Review Article

Duchenne and Becker muscular dystrophies: An Indian update on genetics and rehabilitation

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The application of molecular diagnostic techniques has greatly improved the diagnosis, carrier detection, prenatal testing and genetic counseling for families with Duchenne and Becker muscular dystrophy (D/BMD) in India. The prediction of Duchenne muscular dystrophy (DMD) patients to have out-framed deletions and Becker's muscular dystrophy (BMD) patients to have in-frame deletions of dystrophin gene holds well in the vast majority of cases. Mutation detection is obviously critical for diagnosis but it may also be important for future therapeutic purposes. These factors underscore the need for earlier referral, genetic counseling and provision of support and rehabilitation services which are the main priorities for psychosocial assessment and intervention at medical and social levels.

Key words: Becker's muscular dystrophy, Duchenne muscular dystrophy, genetics, rehabilitation

Introduction

Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy in India, like most other parts of the world. Affected individuals suffer long years of increasing disability and their parents go through psychological trauma and face hardships of managing a physically challenged child on day to day basis. The information on the molecular pathology and genetics has been available for over two decades and genetic counseling and prevention can be offered to families at risk. However, issues of illiteracy, social and religious beliefs and the strong Indian desire to have a normal male child pose difficulties in achieving this objective. In the past few years, Polymerase chain reaction (PCR) -based genetic studies have become available in many parts of the country and support groups of parents and medical personnel have begun the efforts to diagnose and rehabilitate the sufferers. This review will discuss the available genetic information and the social and rehabilitative aspects of DMD/ Becker's muscular dystrophy (BMD) in India.

Genetic Aspects of DMD and BMD

Both DMD and BMD are caused by a functionally abnormal dystrophin gene which encodes the dystrophin protein.[1] Dystrophin, a rod-shaped 427 kd cytoskeletal protein, regulates the influx of ions into the muscle cell. Absence or inactivation of this protein allows the free inflow of ions into the cell causing severe muscle degeneration.[2] Dystrophin is encoded by dystrophin gene located on the X-chromosome (Xp21.1) and is one of the largest known genes in the human genome (2.5MB gene) having 79 exons and codes for a 14kb mRNA.[1] Probably, due to the large size of the gene, the rate of mutations is high with the majority of mutations being intragenic deletions or duplications. Mutations in this gene result in the most severe lethal form of dystrophy – DMD and its milder variant – BMD.[3]

Duchenne Muscular Dystrophy Gene deletions in the Indian Context

The PCR screening of DMD gene analysis identified the deletion frequency of 72% in 25 unrelated patients from Western India population using 14 exons and the promoter region.[4] Intragenic deletions were detected in 18 patients and most of them were located at the 3' hotspot region of the gene indicating that this part of the gene is more deletion prone in the Indian population from Western India.[4] Similarly, the frequency of deletion of 74% was observed in 100 unrelated patients from Western India screening 19 exons including the promoter region by multiplex PCR.[5] Further studies on

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382 patients with 312 DMD and 70 BMD from Western India[6] were carried out using 32 exons and the majority of the deletions i.e. 78.7% were seen in 3' downstream region indicating that this part of the gene is more deletion prone in the population of Western India.

The deletion analysis of 121 unrelated DMD/BMD patients by PCR analysis as well as Southern blot hybridization from Northern India,[7] observed the frequency of deletion to be 72% although a smaller number of patients was studied. Another group from Northern India which screened 160 cases of DMD from all over India showed 64.4% of patients with intragenic deletions of single exon that did not differ from those with deletion of multiple exons.[8] Most (69.7%) of the deletions involved exons 45-51. Their observations concluded differences no ethnic difference with respect to deletions in the DMD gene. The group from Southern India studied 66 unrelated patients of DMD and observed intragenic deletions in 18 exons and Pm region of the DMD gene using multiplex PCR.[9] Of these, 62.1% showed intragenic deletions. Seventy-eight per cent of the deletions were located at the distal hotspot region (exons 44-55) and 22% of the deletions were located at the proximal region (exons 2-19). Exon 50 was most frequently deleted. They reported that the lower incidence from South India compared to North India, is suggestive of variations in the Southern and Northern population.

