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REVIEW ARTICLE

Current Concepts in Dystrophinopathies

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Abstract Dystrophinopathies comprise a group of hereditary muscle disorders characterized by progressive wasting and weakness of skeletal muscle, as a result of degeneration of muscle fibers, and can be distinguished by the mode of transmission, age at onset and pattern of muscle weakness. The range of phenotypes associated with the region Xp21 has been expanding since identification of the gene in 1987. The mild end of the spectrum includes the phenotype of the muscle cramps with myoglobinuria and isolated quadriceps myopathy, while at the severe end, there are progressive muscle diseases that are classified as Duchenne / Becker muscular dystrophy (DMD/BMD).

Keywords Duchenne muscular dystrophy · Becker muscular dystrophy · MLPA testing · Immuno-histochemistry · Carrier analysis · Cardiomyopathy · Kyphoscoliosis

Introduction

In 1861, a French physician had described the first case of muscular dystrophy in his book under the name 'paraplegie hypertrophique de l' enfance de cause cerebrale' [1]. In 1868 Duchenne gave a comprehensive account of the disease based on the study of 13 cases. Gowers was the first to deduce the genetic basis of the disease and also describe the patients with delayed onset. It was Becker who proposed that the less symptomatic patients reflected the milder mutations of the same gene. The major breakthrough about the understanding of the disease came about in 1986 when the gene was identified by Kunkel—gene located at band Xp21. This method has since been used for diagnosing hundreds of other inherited conditions [2–4]. To date the dystrophin gene remains one of the largest gene identified.

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Genetics and Patho-physiology

Dystrophin gene is the largest human gene known, spanning 2.4 Mb of DNA in Xp21, and is comprised of 79 exons. The protein product, dystrophin, has a molecular weight of 427 kDa [5]. Dystrophin is expressed in the sub-sarcolemmal region of the skeletal and cardiac muscle. Deletions of single or multiple exons within the dystrophin gene in Duchenne/Becker's dystrophinopathies are responsible in about 65–70% of the cases; the remaining have point mutations (30%) or duplications (6%) [6, 7].

The defect causes abnormal expression of the protein product; dystrophin which is less than 3% of the normal in Duchenne muscular dystrophy and in BMD it is 10–40% of the normal. Prevalence of DMD and BMD are 1 in 3,500 and 18,500 live born males, respectively [8]. There is very limited data from India on the prevalence of Duchenne muscular dystrophy (DMD) and also there is very scanty information regarding the type of genetic defect, clinical features and the mental status in DMD children [9]. The main reason why the diagnosis of Duchenne muscular dystrophy is delayed or not clearly made in India is due to the lack of availability for molecular diagnosis for DMD locally as there are only very few centers in India performing this test. The costs of the tests are high and this makes it difficult for families to afford them. This in turn results in difficultly with counseling and prenatal testing during the subsequent pregnancies and therefore, the recurrence of a sibling or relative with the same disease is not

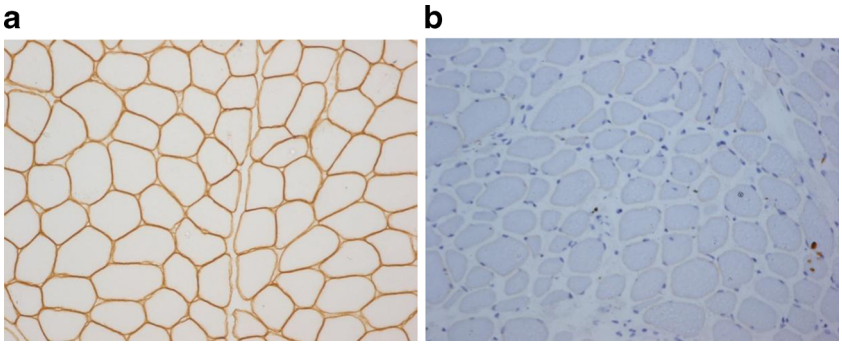

Fig. 1 **a**) Dystrophin rod domain — normal (**b**) Dystrophin — absent in DMD (Picture courtesy of Dr. Gayathri – NIMHANS, Bangalore)

being prevented. All this results in increase in the number of children with DMD being born in our country year after year.

The hallmark of Duchenne muscular dystrophy is the absence of protein called dystrophin in the sub-sarcolemmal region of the muscle cell [10] (Fig. 1). This protein serves both to stabilize the myofibre plasma membrane during myo-fibre contraction and to anchor signal transduction proteins involved in responses of the vasculature to muscle activities and others [11, 12].

