REVIEW ARTICLE DOI: 10.15713/ins.bhj.80

Impact of Evolution of Molecular Technologies on Evaluation of Neuromuscular Disorders in India at a Centre in Mumbai

ABSTRACT

Advances in molecular methods have made genetic testing as an imperative parameter, to be used in research studies and clinical practice. The present article includes the evolution of molecular diagnostic techniques over a period of two decades leading to the corresponding expansion in identification and classification of various neuromuscular disorders (NMDs) in the population of mainly Western India. Initially, conventional testing was done by multiplex polymerase chain reaction (PCR), restriction fragment length polymorphism-PCR for single gene disorders such as Duchenne muscular dystrophy/Becker muscular dystrophy and spinal muscular atrophy, respectively; followed by multiplex ligation-dependent probe amplification technique, for deletion duplication analysis. Under a research study, immuno-specific Western blot was used for protein analysis as an initial screening tool for Limb Girdle Muscular Dystrophy 2B (LGMD2B). Sangers sequencing was done for single gene (for a few LGMD2B cases) and known familial variants of LGMD2B and LGMD2A and GNE myopathy. Availability of next generation sequencing technique involving massive parallel sequencing of multiple genes, improved the possibility of identifying the disease causing pathogenic mutations in phenotypically overlapping NMDs such as LGMDs and hereditary neuropathies. Since the past 12 years, over 2600 patients from mainly Western India have been analyzed for various NMDs. Carrier status of relatives of the probands was also determined in many of the cases. These molecular technologies have enabled to attained definitive diagnosis of different NMDs in India. The diagnostic and predictive data generated helps in better management of the disease. It also has immense relevance in carrier and prenatal testing for the families at risk, thereby limiting the occurrence of the diseases in the population.

Key words: LGMD, Molecular genetics, Neuromuscular, Next generation sequencing, Polymerase chain reaction

INTRODUCTION

Diagnosis and classification of neuromuscular disorders (NMDs) before 2001 were limited to histopathology on muscle biopsy, later followed by immunohistochemistry for a few of the disorders.[1,2] With the advent of molecular diagnostic techniques and with the identification of various genes for NMDs, there was an exponential increase in classification and diagnosis of types of NMDs in Indian population.

PCR TECHNOLOGY

Since the introduction of PCR methods, due to its sensitivity and relatively low cost, it has become a standard tool for preliminary diagnosis of certain disorders. Therefore, the conventional testing by multiplex PCR (mPCR) and restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) for single gene disorders such as DMD/BMD and Spinal muscular atrophy (SMA), respectively, was initiated, followed by multiplex ligation-dependent probe amplification (MLPA) technique, for the detection of deletion duplication and copy number analysis.

PCR technology enabled cost-effective molecular diagnosis of single gene NMDs such as DMD/BMD and SMA to identify deletions only.

Rashna S. Dastur[1], Aisha F. H. Zakaria[1], Satish V. Khadilkar[2], Madhuri R. Hegde[3], Pradnya S. Gaitonde[1]

[1]Centre for Advanced Molecular Diagnostics in Neuromuscular Disorders, Mumbai, Maharashtra, India, [2]Department of Neurology, Bombay Hospital Trust, Mumbai, Maharashtra, India, [3]Global Lab Services at Perkin Elmer, Inc., Atlanta, USA

Corresponding Author:
Dr. Rashna S. Dastur, Centre for Advanced Molecular Diagnostics in Neuromuscular Disorders (CAMDND), C/o IATRIS (TDM Lab), Plot No. 194, Scheme No. 6, Road No. 15, Sion (East), Mumbai - 400 022, Maharashtra, India. Phone: +91-9820304478. E-mail: rdastur@hotmail.com

mPCR and MLPA

DMD and BMD are X-linked disorders having an incidence of one in 3500–4500 live male births, caused due to mutations in the dystrophin-encoding *DMD* gene, on Xp21.2 region containing 79 exons.[3,4]

The majority of mutations in DMD/BMD are deletions in the "hot spot" region and could be identified easily using mPCR technique. Large deletions are most common (approx. 70%), followed by duplications (10%) and point mutations (20%).[5,6]

Since the past 20 years, genetic diagnosis is being carried out at different centers in India, using whole blood sample, thereby preventing the need for invasive procedure of muscle biopsy in DMD/BMD patients.

Initially, deletions were identified in the *dystrophin* gene using mPCR covering 19 exons which were later upgraded to 32 exons [Figure 1a].[7] This method of PCR has become a standard tool for preliminary diagnosis for DMD/BMD due to its relatively low cost and sensitivity, as it picks up deletions in 65% of cases. This technique is also of use in prenatal diagnosis, only in cases with known familial deletions.