Molecular basis for reading frame hypothesis

The reading frame hypothesis was proposed to explain the molecular basis of two allelic forms of muscular dystrophies DMD/BMD as described by Koenig *et al.* [2] Pandey *et al.* evaluated this hypothesis in Indian DMD/BMD patients by analyzing deletion of dystrophin exons in 147 DMD and 19 BMD patients.[10] Studies showed deviation of more than 30% from the reading frame hypothesis in DMD patients and suggested a need to re-evaluate the reading frame hypothesis. The patients from Eastern India were analyzed using multiplex PCR.[11] Out of 70 patients, 46 (63%) showed large intragenic deletions in the dystrophin gene as detected by multiplex PCR for 18 exons. About 79% of these deletions are located in the hotspot region i.e. between exons 42 to 53. They also observed a higher incidence of cases in Muslims, and further suggested that molecular detection of dystrophin gene mutation is essential for carrier detection and genetic counseling.

The studies on the deletion patterns of BMD and its correlation with reading frame rule and different phenotypes was carried out for 222 patients showing deletions; 88.1% (out-frame) of DMD and 89.1% (in-frame) of BMD conformed to the reading frame rule.[6] However, a North Indian study showed more than 30% deviation from the reading frame rule.[10] The Western Indian study also showed that different phenotypes were sometimes found within families as seen in an index case of a six-year-old boy with DMD phenotype and his maternal uncle with BMD phenotype both with in-frame deletions of exons 45 to 48.[6] Mital *et al.*, have analyzed the dystrophin gene in 32 unrelated DMD families for the presence of deletions by multiplex PCR for 27 exons and DNA probes for the entire gene. Deletions were identified in 32 patients and the concordance between the clinical phenotype and "reading frame" hypothesis was observed in 24 (75%) cases.[12] Viswanathan *et al.* (unpublished data) have shown that 60 out of 72 children (83.3%) had DNA deletions involving the central hotspot region of the dystrophin gene (exons 44-45) as carried out by multiplex PCR. Exon 44 followed by 45 was the most common site of deletions. The deletion patterns were studied with reading frame rule and it was observed that 38 out of 49 children (77.5%) showed "out-frame" deletions and 11 (22.44%) showed "in-frame" deletions. The remaining 23 cases had deletions but could not be classified. Thus in this study 76.6% of the patients showed deletions of selected exons as compared to 63.7% shown by Ulgenalp *et al.*[13] Hence, one of the reasons for deviation from the reading frame hypothesis seems to be at the DNA level which also concurs at the RNA level. Therefore, it is advisable to confirm the mutation on muscle RNA or at the protein level. In a collaborative study, the technique of Multiple Ligation-dependent Probe Amplification (MLPA) has been applied to discern deletions and duplications for all 79 exons. Further, cDNA sequencing was done on MLPA-negative patients to detect mRNA splicing changes. A high rate of large duplications, high rate of exceptions to the reading frame rule and different distribution of mutations in 75 cases of BMD has been reported.[14] Correlation between phenotype and genotype of these DMD patients demonstrates that genetic studies of lymphocyte DNA may not always reflect the situation in the tissue i.e. muscle involved in dystrophin. Whether this is a polymorphism or, related to the disease phenotype needs further confirmation.[14]

Carrier detection In DMD

In DMD/BMD due to X-linked nature, males carrying the mutated gene are affected, while females become carriers of the disease, and are usually asymptomatic. Several molecular methods have been suggested to solve this dilemma. Various point mutation screening protocols such as SSCP (single strand conformation polymorphism, HA (Heteroduplex Analysis), CMC (Chemical mismatch cleavage) etc. were designed but the success rate was limited due to large size of the gene. Sinha *et al.*, studied the presence of gene deletions in some families afflicted by DMD/BMD, and demonstrated deletions in the central part of the DMD gene in two of the three families studied.[15] This information can be

useful for genetic counseling with particular reference to prenatal diagnosis and carrier analysis. Another method for carrier detection is by DNA-based linkage analysis using highly polymorphic intragenic made short tandem repeat (STR) markers. It has 95% accuracy due to the possibility of recombination.

