DOI: 10.7860/JCDR/2024/67604.19298

Original Article

Genetics Section

The Spectrum of Deletion Pattern in the Dystrophin Gene in Duchenne Muscular Dystrophy Patients: A Cross-sectional Study from Northeast India

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ABSTRACT

Introduction: Duchenne Muscular Dystrophy (DMD) is an X-linked recessive neuromuscular disorder characterised by progressive, irreversible muscle weakness. It is caused by a mutation in the dystrophin or DMD gene, leading to the absence of the essential muscle protein Dystrophin in DMD. Muscle strength continually diminishes, and death usually occurs from chronic respiratory insufficiency and/or cardiac failure. Due to the high mortality rate, early diagnosis is crucial to allow appropriate planning for patient care and treatment. In India, the traditional multiplex Polymerase Chain Reaction (PCR) assay is the most common method to detect Dystrophin gene deletion mutations.

Aim: To describe the spectrum of deletion patterns in the dystrophin gene in muscular dystrophy patients attending a tertiary care hospital in Northeast India.

Materials and Methods: A hospital-based cross-sectional study was conducted on a total of 53 suspected DMD patients attending the Department of Neurology and Department of Paediatrics at Assam Medical College, Dibrugarh, Assam, India from January 2016 to December 2022. Multiplex PCR was performed to study the deletion patterns for the 25 most common exons of the DMD gene for all patients at the Genetic lab, Department of Anatomy. Deletion mutations at different multiple exons were found, and the results were statistically analysed using Statistical Package for Social Sciences (SPSS) to calculate the mean and standard deviation. The results were presented in tabular form as percentages.

Results: Out of the 53 cases suspected to have DMD on the basis of clinical presentation and high serum Creatine Kinase (CK) levels, 34 patients (64.2%) showing deletion mutations in the 25 most common exons of the DMD gene were included in the study. Deletions were most common in 15 (44.1%) patients in the distal hotspot region of exons 44-55. The most common gene deleted was exon 50 in 11 (32.4%) patients. The age at which symptoms were noticed was 4.7±2.01 years. The mean age at diagnosis was 8.4±2.4 years.

Conclusion: In the present study, most patients suspected of DMD based on clinical and laboratory findings had deletions in the DMD gene, with the most common region for deletion in the dystrophin gene being the distal hotspot region, and exon 50 being the most commonly deleted.

Keywords: Deletion mutation, Polymerase chain reaction, X-linked recessive

INTRODUCTION

Duchenne Muscular Dystrophy (DMD; OMIM 310200) and Becker Muscular Dystrophy (BMD; OMIM 300376) are X-linked recessive disorders [1] characterised by progressive muscle wasting [2] that affect both skeletal as well as cardiac muscles. DMD is the most common and fatal type of muscular dystrophy, affecting 1 in every 3500-5000 male birth [1]. DMD is caused by mutations in the largest known human gene [3], the dystrophin gene (encoding dystrophin) and is located in the short arm of the X chromosome, in the Xp21.2 locus. The DMD gene is the largest human gene, consisting of 79 exons that encode a 14 kb mRNA and produce the 527 kDa dystrophin protein, a cytoskeletal protein than enables the strength, stability and functionality of myofibrils [4]. Mutation of the gene prevents the production of the muscle isoform of dystrophin (Dp427m) [5]. Muscle strength progressively diminishes, leading to wheelchair dependency by around 12 years of age, with death usually resulting from chronic respiratory insufficiency and/or cardiac failure. Early diagnosis is crucial due to the high mortality rate, allowing appropriate planning for patient care and treatment. Patients usually attend the Out Patient Department Services (OPDs) with delayed motor milestones, muscle weakness, hypertrophic calves and the Gowers sign (where patients use their hands to 'walk' on their lower limbs when getting up from the floor to compensate for weakness in the upper leg and hip muscles) [6]. Plasma CK levels should be assessed in boys exhibiting symptoms suggestive of DMD, as CK levels typically show significant elevation in individuals [6]. Mutations, including gross deletions (55-65%), duplications (6-11%), point mutations (20-30%), and deep intronic mutations, are more common in the "proximal" or distal "hot spot" regions of the gene [7]. The mutation spectrum of the dystrophin gene varies greatly depending on the population, which could be explained by the role of ethnic origin in mutagenesis [8].

