

## A Study on Triplet Repeat Expansion Disorders in Western Indian Population

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### Abstract

Triplet repeat expansion disorders (TRED) are one of the commonest causes of hereditary neuropathy with thirty different heredity diseases due to increased number of trinucleotide repeats above certain threshold in the genomic DNA. The degree of disease aggressiveness depends on the number of repeats and resultant alteration in the expression of several genes. Moreover, mechanism of these disorders differs depending on the region of trinucleotide expansion (coding vs. non-coding regions). Present study comprises of 172 individuals suspected of triplet repeat expansion disorders such as Huntington's disease (HD), Spinocerebellar ataxia (SCA), Friedreich's ataxia (FA) and Myotonic dystrophy (MD) from Gujarat (Western Indian population) to know its frequency and clinical manifestations. Among them, 78 (45.34%) individuals were confirmed to have one of these four disorders. The age of onset varied from 11-45 years. SCA was the most common triplet repeat expansion disorder with a frequency of 43.60% followed by HD in 23.1%, MD in 21.8% and FA in 11.5% of patients. In addition, SCA2 was found to be the most common hereditary neuropathy in Western India.

**Keywords:** Triplet repeat expansion disorders; Huntington's disease; Spinocerebellar ataxia; Friedreich's ataxia; Myotonic dystrophy

### Introduction

Triplet repeat expansion disorders (TRED) constitute a class of genetic diseases caused by slippage during DNA replication that results in the expansion of nucleotide repeats. These disorders are usually associated with anticipation where age of onset decreases and extended repeats tend to increase in length from generation to generation [1].

They are commonly classified in two groups; (i) those with moderate CAG expansions that result in a polyglutamine stretch in gene sequence, hence termed as 'Polyglutamine disorders' like Huntington's disease (HD) and Spinocerebellar ataxia (SCA) type 1, 2, 3, 6 and (ii) those with very long expansions (usually non-CAG repeats) known as 'Non-Polyglutamine disorders' like Friedreich's ataxia (FA) and Myotonic Dystrophy (MD) [2,3]. Different mechanisms of triplet repeat expansion exist that depends on the presence of expansion in coding or non-coding regions of the genome. In disorders such as FA, expansions in non-coding regions cause loss-of-function mutations. In contrast, tri-nucleotide expansion in the protein coding region causes an abnormally long stretch of a glutamine into the associated protein which leads to gain-of function mutation in HD [4,5].

Majority of these disorders are autosomal dominant (except FA which is autosomal recessive) with variable age of onset and severity. An estimated prevalence is 7-10/100,000 population for HD [6], 1-5/100,000 for SCA [7], 1/50,000 for FA [8] and 1/8000-10,000 population for MD [9]. The most common phenotypes observed are ataxia, dysarthria, sensorimotor neuropathy, chorea, nystagmus with restricted phenotype of cataracts, cardiac arrhythmias, and cognitive impairment in MD [6,7,9,10].

With increasing life expectancy and availability of diagnostic facility, it is highly likely to observe a surge in the incidence of these disorders in general population posing a greater challenge in patient care and management. An early diagnosis with advanced molecular diagnostic techniques would offer a better therapeutic and rehabilitation possibilities. Present study was aimed to know the frequency of TRED in referrals from Gujarat (Western India) and its vicinity.

### Materials and Methods

The present prospective study was aimed at screening for TRED in 172 patients referred during March 2008 to July 2014. This includes 50 suspected patients for HD, 79 for SCA, 20 for FA and 23 for MD. After obtaining informed written consent and approval from the Institutional Ethics Committee, blood samples were collected in EDTA vacutainer. Clinical details and other relevant information were noted for all subjects in a clinical record form. The study included 96 males (55.8%) and 76 females (44.2%) in the age range of 11-85 years (mean age 40 years) with one child of 7 years having family history of FA. The clinical symptoms were ataxia, dysarthria, difficulty in walking and writing, gait disturbance, sensorimotor neuropathy and chorea. Those suspected with MD had additional phenotypes of myotonia, nystagmus, cataract, cardiac arrhythmias, dementia and cognitive impairment. DNA was isolated from EDTA blood by standard salting out method [11].

### PCR conditions and amplification

Amplification of HD gene was carried out using Thermal cycler (Applied Biosystems-2720) in 25 µl final volume containing genomic DNA (100 ng), dNTPs (2 mM), forward and reverse primer (1 µM), Taq polymerase (5 U), Tris-HCL (75 mM), MgCl<sub>2</sub> (2 mM), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (25 mM) and DMSO (10%). Thirty cycles of amplification were

carried out, each consisting 30s denaturation at 94°C, 30s annealing at 65–70°C and 45s elongation at 72°C. Electrophoresis of amplified PCR product was carried out on 3% agarose gel along with appropriate negative controls, positive controls and DNA ladder (100 bp).

