Neurological Disorders

Lone et al., J Neurol Disord 2014, 2:5
DOI: 10.4172/2329-6895.1000176

Research Article Open Access

Molecular Analysis of CTG/CTA Repeats at SCA8 Locus in South Indian Population

Waseem Gul Lone[1,2], **Subhadra Poornima**[3], **Kaipa Prabhakar Rao**[2], **Angmuthu Kanikannan Meena**[4] **and Qurratulain Hassan**[3*]

[1]*Department of Genetics, Mahaveer Hospitals, AC Guard, Hyderabad-500 004, India*

[2]*Department of Genetics, Osmania University Hyderabad, 500 007, India*

[3]*Department of Genetics and Molecular Medicine, Kamineni Hospitals, LB Nagar, Hyderabad-500 068, India*

[4]*Department of Neurology, Nizam's Institute of Medical Sciences, Panjagutta, Hyderabad, India*

[*]**Corresponding author:** Quratulain Hassan, Senior Consultant, Kamineni Hospitals, LB Nagar, Hyderabad-500 068, India, Tel: +91-40-39879999, Ext 210; Fax: 091-040-24022277; E-mail: qhasan2000@yahoo.com

Rec date: May 14, 2014; **Acc date:** Aug 26, 2014; **Pub date:** Sep 02, 2014

Abstract

Spinocerebellar ataxias are a group of phenotypically and genetically heterogenous disorders characterized by progressive degeneration of the cerebellum with overlapping symptoms. A novel form of SCA has been described with triplet repeat expansions in the 3 UTR of the SCA8 gene and is caused by expansion of a CTG/CTA repeat in the ataxin-8 opposite strand gene (ATXN8OS) located on chromosome 13q21. Analysis of CTA/CTG repeats in SCA8 gene was performed in 188 ataxia patients and 100 healthy volunteers without any neurological signs or family history. The repeat length was found to be highly polymorphic. We were unable to find any individual with pathogenic repeat length in SCA8 gene, when we used the already established pathogenic repeat criteria. However, repeats >35 (4.7%) were exclusively found in patients only and none of the controls suggesting that these repeat sizes could be pathogenic for our population. The frequency of LN alleles was also found to be higher than reported for other populations. The percentage of LN alleles at SCA 8 locus was 65% and 47% in patients and controls. In the present study three patients also exhibited repeats lower than the normal range and the pathological implications of these needs to be explored.

Keywords: Spinocerebellar Ataxia (SCA); SCA 8 gene; Large Normal Alleles (LNA); Pathogenic Repeats

Introduction

Spinocerebellar ataxias (SCAs) are a heterogenous group of disorders characterized by progressive cerebellar ataxia of gait and limbs, dysarthria, dysphagia, and other neurological signs [1]. The genetic classification of the autosomal dominant types of SCAs is associated with 20 loci. Several of these SCAs (SCA1, 2, 3, 6, 7, 17) are due to CAG repeat expansions in the coding regions of the corresponding genes, translated into abnormally long polyglutamine stretches [2,3]. Apart from these a novel type of mutation in SCAs was described which consisted of trinucleotide repeats expansions in the non-coding regions of certain genes, these include the CAG expansion in SCA12 (OMIM 604326) and CTA/CTG in SCA8 (OMIM 603680) [4-6].

The combined CTA/CTG repeat expansion in 3 UTR of the SCA8 gene present on 13q21 (a gene of unknown function) is controversial with regard to its pathologic significance. The defined normal range for SCA8 gene is 15-50 CTG/CTA repeats and pathogenic range is 80-250 CTG/CTA repeats [7-9]. Evidence from several populations has suggested that the disease prevalence in a population may be associated with the presence of large normal alleles at the respective loci [8]. The CAG/GAA/CTG/CAA/ATTCT repeats at different loci are highly polymorphic in normal individuals. Once the repeat number crosses a particular threshold, specific to an individual locus, instability is observed leading to disease manifestation. Normal and disease ranges for triplet repeat disorders are reported to vary considerably between populations. In different populations, the disease prevalence of these ataxias has been shown to be associated with the presence of large normal alleles (LNAs) at the respective loci [10,11]. Screening of populations for establishing normal and expanded ranges for that particular geographical region will help in proper molecular diagnosis So, we elucidated the normal, pathological and LN allele frequencies of triplet repeats in SCA8 gene, in both ataxia patients and healthy controls from the south Indian population.