As there exists possibility of the treatment for the disorder based on skipping certain exons and premature stop codon skipping [7] of the gene, a specific genetic diagnosis has become important for selection of therapeutic options [9], so knowledge of deletion patterns in a population will help the medical fraternity to plan a line of action. Upper Assam region of Northeast India, is an admixture of different population groups, with a genetic makeup peculiar for the region [10]. Study of the mutations in dystrophin gene has not been carried out in this part of Northeast India. The aim of the study was to analyse the deletion pattern of the DMD gene in patients attending a referral hospital from Northeast India.

MATERIALS AND METHODS

The present study was a cross-sectional study conducted at the OPDs of the Department of Neurology and Department of Paediatrics at

Assam Medical College, Dibrugarh, a tertiary care hospital in Northeast India. A total of 53 male children were enrolled from January 2016 to December 2022 for the study. The study protocol was approved by the Institutional Ethics Committee (IEC) vide no. AMC/EC/12330 dated 06.09.2012. Written consent for the study was obtained from all parents or family members of the patients.

Inclusion criteria: Male children with muscular dystrophy presenting with a positive Gower sign and elevated serum CPK were included in the study.

Exclusion criteria: Parents or family members of the patients unwilling to participate in the study were excluded from the study.

Methodology: A total of 53 patients attending the OPDs of the Department of Neurologyand Department of Paediatrics at Assam Medical College, Dibrugarh, Assam, India with a history of muscle weakness, frequent falls, and abnormal gait were included in the study. A detailed history of all patients, including the age of onset of symptoms, was recorded. Biochemical tests, including CK levels, were measured in all patients, with a reference range of CK for provisional diagnosis set at 970-19200 U/L [11].

As deletions are the most common cause of DMD, multiplex PCR was used to diagnosis of deletions. Out of the 79 exons, the 25 most commonly deleted exons were included in the study. Nucleic acid extraction was performed by collecting 2 mL of whole blood in an Ethylenediaminetetraacetic Acid (EDTA) vial and extracting DNA using the QIAamp DNA Mini Kit method. The extracted DNA samples were quantified using a NanoDrop spectrophotometer, with a usable concentration of 20-100 ng/µL.

Multiplex PCR for the DMD gene was conducted using the 25 most common exons deleted in DMD. The tested exons for the dystrophin gene were 3, 4, 6, 8, 12, 13, 17, 19, 21, 34, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 55, 60, and Pm [Table/Fig-1]. The primers were combined into six primer sets based on their size, and a 25 µL PCR mix for each primer set was prepared and placed in a thermal cycler for PCR reaction [Table/Fig-2].

