ORIGINAL ARTICLE

Prevalence Study of Duchene Muscular Dystrophy and its Genetic Sequence in Southern India

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Abstract

Objective

Duchene Muscular dystrophy (DMD) is the common X-linked heterogenous progressive muscular dystrophy characterized by mutations in the DMD gene. The frequency of dystrophin gene mutations is varied in different DMD population. A precise diagnosis can help to reduce the severity of DMD since it aids in planning of targeted medical treatment and required therapies. This study was aimed to investigate the mutation type, their rate and distribution of DMD'S in southern India.

Materials & Methods

An observational study was conducted on 250 genetically confirmed DMD patients from March,2019 to March,2021. The distribution pattern and rate of mutations (deletion, duplication, nonsense mutations, minor mutations) were investigated.

Results

Mutation spectrum was studied on 250 DMD patients, of which 63% exon deletion pattern were reported. 16% deletions were detected in proximal hot region (exons 3-28). The duplications were found 21% in the proximal hotspot largest region (exon 3-25). 16% of the patients reported single deletion (45 exon), 10.7% reported deletions of exon 44. Point mutations detected in 6%, small mutations were detected in 1.2%, non-sense mutations were detected in 2% of study population respectively. Missense Mutations were detected in 0.8% of study population.

Conclusion

This study estimates mutation spectrum of exon deletion pattern (63%) was predominantly identified in distal region; duplication was most frequent in proximal region. Point mutations, Nonsense mutations and small mutations have a least accountability. This study adds a real world evidence for developing research therapies in DMD.

Key words: Duchene muscular dystrophy; Dystrophin; Exon deletion; Genetic diagnosis

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Introduction

Muscular Dystrophy (MD) is an inherited group of rare genetic disorders. The protein production is interfered by the abnormal genes which cause muscle weakness. However, the symptoms, severity and prognosis of the disorder vary with the type of dystrophy. Duchene Muscular dystrophy (DMD) is the most common among the different types of MD and life-threatening disorder predominantly identified in young boys (1). Progressive muscle wasting, difficulty in standing up, frequent falls, waggling gait, inability to sit or stand without support, cardiac and respiratory muscle weakness are the dominant symptoms involved. DMD is a recessive X-linked progressive genetic disorder (2). There is an incidence of 1 in 5000-6000 boys (3). The life expectancy of DMD's is attenuated to 20's or early teens. DMD is caused by mutations in the dystrophin gene that is the largest human gene with 79 exons located at Xp21 locus spanning 2.2mb (mega base pair) of genomic DNA (4-6). There is a high mutation rate due to its enormous size and the deletions observed in the gene are non-randomly distributed. The majority of the cases are due to gross rearrangements (duplications and deletions). Identification of hotspots and the genetic mapping propels the clinical trials that reflect prevention (7), diagnosis and therapy management.

Cure and therapies are not currently unavailable for DMD. However, management therapies are available that reduce the progression of the disease. Mutation-specific therapies yield highly promising outcomes which is an ongoing concern in research and development. There are varied molecular and genetic diagnostic techniques followed by different set of patients for the analysis of gene patterns such as Southern blot technique locates the duplication/deletion origin of the gene for the identification of duplication/deletion sequence. Some diagnostic procedure's used Southern blot which is difficult and time-consuming (8). Although, southern blot predicts the severity by distinguishing between frameshift and in-frame mutations in most of the cases, Multiplex ligation-dependent probe amplification (MLPA) is commonly used with its added advantages such as cheaper, faster rate and easier to perform while compared to Southern blot (9). It is advised to use both tests for better

results. polymerase chain reaction (PCR) method is another technique that determines carriers. Also, there is Multiplex polymerase chain reaction (MPCR) technique available which operates at high speed due to which it is ideal for Prenatal diagnosis. Quantitative polymerase chain reaction (qPCR) is another method used for measuring gene sequence. This method also helps to determine gene dosage and carriers (but need to identify deletion/ duplications at first) (10). MLPA technique asses the possible deletion and duplication of the dystrophin gene. MLPA techniques have added advantages such as it helps to study intragenic deletions (11), correlation of phenotype and genotype. The targeted molecular therapies necessitate precise DMD mutation analysis (12). To emphasize a few, exon skipping trials are suitable for patients with exon deletions, read-through therapy of 'Nonsense codon' can produce full - length in patients with nonsense mutation harbor (13). Hence, this study investigates the rate and type of distribution pattern of gene deletions/ duplications/ mutations of the Southern region of India. This report can be a reference of genetic mutation pattern distribution that support the identification of diagnosis, prevention and research therapy of DMD's (14).