With the availability of MLPA technology (Multiple Ligation dependent Probe Amplification), using capillary electrophoresis, for identification of deletions/duplications of all the 79 exons of DMD gene, became possible in Indian laboratories.[8] This technique of MLPA also allowed to determine the carrier status of this X-linked disease in females having family history of DMD individuals, thereby estimating the risk in the future pregnancies and prenatal testing, to limit the burden of the disease in the population.[9,10]

However, since the past few years, due to the introduction of comprehensive next generation sequencing (NGS) panels such as focused exome panel, deletion/duplication, and point mutation is covered for dystrophin genes. However, NGS panels being a high-end test, mPCR/MLPA remains a cost-effective method of choice for identifying the deletions, in Indian scenario especially for patients referred from public hospitals.

In our center alone in the past 12 years, over 1200 samples were analyzed by mPCR and MLPA, out of which 69% showed deletions/duplications of various exons [Figure 1]. Over the period of time, MLPA has become a method of choice for patients and for carrier testing [Figure 1b].[11]

PCR-RFLP

SMA is one of the most common genetic cause of infant mortality with an incidence of one in 6000–10,000 live births and a carrier frequency of 1:50 worldwide. SMA is an autosomal recessive disorder associated with a mutation in the Survival Motor Neuron (*SMN1*) gene, located on chromosome 5.[12] About 95% of people with SMA have homozygous deletion of exon 7 of the *SMN1* gene. There are four types of SMA, classified clinically according to the age of onset, mode of inheritance, distribution of muscle weakness, and progression of symptoms. The most common cause of SMA is due to deficiency of a motor neuron protein called SMN.[13,14]

SMN gene deletion studies have become the first line of investigation for confirmation of a clinical diagnosis of SMA. The findings of homozygous deletions of exons 7 and/or 8 of *SMN1* gene confirm the diagnosis of SMA, even in patients with atypical clinical features.

Patients are tested for homozygous deletion of exons 7 and 8 of *SMN1* gene by a PCR-RFLP method [Figure 2]. More than 600 patients suspected of SMA were analyzed during the past 12 years, out of which more than 350 were diagnosed with SMA of various types.[15]

Hence, absence of exon 7 of *SMN1* gene has been used as a diagnostic tool for confirmation of the disease as well as for the prenatal diagnosis. In recent years, identification of SMA carrier is being made possible by determining *SMN1* and *SMN2* copy numbers, using quantitative PCR methods. This also helps in predicting the progression of the disease.[16]

The disease severity causing different phenotype but with same genotype is also known to depend on disease modifiers,

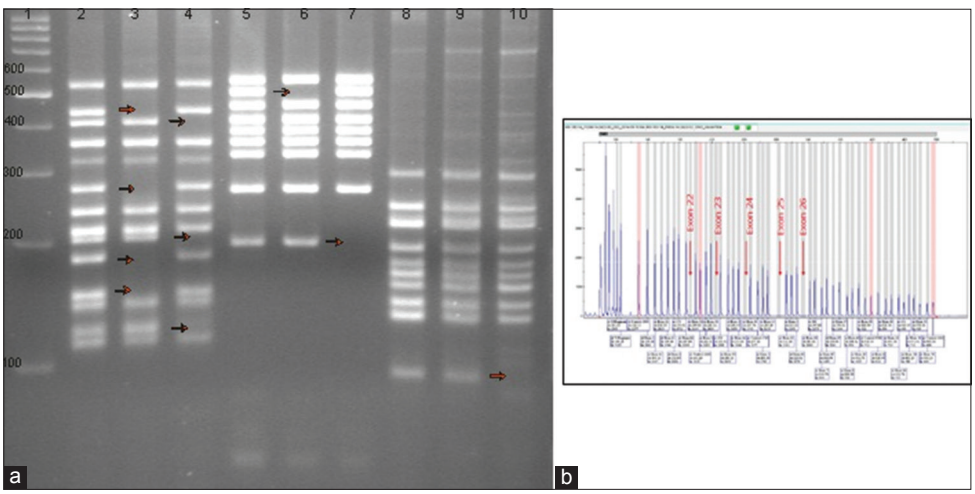

Figure 1: Agarose gel electrophoresis of PCR products and multiplex ligation-dependent probe amplification (MLPA) electropherogram of DMD patient (a) Agarose gel electrophoresis of PCR products of 32 exons of *dystrophin* gene; Pt. 1- Deletions Exons 46 – 50, Lane 3 – Exons 46, 47, 50, 49, Lane 6 – Exon 48; Pt. 2- Deletions Exons – 3-7, Lane 4 – Exons 7, 6, 3, Lane 7 – Exon 4, Lane 10 – Exon 5. (b) MLPA electropherogram of DMD male patient showing absence of peaks in DMD Probe-P034 (MRC-Holland) representing deletions of the exons 22–26 of the dystrophin gene (arrow indicating absence of peak corresponding to exons 22, 23, 24, 25, and 26 from the left)

