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REPORT OF AN UNUSUAL FAMILY OF MYOTONIC DYSTROPHY WITH POSSIBLE EXTENDED APPROACH FOR GENETIC COUNSELLING: A CASE REPORT

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Abstract: Myotonic Dystrophy Type 1 is caused by an expanded number of CTG repeats in the 3′ UTR of a DMPK gene and has an incidence of ~1 in 7500 adults. It varies in normal population from 5-34 repeats to > 40 repeats in affected individuals. We report the CTG repeat pattern of a DM1 family in which three patients with their asymptomatic or normal family members. The intention of this case report is the diagnosis of a DM1 family and the establishment of TP-PCR methodology and genetic counseling approaches in early screening of affected patient and their family members. Clinical and biochemical testing involve in the initial prediction and diagnosis of the disease which is confirmed by molecular testing. Thus, TP-PCR could be successfully used for the identification of repeat size in DM1 and enable us to prediction of those myotonic cases where presymptomatic and prenatal diagnosis is required.

Keywords: Myotonic Dystrophy, Presymptomatic, TP-PCR

INTRODUCTION

Trinucleotide repeat disorders are a set of genetic disorders caused by expansion in certain genes exceeding the normal, stable, threshold and which differs per gene. These disorders classified into 2 major category: (i) Coding expansion disorder (Huntington disease and different spirocerebellar ataxia) (ii) Non-coding expansion disorder (Myotonic Dystrophy (DM) and Friedreich ataxia). Disorders that have triplet repeat expansions in non-coding regions typically cause a loss of gene function or toxic effect at the mRNA level whereas those that occur in coding regions result in an expanded polyglutamine or polyalanine tract in the protein product which causes the protein to become toxic with or without the loss of its normal function.

Myotonic Dystrophy (DM) is a chronic, slowly progressing, highly variable inherited multisystemic autosomal-dominant disease characterized by marked intrafamilial and clinical variability and mainly categorized into Myotonic Dystrophy Type1 and 2 (DM1 and DM2). It affects approximately 1 in 7,500 individual worldwide [1] and the prevalence of DM1 and DM2 is 98% and 2% respectively. The disorder shows genetic anticipation with expansion of the repeat number dependent on the sex of the transmitting parent.

On the basis of clinical symptoms, severity and age-of onset of disease myotonic dystrophy patients are often divided into three groups congenital, mild and juvenile. The most severe form is congenital myotonic dystrophy (CDM) which is associated with generalized muscular hypotonia, talipes and mental retardation while mildest form (seen in middle-to-old age) is characterized by cataracts, baldness and minimal or absent muscle involvement and juvenile/adult form is phenotypically variable with myotonia, muscle weakness, cardiac arrhythmias, male balding, hypogonadism and glucose intolerance etc. The DM1 mutation involves an expanded trinucleotide repeat (CTG) in the DMPK (Dystrophia Myotonica Protein Kinase) gene in the 3′-untranslated region [2, 3-4] and varies in the normal population from 5-34 and repeat >40 CTG (5-34 CTG repeat for normal individual, 35-49 repeat for asymptomatic individual and >50 repeat for patients) is associated with DM1 severity [2, 5]. The correlation between CTG repeat size observed in one tissue (eg. blood) often does not match the severity of the disease and the CTG repeat size in other organs (eg. muscle). The DMPK gene is ~14 kb and encodes 2.3 kb of mRNA with 15 exons and a protein (cAMP-dependent serine-threonine kinase) of 624 amino acids [6-7]. Offspring of an individual with an expanded allele have a 50% chance of inheriting the mutant allele.