Materials and Methods

Sample collection and size

The present study was carried on 288 cases which included 186 males and 102 females in the age group of 5-76 years with the mean age of 35.6 15.9 years. A three generation pedigree was recorded with relevant medical history of the family members. Individuals include one hundred eighty eight (n=188) SCA patients (117 males and 71 females) and one hundred (n=100) controls with no symptoms or family history of any neurological disease or ataxia. These patients were clinically diagnosed by a neurologist and detailed information was provided by the referring physician along with radiological and ophthalmological results. Autosomal dominant family history was observed in 28.1% (53/188) and sporadic in 71.8% (135/188) cases. All the cases were ataxic, 29% of patients had slurred speech and deteriorated writing. 22% had optical atrophy with tremor while as speech disturbance with slow eye movements was found in 18% of patients. The remaining cases had imbalance walk with dysarthria or/and dysphagia.

DNA extraction

DNA was obtained from peripheral blood by salting out method using ammonium acetate and ethanol to precipitate the DNA [12].

Polymerase chain reaction

The molecular identification of the genes associated with these neurodegenerative diseases is based on the observation that mutational event consists of the expansion of trinucleotide repeats, hence rapid and specific tests based on the polymerase chain reaction was developed for diagnosis. The application of this technique allows the differentiation of normal and mutated alleles by determining the number of repeats which is adequate for management, as well as, genetic counseling. PCR reactions were performed with specific primers flanking CTA/CTG regions of SCA8 genes, respectively. Primer sequences for these genes were taken from the literature [13]. A three step PCR was carried out by the method published by our group [14]. Briefly 35 cycles with an initial denaturation at 95°C for 5 min followed by cycling at 95°C for 30s, annealing for 40 s at the appropriate temperature, extension at 72°C for 1 min and a final extension at 72°C for 5 min. The amplified products were initially electrophoresed on a 2% agarose gel and to establish the repeat numbers, PCR products were subsequently separated in denaturing 10% polyacrylamide gels . Molecular weight analysis and sizing of alleles was performed by using UVI Tech software (UK).

Results

Two factors make clinical diagnosis difficult for spinocerebellar ataxia. (i) Within a genetic subtype, the clinical signs are highly variable; (ii) there is a striking phenotypic overlap between each type of SCA. As a result, the diagnosis cannot rely on sole clinical evaluation and a molecular diagnosis is required. The SCA8 PCR products were assessed (Figure 1) and none of the cases had SCA 8 expansion in based on the defined pathological range which is 81-250 CTG/CTA repeats.

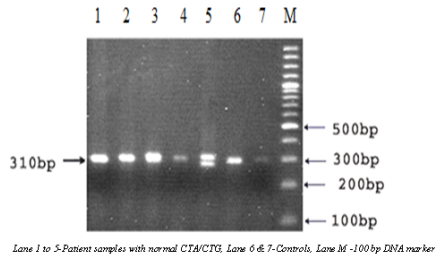

Figure 1: Ethidium bromide stained 2% Agarose gel with PCR amplified products of SCA 8 gene of ataxia patients and a control

A total of 576-pooled chromosomes from 288 individuals were analyzed for the distribution of alleles at the SCA 8 locus. Alleles in the present study ranged between 16-37 CTG/CTA repeats (Figure 2). Heterozygous alleles were seen in 40% (76/188) and 53% (53/100) of patients and controls, respectively. The mode for the current study was 28 and 31 CTA/CTG in controls and 26 in patients. 35-37 and 16-18 repeats were seen in only patients, while 22, 28 and 31 had the highest frequency.