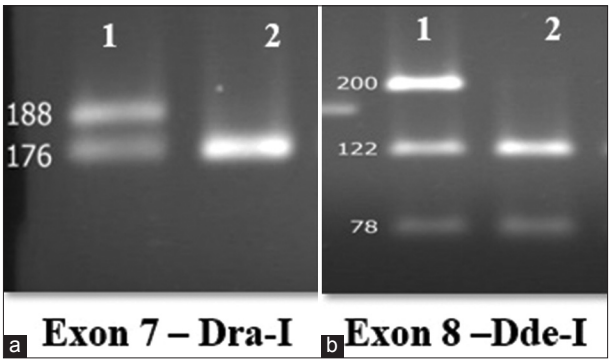

Figure 2: Detection of Survival Motor Neuron (SMN1) gene deletion using restriction fragment length polymorphism-polymerase chain reaction: Agarose gel showing *SMN 1* gene exon 7 and 8 (a) Exon 7 – Dra-I; Lane 1: Control-no deletion, Lane 2: Patient-deletion of *SMN 1* Exon 7 (b) Exon 8 –Dde-I; Lane 1: Control-no deletion, Lane 2: Patient-deletion of *SMN 1* Exon 7

which do not cause disease but can affect (modify) the onset and progression by influencing various biological pathways. *SMN2* gene copy number is one such factor altering the prognosis of the disease. Identifying the deletion helps in carrier and prenatal diagnosis to prevent the birth of affected individuals. Recent advances in the treatment for SMA make genetic confirmation imperative for further management.[17]

Trinucleotide repeat studies for spino-cerebellar ataxia (SCA)

SCA is a neurodegenerative disorder affecting cerebellar, extrapyramidal, and autonomic system leading to progressive incoordination of gait and other movement deficits. Incidence of these ataxias varies from 1 to 5/100,000 individuals.

More than 43 genes have been recognized to be associated with SCAs, which are known to show dominant or recessive pattern of inheritance. SCAs are caused either due to specific mutations or a pathological expansion of trinucleotide repeats, in the specific gene. Hence, genetic testing for ataxia is complicated and requires two molecular testing methods, that is, trinucleotide repeat studies or Exome sequencing (ES), which are to be opted for, to obtain a definitive diagnosis. In routine molecular laboratories, method of PCR and capillary electrophoresis is used to identify expansion repeats in SCA1–SCA7 as NGS fails to detect the repeats.[18]

The high incidence of SCA1 has been reported in Southern India, whereas SCA2 type has been identified more in Northern and Eastern India.[19] Trinucleotide repeat studies on SCA type 12 with founder mutation in an ethnic group have also been reported in North Indian population. SCA12 has also found to be one of the common disorders seen in Agarwal community.[20]

DNA SEQUENCING METHODS

In India, LGMDS such as dysferlinopathy (DYSF), calpainopathy (CAPN3), sarcoglycanopathy, and myopathy like GNE is common muscle disorders seen phenotypically.[21] In a few of these, depending on the clinical work up, the specific single gene suspected to be involved was sequenced by Sanger method.

Sanger sequencing method to sequence the entire gene was initially available in India since the past two decades and was restricted to research institutes and could not be used as a regular diagnostic tool due to its laborious protocol and high cost.

CAPN3 (LGMD2A) is an autosomal recessive muscular dystrophy, caused by mutations in the *CAPN3* gene (24 exons) located on chromosome region 15q15.1–q21.1. During the course of research studies, founder mutations were detected in cases of CAPN3. In "Agarwal community," two ancestral founder mutations in calpain gene (exon 22 c.2338G>C, p.D780H and a splice site variant in intron 18/exon 19 c.2051-1G>T) were detected.[22] Targeted testing for these two variants used be a cost effective method in diagnosing CAPN3 for affected individuals belonging to "Agrawal community."

This target sequencing test was used initially as a diagnostic tool at our center, till the advent of NGS technology. Founder alleles were detected in 89% of patients from Agarwal community showing LGMD phenotype and unaffected carriers were also detected using target sequencing test for these two founder mutations.[23]

The Agarwal's practice intra-communal exogamy is known to harbor autosomal recessive diseases. These founder variant analysis can be utilized as the initial non-invasive diagnostic step for index cases, carrier detection, and counseling with almost 90% yield.[23]

DYSF, LGMD2B, and Miyoshi Myopathy: During the year 2012–2016, a project was undertaken, funded by the Jain Foundation (USA) and in collaboration with Emory Genetics (USA), to identify DYSF cases from the large cohort of LGMD cases available in India.[24]

DYSF is an autosomal recessive type of muscular dystrophy caused by pathogenic variants in the *dysferlin* gene (*DYSF* gene) located on chromosome 2p13. The *dysferlin* gene consists of 55 exons, and encodes transmembrane protein which is highly expressed in muscle and also in blood monocytes.[25] Dysferlin protein is known to be either absent or substantially reduced in cases of DYSF. Hence, an alternative screening method of Western blot was adopted to check the status of protein in peripheral blood monocytes in suspected cases of LGMDs.[24]

