Brief Report

DOI: 10.5582/irdr.2019.01063

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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Summary **Spinocerebellar ataxia (SCA) is a rare, heterogeneous genetic group of disorders with overlapping clinical features that arises as a result of the degeneration of Purkinje cells. The most prominent clinical feature of SCA is difficulty with whole body movements. The aim of the current study was to analyze the allelic frequency of normal repeat sizes in different SCA subtypes in the north Indian population. Blood samples were collected from 200 subjects, DNA was extracted, and then multiplex PCR and fragment analysis were performed using the ABI-310 genetic analyzer. The prevalent cytosine-adenine-guanine (CAG) repeat size or allelic frequency for SCA1, 2, 3 , 6, and 7 were 29 repeats (59%), 21 repeats (72.5%), 23 repeats (13.1%), 9 repeats (30%), and 3 repeats (75%), respectively. Results indicated that the normal repeats are shifting to lower or upper ranges in the Indian scenario, and similar findings have been reported in other previous studies. Thus, this and other studies have suggested that the normal range of repeats for various SCA in the Indian scenario needs to be redefined and should be confirmed by studies with larger samples and by functional studies.**

Keywords: Spinocerebellar ataxia, ataxia, triplet repeat disorder, repeats

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 subtypes of SCA 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.

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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	gcggtcccaaaagggtcagt AAC TGG AAA TGT GGA CGT AC	124+CAG
SCA1 R		ggtcccaaaagggtcagtCAA CAT GGG CAG TCT GAG	
SCA2 F	PET	aaaagggtcagt GGG CCC CTC ACC ATG TCG	59+CAG
SCA2 R		caaaagggtcagtCGG GCT TGC GGA CAT TGG	
SCA3 F	VIC	gcggtcccaaaagggtcagt CCA GTG ACT TTG ATT CG	161+CAG
SCA3 R		gcggtcccaaaagggtcagt TGG CCT TTC ACA TGG ATG TGA A	
SCA6 F	NED	caaaagggtcagt CAG GTG TCC TAT TCC CCT GTG ATC C	102+CAG
SCA6 R		aaagggtcagtTGG GTA CCT CCG AGG GCC GCT GGT G	
SCA7 F	FAM	gcggtcccaaaagggtcagtTGT TAC ATT GTA GGA GCG GAA	277+CAG
SCA7 R		gtcccaaaagggtcagtCAC GAC TGT CCC AGC ATC ACT T	

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 *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, and *ATXN7* gene primers (1.5 pmol each), 2 ul of dNTPs, 1.5 X buffer#1, Taqpolymerase (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, 58°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.) with a 47 cm long and 50 micrometer diameter capillary containing Performance Optimized Polymer-4 (POP-4, Applied Biosystems Inc.) for 5 seconds with an injection kV of 15.0, and samples were electrophoresed at 15 kV for 40 minutes at 65°C. Amplicon length was calculated in comparison to the GS500-LIZ molecular weight standard using the program Genescan (Applied Biosystems Inc.)

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 amplicon 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.

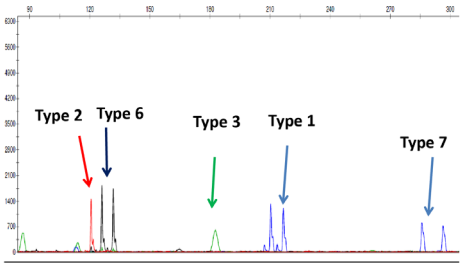

Figure 1. Representative result of multiplex PCR for five subtype of SCA.

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

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 amplicon 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. The allelic frequency of SCA subtypes 2, 3, 6, and 7 was 72.5% (21 repeats), 13.1% (23 repeats), 30% (9 repeats), and 75% (3 repeat). Allelic frequencies are indicated in Figure 2.

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 are 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

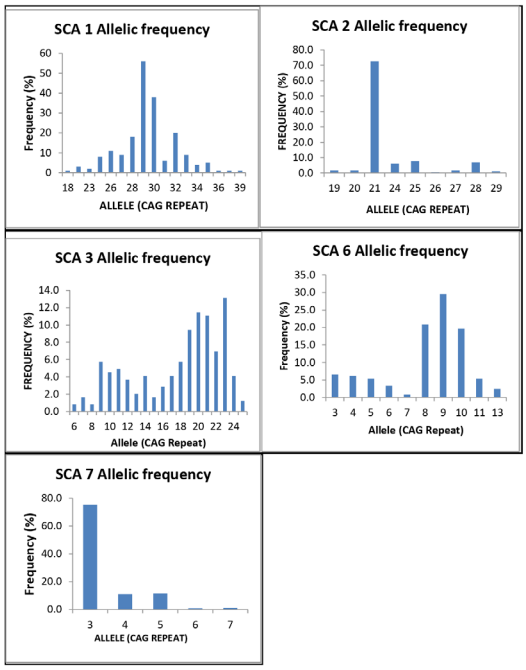

Figure 2. Allelic frequency of different subtypes (1, 2, 3, 6, and 7) of SCAs/.

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 i some subtypes are more prevalent n 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 *ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A*, and *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 *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.

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