The genetic testing of DM1 plays a critical role in characterizing them and to direct clinical person to cater them for the appropriate therapy and management. Clinically myotonic dystrophy is diagnosed by the elevated level of muscle enzyme SCK (Serum Creatinine Kinase), characteristics pattern of electromyography peaks, nerve conduction velocity (NCV) and various other parameters. PCR-RFLP [8-10] followed by southern blotting [11-12] are used for the detection of CTG repeats. The utility of TP-PCR (Triplet

Primed Polymerase Chain Reaction) in DM1 was shown by the Paola Amicucci et al [13] and it was developed by Warner et al [14] for screening the CAG repeat expansion in myotonic dystrophy. It provides characteristics peaks pattern which confirms the existence of triplet repeats in the DMPK gene [14].

The intention of the present case study is (i) evaluation at clinical, biochemical and molecular level (ii) to establish PCR methodology for early screening in DM1 patient and similar repeat expansion disorder (iii) detection of presymptomatic carriers in affected family members (iv) to provide genetic counseling in family members having affected probands and asymptomatic carriers (v) to provide prenatal diagnosis if and when required.

As per our knowledge, this is the first report that engages TP-PCR as a method of choice for detection of premutation in DM1 family members in India and exploring the possibilities of genetic counseling and prenatal diagnosis.

CASE PRESENTATION

Here we are discussing a myotonic dystrophy type 1 (DM1) brahmin family case in which three member, first 50 year old female (proband 1, II-1) second 28 year old boy (proband 2, III-1) and third 17 year old boy (proband 3, III-4), are affected with DM1 (Figure.1). The first and second proband hailed from Delhi while third from Uttar Pradesh (UP) visited Neurology OPD in SGPGIMS with a complaint of muscle weakness. They are vegetarian and non-vegetarian with no habit of any addiction. On presentation the physician detected muscle wasting (MW), jaw and temporal wasting (JTW) and facial weakness (FW) in the probands (Table.1 and 2) however, family members of the family was found normal. The height, weight and BMI (Body mass index) of the patients (II-1, III-1, III-4) were 155cm, 38Kg, 15.81Kg/m^2; 180cm, 55Kg, 16.97Kg/m^2; 155cm, 45Kg, 18.73Kg/m^2 respectively. They had no learning, writing, speech/languages, dysphasia and dyspepsia, sleep apnea, respiratory insufficiency, abnormal physical activity and behavioral problems on presentation (Table.2).

The proband first (II-1) and second (III-1) affected while third proband (III-4) was non-diabetic but all are clinically diagnosed as DM1. The Electrocardiogram (ECG) and Echocardiogram were found normal for all the DM1 affected proband (patients) and there were no conduction block. The characteristics pattern of EMG (Electromyography, Figure.2) and high level of SCK (220U/L, 325U/L, 475U/L) confirmed the clinical finding. The patients were referred to Genetics Department for molecular evaluation. The TP-PCR analysis was performed in probands and family members. The TP-PCR analysis revealed a large expansion of CTG repeat in probands (CTG repeat no.~ 50, 50, 60 in proband first (II-1), second (III-1) and third (III-4) respectively however, other family members like 74 year old female (I-2, CTG repeat~ 10), 26 year old boy (III-2, CTG repeat~ 10) and 20 year old boy (III-3, CTG repeat ~ 20) were found normal while 45 year old man (II-3, CTG repeat~ 35) and his 41 year old wife (II-4, CTG repeat~ 40) were found asymptomatic carrier for the disease and 78 year old pathogenic male (I-1), 48 year old male (II-2) and 42 year old pathogenic male (II-5) had died before testing for CTG repeat.