In Indian patients, new mutations have been observed including germline mosaicism in 20 out of 29 sporadic mothers, using polymorphic dinucleotide loci for carrier analysis and prenatal diagnosis in deletional DMD families with no previous history of the disease.[16]

The carrier detection by DNA-based linkage analysis using highly polymorphic intragenic STR markers was carried out for 327 DMD/BMD patients and out of these, 220 (67.3%) were found to have intragenic deletions and 107 (32.7%) were non-deletion cases. Carrier detection in the female relatives has been carried out in about 25 DMD/BMD families. The authors observed 58-70% informative-ness for central region STRs and 27-47% for 5'/3' region STRs. They claimed that prenatal diagnosis was successfully provided to five families.[17]

Kumari *et al.*, have analyzed the dystrophin gene in eight DMD and 10 BMD unrelated families (22 subjects) for the presence of deletions by multiplex PCR using 27 exons and Southern hybridization using eight cDNA probes.[18] Deletions were identified in five DMD and seven BMD patients. The concordance between the clinical phenotype and "reading frame hypothesis" was observed in 11/12 patients (92%). Thus the molecular characterization of the dystrophin gene in this study has been useful in advising the patients regarding the mode of inheritance and carrier diagnosis of female relatives, and should also prove useful for prenatal diagnosis.

Frequency of Deletions In other Asian Populations

The frequency and distribution of deletions of 19 deletion-prone exons clustered in two hotspots in the proximal and central regions of the dystrophin gene were compared in three populations from Singapore, Japan and Vietnam by Lai *et al.*[19] The most commonly deleted exons at the central deletion hotspot were exon 50 in the Singaporean, exons 49 and 50 in the Japanese and exon 51 in the Vietnamese population. At the proximal deletion hotspot, the most commonly deleted exons were exons 6 and 8 in the Singaporeans, exons 12 and 17 in the Japanese and exons 8 and 12 in the Vietnamese. Similarly, the distribution of dystrophin gene deletions in northeastern China also clusters mainly in two hotspots with neighboring regions of exon 8, which indicates this to be a real hotspot region prone to deletions.[20] The results suggested that although the presence and frequency of the deletions in the two hotspot regions may be similar in the four Asian populations analyzed, the distribution and frequency of deletions among the different exons can vary as a result of population-specific intronic sequences that predispose individuals to preferential deletion breakpoints.

Hassan *et al.*, have studied dystrophin gene deletions in DMD/BMD patients in a Pakistani population and analyzed the frequency and distribution of deletions of 18 exons within the dystrophin gene in 211 unrelated DMD patients.[21] The authors observed that the proportion of intragenic deletions in the Pakistani population is relatively low which is comparable with most of the Asian data.

Psychosocial and Rehabilitative Aspects

In India the situation faced by DMD patients is afflicted with the unique psychosocial characteristics of Indian society—lack of needed infrastructure to aid these patients in daily living and low level of awareness about the disease amongst medical practitioners and society at large. In the Indian setting, the strong desire to have a normal male child is coupled with religious beliefs and taboos, and poses difficulties for counseling. Many families are willing to look after their DMD sufferers but continue to procreate with the fervent wish for a normal son. Thus, authors have seen up to six DMD siblings in one family. Alternative medical systems like Ayurveda, Unani medicine, Homeopathy and other folk medicines are resorted to along with allopathy and it is very common for patients to turn to one or more of them when allopathy does not offer a cure.