In this study, out of a total of 317 clinically suspected LGMD patients, 166 were analyzed using monocyte assay/Western blot, while the balance 151 LGMDs were screened using only a predictive algorithm called ALDA, predicting possibilities of LGMD subtypes based on clinical symptoms (http://www.jain-foundation.org/lgmd-subtyping-diagnosis-tool). A total of 125 screened and predicted LGMD2B patients were sequenced. Due to cost restrains, initially 15 cases showing absence of protein were analyzed by Sanger sequencing. Two pathogenic variants were detected in all of them. With the subsequent availability of

NGS technology, 110 cases were later sequenced and studied. In this specific study, 98.2% of patients with absence of the *dysferlin* protein showed one or more variants in the *dysferlin* gene; hence, the assay has a high predictive significance in diagnosing dysferlinopathies. At present, identification of *dysferlin* pathogenic variants by NGS is the ultimate method for diagnosing dysferlinopathies, though follow-up with the monocyte assay can be useful to understand the phenotype in relation to the *dysferlin* protein expression and also be a useful biomarker for future clinical trials.[24]

GNE myopathy/Nonaka myopathy is an autosomal recessive disorder caused due to mutations in the *GNE* gene located on chromosome 9p13.3. The gene encodes an enzyme glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase responsible for sialic acid production.[26] On Sanger sequencing, a common mutation in exon 10 (c.1853T>C) was identified in the *GNE* gene in six patients belonging to the Rajasthan ancestry.[27,28] Before the introduction of NGS, target sequencing was carried out in a very few cases, to identify GNE myopathy.

NGS

Overlapping clinical phenotype and heterogeneity of the most NMDs lead to a suspicion of more than one gene. Analysis of more than one gene is very tedious and expensive for clinical diagnosis. The high demand for low-cost sequencing has led to the development of the high-throughput sequencing technologies. NGS is a high-throughput DNA sequencing technology, enabling high speed, and simultaneous sequencing of many genes for many patients. NGS is highly accurate, as mutations as small as a single base pair are detected with minimal amount of DNA required.[29]

The use of NGS techniques has improved diagnosis and understanding of NMDs and has provided insight to correlate between clinical phenotype and pathogenic mutation.[30] Since the past 6 years, there has been an exponential increase in the identification, classification, and diagnosis of NMDs in India. This was due to the availability of NGS in the centralized diagnostic centers.

Genes involved in the same disease phenotype are grouped together as one panel making it cost effective. The NGS platform can be employed to effectively target a large number of genes related to NMDs for accurate diagnosis.

Between the span of 2 years, the NGS methods have evolve rapidly from disease specific panel test covering 60 plus genes, to focused exome spanning more than 6000 genes related to NMDs. Focused exome is a highly targeted design that enables analysis of only disease-associated targets, enabling superior coverage of disease-associated genes. The cost of sequencing technologies has also shown rapid decline and as a result, there is an increase in affordability to undergo genetic testing and is the diagnostic method of choice. Although, in Indian scenario, whole genome sequencing is yet to be used as routine practice in diagnosis of NMDs due to its high cost.

NMDs such as LGMDs, myopathies, and neuropathies are rare inherited disorders, affecting muscle and/or nerves with neonatal, childhood, or adulthood onset. Clinical heterogeneity and unidentified genes require multiple biochemical and molecular methods to reach a definitive diagnosis.[31] The application of NGS, as a routine diagnostic strategy, has led to the identification of variants (mutations) in rare genetic disorders such as NMD. To date, >250 genes are associated with various inherited NMD's.[25]

Inherited myopathies combined have a high prevalence of one in 6000 individuals across the world, comprising of more than 200 different rare disease-subtypes.[32] Clinical-correlation driven definitive molecular diagnosis became possible due to NGS technology.

Initially at our center, NGS was carried out on 110 suspected LGMD2B patients as continuation of the study funded by the Jain Foundation (USA) and in collaboration with Emory Genetics (from 2012 to 2017). Diagnosis of DYSF (LGMD2B) was confirmed by LGMD NGS panel testing and two variants were identified in 100 Indian patients.[24]

The NGS analysis performed at PerkinElmer Genomics Laboratory (USA), initially sequenced patients using LGMD/NMD panel tests covering over 60 genes, but now a comprehensive focused exome panel covering over 6000 genes of NMDs is being carried out.

In our 335 patients sequenced (PerkinElmer Genomics laboratory [USA]), since the past 7 years, so far DYSF followed by CAPN3 was found to be most common LGMD. Although number of patients showing variants in *GNE* gene was more, about 26% showed only one pathogenic variant in the GNE gene. About 24% of patients remained unclassified showing variants of uncertain significance (VUS) in multiple genes. Further analysis of these patients is required.