Table.1. Demographic characteristics of DM1 affected individuals

Patients	Sex	Origin	Caste	Age at onset	Age at presentation	Dur.	Nut.	Add.	Occ.	Marital status
1(II-1)	F	Delhi	B	48	50	2	Both	N	Teacher	M
2 (III-1)	M	Delhi	B	26	28	2	Both	N	Student	U
3(III-4)	M	UP	B	13	17	4	Both	N	Student	U

Dur, Duration of the disease; Nut., Mode of the nutrition; Add, Addiction (N=No); Occ, occupation of the patient; M, Male; F, Female; B, Brahmin caste; Both, Vegetarian and Non-Vegetarian; U, Unmarried; M, Married

Table.2: Clinical Profile of disease for DM1 affected individuals

Patients	L	W	S/L	MW	JTW	FW	Behavior	RI	HYS	E	ADL	DYH	DYP	DM
1(II-1)	N	N	N	Y	Y	Y	Normal	N	Y	Y	Y	N	N	Y
2(III-1)	N	N	N	Y	Y	Y	Normal	N	Y	Y	Y	N	N	Y
3(III-4)	N	N	N	Y	Y	Y	Normal	N	Y	Y	Y	N	N	N

L; Learning problem; W, Writing problem; S/L, Speech/Languages problem; MW, Muscle wasting; JTW, Jaw and temporal wasting; FW, Facial weakness; RI, Respiratory insufficiency (Problem in respiration); HYS, Hypersomnia (excess sleep or day time sleep); E, Physical activity/Exercise; ADL, Activity of daily living; DYH, Dysphasia (Problem in swallowing of food); DYP, Dyspepsia (Indigestion); DM (Diabetes mellitus); N, No; Y, Yes.

DISCUSSION

TP-PCR gives characteristics peak pattern of triplet base pair size for DM1 patient and their family members. All three probands (patients) study indicates that at the younger age the CTG repeats have large expansion in comparison to older age (Table.4, Figure.3). This report has a strong concordance with the previous studies [10, 14]. When one parent is a carrier of an autosomal dominant faulty gene, there is 50% chance in every pregnancy that their child will be affected by or predisposed to developing the condition. So there is an equal chance that they will neither be affected nor predisposed to developing the condition (II-1). When both parents are carriers of an autosomal dominant faulty gene, there is 25% chance in every pregnancy that their child will receive the faulty gene copy from both parents and be more severely affected by the condition as patient III-4 [15].

Table.3. Clinical and biochemical testing of DM1 affected individuals

Patients	Electrocardiogram	Echo	Cardiac involvement	EMG	SCK (U/L)
1(II-1)	N	N	No	+	▲
2(III-1)	N	N	No	+	▲
3(III-4)	N	N	No	+	▲

Echo, Echocardiography; EMG, Electromyography; SCK, Serum creatinine kinase concentration (normal range: 25-200 U/L); N, Normal; +, positive for myotonic dystrophy type 1 (DM1), High SCK concentration.

Table.4. Repeat size in family member of Probands

Sample DM1 Family	Age (year)	Status	Clinical feature	Repeat Size
Proband 1 Mother (I-2)	74	Normal	All 3 Patient (Proband) have MW, JTW, FW, Hypersomnia	10
Proband 1 (Female, II-1)	50	Affected		50
Proband 1 Brother (II-3)	45	Normal		35
Proband 1 Brother's wife (II-4)	41	Normal		40
Proband 2 (Male, III-1)	28	Affected		50
Proband 2 Brother (III-2)	26	Normal		10
Proband 3 (Male, III-4)	17	Affected		60
Proband 3 Brother (III-3)	20	Normal		20

MW, Muscle wasting; JTW, Jaw and temporal wasting; FW, Facial weakness

SCK and diabetic condition may or may not be involve in the progression of the disease (Table.2 and 3) because the impact of various other parameter on the disease. Both sexes are affected with the disease and the undiagnosed cases take more time from overcome of the disease severity due to unavailability of proper diagnosis and treatment. So, if there is a large gap between age at presentation and age at onset of disease (i.e. from occurrence to hospitalization / treatment of the patient), the duration of the disease is higher and thus patient will take more time from overcome of the disease (Table.1).

