Case Studies

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EVALUATION OF A NOVEL MOLECULAR TP-PCR TECHNIQUE FOR ITS DIAGNOSTIC PERFORMANCE IN MYOTONIC DYSTROPHY TYPE 1 (DM1): A CASE SERIES

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Abstract: Myotonic Dystrophy type 1 (DM1) is an autosomal dominant neuromuscular disorder occurred due to an expanded number of CTG repeats in the 3′ UTR of dystrophia myotonica protein kinase (DMPK) gene with an incidence of ~1 in 7500 adults. The number of repeats varies in normal population from 5-34 repeats and to > 40 repeats in affected individuals. The intention of the present case report is the evaluation of Triplet Primed PCR (TP-PCR) analysis which is a robust, rapid and non-radioactive technique in clinical application. The present study reports the CTG repeat pattern of three cases of DM1 patients and their family members. All 3 cases were provisionally diagnosed as DM1 and later were confirmed for expanded CTG repeats by TP-PCR. The reports confirm that TP-PCR could be successfully used for the identification of CTG repeat expansion in DM1. However, detailed clinical workups along with molecular diagnosis are needed to establish the genetic counseling and prenatal diagnostic clinics.

Keywords: Myotonic Dystrophy Type 1 (DM1), Triplet repeat disorder, CTG repeat, TP-PCR

INTRODUCTION

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 Type1 and Type 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.

The DM mutation involves an expanded trinucleotide repeat (CTG) in the DMPK gene in the 3′-untranslated region [2-4] and repeats varies in the normal population from 5-34 and >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 genetic testing of DM1 plays a critical role in directing the person for appropriate therapy and management. Muscle wasting, Jaw and temporal wasting, facial weakness and hypersomnia are the major clinical features of DM1 [2, 5-7] and associated with elevated level of muscle enzyme Serum Creatinine Kinase (SCK), characteristics pattern of electromyography peaks, nerve conduction velocity (NCV) and various other parameters. In past years PCR-RFLP [8, 9] and southern blotting [10, 11] were used for the detection of CTG repeats. The utility of TP-PCR was first shown by the Paola *et al.*,[12] and later Warner et al.,[13] used this technique for screening of CTG repeat expansion on the basis of characteristics peaks pattern

MATERIAL AND METHODS

In this case report we used the primers described by Warner *et al.*, [13] for our TP-PCR assay. The assay carried out in a reaction volume of 25µlitre with P1, P3 and P4 primers in the concentration of 10pmol, 10pmol and 1pmol respectively with 50ng of genomic DNA, 1U of Taq Polymerase and 200µM of dNTPs. The temperature profile adopted in TP-PCR cycling was 4 min at 94°C followed by 30 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 2 min and one cycle of 72°C for 10 minutes. The final products were analysed on 2% agarose gel and fragment analysis was performed on ABI-310 Genetic Analyser by the use of polymer (POP-4), Liz-500 and Hi-di formamide. The intention of the present case study is to establish the TP-PCR methodology in early screening of DM1 patient and family members in Indian setup and to provide genetic counseling. As per our knowledge, this is the first study that engages TP-PCR as a method for screening of DM1 patients and their family members in India.

Case presentations:

Case.1, A 29 year old unmarried proband (II-1) of Brahmin family visited SGPGIMS Neurology OPD with a complaint of muscle wasting and facial weakness. He was non-diabetic vegetarian and had no addictions. The height, weight and Body mass index (BMI) of the patient was 160cm, 67Kg and 26.17Kg/m^2 respectively. Electromyography (EMG) and SCK levels are mentioned in table 1. On the basis of clinical symptoms he has been advised for Phenytoin sodium (sodium 5, 5-diphenyl-2, 4-imidazolidinedione) with a dosage of 100 mg B.D.

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The patient and his family members were referred to Genetics Department for molecular evaluation. Blood sample were drawn from patient and his family members. The DNA was extracted from 2 ml EDTA blood and was subjected for TP-PCR analysis. The TP-PCR analysis revealed an expansion of CTG repeat in patient while his father (I-1) was found normal for CTG repeat. However, his mother (I-2) and his sister (II-2) were diagnosed as asymptomatic carriers for the disease (Figure 1). The family members had not given the consent for analysis of another 23 year old male child (II-3).