The most intriguing factor about the genetics of the disease is the fact that there appears to be no simple relation between the size of the deletion and the resultant clinical disease. For example, the deletion of small exons such as exon 44, typically results in classic Duchenne muscular dystrophy. However, large deletions, which may involve nearly 50% of the gene, have been described in patients with Becker muscular dystrophy [13–15]. In some patients there was an isolated increase in creatinine kinase concentration with normal or near normal dystrophin concentrations and these patients were shown to have "in frame" deletions involving exons 32–44, 48–51 or 48–53 [16]. It is therefore clear that the phenotype depends not on the extent of the deletion but on the whether it disrupts the reading frame or not. To make matters more complex, very different deletions in size and position may give rise to identical clinical phenotype. In patients with Duchenne muscular dystrophy the deletions or duplications disrupt the reading frame, resulting in unstable RNA that eventually leads to the lack of production of dystrophin. This reading frame hypothesis holds true for over 90% of the cases and is commonly used both as a diagnostic confirmation of dystrophinopathies and for the differential diagnosis of DMD and BMD [10, 11, 17]. Mutations which change the translational reading frame cause severe form DMD, whereas the ones that maintain the translational frame result in the milder form BMD. The patterns of gene deletions and their distribution are known to vary in different ethnic groups [12, 18].

Figure 2 shows the detailed mapping of the various domains in the gene and how the clinical picture may vary depending on where the deletions are [19]. Muntoni and his colleagues have described [20] that the most commonly mutated region includes exons 45–55 with genomic break points (*i.e.*, end points where the deletions actually occurs) lying within intron 44, while the 5' end hot spot includes exons 2–19 with genomic breakpoints found in introns 2 and 7 and extending towards the downstream introns. The clusters of these two hot spots represents the basis for the use of multiplex PCR techniques, that by screening only 19 exons identifies about 98% of all deletions [21–23].

Twenty to thirty five percent of the patients with DMD or BMD do not have deletions or duplications of the dystrophin gene. Point mutations or small frame shift deletions or

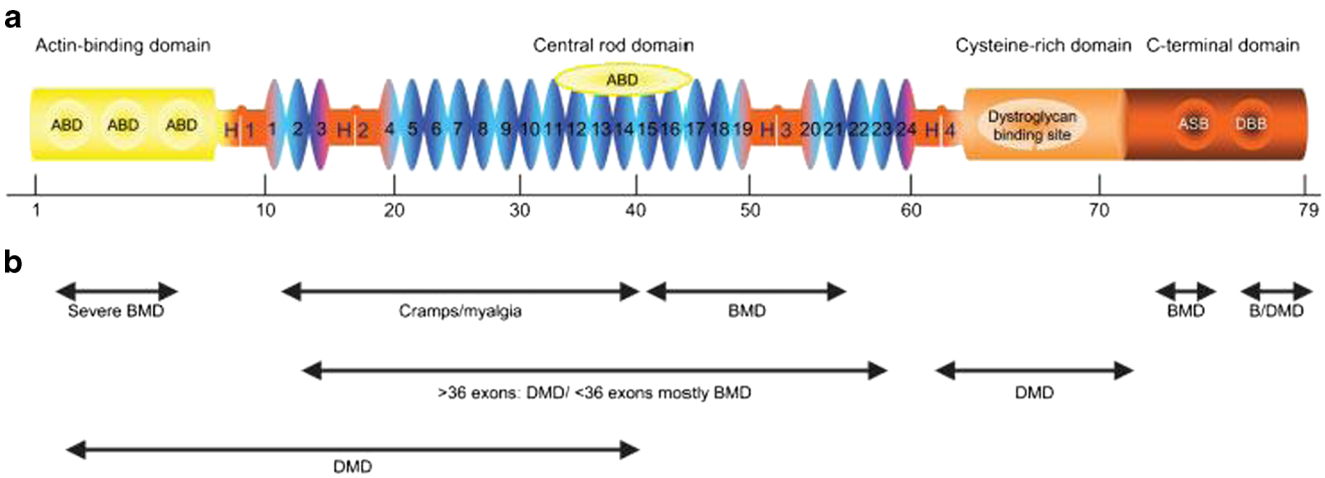

Fig. 2 Schematic drawing of the dystrophin protein [19]