Study of the psychosocial aspects of DMD

Psychosocial aspects of 25 DMD families from the registry of muscular dystrophy society of Mumbai have been studied. Our stress curve showed a bimodal peak. The first smaller peak was seen at the time of diagnosis and the second higher one when the boys become immobile. This is a departure from Western studies which propose that stress is generally highest at the time of diagnosis and decreases with later adjustment.[22] This difference may be related to the psychological background of the Indian parents or may be simply a manifestation of increased difficulties of caring for the immobile patients. Parents in our cohort were in denial for unusually long periods of time. Even in acceptance they experienced chronic guilt and helplessness, the degree of which was in direct proportion to the educational level of the parents. The traditional beliefs also had a role to play, 94% parents resorted to seeking religious penance to alleviate guilt.

Patients exhibited poor self-image and mild to moderate depression. Immobility led to social alienation and profound isolation. In turn this led to increase in perceived parental stress and diminished the effectiveness of parental support and encouragement.

This put them at greater risk of psychological maladjustment and this second handicap posed a significant mental health problem. Impact of the second handicap has been elucidated previously in the Western literature.[23,24]

Interpersonal relationships in these families were peculiar in that there was strong identification forming a close-knit unit, which nonetheless suffered social isolation. However, such preoccupation with the family, with its pervasive sharing of feelings of helplessness and guilt suppressed open communication about the disease implications.[22] All these factors underscore the need for psychosocial assessment and intervention at medical and social levels.[24]

In the rehabilitation of patients with DMD, proper assessment of disabilities is essential. This requires specialized approach. In a study form Bangalore, 31 patients with DMD of age four years and above were studied. The motor functions were evaluated using total motor score, upper and lower extremity function grades and timed function tests. Disability was quantitated with Barthel index. Children were found to have disabilities in multiple spheres of life, which were significantly influenced by the motor power. Barthel index was useful in identifying and quantifying specific areas of disabilities in these children.[25]

Support groups have formed to help the rehabilitation of DMD and BMD patients in India. The groups undertake camps for the affected individuals and their caregivers provide physiotherapy and orthotics services and liaise with the medical specialists for advice. A list of Indian organizations working for muscular dystrophy, appearing on the internet, has been presented in Table 1.

Rehabilitation program for Duchenne muscular dystrophy at Mumbai

The muscular dystrophy society has been active in the field of rehabilitation since 1973. Patients are assessed and provisional diagnosis is provided by the neurologists. Multiplex PCR technique is used for dystrophin gene analysis. Patients with strong clinical signs but no deletions are subjected to muscle biopsy and immunostaining for dystrophin. The physiotherapists and occupational therapists then carry out a rehabilitation program, which increases the duration of the ambulatory stage. Due to the structure of the metropolitan setting in Mumbai, dwellings are small and transportation of patients to the centre becomes difficult for the parents. Keeping this in mind, a home-based rehabilitation approach has been developed wherein the team visits homes at regular intervals.