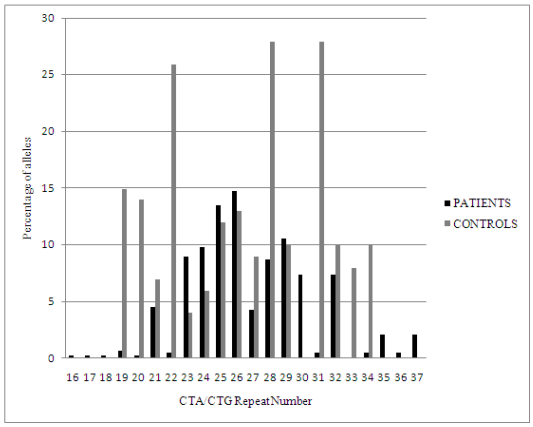

Figure 2: Distribution of CTG/CTA repeats in different alleles at the SCA 8 locus

No. of Repeats	Patients	Controls
25	CTG-13 CTA-12	CTG-15 CTA-10
26	CTG-15 CTA-11	CTG-16 CTA-10
28	CTG-15 CTA-13	CTG-16 CTA-12

Table 1: Analysis of CTG/CTA repeats after sequencing at different repeat number of SCA8 gene in patients and controls

The pathogenic (CTG)n repeat in SCA8 gene is adjacent to a non-pathogenic but highly polymorphic, stably transmitted (CTA)n repeat, which makes it technically difficult to determine the precise number of pathogenic (CTG)n repeats. Therefore, the current reference ranges are based on the total number of both the (CTA)n and (CTG)n repeats. Hence sequencing was carried out in random cases and was found that CTA repeats were usually more in patients than controls studied (Table 1). The pathogenic criteria of SCA 8 is > 80 CTG repeat and in the present study none of the patients falls in this criteria , however, 9 patients with >35 CTG repeats exhibited symptoms of SCA8 type (Table 2) indicating that this may be the pathogenic repeat number for the South Indian patients.

The prevalence of SCAs in various populations has been correlated with the percentage of large normal alleles. To study the frequency of triplet repeat expansion in our two groups relative to the frequency of normal alleles of larger size at the SCA 8 locus, we used the criteria of Takano et al. [8]. In the present study LNA allele distribution were assessed from the healthy individuals (n=100) and from non-disease alleles (n=188). The frequency of large normal alleles >28 repeats at SCA 8 gene in the present study was 0.58. The percentage of large normal at SCA 8 locus was 65% and 47% in patient group and control group respectively (Figure 3). There is no published literature on the frequency of LN alleles at SCA 8 locus from India. The frequency of large normal alleles in the present study is significantly higher than the frequencies reported in Portuguese and Brazilian populations (Table

3). In the present study lower repeats at SCA 8 locus i.e. 16-18 repeats at SCA 8 gene were identified only in the patients (Table 4). The pathological implications of lower repeats than the normal range needs to be explored.

S.No	Age	Sex	Onset	Family history	Consan-gunity	Repeats	Clinical Details			
						Observed	Gait	Speech	Eye	Any other
1	45	M	40	No	No	35/28	+	-	-	-
2	26	F	24	No	Yes	35/25	+	-	+	CT- Normal
3	25	M	19	No	No	35/28	+	+	+	-
4	33	F	25	No	Yes	35/26	+	+	-	Olivopentocerebellar degeneration
5	11	F	7	No	No	35/23	+	+	-	Walking with support
6	19	F	17	No	No	35/24	+	+	-	
7	34	M	29	No	Yes	35/25	+	+	+	Cerebellar atrophy
8	55	M	52	No	No	35/25	+	+	-	Motor sensory axonal neuropathy
9	22	M	20	Yes	No	35/24	+	+	+	
M=Male; F=Female										