To the best of our knowledge, we firstly report the cases related to the TP-PCR testing of DM1 patient and their family members. The present cases report indicates that TP-PCR could be successfully used for the identification of repeat size in DM1 and other repeat expansion disorder following similar mutational mechanism. It can be used successfully in place of southern blot which is radioactive and time consuming technique. Clinical and biochemical testing involve in the initial prediction and diagnosis of the disease which is confirmed by molecular testing. The enhanced application of this present technique enables us to prediction of those myotonic cases where presymptomatic and prenatal as well as postnatal diagnosis is required.

Figure.1: A 17 year old DM1 proband (III-4) showing muscle wasting, jaw and temporal wasting and facial weakness. He had no learning, writing, speech/languages, dysphasia and dyspepsia, sleep apnea, respiratory insufficiency and abnormal physical activity.

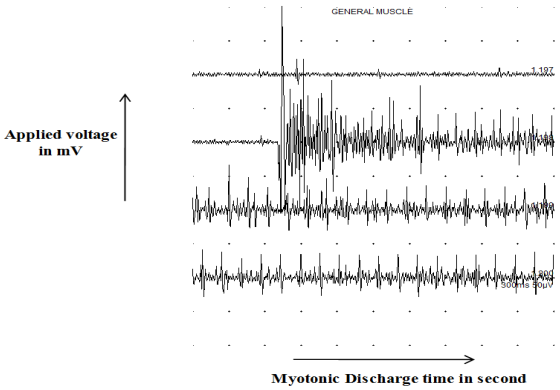

Figure.2: Concentric needle electromyography showing a typical myotonic discharges (20 second) in a myotonic dystrophy proband (III-4) with variation in amplitude as well as frequency, triggered by mechanical stimulation of abductor pollicis brevis muscle.

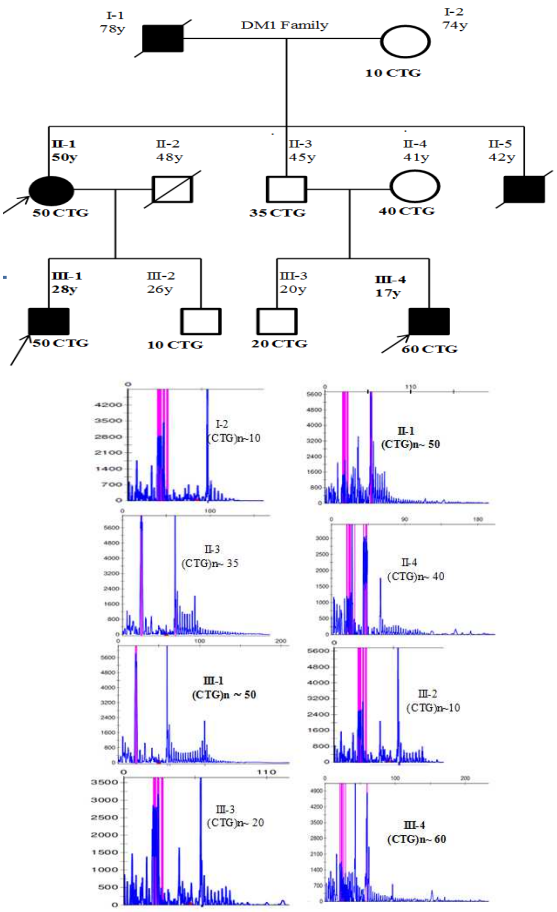

Figure.3: TP-PCR result of a DM1 family. Individual (I-2, III-2, III-3) were normal for CTG repeat while II-3 and II-4 were asymptomatic carrier for the disease. Probands (II-1, III-1 and III-4) were affected however, I-1, II-2 and II-5 were either pathogenic or normal but died before testing for CTG repeats.

AUTHORS' CONTRIBUTIONS

Ashok kumar is the first author and is responsible for the conception and design of the case report. Contributing authors Sarita Agarwal, Shubha Phadke and Sunil Pradhan made substantial contributions to the design of the manuscript and the acquisition, analysis and interpretation of the data.

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