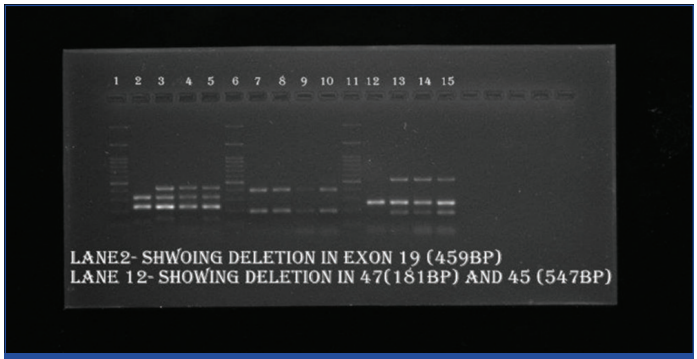
S. No.	Name	Primer sequence	# Of bases
1	53F	TTGAAAGAATTCAGAATCAGTGGGATG	27
2	53R	CTTGGTTTCTGTGATTTTCTTTTGGATTG	29
3	47F	CGTTGTTGCATTTGTCTGTTTCAGTTAC	28
4	47R	GTCTTACCTTTATCCACTGGAGATTTG	27
5	42F	CACACTGTCCGTGAAGAAACGATGATG	27
6	42R	TTAGCACAGAGGTCAGGAGCATTGAG	26
7	60F	AGGAGAAATTGCGCCTCTGAAAGAGAAAG	29
8	60R	CTGCAGAAGCTTCCATCTGGTGTTCAGG	28
9	45F	AAACATGGAACATCCTTGTGGGGAC	26
10	45R	CATTCCTATTAGATCTGTCGCCCTAC	27
11	48F	TTGAATACATTGGTTAAATCCTTGTGGGGAC	28
12	48R	CCTGAATAAAGTCTTCCTTACCACAC	26
13	49F	GTGCCCTTATGTACCAGGCAGAAATTG	27
14	49R	GCAATGACTCGTTAATAGCCTTAAGATC	28
15	43F	GAACATGTCAAAGTCACTGGACTTCATGG	29
16	43R	ATGTATGTGTTACTGCAAGATGCATGCCA	29
17	44F	CTTGATCCATATGCTTTTACCTGCA	25
18	44R	TCCATCACCCTTCAGAACCTGATCT	25
19	PmF	GAAGATCTAGACAGTGGATACATAACAAATGCATG	35
20	PmR	TTCTCCGAAGGTAATTGCTCTCCAGATCTGAGTCC	35
21	19F	TTCTACCACATCCCATTTTCTTCCA	25
22	19R	GATGGCAAAAGTGTTGAGAAAAAGTC	26
23	3F	TCATCCATCATCTTCGGCAGATTAA	25
24	3R	CAGGCGGTAGAGTATGCCAAATGAAAATCA	30
25	8F	GTCCTTTACACACTTTACCTGTTGAG	26
26	8R	GGCCTCATTCTGTTCTTATTAG	25
27	13F	AATAGGAGTACCTGAGATGTAGCAGAAAT	29
28	13R	CTGACCTTAAGTTGTTCTTCCAAAGCAG	28
29	51F	GAAATTGGCTCTTTAGCTTGTGTTTC	26
30	51R	GGAGAGTAAAGTGATTGGTGGAAAATC	27
31	50F	CACCAAATGGATTAAGATGTTCATGAAT	28
32	50R	TCTCTCTCACCCAGTCATCACTTCATAG	28
33	6F	CCACATGTAGGTCAAAAATGTAATGAA	27
34	6R	GTCTCAGTAATCTTCTTACCTATGACTATGG	31
35	21F	GATGAAGTCAACCGGCTATC	20
36	21R	GTCTGTAGCTCTTTCTCTC	19
37	55F	GGCTGCTTTGGAAGAAACTC	20
38	55R	TTACGGGTAGCATCCTGTAGGA	22
39	17F	GACTTTCGATGTTGAGATTACTTTCCC	27
40	17R	AAGCTTGAGATGCTCTCACCTTTTCC	26
41	4F	TTGTCGGTCTCCTGCTGGTCAGTG	24
42	4R	CAAAGCCCTCACTCAAACATGAAGC	25
43	46F	AAGAACAAAAGAATATCTTGTCAG	24
44	46R	GACTTGCTCAAGCTTTTCTTTTA	23
45	34F	GAAATTGTCCCGTAAGATGCG	21
46	34R	AGGCATTCCTTCAACTGCTG	20
47	52F	AATGCAGGATTTGGAACAGAGGCGTCC	27
48	52R	TTCGATCCGTAATGATTGTTCTAGCCTC	28
49	12F	GATAGTGGGCTTTACTTACATCCTTC	26
50	12R	GAAAGCACGCAACATAAGATACACCT	26

[Table/Fig-1]: Primer sequence for the 25 different exons screened for deletion by multiplex PCR.