For SCA, study was carried out by amplifying different genes (*ATXN1*, *ATXN2*, *ATXN3* and *CACNA1A*). DNA amplification was carried out in a final volume of 19 µl containing genomic DNA (500 ng), 10x PCR buffer, dNTPs (2 mM), forward and reverse primer (12 pM), Taq polymerase (5 U). In addition, formamide (2%) for SCA1, DMSO (10%) and MgCl<sub>2</sub> (2 mM) for SCA2, DMSO (8%) for SCA3 and glycerol (3.5%) for SCA6 were added. Thirty cycles of amplification were carried out, each consisting of 60s denaturation at 95°C, 60s annealing at 63°C and 60s elongation at 72°C. PCR products were then electrophoresed on 3% agarose gel along with the appropriate negative controls, positive controls and DNA ladder (100 bp).

For FA, amplification of *Frataxin (FXN)* gene was carried out in a 25 µl final volume containing genomic DNA (100 ng), dNTPs (2 mM),

forward and reverse primer (20 pmol), hotstar Taq polymerase (5 U), 10x PCR buffer and 5x-Q solution. Thirty cycles of amplification were used; each consisting 45s denaturation at 95°C, 30s annealing at 60°C and 1 min elongation at 72°C. PCR products were electrophoresed on 1.5% agarose along with the appropriate negative controls, positive controls and DNA ladder (100 bp).

For MD, amplification of *DMPK1* gene was carried out by triple repeat primed polymerase reaction (TP-PCR) in a 25 µl final volume containing genomic DNA (50 ng), primers P1, P2 and P3 (P1 and P2 are flanking primers and P3 is universal primer) (10 pmol), Taq polymerase (1 U), and dNTPs (200 µM). Thirty cycles of amplification were used; each consisting of 1 min denaturation at 94°C, 1 min annealing at 60°C and 2 min elongation at 72°C. PCR products were then electrophoresed on 2% agarose along with the appropriate negative controls and segment analysis was carried out by ABI-310 Genetic Analyzer.

The Primer sequences used for amplification and amplicon sizes for different TRED (Table 1).

Disorder	Type of repeat	Gene name	Primer sequence (5'-->3')	Amplicon size
HD	CAG	<i>HD</i>	F.P: ATGAAGGCCTTCGAGTCCCTCAAGTCCTTC R.P: GGCGGTGGCGGCTGTTGCTGCTGCTGCTGCTG	48 bp
SCA1	CAG	<i>ATXN1</i>	F.P: CAACATGGGCAGTCTGAG R.P: AACTGGAATGTGGACGTA	124 bp
SCA2	CAG	<i>ATXN2</i>	F.P: GGGCCCCTCACCATGTCCG R.P: CGGGCTTGCGGACATTGG	59 bp
SCA3	CAG	<i>ATXN3</i>	F.P: CCAGTGACTACTTTGATTCCG R.P: TGGCCTTTCACATGGATGTGAA	162 bp
SCA6	CAG	<i>CACNA1A</i>	F.P: CACGTGTCCTATTTCCCTGTGATCC R.P: TGGGTACCTCCGAGGGCCGCTGGTG	102 bp
FA	GAA	<i>FXN</i>	F.P: GGCTTAAACTTCCCACACGTGTT R.P: AGGACCATCATGGCCACACTT	500 bp
MD	CTG	<i>DMPK</i>	P1 - F.P: GAAGGGTCTCTGTAGCCGGGAA P2 - R.P: TACGCATCCCAGTTTGAGACGCAGCAGCAGCAGCAG P3 - Universal Primer: TACGCATCCCAGTTTGAGACG	

**Table 1:** Primer sequence and amplicon size in trinucleotide repeat expansion disorder

## Results

Of 172 referrals, 78 patients (45.3%) were found to be affected with one of the TRED with variable frequency and age of onset (Table 2). HD was suspected in 50 patients of which 18 (36.0%) were found with >35 CAG repeats [Age range: 27-73 years; Males (N=7, 38.8%), Females (N=11, 61.1%)]. Pre-mutation (26-34 repeats) was seen in six patients (12%) with age range of 12-70 years; Males (N=4; 66.7%), Females (N=2; 33.3%).

Out of 79 patients investigated for SCA, 34 patients (43.0%) [18 males (52.9%) and 16 females (47.0%)] in the age range of 14-85 years and with age of onset of 13-78 years respectively showed an increased number of CAG repeats (SCA1: 44-83 repeats; SCA2:35-400 repeats;

SCA3: 55-86 repeats). The frequency of SCA subtypes were: SCA1 (8.8%), SCA2 (53.0%) and SCA3 (38.2%). SCA6 was not observed in any of the patients.

Of 20 patients investigated for FA, 9 patients (45.0%) in the age range of 7 to 30 years [5 males (55.6%) and 4 females (44.5%)] were found to have increased number of GAA repeats with an age at onset of 2 to 18 years.