Sarcoglycanopathies, a subtype of LGMDs is comparatively uncommon, and caused due to alterations in any of the genes associated with Sarcoglycan complex leading to absence of one of the four transmembrane glycoproteins (alpa-, beta-, gamma-, or delta-). In India, many studies on sarcoglycanopathies have been cited in the literature, done on the bases of histopathological and immunohistochemical findings.[33,34] Mutation analysis on 18 cases of suspected sarcoglycanopathies from Western India, has been reported, only by Khadilkar *et al*. in 2009.[35] From the recent NGS data and our observations, sarcoglycanopathies are rare in the Indian population.

A large clinical ES study with phenotype correlation was performed on 207 clinically well-characterized inherited myopathy-suspected patients from the Indian subcontinent establishing clinical-correlation driven definitive molecular diagnosis. The majority of pathogenic variants were identified in either of three genes, GNE (GNE-myopathy), DYSF, and CAPN3, indicating that these genes are the major contributors to genetic myopathy in India.[36]

Inherited neuropathies are large group of disorders affecting peripheral nervous system, having variable genotypic and phenotypic features. These are classified according to the

involvement of type of deficit, either in motor, sensory, and/or autonomic never fibers.[37]

One of the most common inherited neuropathies is Charcot-Marie-Tooth disease (CMT) having prevalence of one in 2500 individuals. More than 30 genes have been known to be involved in causing CMT. There are three patterns of CMT inheritance X-linked, autosomal dominant, and autosomal recessive.[37]

The duplication of 1.5 Mb DNA fragment of *Peripheral Myelin Protein 22* (*PMP22* gene) on chromosome 17p.11.2 is associated with over 70% of CMT1 cases.

The genes associated with axonal type of CMT2 are mapped on first, third, and seventh chromosome following autosomal dominant pattern of inheritance whereas CMT4 type affects either exons or the myelin and have an autosomal recessive pattern.[38] Focused ES test remains the first choice of testing option to identify pathogenic variants in the genes causing these different types of CMTs which are included in the panel.

FASCIOSCAPULOHUMERAL MUSCULAR DYSTROPHY (FSHD) 1 MOLECULAR DIAGNOSTIC TEST

FSHD is one of the autosomal dominant types of muscular dystrophy, with prevalence of one in per 10,000 individuals. It is the third common form of muscular dystrophy, manifested by scapular wing shoulder, girdle weakness, facial weakness, lower limb weakness causing foot drop, and abdominal weakness causing lordosis; age of onset is usually around adolescence.

There are two types of FSHD's, FSHD type 1 (FSHD1), and FSHD type 2 (FSHD2). FSHD1 being the most common and found in 95% of patients. These two types are linked to two different gene mechanisms.

FSHD1 is known to be associated with contraction of chromosomal repeat called D4Z4 at 4q35 location of chromosome 4. Normal individual has 11–150 D4Z4 repeats which are hypermethylated. Individual with FSHD1 has contracted repeat array arranged between 1 and 10 contiguous units and these are hypomethylated. There are two alleles of chromosome 4, the 4qA variant and 4qB variant. The 4qA variant with shortened D4Z4 repeat causes FSHD1. Individuals having contracted D4Z4 repeats on 4qB allele are not associated with FSHD and are unaffected.[39]

Similar D4Z4 repeat array exists on chromosome 10 but shortening of these repeats does not cause FSHD. FSHD2 though clinically similar to FSHD1 is caused due to detection of pathogenic variants in *SMCHD1* gene.[39]

Pattern of inheritances of FSHD is autosomal dominant and single gene pathogenic variant causes a disease. Hence, even if one parent is affected, there is 50% chance of each offspring to inherit FSHD.

Conventional testing for FSHD is done by Southern blot analysis using pulse field gel electrophoresis after digestion with a set of restriction enzymes to determine the size of D4Z4 repeat array. Other methods include long range PCR, molecular combing, and marker analyzes having used by different laboratories. Southern blot has its own limitation as the method is laborious, requires large amounts of DNA and provides approximate size of the D4Z4 repeat.[40]

Recent testing method has been developed by PerkinElmer using whole genome optical mapping (Bionano Genomics, USA) assays. This method is used to determine the repeats of D4Z4 region precisely even to one repeat of D4Z4 size, thereby imparting diagnostic accuracy to identify FSHD1 patients (unpublished data).

CONCLUSION

Since the past two decades, evolution of molecular technologies has facilitated evaluation and delimitation of NMDs resulting in their improved identification, classification, and diagnosis. At our center, molecular diagnosis was initiated in 2002, with just PCR for single gene deletions, after two decades with the availability of NGS, there was an exponential increase in classification and diagnosis of types of NMDs in Indian population [Figure 3].

The emergence and development of various molecular techniques have enabled definitive diagnosis of NMDs, in India [Figure 4].