Materials & Methods

An observational study was conducted through a detailed phone interview and sharing questionnaires related to clinical variables with the patient group. The purpose of the study was clearly explained to every patient and their consent was obtained from all the participants. In case of young DMD boys, the study was explained to their parents and consent was taken from them as well.

The study was conducted on clinically confirmed DMD patients registered with Amaravathi Muscular Dystrophy Association (AMDA) [Registration. No. 33 of 2019] between March,2019 to March,2021. Clinical and pathological parameters such as pattern of muscle weakness, Gower's sign, frequency of falls, ambulation status, calf hyper trophy, serum creatinine activity were considered as the screening parameters for inclusion criteria of patients. Frequency and distribution pattern of exon deletions, exon duplications, point mutations, non-sense mutations, small mutations, missense mutations, Single exon deletion distribution were studied. The data was reviewed and interpreted through statistical methods mean ± SD represented as percentage.

Results

The study investigated 250 DMD patients who have undergone the genetic sequencing technique. The Genetic diagnosis method used is MLPA, Sanger sequencing or/and multiplex PCR depending on the exon harbor. In this study, correlations were made between gene sequence patterns and the rate of deletions.

Deletions were detected in 158 patients (63%) and duplications were detected in 63 patients (25.2%). The rate and distribution of mutation pattern identified was represented in by plotting total number of study Patients and genetic mutation pattern detected. Among these 158 patients, 56 patients (22.4%) have single exon deletion, 47 patients (18.8%) have two exon deletion, 8 patients (3.2%) have three exon deletion, 18 patients (7.2%) have four exon deletions, 8 patients (3.2%) have five exon deletions, 3 patients (1.2%) have six exon deletions, 6 patients (2.4%) have seven exon deletions, 8 patients (3.2%) have eight exon deletions, 2 patients (0.8%) have 10 exon deletions, only 2 patients (0.8%) show eleven

exon deletions. There are 4patients (1.6%) who reported both exon deletion and duplication. 21 patients (8.4%) reported large duplications, 15 patients (6%) reported two exon duplications, 12 patients (4.8%) reported eleven exon duplications, 10 patients (4%) reported seven exon duplication and only 5 patients (2%) reported single exon duplication. Other mutation distributions such as point mutations, missense mutations, etc. account were reported in 25 patients (10%) which is explained further. (Figure 1).

Mutation spectrum also includes point mutations that were observed in 15 patients (6%), small mutations observed in 3 patients (1.2%), non-sense mutations observed in 5 patients (2%). Missense Mutations were observed only in 2 patients (0.8%) (Figure 1). This was reflected in that represents rate and distribution of mutation pattern identified (Figure 1).

A plot was constructed between the total number of study patients and specific duplication affected (Figure 2). A total of 21 patients have shown duplication between 3 and 25 exons (21%). There are 12 patients (19%) who reportedly shown duplications in the exons between 45-55, 15 patients (23.8%) reported duplications between exon 8 to exon 9, 10 patients (15.8%) reported duplications between exon 60 to exon 66, 4 patients (6.3%) reported duplications on exon 63 and only one patient reported duplications on exon 66 (1.6%) (Figure 2). On the other hand, Exon deletion of 3 to 28 is identified in 17 patients (16.6%), 4 patients (3.9%) reported exon deletions between 53 and 60, 3 patients (2.9%) reported deletions in the region of 61 and 79, 32 patients (31.3%) reported exon deletions between 40 and 45, 42 patients (41.1%) reported exon deletions in the hotspot region of 45 and 52 (Figure 3). Deletions were found in 2 patients (1.9%) each in the regions of 29 to 35 and 35 to 40 respectively. These were reflected in Figure 3 with a plot between exon deletion pattern and total number of study patients.