Table 1: Muscular Dystrophy- Indian Organizations

Name	Address and contacts
Muscular Dystrophy Association India	C/O Molecular Diagnostic Facility, Sundaram Medical Foundation Dr. Rangarajan Memorial Hospital, Shanthi Colony, 4th Avenue, Anna Nagar, Chennai - 600 040 Tel: +91-44-26268844 Extn: 349, 350 Fax: +91-44-26284257, Website: www.mdaindia.org
Indian Association of Muscular Dystrophy	Ms. Sanjana Goel: President, IAMD, C/O M/s Stich-n-Style, Hospital Road Solan Distt. Solan, Himachal Pradesh, India, Pin-173212, Phone: +91-1792-223183/220212 Website: www.iamd.org
Indian Muscular Dystrophy Association (IMDA)	R. Jananrdana Rao, 21-136,Batchupet,Malchilipatnam(AP) 521001 India, Tel:+91-8672-2817
Muscular Dystrophy India`	Website: www.mdaindia.com Phone: +91-0715-2215786 Mobile Contacts: Mr. Amardeep Singh Hira: 9815228877 Mr. Manu Kapila: 9871266666 Mr. Swatentar Bansal: 9815215786 Dr. Amit: 9876416000
All India Muscular Dystrophy Association	Anaj Mandi, Nabha Gate, PATIALA 147001, Phone:+91-175-215786 - Fax: 91.175 200 786
Muscular Dystrophy Society, Mumbai	Coordinator: Mrs. Bharti Chabbria Neuromuscular clinic, Department of Neurology, Fourth Floor, Grant Medical College and Sir JJ Group of Hospitals, Byculla, Mumbai Tel: +91-22-23753875 Email:khadilkar@vsnl.com Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences. Raebareli Road, Lucknow 226014, India Phones: +91-522-2668004 to 2668008 +91-522-2668700 +91-522-2668800 +91-522-2668900, Fax: +91-522-2668017 or 2668078 Website: www.sgpgi.ac.in Mr. Sharma S and Ms. Namrata Department of Neurology, MMI Hospital Lalpur, Raipur (C.G.) India Pincode - 492007 E-mail - drsanjaysharma123@rediffmail.com

This approach has proven to be more beneficial to the families and is being explored further.[26]

Management of Duchenne Muscular Dystrophy at Chennai

A large number of families of children with DMD are registered with MDA INDIA group (Chennai). This NGO was formed on 4 February 2000 to assist the families of children with MD. This has grown over the years in numbers and activities with a lot of help from philanthropists from all over the world. The Association is involved in helping families with information about all aspects of MD including physiotherapy, diet, monitoring the breathing, and updates about what is new in the form treatments that may be in the pipeline. Regular meetings with the families are held to identify their needs like wheelchairs, ankle foot orthosis, spinal braces, cough-assistive devices for helping to clear secretions, motorized wheelchairs, hoists to help them move from the bed to the toilets at home, adaptations in the wheelchairs to prevent scoliosis, special spike cushions to prevent ulcers at the seating area as they sit on the wheelchair for long hours and also water beds for children who are unable to move around in the bed and need to be turned by parents regularly. Through MDA India, these accessories are given free for the children who need them.

On the clinical side MDA India is fortunate to be part of the Cooperative International Neuromuscular Research Group (CINRG) over the last five years and has been participating in some multi-center clinical trials. This collaboration has helped us in setting up a molecular diagnostics facility at Chennai for the diagnosis of DMD as well as carrier analysis.

All this had led to the formation of a vibrant team involving physiotherapists, dietician, psychologist, molecular biologists, clinical research coordinator, social workers and volunteers apart from the pediatric neurologist. Most of the personnel involved have had training and exposure to what happens in some of the best centers of excellence in the world as regards each of their disciplines. This team is therefore able to share their knowledge and skills with the families of children with DMD in their area of expertise. Hence, these studies on molecular diagnostic approaches and the usefulness of the rehabilitation programs will be very beneficial to analyze the needs of patients with DMD/BMD and to develop strategies to improve the management of the disease.

Conclusion

DMD and BMD, common and important muscular dystrophies in India, have been characterized genetically in various centers in India. The results of the genetic information are largely similar across the country with some regional variations and follow the pattern seen in other Asian countries. The information on reading frame hypothesis is being explored with local and collaborative efforts and carrier detection has become available in select centers. Support groups are vital in the management of this disease and its chronic disability. Regional groups are currently working for this objective. Illiteracy, cultural and social issues in India pose unique challenges in these disorders. There is a need for further comprehensive efforts for the accurate molecular diagnosis, carrier detection, prenatal diagnosis, genetic counseling and rehabilitative measures on a large scale in India.

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