For single gene disorders, PCR methods still remain a cost-effective method of choice for molecular diagnosis. With decline in cost and increase in gene coverage, by NGS platforms and focus ES, single gene sequencing has been replaced by parallel sequencing technologies. The data obtained provide valuable information for affected individuals for the management and future gene-based treatment approaches. Carrier status for other unaffected family members can be determined; thereby preventing the transmission of the disorders by offering prenatal diagnosis. Overall, molecular diagnostics have improved our knowledge of the gene variant spectrum, mutational hotspots, genetic etiologies, and prevalence of different NMD subtypes across India, thus allowing timely management of the disease.

As next generation DNA sequencing testing is being offered by many centers in India, the data generated by these tests reveal a spectrum of gene variants associated with NMDs in different cohorts of the population, some of them identified as "founder mutation" in certain ethnic groups. Such studies eventually are useful in more accurate classification and helps creating a database. Large number of VUS is also identified in the gene associated with the disorders but a sufficient statistical data are yet to be achieved to reclassify them to attain a definitive diagnosis. Therefore, more research based subsidized and cost effective, ES studies are to be made available in India to encourage gene testing where 50% patients approaching are from public hospital.

Classification of different LGMDs and other NMDs on the basis of genetically diagnosed cases, has initiated, formation of registries for respective subtypes. These registries having the gene variant and clinical data of patients serve as a guide to understand treatment options, to design clinical trials, patient recruitment, and to evaluate patient outcomes in the future.

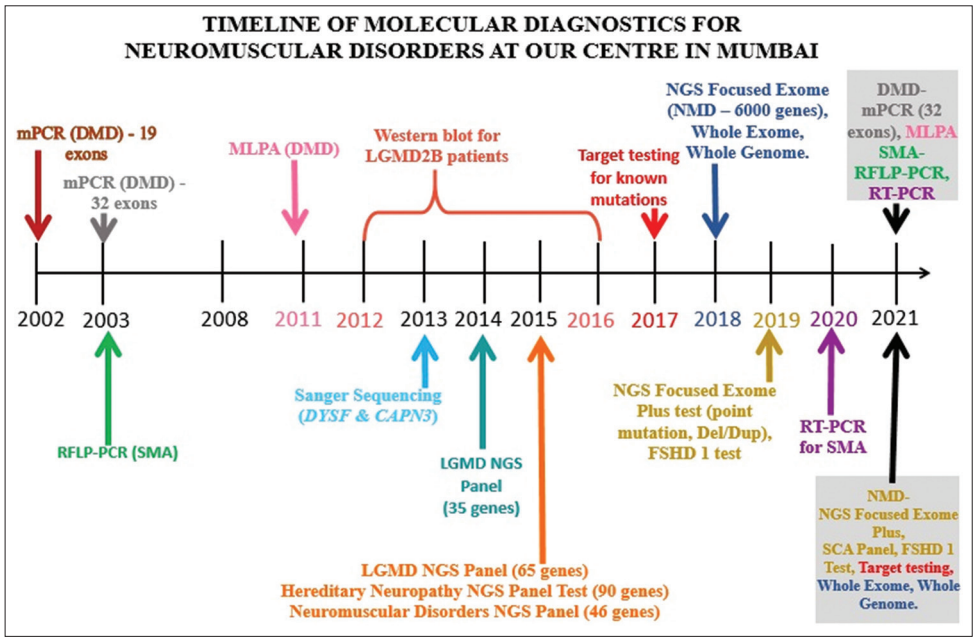

Figure 3: Timeline of molecular diagnostics for neuromuscular disorders at our center in Mumbai

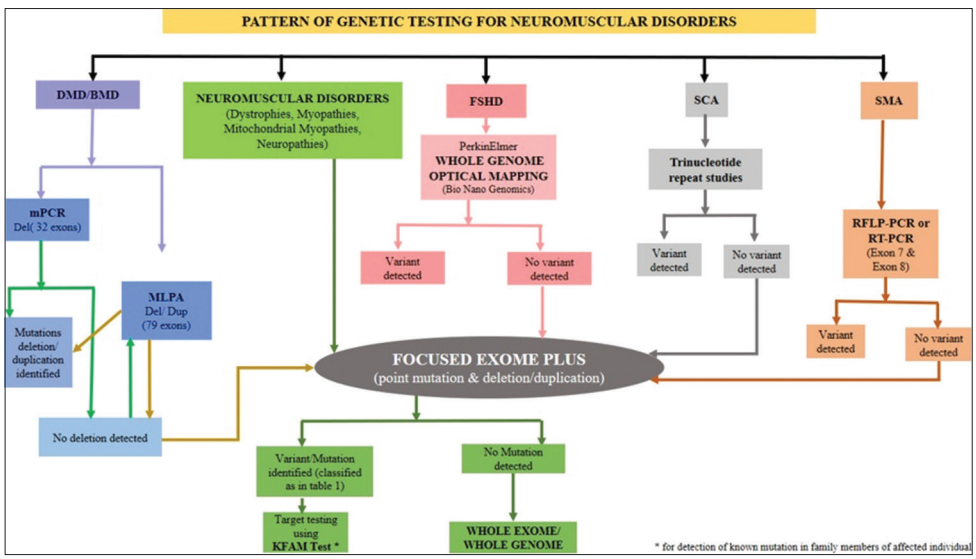

Figure 4: Pattern of genetic testing for neuromuscular disorders

ACKNOWLEDGMENTS

We acknowledge and thank The Jain Foundation, USA, for funding the entire study on Dysferlinopathies. Special thanks are due to Dr. Sasikumar K. Menon, Director, IATRIS (TDM Lab), Mumbai, for providing the infrastructural facilities. We also thank Ms. Mugdha Devalkar, Research Assistant and Ms. Jayashree Thorat for data entry.