insertions may be identified using special techniques like the protein truncation tests, which can be applied to muscle RNA [24]. The technique of multiplex ligation-dependent probe amplification (MLPA) assay is an advanced technique to identify deletions and duplications of all the 79 exons of DMD gene in patients with Duchenne/Becker muscular dystrophy (DMD/BMD) and female carriers. MLPA is a modification of polymerase chain reaction and permits multiple targets to be amplified with only a single primer pair [25]. This method detects deletions in the remaining non-hotspot regions or duplications, if any. Duplications of exons cannot be seen by mPCR-based assay, due to non-quantitative nature of the technique [26]. Combining the two techniques, mPCR followed by MLPA assay, has enabled more accurate detection and extent of deletions and duplications which otherwise would have remained unidentified, thereby increasing the mutation pick up rate. These findings have also allowed prediction of expected phenotype. Determining carrier status has a considerable significance in estimating the risk in future pregnancies and prenatal testing options to limit the birth of affected individuals [27]. With the possibility that exon skipping techniques may help in some of these children as a therapeutic option, it becomes important that the borders of the deletions are clear and this is possible only by analyzing all the 79 exons in the DMD gene using MLPA technique.

Dystrophin-Glycoprotien Complex (DGC)

The DGC is an oligomeric complex composed of dystrophin and three major sub complexes that are composed of dystrophin associated proteins. Dystrophin is on the cytoplasmic aspect and has four functional domains—the N terminal, the C terminal, the cysteine rich domain and the rod domain as in (Fig. 2). The N terminal binds with the actin cytoskeleton, C terminal with the syntrophin complex and the cysteine rich region probably binds with the dystroglycan complex. This is most abundant at the neuromuscular junction. In Duchenne muscular dystrophy patients, antibodies directed against the C terminal detect absence of dystrophin and antibodies directed against the N terminal or the rod domain detect truncated dystrophin with decreased amounts of dystrophin. Antibodies against the C terminal are useful in separation of Duchenne and Becker muscular dystrophy patients.

Muscle biopsy with immunocytochemistry seems to be therefore important whenever one is confronted with an in frame mutation, no deletion or a clinical phenotype which is not typical of Duchenne muscular dystrophy and this then helps to quantify the amount of dystrophin present in the muscle tissue. Western blot analysis has been the technique used traditionally for quantification of dystrophin. One of the biggest challenges that is being faced now is in the quantification of dystrophin in the muscle biopsy specimens, given the setting of various clinical trials looking for even marginal increase in dystrophin expression in the muscle. A technique using image analysis that allows reliable and semi-automated immune-fluorescent quantification of low level dystrophin expression in sections co-stained with spectrin has been developed [28].

Clinical Features

The most important clinical distinction has to be between Duchenne and Becker muscular dystrophy

Children with *Duchenne muscular dystrophy* have the following main features

- Onset of symptoms before 5 y of age
- Progressive symmetrical proximal muscle weakness than distal with calf hypertrophy
- Wheel chair bound before 13 y of age

Children with *Becker muscular dystrophy*

- Progressive symmetrical proximal muscle weakness than distal with calf hypertrophy
- Activity induced cramping in some children
- Flexion contractures of the elbow may occur later in the course
- Wheel chair dependency if present after 16 y of age
- Preservation of neck flexor muscle strength [29]

The *clinical course in Duchenne muscular dystrophy* (DMD) follows a set pattern.

Stage 1 Presymptomatic: One may detect a high CPK usually due to a positive family history but the child appears well.

Stage 2 Early ambulatory phase: Waddling gait starts usually between 2 to 6 y of age and is secondary to hip girdle weakness. Usually most of these children start to walk independently later than 15 mo. The affected children experience more falls than their peers. Gait abnormalities like difficulty in getting up from floor, difficulty with climbing stairs become obvious by about 3 to 4 y of age. The neck flexors and rectus abdominis muscles are weak and that is why these children tend to turn to their side and get up (which is the initial component of gower's manouvre) when getting up from a supine position on the floor. Calf hypertrophy is usually obvious by 3 y of age. Hip girdle muscles are affected earlier than the shoulder girdle muscles. Hip girdle muscle weakness is the cause for the difficulty in getting up from the floor and waddling gait. Increased lumbar lordosis

ensues, which is necessary to keep the center of balance stable. Complete Gower's sign is present usually by the time the child is around 5 y of age. Contractures of the heel cords may be a significant problem as early as 4 to 5 y of age.

Stage 3 Late ambulatory phase: Iliotibial bands and hip flexor contractures develop. If ankle contractures advance to a fixed equinovarus position, the patient walks on his toes and often falls. They have increasing difficulty with ascending stairs. The respiratory muscles become weaker and forced vital capacity gradually wanes.