Of 23 patients suspected with MD, 17 patients (73.91%) were confirmed with the disease having expanded CTG repeats [11 males (64.7%), 6 females (35.3%) in the age range of 15 to 78 years]. The age at onset was in the range of 21 to 50 years.

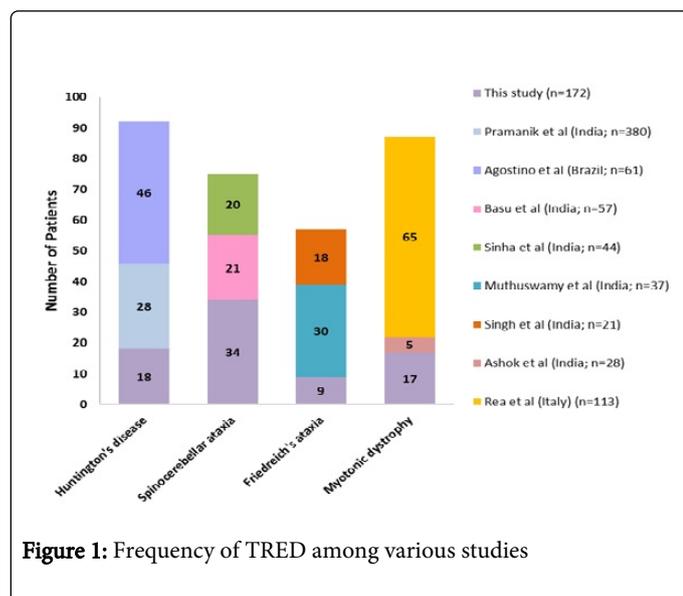
## Discussion

Our study has shown that 45% of patients suspected with TRED had any one of the aforementioned neurodegenerative disorders (Figure 1).

Among all, SCA was the most frequently observed CAG repeat disorder with SCA2 as the most common followed by SCA3 and SCA1. Several reports from India had similar observations [12-14]. Though none of our patients showed the presence of SCA6 while study in Korean patients showed the highest frequency of SCA3 and SCA6 which further suggests the geographical influence on TRED in different populations. Nonetheless, clinical presentation with ataxia, dysarthria and tremors were almost similar as has been observed by other group [12,15].

Disorder	No. of Pts Screened	Affected Pts N (%)	Age Onset at Years	Affected Males (%)	Affected Females (%)
HD	50	18(36.0)	32-75	7(38.8)	11(61.1)
SCA	79	34(43.0)	13-78	18(52.9)	16(47.0)
FA	20	9(45.0)	02-18	05(55.5)	04(44.4)
MD	23	17(73.9)	21-50	11(64.7)	06(35.2)

**Table 2:** Frequency of TRED Disorders and age of onset



**Figure 1:** Frequency of TRED among various studies

HD was the second most common neurodegenerative disorder and detected in 36% of the cases. Number of triplet repeats and disease onset varies in different a population [6] which is likely to be due to environmental and other unknown influences on gene expression [16]. In our study, CAG repeats varied from 37-51 with age of disease onset varied from 2nd to 8th decade of life. Another study from India reported similar results with CAG repeats in the range of 41 to 56 [17]. A recent report from Brazil had shown minimal number of 37 CAG repeats [18] with presence of chorea in more than 80% of patients as has been observed in the present study.

Though the number of non-polyglutamine neurodegenerative disorder were less frequently seen as compared to polyglutamine, MD

type I was seen with the highest frequency followed by FA. In the current study, nearly 74% patients suspected with MD had shown the presence of expanded CTG repeats in *DMPK1* gene. Another study could demonstrate the presence of CTG repeats in only 17.8% of the subjects [19]. This could be due to referral bias in both the study as ours were the patients with high degree of clinical suspicion presented with myotonia and wasting of muscles with or without presence of ocular signs like dropping of eye lids and ptosis.

Last group of neurodegenerative disorder was FA due to GAA repeats with nearly 45% of affected patients from a pool of 20 suspected subjects. Similar study was carried out in 63 individuals of North Indian origin and 16 of South Indian origin with increased GAA repeats in 33% of patients [20]. Another study showed the presence of expanded GAA allele in 81% of their patients [10]. This could be due to very stringent criteria of study selection by the authors. The most common clinical presentation was gait disturbance, dysarthria and ataxia in 66% of our subjects against nearly all subjects with these phenotypes in a study by Potdar [21].

Present study demonstrates SCA2 as one of the most common hereditary neurodegenerative TRED of SCA panel followed by HD, MD (Type I) and FA in our population. The most common clinical manifestation ranged from mild to severe muscle wasting to sensory motor neuropathy that aid differential diagnosis in these autosomal diseases with age of onset from 2nd to 5th decade of life. A combination of all these would offer a confirmed diagnosis with a better therapeutic option and genetic counseling that is likely to relieve them from a great mental agony.

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