REFERENCES

1. Khadilkar S. Bird's eye view of myopathies in India. Neurol India 2008;56:225-8.
2. Nalini A, Polavarapu K, Preethish-Kumar V. Muscular dystrophies: An Indian scenario. Neurol India 2017;65:969-70.
3. Emery AE, Muntoni F, Quinlivan RC. Duchenne Muscular Dystrophy. 4th ed. Oxford: Oxford University Press; 2015.
4. Worton RG, Thompson MW. Genetics of Duchenne muscular dystrophy. Annu Rev Genet 1988;22:601-29.
5. Beggs AH, Koenig M, Boyce FM, Kunkel LM. Detection of 98% of DMD/BMD gene deletions by polymerase chain reaction. Hum Genet 1990;86:45-8.
6. Chamberlain JS, Gibbs RA, Ranier JE, Caskey CT. Multiplex PCR for the diagnosis of Duchenne muscular dystrophy. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR Protocols: A Guide to Methods

and Applications. San Diego: Academic Press; 1990. p. 272-81.
7. Dastur RS, Gaitonde PS, Khadilkar SV, Nadkarni JJ. Becker muscular dystrophy in Indian patients: analysis of dystrophin gene deletion patterns. Neurol India 2008;56:374-8.
8. Kohli S, Saxena R, Thomas E, Singh J, Verma IC. Gene changes in Duchenne muscular dystrophy: Comparison of multiplex PCR and multiplex ligation-dependent probe amplification techniques. Neurol India 2010;58:852-6.
9. Nadkarni JJ, Dastur RS, Viswanathan V, Gaitonde PS, Khadilkar SV. Duchenne and Becker muscular dystrophies: An Indian update on genetics and rehabilitation. Neurol India 2008;56:248-53.
10. Singh RJ, Manjunath M, Preethish-Kumar V, Polavarapu K, Vengalil S, Thomas PT, *et al*. Natural history of a cohort of Duchenne muscular dystrophy children seen between 1998 and 2014: An observational study from South India. Neurol India 2018;66:77-82.
11. Dastur RS, Kachwala MY, Khadilkar SV, Hegde MR, Gaitonde PS. Identification of deletions and duplications in the Duchenne muscular dystrophy gene and female carrier status in western India using combined methods of multiplex polymerase chain reaction and multiplex ligation-dependent probe amplification. Neurol India 2011;59:803-9.
12. Melki J, Abdelhak S, Sheth P, Bachelot MF, Burlet P, Marcadet A, *et al*. Gene for chronic proximal spinal muscular atrophies maps to chromosome 5q. Nature 1990;44:767-8.
13. Lefebvre S, Burglen L, Reboullet S, Clermont O, Burlet P, Viollet L, *et al*. Identification and characterization of a spinal muscular atrophy-determining gene. Cell 1995;80:155-65.
14. Monani UR, Lorson CL, Parsons DW, Prior TW, Androphy EJ, Burghes AH, *et al*. A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN 1 from the copy gene SMN2. Hum Mol Genet 1999;8:1177-83.
15. Dastur RS, Gaitonde PS, Khadilkar SV, Udani VP, Nadkarni JJ. Correlation between deletion patterns of SMN and NAIP genes and the clinical features of spinal muscular atrophy in Indian patients. Neurol India 2006;54:255-9.
16. Niba ET, Rochmah MA, Harahap NI, Awano H, Morioka I, Iijima K, *et al*. Spinal muscular atrophy: Advanced version of screening system with real-time mCOP-PCR and PCR-RFLP for SMN1 deletion. Kobe J Med Sci 2019;65:E49-E53.
17. Khadilkar SV, Singh RK. Nusinersen and spinal muscular atrophies: Where are we in 2020? Ann Indian Acad Neurol 2020;23:743-4.
18. Paulson HL. The spinocerebellar ataxias. J Neuroophthalmol 2009;29:227-37.
19. Venkatesh SD, Kandasamy M, Moily NS, Vaidyanathan R, Kota LN, Adhikarla S, *et al*. Genetic testing for clinically suspected spinocerebellar ataxias: Report from a tertiary referral centre in India. J Genet 2018;97:219-24.
20. Choudhury S, Chatterjee S, Chatterjee K, Banerjee R, Humby J, Mondal B, *et al*. Clinical characterization of genetically diagnosed cases of spinocerebellar ataxia type 12 from India. Mov Disord Clin Pract 2017;5:39-46.
21. Khadilkar SV, Faldu HD, Patil SB, Singh R. Limb-girdle muscular dystrophies in India: A review. Ann Indian Acad Neurol 2017;20:87-95.