Groups	Primer sets for exons	Band size (bp)
Group-I	53, 47,42 and 60	212,181,155,139
Group-II	45, 48, 49, 43 and 44	547,506,439,357,268
Group-III	Pm*, 19, 3, 8 and 13	535,459,410,360,238
Group-IV	51, 50, 6 and 55	388,271,202,119
Group-V	21, 52 and 12	178,113,311
Group-VI	17, 4, 46 and 34	416,196,139,102

[Table/Fig-2]: Distribution of six primer sets in different groups (on the basis of band size).
*Pm- Promoter region

The bands were visualised using a 2% agarose gel and analysed using a Gel documentation system [Table/Fig-3]. The 79 exons of the dystrophin gene were divided into four regions: the first region from exon 1 to exon 20 (proximal hotspot region), the second region from exon 21 to 44, the third region from exon 45 to 55 (distal hotspot region), and the fourth region from exon 56 to 79 [12].



[Table/Fig-3]: Agarose gel showing the deletion of multiple exons (19,47,45) in gel documentation system.

STATISTICAL ANALYSIS

The deletions in all 34 patients were presented in tabular form, classified according to the hotspot regions. Statistical analysis

was conducted using SPSS software version 21.0, calculating the mean and standard deviation to summarise the central tendency and variability, respectively. The frequencies of the deleted individual exons and their respective hotspot regions were expressed as percentages.

RESULTS

The mean age of initial symptoms and the mean age at diagnosis are shown in [Table/Fig-4]. The most common presentation was frequent falls in 47 (88.67%) patients, followed by muscle weakness, abnormal gait, and calf muscle hypertrophy [Table/Fig-5]. All patients had elevated CK levels, with a mean value of 1540.64±834.24 U/L.

Total cases	Mean age of symptoms	Range	Mean age of diagnosis	Range
53	4.7±2.01 years	2-11 years	8.4±2.4 years	4-17 years

[Table/Fig-4]: Showing the mean age of initial symptoms and mean age at diagnosis.

Clinical features	n (%)
Frequent falls	47 (88.67)
Muscle weakness	42 (79.24)
Abnormal gait	37 (69.81)
Calf muscle hypertrophy	18 (33.96)

[Table/Fig-5]: Showing the different types of clinical presentation.

In the present study, 34 patients were diagnosed as positive for deletion of exons (64.2%, 34/53) based on multiplex PCR. Out of these 34 patients, one DMD positive patient had a positive family history (serial no-23). A total of 121 exons were deleted among the 34 patients [Table/Fig-6].

S. No.	Age (years) at onset	Age (years) at diagnosis	Deleted exons (Total number of exons deleted)
1	11	17	12,13,17,19 and 21 (5)
2	4	7	49, 50 (2)
3	5	8	47,48,45,46 (4)
4	7	11	49,48,50 (3)
5	4	9	48,49,50,51,52 (5)
6	5	10	42, 43, 44 (3)
7	3	8	49,47, 21, 52, 53 (5)
8	3	7	51, 45, 43, 49 (4)
9	3	6	47, 55, 19 (3)
10	2	4	42,43,34 (3)
11	3	8	47,44,48,45,46 (5)
12	4	7	43 (1)
13	3	6	13,8,3,6,12,4,17 (7)
14	4	10	47,53,49,48,50,51,52,46 (8)
15	5	8	50 (1)
16	3	8	42,44,43,13,8,3,19,6,21,12,34,4,17 (13)
17	3	7	13,19,21,12,17 (5)
18	4	7	13,8,19,12,21,17,34 (7)
19	5	7	42,43,34 (3)
20	5	8	53,19,3,51, Pm (5)
21	4	6	50, 13, 8 (3)
22	10	13	47, 45 (2)
23*	4	6	19, 12, 13, 17 (4)
24	6	8	21, 43, 51 (3)
25	8	12	Pm, 50 (2)
26	3	7	8, 48 (2)
27	5	9	45 (1)
28	8	13	48, 49 (2)
29	4	7	34, 50 (2)
30	4	8	51 (1)
31	5	10	45 (1)
32	3	8	45, 50 (2)
33	3	8	45, 50 (2)
34	4	8	50, 48 (2)

[Table/Fig-6]: Distribution of patients according to age at onset and age at diagnosis with the list of deleted exons in the dystrophin gene.
*Patient with positive family history

The deletions in the present study were most common (44.1%) in the distal hotspot region of 44-55 and absent in the distal region of 56-79 in the dystrophin gene. Among all the multiple exon deletions, a two-exon deletion was the most common, found in 9 (26.5%) cases, while single exon deletions were found in 5 (14.7%) cases. The most commonly deleted gene was exon 50 (n=11;32.4%), followed by exon 45 and 48 (n=8; 23.5% of patients) [Table/Fig-7].