Table 2: Clinical details of the patients with >35 CTG/CTA repeats at SCA8 locus

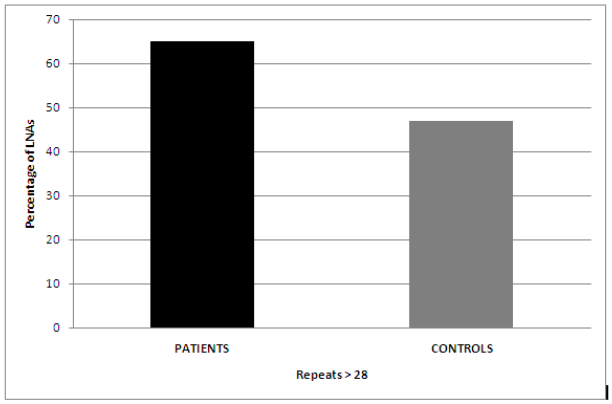

Figure 3: LNAs at SCA 8 locus based on published literature (Takano et al.)

Discussion

Earlier our group had established the methods of molecular diagnosis, prevalence and role of LN alleles for a panel of ataxias, which includes SCA 1, 2, 3, 6 and 7 in 124 patients with clinical diagnosis of SCA. However only 8% of the cases could be diagnosed as per the established pathogenic range at 5 SCA loci [14]. Hence, establishing non pathological ranges/Pathological range of the trinucleotide repeats in SCA 8 gene characteristics of the south Indian ethnical background, allowed us to introduce in our patients a new test with SCA.

The combined CTA/CTG repeat in the 3 UTR of ATXN8OS gene, present on chromosome locus 13q21, is characterized by a slowly progressive ataxia with disease onset typically occurring in adulthood. The progression is typically over decades regardless of the age of onset. Common initial symptoms are scanning dysarthria with a characteristic drawn-out slowness of speech and gait instability. Some individuals present with nystagmus, dysmetric saccades and, rarely, ophthalmoplegia. Tendon reflex hyperreflexity and extensor plantar responses are present in some severely affected individuals [15]. In the present study >35 repeats were found only in patients only with clinical symptoms of SCA 8 type (Table 2). Hence there is a need to re-evaluate the threshold of abnormal repeat expansion and it can be lowered to 35 repeats.

Its pathogenesis has been distinguished among triplet disease disorders both in terms of the transcribed repeat motif (CTG) and for its location in the noncoding region of the gene, resulting in an untranslated expansion at the 3[5,9,16]. However, in the present study CTG repeats were found less in patients as compared to controls (Table 1). The existence of an antisense transcript, encoding a novel actin-binding protein (KLHL1), has recently been reported [17,18] suggesting that the pathogenic effect of SCA8 expansion may result in an alteration of KLHL1 messenger RNA stability or processing. However, the role of CTG expansion in the pathogenesis of SCA8 and the molecular mechanism responsible for the disease remain to be clarified [19].

SCA8 form of ataxia accounts for 2-5% of ADAs. SCA8 expansion was found in 4.7% (8/167) ataxia patients in Italy [13]. It is infrequent in Japan and its prevalence is 2.7% in Taiwanese [20,21]. None of the cases in the present study showed expansion at the SCA 8 locus as per the defined pathogenic range of 80-250 CTG/CTA repeats. However, 9 patients with >35 CTG repeats exhibited symptoms of SCA 8 gene (Table 2). This implicates a more sophisticated interpretation of SCA8

gene and raise the question about the diagnostic thresh hold between normal and expanded repeats in our population.

LNA	Present Study	Portuguese Group (Silveira et al.)	Brazilian Group (Silveira et al.)
>28	0.58	0.11 (<0.0001)	0.07 (<0.0001)
The values which are indicated in parentheses are P-values which were obtained from chi-square analysis when comparison was between the present study and the other studies using MedCalc 7.6.0.0.			