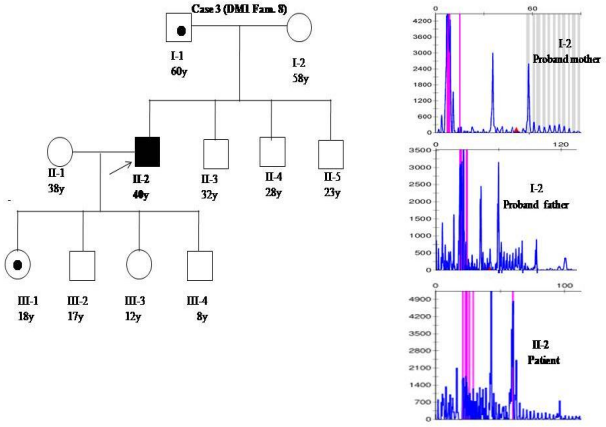

Figure.1: Case 1: I-1, I-2, II-2, II-3 were proband's father, mother, younger sister, younger brother and II-1 was DM1 Proband. Square = male; circle = female; black symbol= Proband (II-1); black dot in symbol represent asymptomatic carriers (I-2 and II-2).

Case.2, A 45 year old married female (II-3) of Kayastha family hailed from Madhya Pradesh visited Neurology OPD in SGPGIMS with a complaint of muscle weakness. She was non-diabetic vegetarian and had no addictions. On examination muscle wasting, jaw and temporal wasting and facial weakness were documented. The height, weight and BMI of the patient were 155cm, 40Kg and 16.64Kg/m^2 respectively. She had complained for hypersomnia and respiratory insufficiency problems. The ECG and SCK levels are mentioned in table 1. On the basis of clinical symptoms she has been advised for Phenytoin sodium (sodium 5, 5-diphenyl-2, 4-imidazolidinedione) 100mg twice a day.

Her father (I-1) and two brothers (II-4 and II-5) had died due to cardiac arrests. Her one son (III-1) and two daughters (III-2 and III-3) died at an early age. The patient and her husband were referred to Genetics Department for molecular evaluation. The TP-PCR analysis revealed expansion of CTG repeat in patient while her husband (II-2) was found normal for CTG repeat (Figure 2). Patient has not given the consent for molecular analysis of her mother (I-2) and unmarried sister (II-1).

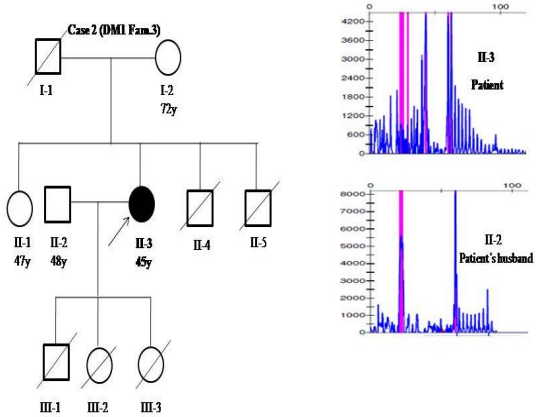

Figure.2: Case 2: I-1, I-2, II-1, II-2, II-4, II-5, III-1, III-2, III-3 were proband's father, mother, elder sister, husband, first brother, second brother, first child, second child, third child and II-2 was DM1 Proband. Italics line in square and / or circle represent death individuals (I-1, III-1, III-2 and III-3).

Table.1: Clinical features, Biochemical investigations and CTG repeat expansion in myotonic dystrophic patients

S.N.	Sex/Age (Year)	Disabilities			Clinical features								Activity		Biochemical Investigations		CTG repeat expansion (P=Present, A= Absent)
		L	W	S	MW	JTW	FW	RI	HYP	DYP	DYH	DM	E	ADL	SCK (U/L)	EMG	
1	M/29	N	N	N	Y	N	Y	N	N	N	N	N	Y	Y	272	+	P
2	F/45	N	N	N	Y	Y	Y	Y	Y	N	N	N	Y	Y	88	+	P
3	M/40	Y	N	Y	Y	Y	N	N	N	N	N	Y	Y	Y	73	+	P

L; Learning disability; W, Writing disability; S/L, Speech problem; MW, Muscle wasting; JTW, Jaw and temporal wasting; FW, Facial weakness; RI, Respiratory insufficiency; HYS, Hypersomnia (excess sleep or day time sleep); DYP, Dyspepsia (Indigestion); DYH, Dysphasia (Problem in swallowing of food); DM (Diabetes mellitus); E, Physical activity/Excercise; ADL, Activity of daily living; SCK, Serum creatinine kinase concenteration (normal range: 25-192U/L); EMG. Electromyography; N, No; Y, Yes; +, positive for myotonic dystrophy;

Case.3, A 40 year old married male (II-2) of Brahmin family hailed from Uttar Pradesh visited Neurology OPD in SGPGIMS with a complaint of muscular weakness. He was diabetic and had muscle wasting, jaw and temporal wasting on evaluation. The height, weight and BMI of the patient were 155cm, 65Kg and 27.05Kg/m^2 respectively. The ECG and SCK levels are mentioned in table 1. On the basis of his clinical symptoms he has been advised for Phenytoin sodium (sodium 5, 5-diphenyl-2, 4-imidazolidinedione) 100 mg

BD along with vitamin supplementations, Calcium citrate, L-Carnitine and Zinc Sulphate.