Region	n (%)
1-20	2 (5.9)
21-44	4 (11.7)
45-55	15 (44.1)
56-75	Nil
1-20 and 21-44	4 (11.7)
21-44 and 45-55	5 (14.7)
1-20 and 45-55	4 (11.7)
Total patients	34
Numbers of exons deleted in patients	
No. of exons deleted	**Number of patients**
Single exon	5 (14.7)
Two exons	9 (26.5)
Three exons	7 (20.6)
Four exons	3 (8.8)
Five exons	6 (17.7)
Seven exons	2 (5.9)
Eight exon	1 (2.9)
Thirteen exons	1 (2.9)
Common exons deleted	
Exon number	**n (%)**
Exon 50	11 (32.4)
Exon 45,48	8 (23.5)
Exon 12,13,19,43,49	7 (20.6)
Exon 17,21,51	6 (17.7)
Exon, 8,34,47	5 (14.7)
Exon 42,52	4 (11.8)
Exon 3,44,46,53	3 (8.8)
4,6, Pm	2 (5.9)

[Table/Fig-7]: Pattern of deletion in different exons of the dystrophin gene in DMD positive patients.

DISCUSSION

The DMD and BMD are an inherited progressive muscular wasting disease. It is primarily seen in males and is maternally inherited [9]. Gross gene deletions (55-65%), duplications (6-11%), point mutations (20-30%) and deep intronic mutations are the reason for its occurrence [7]. As the most common cause of DMD was deletion and multiplex PCR allows the detection of 98% of the deletions [13], the method was an alternative and cheaper approach for genetic diagnosis of most of the cases of DMD. Use of multiplex PCR in the study was justified in view of the high sensitivity of the procedure and in absence of facility for MLPA in the institute required for the diagnosis of the other causes of DMD.

In present study, the mean age of initial symptoms was 4.7±2.01 years (range 2-11 years), and the mean age at diagnosis was 8.4±2.4 years (range 4-17 years). In the present study, age of initial symptoms and mean age at diagnosis was delayed by about 3-4 years compared to other studies from India [Table/Fig-8] shows the comparison between different studies and present study for age [12,14-18]. DMD is a rare disease and 70% of people in India do not have any basic knowledge of rare diseases irrespective of their formal education level [19]. Lack of awareness about the disease may be the reason for delay in presentation of the patient to the hospital.

Author	Year	Place of the study	Sample size	Mean age of onset (years)	Mean age at diagnosis (years)
Basumatary LJ et al., [12]	2013	India	69	1.72±0.69	6.24±1.76
Dey S et al., [14]	2015	India	81	3.93	7.74
Li Y et al., [15]	2016	Chine	238	---	6.6±2.9
Elhawary NA et al.,[16]	2018	Saudi Arabia	45	3.5	11.5
Iskandar K et al., [17]	2019	Indonesia	34	4.8±2.1	6.8±2.8
Goyal M et al., [18]	2021	India	120	3.2	7.2
Present study	2024	India	53	4.7±2.01	8.4±2.4

[Table/Fig-8]: Findings of the present study are compared to different studies worldwide [12,14-18].

Present study aimed to investigate the prevalence and characteristics of deletion mutations in a specific population in India. Deletion mutations are known to play a significant role in DMD, and understanding their distribution and impact can provide valuable insights into disease development and potential therapeutic strategies. In the present study, 34 (64.2%) patient had deletion mutation in the DMD gene. Present study findings revealed that deletion mutations were observed in a considerable proportion of present study population [Table/Fig-9] shows the comparison between different studies and present study for region of deletion and exon deletion [7,12,14-18,20-24]. The deletion