A plot was constructed between the specific exon affected and the total number of study patients. Single exon deletion is the most common deletion pattern observed in about 56 patients. Out of which, Exon 10 deletion are the observed in 2 patients (3.5%). Only 1 patient (1.8%) reported deletions in Exon 17, 18, 53, 54 respectively. 6 patients (10.7%) reported deletions of exon 44, 9 patients (16%) reported deletions of exon 45, 5 patients (8.9%) reported deletions of exon 46, 6 patients (10.7%) reported deletions of exon 47, 7 patients (7%) reported deletions of exon 48, 6 patients (6%) reported deletions of exon 49, 6 patients (6%) reported deletions of exon 50, 2 patients (3.5%) reported deletions of exon 51, 3 patients (5.3%) reported deletions of exon 52(Figure 4).

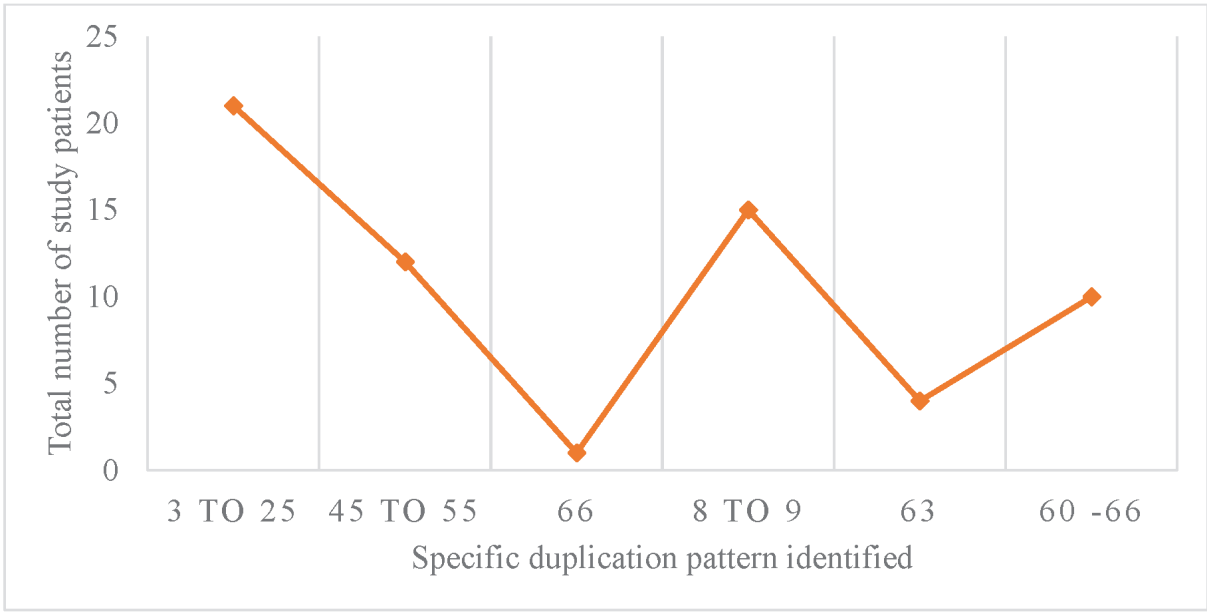

Figure 2. *Exon duplication Pattern in the DMD patients of the study*

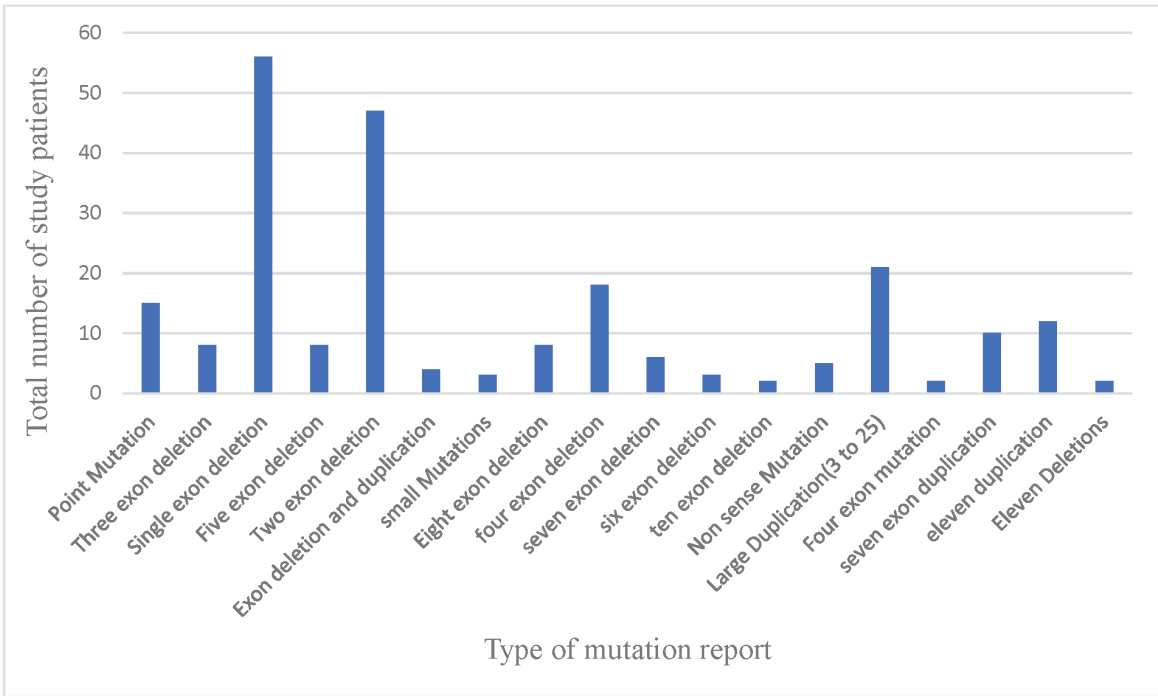

Figure 1. *Distribution of Gene Mutation in the DMD patients of the study*

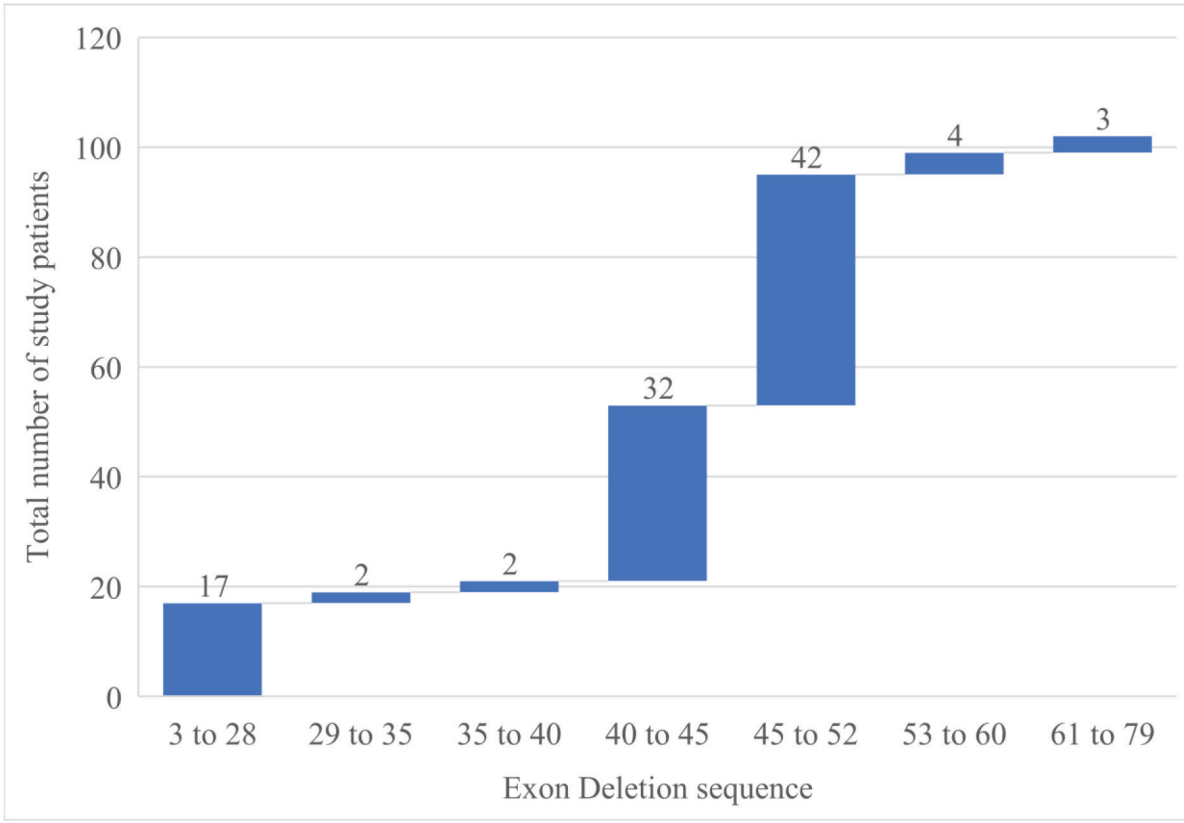

Figure 3. *Multiple Exon deletion Pattern in the DMD patients of the study*

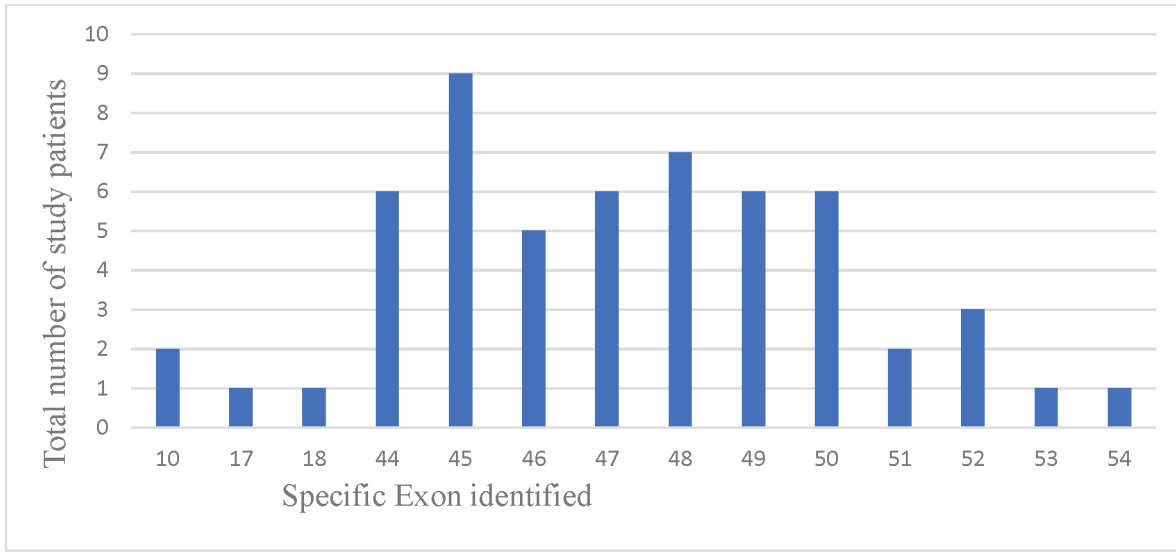

Figure 4. *Single Exon deletion Pattern in the DMD patients of the study*

Discussion

DMD is the most common X-linked inherited progressive disease. The objective behind the study is to understand the gene deletion patterns that can support research studies and also patients can identify the new clinical trial application for the specific gene pattern. Most of the clinical trials focus on exon skipping, dystrophin production. The approach behind exon skipping is mutation specific (15). The severity of the DMD is reduced by dystrophin introduction that converts severe DMD to milder. Also, exon skipping is achieved

by antisense mediated in the current ongoing trials that restores the disrupted frames leading to reduced muscle wasting.