Stage 4 Early non ambulatory stage: The loss of ambulation often happens around 10 y of age. Steroid treated DMD boys remain ambulant definitely longer for another 2 to 3 y [28].

Stage 5 Late non ambulatory stage: Once the child is wheel chair bound they develop contractures of the lower extremities followed by upper limb contractures [30]. Kyphoscoliosis often develops around the same time. Proper wheel chair sizing, positioning, seating and strapping so that scoliosis is prevented would be important. It is also important to ensure that the foot rest / arm rest are at the appropriate level to help prevent deformities to an extent. Arm straps / restraints may be needed in the wheel chair to prevent fingers getting stuck on the spokes of the wheel chair.

The Use of Corticosteroids in Duchenne Muscular Dystrophy

Timing Many centres in the world use steroids typically around the age of 4–6 as they feel that the benefits of this may be better. Less functional gain may be seen if initiation of steroids is delayed until close to the loss of ambulation. In authors' personal practice they find families whose children are around 4 to 6 y of age are reluctant to give steroids to their children in India due to side effects but are more favourable if prescribed the same around 7 to 9 y. The main concern for the families is the immuno-suppression and exposure of the child to infectious diseases like chicken pox and tuberculosis.

Regimes The most common daily dosage regimes are Prednisolone 0.5 to 0.75 mg/kg/d. Some centers use Deflazocort at a dose of 0.8 mg /kg/d but it is a lot more expensive than Prednisolone. They are likely to be equally effective, but have slightly different side-effect profiles. Deflazacort may produce less weight gain but has a higher risk of asymptomatic cataracts. The response to steroids and the side effects like weight gain appear to be variable in different children and some gain a lot of weight and others tolerate higher doses very well without any side effects. So the exact dosage needs to be titrated depending on the child's response and side effects seen.

Monitoring Monitoring of the child periodically for improvement in the clinical symptoms like time taken for getting up from the floor, ability to climb stairs, not falling as frequently as before, muscle strength testing, Forced Vital Capacity evaluation by lung function testing and parent and child perception of the value of the treatment are all important.

Side Effects Major side effects of steroids to consider are behavioral changes, failure to gain height, excessive weight gain, osteoporosis, impaired glucose tolerance, immune/ adrenal suppression, dyspepsia/peptic ulceration, cataract, and skin changes. It is for this reason it is important to monitor weight, height, blood pressure, urinary dipstix (glucose), cushingoid features, mood/behavior/ personality /GI skin changes, red reflex of eyes, bone fractures, and recurrent infections. Many of the side effects can be dealt with easily if closely monitored.

Tapering If corticosteroids need to be stopped, they should be tapered/stopped slowly over weeks and not suddenly. Suggested tapering of drug dosage is to take 1/2 the regular corticosteroid dose the first week, 1/4 the dose during the second week, 1/8 the dose during the third week and thereafter stop corticosteroid medication.

How Long to Continue Steroid Treatment Most families in India are quite reluctant to continue steroids beyond the stage when the child becomes non ambulant. There is some evidence that steroids do improve hand function and lung function in wheel chair bound children and so steroids are routinely prescribed / continued in children who become non-ambulant. However, some patients may experience excess weight gain and osteopenia and this needs to be discussed with the family before prescribing steroids to non ambulant children [31].

Respiratory Issues in Children with Duchenne Muscular Dystrophy

Shoulder weakness is the earliest sign towards the onset of respiratory muscle weakness. It is usually the expiratory muscles that are first affected, followed by the inspiratory muscles. Even the surrounding muscles of neck and back are affected, resulting in drooping or sagging of shoulders. This results in the patients being unable to expand their chest to maximum limit and also causes poor clearance of mucus, which is

normally expected to be cleared of the airways. All these together contribute towards collapse infection and poor lung function. Additional problems that contribute to respiratory muscle weakness in these children are kypho-scoliosis and obesity.

Regular breathing exercises using incentive spirometers (Fig. 3) help the children to clear the mucus and this should be advised early so that these children learn how to use this effectively at home. Help of a physiotherapist who is familiar with these would be of major value. Similarly, regular chest physiotherapy with postural drainage may be of value in children who already have developed scoliosis.