Table 3: Frequency of LNA at SCA 8 locus in the present study compared with other populations

S. No	Age	Sex	Onset	Family history	Consa-gunity	Repeats Observed	Clinical Details			
							Gait	Speech	Eye	Any other
1	8	F	7	No	No	22/16	+	+	+	-
2	36	F	32	No	No	24/17	+	+	+	-
3	40	M	30	No	No	22/18	+	+	-	-

Table 4: Clinical details of the patients with lower repeats than the normal range at SCA 8 locus

At SCA8 locus repeats varied from 18-33 with 90% of the alleles having less than 27 repeats in northern Indian population [22] and in the present study repeats varied from 16-37 (Figure 2) with 44% of the alleles having >27 repeats which indicates that greater repeat size at SCA 8 locus is more in southern population of India. The frequencies of large normal alleles at SCA 8 locus were quite high when compared with other populations (Table 3). LN alleles are relatively unstable and undergo expansion to reach an intermediate range from which further expansion to the disease range take place. Therefore a study on the percentage of large normal alleles in any population would be an indirect reflection of the prevalence of the disease in that population. This was found to be true in African and Israeli populations [23,24]. A similar study involving White and Japanese ADCA patients and normal individuals observed that the prevalence of SCAs is highly correlated with the frequency of large normal alleles in the normal population [8].

The role of triplet repeat expansion beyond thresh hold size in disease pathogenesis has been defined, however the exact role of lower repeats in disease pathogenesis is still unknown in these type of disorders. Notably, the lower repeats may also result in abnormal phenotype, as has been noted in diseases such as SBMA. In the present study lower repeats were not found in the controls which indicate that lower repeats at these loci may play a role in diseases pathogenesis.

Conclusion

SCA 8 gene constitutes a high degree of genetic heterogeneity in our population. It is proposed that pathogenic repeats can be redefined (>35 repeats) based on case/control data of this study at SCA 8 locus, using this criteria 4.7% (n=9) patients can be diagnosed for SCA 8 type. The redefined pathogenic repeats were seen in patients only with clinical characteristics of SCA 8 type. Hence, it is proposed that repeats pathogenic for the South Indian population should be >35 CTG repeats. The higher frequency of LNAs at SCA 8 gene also indicates that it could be more prevalent in our population. Protein misfolding due to expansion of polyglutamine tracts may not be the only mechanism for neurodegenerative diseases, repeats lower than normal range may also result in abnormal phenotype. The repeat range and LN allele frequency of the present study can be of significant help in proper molecular diagnosis at SCA 8 locus.

Acknowledgement

The authors are grateful to all the patients and volunteers who formed the study group. We thank Department of Genetics and Molecular Medicine Kamineni Hospitals, LB Nagar, Hyderabad for providing the facilities.

References

1. Barnes JA, Ebner BA, Duvick LA, Gao W, Chen G, et al. (2011) Abnormalities in the climbing fiber-Purkinje cell circuitry contribute to neuronal dysfunction in ATXN1[82Q] mice. J Neurosci 31: 12778-12789.
2. Stevens JR, Lahue EE, Li GM, Lahue RS (2013) Trinucleotide repeat expansions catalyzed by human cell-free extracts. Cell Res 23: 565-572.
3. Stevanin G, Dürr A, Brice A (2000) Clinical and molecular advances in autosomal dominant cerebellar ataxias: from genotype to phenotype and physiopathology. Eur J Hum Genet 8: 4-18.
4. Ushe M, Perlmutter JS (2012) Oromandibular and lingual dystonia associated with spinocerebellar ataxia type 8. Mov Disord 27: 1741-1742.
5. Koob MD, Moseley ML, Schut LJ, Benzow KA, Bird TD, et al. (1999) An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8) Nat Genet 21: 379-384.
6. Holmes SE, O'Hearn EE, Mcinnis MG, Gorelick-Feldman DA, Kleiderleini JJ, et al. (1999) Expansion of a novel CAG trinucleotide repeat in the 5'region of PPP2R2B is assiociated with SCA12. Nat Genet 54: 391-392.
7. Faruq M, Scaria V, Singh I, Tyagi S, Srivastava AK, et al. (2009) SCA-LSVD: a repeat-oriented locus-specific variation database for genotype to phenotype correlations in spinocerebellar ataxias. Hum Mutat 30: 1037-1042.