The mutation spectrum was investigated on 250 DMD patients, of which exon deletion pattern (63%) was predominantly identified. Exon duplication was identified as the second predominant mutated hotspot. A very few patients (1.5%) exhibited both deletion and duplication sequence.

Duplication pattern of exons was represented in Figure 2 that reports specific duplication identified. DMD patients exhibit the largest duplication hotspot region between exon 3 and 25. Minimal duplications were found on exon 66. The next frequent duplication was found between 8 and 19 exons. Hence, exon duplication of 3 to 25 region is the largest hotspot identified. The Second hotspot is identified between 8 and 9 exons. It can be understood that the proximal region is acting as the hotspot region for exon duplication. Duplications were not identified in 30-42 and 75 – 79 regions. On the other hand, Exon 45-52 is the highest stereotype deletion identified (42 patients). Hotspot region of exon deletion was observed between exons 40 and 52. The next frequent deleted hotspot is between exon 3 and 28. The least frequent exon deletion found is between 29 to 35 and 61 to 79 exon deletions. Exon deletions were identified to be more in the distal region and the exon sequence between 40 to 52 is identified as the hotspot region. The distal region (3 to 28) is identified as the second hotspot for exon deletion. The differences in the deletion/duplication pattern with different techniques used. The differences in the sequence may be attributed to the study of exon number, intron differences and also the genetic distance agreement among the population in specific techniques such as MLPA, southern blot, cDNA or a combination of both.

The rate of point mutations account to 6% which is the highest spectrum identified. The distribution of missense mutations is very low. There is no particular reason attributed to explain the rate and distribution differences in the mutations of dystrophin gene but it assumed that due to intronic sequence differences, their accumulation and genetic drift might cause the disproportionality.

The distribution of exon and their deletions were studied for a better understanding of the recurrent hotspot regions. Predominantly, exon-specific therapies such as an exon-skipping focus on the specific or single exon alternation/skipping; hence our study focus on the identification of such exons. Exon 45 is identified as the first hotspot region and the proximal region (exon 17,18) has the least deletions. Nine exon Deletions were not reported in the patients of the study.

The rate and distribution of gene deletions analyzed show that single exon deletion is the recurrent deletion and combination of deletion and duplication spectrum are the least occurring. Identification and understanding the gene deletion patterns is the primary applied objective of the study in order to enhance, enable patients and other research groups to participate in the ongoing and new novel clinical trials. Since, DMD is caused by the deletion/mutation of one or more dystrophin gene of 79 exons. The protein synthesis is interfered due to mutations of one or more dystrophin gene which leads to specific skipping of one or more exons and fringing to specific deletion that allows to reduce the severity of DMD by converting its phenotype (16).

In Conclusion

our study suggests the type of pattern and rate of distribution of Gene Duplications, deletions, mutations in the DMD population of South India. A systematic questionnaire and interview were conducted to understand the gene sequence. The results are similar to the studies carried out in different countries. It was concluded that mutation spectrum of exon deletion pattern (63%) was predominantly identified. A very few patients (1.5%) exhibited both deletion and duplication sequence. We believe reporting this data could be used as a reference to understand the DMD gene distribution pattern in South India and might add up for a clinical trial and its approach. Due to the availability of ineffective treatment methods, Genetic diagnosis offers Prenatal diagnosis for DMD families that surges the prevention of occurrence of DMD in off-springs. Also, the early diagnosis of gene frequency and distribution offers the befitting therapy development and implementation of suitable management methods.

Patient Consent

All the clinical information was obtained from the patient with the written consent from patient. Also, due efforts were made in protecting patient's name and conceal their identity.

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Author's contribution

Nigama chandra S: Principal researcher, design of study, sample selection, patient`s follow up, article writing

Rao AA: Data analysis and findings evaluation

Rao GSNK: findings evaluation, article writing

Umasankar k: Data analysis

Alavala RR: Data analysis

Conflict of interest

None

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