXN3}, \textit{CACNA1A}, and \textit{ATXN7} was 29, 21, 23, 9, and 3, respectively. Their allelic frequency was 56%, 72%, 13%, 29.5%, and 75.4%, respectively.

Both the normal and disease range of repeats in SCA vary in the population. According to the ACMG guidelines, the normal range of repeats is 6-36 for SCA1, 15-31 for SCA2, 12-40 for SCA3, 4-18 for SCA6, and 4-19 for SCA7, but different studies of the Indian population have reported varied ranges of repeats. A study by Alluri et al. (10) identified the normal range of repeats of SCAs in 187 samples. According to that study, the range of repeats was 20-37 for SCA1, 14-27 for SCA2, 6-38 for SCA3, and 3-20 for SCA7. The current study obtained a different normal range of repeats for SCA subtypes 1 and 7. A study by Saleem \textit{et al.} (9) identified the normal range of repeats of SCAs in 150 samples. According to that study, the range of repeats was 7-37 for SCA1, 18-30 for SCA2, 14-37 for SCA3, and 9-14 for SCA7. In the current study, the range of repeats was 18-36 for SCA1, 19-31 for SCA2, 6-23 for SCA3, 3-18 for SCA6, and 3-19 for SCA7.

Looking at the size of repeats in different SCA subtypes in the Indian population indicates that the range of repeats varies. This may be because of the heterogeneous population in terms of ethnicity. Studies with a large sample and functional studies need to be conducted to redefine the range of repeats in the Indian population.

Acknowledgements

The authors are grateful to the Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS) Lucknow, Uttar Pradesh, India, for providing the facilities where this study was conducted and to the University Grant Commission (UGC) New Delhi for awarding a fellowship to the first author.

Ethics approval

This study was approved by 103rd Institutional Ethics Committee (IEC) "2017-20-PhD-95 PGI/BE/282/2018" of SGPGIMS Lucknow. Subjects provided informed consent before being enrolled in the study.

References


(Received May 22, 2019; Revised July 25, 2019; Revised August 24, 2019; Accepted August 31, 2019)