22. Ankala A, Kohn JN, Dastur R, Gaitonde P, Khadilkar SV, Hegde MR. Ancestral founder mutations in calpain-3 in the Indian Agarwal community: Historical, clinical, and molecular perspective. Muscle Nerve 2013;47:931-7.
23. Khadilkar SV, Chaudhari CR, Dastur RS, Gaitonde PS, Yadav JG. Limb-girdle muscular dystrophy in the Agarwals: Utility of founder mutations in CAPN3 gene. Ann Indian Acad Neurol 2016;19:108-11.
24. Dastur RS, Gaitonde PS, Kachwala M, Nallamilli BR, Ankala A, Khadilkar SV, *et al*. Detection of dysferlin gene pathogenic variants in the Indian population in patients predicted to have a dysferlinopathy using a blood-based monocyte assay and clinical algorithm: A model for accurate and cost-effective diagnosis. Ann Indian Acad Neurol 2017;20:302-8.
25. Nallamilli BR, Chakravorty S, Kesari A, Tanner A, Ankala A, Schneider T, *et al*. Genetic landscape and novel disease mechanisms from a large LGMD cohort of 4656 patients. Ann Clin Transl Neurol 2018;5:1574-87.
26. Eisenberg I, Avidan N, Potikha T, Hochner H, Chen M, Olender T, *et al*. The UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase gene is mutated in recessive hereditary inclusion body myopathy. Nat Genet 2001;29:83-7.
27. Khadilkar SV, Nallamilli BR, Bhutada A, Hegde M, Gandhi K, Faldu HD, *et al*. A report on GNE myopathy: Individuals of Rajasthan ancestry share the Roma gene. J Neurol Sci 2017;375:239-40.
28. Nalini A, Gayathri N, Nishino I, Hayashi YK. GNE myopathy in India. Neurol India 2013;61:371-4.
29. Ilyas M. Next-generation sequencing in diagnostic pathology. Pathobiology 2017;84:292-305.
30. Shieh PB. Muscular dystrophies and other genetic myopathies. Neurol Clin 2013;31:1009-29.
31. Bushby KM. The limb-girdle muscular dystrophies-multiple genes, multiple mechanisms. Hum Mol Genet 1999;8:1875-82.
32. González-Jamett AM, Bevilacqua JA, Díaz AM. Hereditary myopathies, Muscle Cell and Tissue - Current Status of Research Field. India: IntechOpen; 2018.
33. Khadilkar SV, Singh RK, Katrak SM. Sarcoglycanopathies: A report of 25 cases. Neurol India 2002;50:27-32.
34. Nalini A, Gayathri N, Thaha F, Das S, Shylashree S. Sarcoglycanopathy: Clinical and histochemical characteristics in 66 patients. Neurol India 2010;58:691-6.
35. Khadilkar SV, Singh RK, Hegde M, Urtizberea A, Love DR, Chong B. Spectrum of mutations in sarcoglycan genes in the Mumbai region of western India: High prevalence of 525del T. Neurol India 2009;57:406-10.
36. Chakravorty S, Nallamilli BR, Khadilkar SV, Singla MB, Bhutada A, Dastur R, *et al*. Clinical and genomic evaluation of 207 genetic myopathies in the Indian subcontinent. Front Neurol 2020;11:559327.
37. Tazir M, Hamadouche T, Nouioua S, Mathis S, Vallat JM. Hereditary motor and sensory neuropathies or Charcot-Marie-Tooth diseases: An update. J Neurol Sci 2014;347:14-22.
38. Nagappa M, Sharma S, Govindaraj P, Chickabasaviah YT, Siram R, Shroti A, *et al*. PMP22 gene-associated neuropathies: Phenotypic spectrum in a cohort from India. J Mol Neurosci 2020;70:778-89.
39. Lemmers RJ, van der Wielen MJ, Bakker E, Frants RR, van der Maarel SM. Rapid and accurate diagnosis of facioscapulohumeral muscular dystrophy. Neuromuscul Disord 2006;16:615-7.
40. Tamhankar PM, Phadke SR. Clinical profile and molecular diagnosis in patients of facioscapulohumeral dystrophy from Indian subcontinent. Neurol India 2010;58:436-40.

How to cite this article: Dastur RS, Zakaria AFH, Khadilkar SV, Hegde MR, Gaitonde PS. Impact of Evolution of Molecular Technologies on Evaluation of Neuromuscular Disorders in India at a Centre in Mumbai. Bombay Hosp J 2021;63(4):206-212.

Source of support: Nil, **Conflicts of interest:** None