The TP-PCR analysis revealed a expansion of CTG repeat in proband. His mother (I-2), wife (II-1), three younger brother (II-3, II-4 and II-5), two kids ((III-2, III-3) were found normal for CTG repeat. While, his father (I-1) and one of his daughter (III-1) had expansion of CTG repeats and diagnosed as asymptomatic carriers (Figure.3). The family members have not given the consent for analysis of his son (III-4).

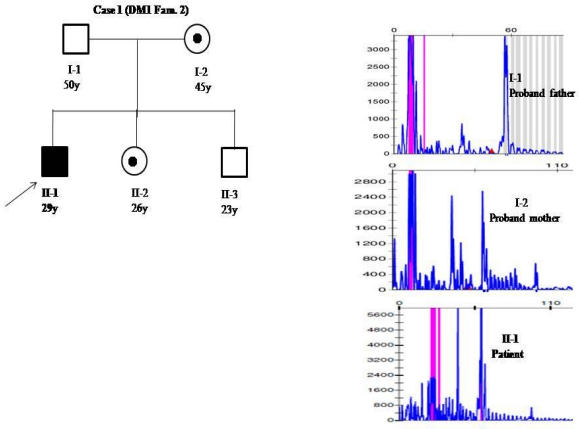

Figure.3: Case 3: I-1, I-2, II-1, II-3, II-4, III-1, III-2, III-3 and III-4 were proband's father, mother, wife, first brother, second brother, first daughter, first son, second daughter, second son and II-2 was DM1 Proband. I-1 and III-1 were asymptomatic carriers.

DISCUSSION

Myotonic dystrophy (DM) is a common neuromuscular triplet repeat disorder comprising at least two genetically different forms. DM1 is caused by expansion of a (CTG)(n) repeat in the DMPK gene, while DM2 is caused by expansion of a (CCTG)(n) part of a complex repetitive motif (TG)(n)(TCTG)(n)(CCTG)(n) in the ZNF9 gene [14]. Trinucleotide repeat disorder caused by increase in the number of triplet repeats occuring in some of the gene and classified into 2 major categories: (i) Coding expansion disorder (Huntington disease and different spirocerebellar ataxia etc.) (ii) Non-coding expansion disorder (Myotonic Dystrophy (DM) and Friedreich ataxia etc.).

Detection of the responsible expansions is complicated in both DM1 and DM2 because of the extremely variable length of the expanded alleles, which can contain even several thousands of repeats in both disorders [15]. In molecular methods, southern blotting, although used widely has a long laboratory turnaround time, is relatively expensive and has got hazards associated with the use of radioisotopes [16]. With respect to the genetic screening as well as diagnostics application, one of the commonly used detection approaches utilizes the combination of conventional PCR and triplet repeat-primed PCR (TP-PCR) [13, 14, 16]. TP-PCR method is routinely used by western countries for the diagnosis of DM1 while in India DM1 is diagnosed by clinically alone.

In the early amplification cycles of TP-PCR, the repeat specific P4 primer binds at multiple sites within CTG repeat alleles at 3′ and give rise to a mixture of products and specificity is dictated by the fluorescent locus specific primer (PI). A 10:1 molar ratio of P3 to P4 ensures that primer P4 is exhausted in the early amplification cycles and primer P3 amplifies from the end of products from previous amplification rounds. A long extension time is used to allow complete extension of the larger sized products within the PCR product mixture and conserve the representation of the longer products [13]. TP-PCR has certain limitation like it can detect the presence of expanded allele size without determining the total size of the expansion or the exact number of triplet repeat expansions like CTG repeat in myotonia.

The present report, which is first of its kind in India, illustrates three case reports characterized at the molecular level. This report has a strong concordance with the previous studies reported by Warner *et al.*, (1996) and Sermon *et al.*, (1998). SCK has no association with the progression of the disease (Table 1) because various other parameters also affect the disease progression. When one parent is asymptomatic carrier (permutation) 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 (Figure 1 and 3). Premutations can be inherited relatively stable for several generations if transmitted by women [17] and passage through the male germline almost invariably results in a large increase into the full disease range [17]. Therefore, it seems unlikely that the DM1 mutation could be stably maintained in the population for any significant length of time in this state.