8. Takano H, Cancel G, Ikeuchi T, Lorenzetti D, Mawad R, et al. (1998) Close associations between prevalences of dominantly inherited spinocerebellar ataxias with CAG-repeat expansions and frequencies of large normal CAG alleles in Japanese and Caucasian populations. Am J Hum Genet 631: 60-66.
9. Silveira I, Miranda C, Guimarães L, Moreira MC, Alonso I, et al. (2002) Trinucleotide repeats in 202 families with ataxia: a small expanded (CAG)n allele at the SCA17 locus. Arch Neurol 59: 623-629.
10. Perissinotti PP, Ethington EG, Cribbs L, Koob MD, Martin J, et al. (2014) Down-regulation of endogenous KLHL1 decreases voltage-gated calcium current density. Cell Calcium 55: 269-280.
11. Squitieri F, Andrew SE, Goldberg YP, Kremer B, Spence N, et al. (1994) DNA haplotype analysis of Huntington disease reveals clues to the origins and mechanisms of CAG expansion and reasons for geographic variations of prevalence. Hum Mol Genet 3: 2103-2114.
12. Chava S, Mohan V, Pasupuleti N, Latha MM, Khan IA, et al. (2011) Evaluation of Aurora-A Gene Polymorphism and Esophageal Cancer Risk in a South Indian Population. Genetic Testing and Molecular Biomarkers 15: 185-189.
13. Cellini E, Nacmias B, Forleo P, Piacentini S, Guarnieri BM, et al. (2001) Genetic and clinical analysis of spinocerebellar ataxia type 8 repeat expansion in Italy. Arch Neurol 58: 1856-1859.
14. Alluri RV, Komandur S, Wagheray A, Chaudhuri J, Sitajayalakshmi S, et al. (2007) Molecular analysis of CAG repeats at five different loci: alternative hypothesis for disease pathogenesis. Molecules and Cells 24: 338-342.
15. Kearse MG, Todd PK (2014) Repeat-Associated Non-AUG Translation and Its Impact in Neurodegenerative Disease. Neurotherapeutics.
16. Martindale JE, Seneca S, Wieczorek S, Sequeiros J (2012) Spinocerebellar ataxias: an example of the challenges associated with genetic databases for dynamic mutations. Hum Mutat 33: 1359-1365.
17. Krysa W, Rajkiewicz M, SuÅ‚ek A (2012) Rapid detection of large expansions in progressive myoclonus epilepsy type 1, myotonic dystrophy type 2 and spinocerebellar ataxia type 8. Neurol Neurochir Pol 46: 113-120.
18. Nemes JP, Benzow KA, Moseley ML, Ranum LP, Koob MD (2000) The SCA8 transcript is an antisense RNA to a brain-specific transcript encoding a novel actin-binding protein (KLHL1). Hum Mol Genet 9: 1543-1551.
19. Day JW, Schut LJ, Moseley ML, Durand AC, Ranum LP (2000) Spinocerebellar ataxia type 8: clinical features in a large family. Neurology 55: 649-657.
20. Sasaki H, Yabe I, Tashiro K (2003) The hereditary spinocerebellar ataxias in Japan. Cytogenet Genome Res 100: 198-205.
21. Soong BW, Lu YC, Choo KB, Lee HY (2001) Frequency analysis of autosomal dominant cerebellar ataxias in Taiwanese patients and clinical and molecular characterization of spinocerebellar ataxia type 6. Arch Neurol 58: 1105-1109.
22. Saleem Q, Sreevidya VS, Sudhir J, Savithri JV, Gowda Y, et al. (2000) Association analysis of CAG repeats at the KCNN3 locus in Indian patients with bipolar disorder and schizophrenia. Am J Med Genet 96: 744-748.
23. Goldman A, Ramsay M, Jenkins T (1996) Ethnicity and myotonic dystrophy: a possible explanation for its absence in sub-Saharan Africa. Ann Hum Genet 60: 57-65.
24. MorCohen R, Magal N, Gadoth N, Shohat T, Shohat M (1997) Correlation between the incidence of myotonic dystrophy in different groups in Israel and the number of CTG trinucleotide repeats in the myotonin gene. Am J Med Genet 71: 156-159.