Author	Year	Place of the study	Deletion mutation	Most common region of deletion	Most common exon deleted
Basumatary L et al., [12]	2013	India	71%	Distal region (44-55)	48
Rao MV et al., [20]	2014	India	73.8%	Distal region (45-52)	50
Barzegar M et al., [24]	2015	Iran	57.3%	Distal region (45-52)	50
Dey S et al., [14]	2015	India	72.6%	Distal region (44-55)	48
Vengalil S et al., [21]	2017	India	91.8%	Distal region (44-55)	50
Li Y et al., [15]	2016	China	59.4%	Distal region (45-52)	49
Elhawary NA et al., [16]	2018	Saudi Arabia	46.3%	Distal region (44-55)	50
Iskandar K et al., [17]	2019	Indonesia	67.6%	Distal region (43-52)	53
Neri M et al., [22]	2020	Italy	57%	Distal region (45-52)	45
Kumar SH et al., [23]	2020	India	64.8%	Distal region (44-55)	45
Goyal M et al., [18]	2021	India	78.5%	Distal region (48-51)	47
Zinina E et al., [7]	2022	Russia	49.0%	Distal region (44-55)	3 and 7
Present study	2024	India	64.2%	Distal region (44-55)	50

[Table/Fig-9]: Patterns of deletion mutation compared to other studies [7,12,14-18, 20-24].

among clinically suspected patient diagnosed by multiplex PCR was 71% in Northeast India, 73% in Eastern India [12,14]. In studies, where Multiplex Ligation-dependent Probe Amplification (MLPA) was used, the deletion was 78.5% in Rajasthan, 91.2% in South India, and 74.5% in Tamil Nadu [18,21,23]. Multiplex PCR may miss some of the deletions hence numbers of patients diagnosed by MLPA are probably more than the findings of present study [16]. This aligns with previous studies conducted in India and other countries, which reported similar frequencies of deletion mutations in different regions. In the present study exon 50, (n=11;32.4%) was the most common exon deleted. It was similar to Elhawary NA et al., Rao MV et al., and Barzegar M et al., done at Saudi Arabia [16,20,24]. The commonest exon deleted in the study did not follow any specific pattern. Difference in population type may be a reason for it.

The distal region (44-55) of the gene was most commonly affected by deletion mutations, aligning with findings from previous studies. Second most common region of deletion reported was the proximal hotspot region of exon 1-20 [7]. In the present study only 5.9% (2/34) deletions were exclusively in the proximal hotspot region, which was similar to Saudi Arabians 7.5% (5/68), Indonesians 6.66% (1/15) [16,17].

In the present study, the sample size was similar to the studies where proximal deletions were less than 20%. The mutation spectrum of the dystrophin gene varies greatly depending on the population, which explains the role of ethnic origin in mutagenesis [22]. Difference in sample size or population type may be the reason for difference in deletion pattern in the proximal region. Therefore, future studies with larger sample sizes and broader mutation profiles are warranted to further elucidate the landscape of genetic alterations in our population.

Limitation(s)

Firstly, the sample size was relatively small, which may limit the generalisability of present study findings to the broader population. Additionally, present study focus was specifically on deletion mutations, and other types of mutations such as duplications or point mutations were not explored.

CONCLUSION(S)

The prevalence of deletion mutations in present study population underscores the significance of considering these genetic alterations in the diagnostic and screening processes for suspected cases of DMD. Present study examination of the DMD mutation spectrum revealed that the distal hotspot region was more commonly affected by deletions compared to the proximal hotspot region. Identifying and characterising specific exons and regions prone to deletion mutations can enhance the development of targeted genetic testing strategies. While present study concentrated on a specific population in India, it is essential to compare and integrate present study findings with those of other studies conducted in diverse regions worldwide to gain a comprehensive understanding of DMD mutations.

Acknowledgement

Authors would like to express their gratitude to the patients and their family members who provided consent for participating in the study and generously contributed their blood samples.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Sep 25, 2023
- Manual Googling: Dec 27, 2023
- iThenticate Software: Jan 26, 2024 (12%)

ETYMOLOGY: Author Origin

EMENDATIONS: 6

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Sep 25, 2023**
Date of Peer Review: **Dec 16, 2023**
Date of Acceptance: **Jan 29, 2024**
Date of Publishing: **Apr 01, 2024**