As age advances breathing muscles of DMD children become weaker and they develop significant CO_2 retention with marked hypoxia particularly when lying down, causing early morning headaches. Pulmonary function testing including Forced Vital Capacity, particularly when they become wheel chair bound, periodically would help to identify early onset respiratory failure. Non invasive positive pressure ventilation (NIPPV) (Fig. 4) is sometimes required for some of these children particularly at night time. Sleep disordered breathing is common in children with DMD which is best detected by polysomnography.

Prevention of Recurrent Respiratory Infections

Good oral and dental hygiene should be maintained in DMD children. Upright posture should be maintained while sitting to prevent kypho-scoliosis. Propped up posture during sleep with an average sized pillow should be maintained to prevent aspiration. Regular breathing exercises like “Pranayama” or deep and forceful breathing through the nose to facilitate nasal breathing and clear all retained secretions should be done. Regular vaccinations against vaccine preventable diseases like BCG, DPT, Measles, Varicella, *Haemophilus influenza B*, pneumococcal vaccine and yearly influenza vaccine should be given. Dietary advice to avoid obesity is also of value [31].

Fig. 3 Incentive spirometer

Fig. 4 Non invasive positive pressure ventilator

Cardiac Involvement in Duchenne Muscular Dystrophy

Children with DMD are prone for cardiomyopathy and periodic screening from the age of 5 or 6 y would be useful to pick up impending cardiac failure. The children require an ECG and echocardiogram atleast once every 24 mo during early years but once they reach 10 y of age, they need cardiac screening every year to look for early evidence of left ventricular dysfunction / appropriate intervention [32].

Physiotherapy Management in Duchenne Muscular Dystrophy

The role of physiotherapist in DMD starts early by educating the family on the need for early, gentle stretching of tendo-achilles and use of orthoses at night time to prevent fixed deformities. Later, stretches for the upper limbs, hips, knees and ankles may be required [31]. Breathing exercises should also be initiated early and continued regularly.

Carrier Analysis / Counseling

Carrier analysis of the mother by molecular genetic testing is an important next step along with genetic counseling. Unfortunately this is again not freely available and sometimes rather expensive. However, it is an important step as it helps to counsel the family about the chance of recurrence during the next pregnancy and to do prenatal testing in the family. Even if the condition has arisen as a result of a new mutation, there is an average 10% risk of recurrence due to germline mosaicism. Genetic counseling should also be offered to sisters and aunts

(mother's side) in reproductive age if the mother carries the mutation.

Role of Muscle MRI in Duchenne Muscular Dystrophy

Muscle MRI is currently being used predominantly to identify which of the muscles is ideal to be biopsied for diagnostic purposes, to look at the differential rates of natural progression to fatty replacement in different groups of muscles and also to identify suitable groups of muscles as biomarkers in clinical trials [33].

Role of Skin Biopsy in Duchenne Muscular Dystrophy

In a study from India [34] the authors have clearly demonstrated that skin biopsy is very useful for the diagnosis of dystrophinopathies and has a high degree of sensitivity, specificity and positive and negative predictive values. It can be useful adjunct / replacement for muscle biopsies particularly when repeated biopsies are required for monitoring therapy or in patients with advanced DMD where extreme fibrosis, adipose tissue infiltration and inflammation make interpretation of the muscle biopsy difficult. Skin biopsy is also simple, cost effective, less invasive and does not require general anesthesia when compared to muscle biopsy.

Societal Needs

The other issue that is predominant in India is lack of support for the families of these children, in terms of transport once the children are unable to walk. Therefore many of the families are not able to bring these children for assessment / follow up to the hospitals and this results in lesser number of cases being seen / reported in India, particularly at major hospitals. When door to door surveys were conducted, it was found that there are a large number of children / families with muscular dystrophy who have never been diagnosed / nor have they sort medical help. Many of these children were misdiagnosed as having other forms of impairment including cerebral palsy. The need for a door to door survey covering large number of population, is therefore very important in order to get the true picture about the incidence / prevalence of this disease in India.

It is known over many years that dystrophin is expressed in the brain and learning disabilities are common in children with DMD. These children require early identification and appropriate support. These children / families require counseling and help to confirm the diagnosis by genetic testing, planning for the future including carrier status in the family *etc*. and also appropriate support in getting hold of all the assistive devices, wheel chairs, splints, physiotherapy, psychological and educational support. Education for all these children becomes a difficult task once they become non ambulant and therefore, it would be important to have more schools with barrier free environment and wheel chair access to the class rooms and toilets to enable more children to continue schools as long as they can. It would also be important that the family is provided awareness about all the existing governmental / non-governmental support schemes and how to apply for the same.

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