CONCLUSION

To the best of our knowledge, this is the first report from India where evaluation of TP-PCR has been done for CTG repeat expansion in DM1 patients and their family members. The present report reconfirms the utility of TP-PCR for screening purposes and thus could be successfully used for the identification of repeat expansion disorders in DM1. The application of TP-PCR will help in detecting premutation in extended family members of the DM1 patients and for prenatal diagnosis, if and when required.

Author's contribution and Acknowledgements:

Ashok Kumar (AK) is the first author and is responsible for the conception and design of the case series. Prof. Sarita Agarwal and Srinivasan made substantial contributions to the design of the manuscript. Authors are thankful to Sanjay Gandhi Post Graduate institute of Medical Sciences, Lucknow for providing infrastructure facility in the department. AK is thankful to DBT, New Delhi for his fellowship.

REFERENCES

1. Harper PS, Myotonic dystrophy 2nd ed. London: Saunders, 1989.
2. Brook JD, McCurrac ME, Harley HG, Buckler AJ, Church D, Aburatani H, Molecular basis of myotonic dystrophy: Expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member, Cell, 1992, 68, 799-808.
3. Buxton J, Shelbourne P, Davies J, Jones C, Tongeren T, Aslanidis C, Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy, Nature, 1992, 355, 547–548.
4. Fu Y, An unstable triplet repeat in a gene related to myotonic muscular dystrophy, Science, 1992, 255, 1256–1258.
5. Redman JB, Fenwick RGJr, Fu YH, Pizzuti A, Caskey CT, Relationship between parental trinucleotide GCT repeat length and severity of myotonic dystrophy in offspring, JAMA, 1993, 269(15), 1960-5.
6. Mahadevan MS, Amemiya C, Jansen G, Sabourin L, Baird S, Neville CE. Structure and genomic sequence of the myotonic dystrophy (DM kinase) gene, Hum. Mol. Genet., 1993, 2, 299–304.
7. Shaw DJ, McCurrach M, Rundle SA, Harley HG, Crow SR, Sohn R, Genomic organization and transcriptional units at the myotonic dystrophy locus, Genomics, 1993, 18, 673–679.
8. Shaw DJ, Meredith AL, Sarfarazi M, Huson SM, Brook JD, Myklebost O, The apolipoprotein gene C2 gene: Sub chromosomal localization and linkage to the myotonic dystrophy locus, Hum Genet, 1985, 70, 271-273.
9. Hamzi K, Bellayou H, Slassi I, Nadifi S. A rapid polymerase chain reaction-based test for screening Steinert's disease (DM1), Neurol India, 2010, 58, 99-102.
10. Sermon K, Seneca S, De Rycke M, PGD in the lab for triplet repeat diseases-myotonic dystrophy, Huntington's disease and fragile-X syndrome, Mol Cell Endocrinol, 2001, 183, S77– S85.
11. Goossens V, DeRycke M, DeVos A. Diagnostic efficiency, embryonic development and clinical outcome after the biopsy of one or two blastomeres for preimplantation genetic diagnosis, Hum Reprod, 2008, 223, 481– 492.
12. Amicucci P, Gennarelli M, Novelli G, Dallapiccola B, Prenatal diagnosis of myotonic dystrophy using fetal DNA obtained from maternal plasma, Clin Chem, 2000, 46(2), 301-2.
13. Warner JP, Barron LH, Goudie D. A general method for the detection of large CAG repeat expansions by fluorescent PCR, J Med Genet, 1996, 33, 1022– 1026.
14. Khajavi M, Tari AM, Patel NB et al. "Mitotic drive" of expanded CTG repeats in myotonic dystrophy type (DM1), Hum Mol Genet, 2001, 10, 855-863.
15. Radvansky J, Ficek A, Kadasi L, Upgrading molecular diagnostics of myotonic dystrophies: multiplexing for simultaneous characterization of the DMPK and ZNF9 repeat motifs, Mol Cell Probes, 2011, 25, 182-185.
16. Addis M, Serrenti M, Meloni C, Cau M, Melis MA. Triplet-Primed PCR Is More Sensitive Than Southern Blotting-Long PCR for the Diagnosis of Myotonic Dystrophy Type1, Genet Test Mol Biomarkers, 2012, 16 (12), 1428–1431.
17. Barcelo JM, Mahadevan MS, Tsilfidis C, Mackenzie AE, Korneluk RG, "Intergenerational stability of the myotonic dystrophy protomutation," Hum Mol Genet, 1993, 2, 705–709.

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