The Muscular Dystrophies — From Genes to Proteins

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INTRODUCTION

Exactly 10 years ago in an update article in the Journal of Association of Physicians of India, we had discussed about the molecular genetics of Duchenne’s muscular dystrophy (DMD) and Becker’s dystrophy (BMD) in the light of the discovery of the gene responsible for DMD/BMD.1

We have walked a long way since then, with explosive growth in our knowledge not only on DMD/BMD, but also of other muscular dystrophies like limb girdle muscular dystrophy (LGMD) and various types of congenital muscular dystrophies (CMD). This knowledge not only identifies the gene, but also the gene product - the protein in the muscle which forms an integral structural component of the muscle fibers. We are now beginning to visualise the individual tiny proteins in the composition of the muscle membrane whose defects result in a particular clinical forms of the muscular dystrophies.

This identification of the various structural component has also given us a new way of classifying the various muscular dystrophies. In the seemingly chaotic scenario we seem to have a new way of looking into various clinical types - an approach with a closer correlation between structure, function and the derangement causing the clinical features. In the near future we are likely to be talking of dystrophinopathies, sacroglycanopathies and lamininopathies rather than DMD, LGMD or CMD.

STRUCTURE OF THE MUSCLE PLASMA MEMBRANE

The structural detail of the muscle plasma membrane is being worked up in considerable depth. Our idea of the plasma membrane being a cell wall which partitions the inside of the cell from the interstitial space is now too simplistic. The trilaminar membrane is actually housing literally thousands of proteins forming various channels, pores, receptors, and anchoring proteins. Each is essential for the function of the muscle fiber. It has been found that in all the muscular dystrophies - i.e. the group of genetically determined progressive, degenerative disease of the muscles, there is a unique pathology and in them the structural proteins, which link the activity contracting action and myosin components to the non-contracting structures in the fiber and outside, are defective. In our article we will strictly confine ourselves to this anchoring device which is called the dystrophin - glycoprotein complex (DGC).2

As shown in Fig. 1 (after Lim and Campbell3) dystrophin, the gene product defective in DMD, is a large rod-shaped cytoskeletal protein, which is entirely intracellular, is bound the F-action contractile protein on its one end, while its other end, near the plasma membrane, is attached to at least three groups of protein molecules which have a complex interrelation amongst themselves.4 The dystroglycan complex is one of these proteins and is composed of two large subunits the α and the β dystroglycans respectively. The α - dystroglycan is entirely extracellular and is attached at one end to laminin which is the actual extracellular anchor protein.5 The other end of α - dystroglycan attaches itself to the β-submit which is the trans-membrane protein binding to the cystein-rich region of dystrophin on the intracellular part and the α-subunit on the extracellular part.6

The dystrophin, near its attachment with β - dystroglycan also binds by its - C-terminus to a second group of proteins called syntrophin which are also entirely intracellular. Two subunits of syntrophin are recognised - the α and the β.7 The intracellular syntrophin itself is associated with an alternatively spliced protein called dystrobrevin,8 and an enzyme protein named neuronal nitric oxide synthase.9 The function of these minor proteins of DGC s not yet known.

An important member of the DGC is the sacroglycan complex which has five subunits, each coded by separate genes.3,10,11 These are the α, β, γ, δ and ε sacroglycans. All these sacroglycans are transmembrane proteins, with a relatively small intracellular portion and larger extracellular portions. Only α-sacroglycan (adhalin) has a N-terminus extracellularly, while others have their C-terminus extracellularly. Gene mutations of these various subunits are seen in the various clinical subtypes of which are generally called the limb-girdle muscular dystrophies.

A few other minor proteins are also cloned and lie near the main DGC. Sacrospan; a 4-transmembrane-domain protein interacts with the dystroglycans12 while various other proteins have their genes identified. But not yet have they found a place in the current conceptual model of the DGC. These proteins are caveolin-3, calpain-3 and dysferin. Although derangements of these result in clinical subtypes of muscular dystrophies, their sites are yet to be identified in the main DGC.
With the data presently available, an unifying hypotheses of membrane instability, in case of quantitative or qualitative defects of the various proteins of the DGC is proposed.

The DGC is seen as the stabilizing factor in the contractile muscle fiber, as it links the contractile protein action with the stable cytoskeleton on the cell and the perimysium. Most muscular dystrophies have elevated creatine-kinase values from birth and so some disruption of the membrane stability is present early. A defect in any one member of the whole complex results in the instability of the membrane with membrane leaks. There is also entry of large amounts of Ca$^{++}$ intracellularly. This becomes toxic by a series of cascading reactions ultimately leading to muscle fiber necrosis and the clinical phenotype results.

**Classification**

The classification of muscular dystrophies, had many changes reflecting the fact that none had been satisfactory. It now encompasses disorders of structural proteins of muscles. In the early years of clinical myology, about 150 years ago, the clinical features were the only way and it took nearly a 100 years for a classification by Walton and Naltrass in 1954 to clinically include four major forms: Duchenne, myotonic, facioscapulohumeral and limb girdle muscular dystrophy. Myotonia is now proved to be a channelopathy and not a disorder of structural proteins. There are a lot of unclassified phenotypes differing inheritance patterns. When children with muscle diseases are analysed, congenital muscular dystrophy with many subtypes become a cumbersome way of looking at muscular dystrophies.

All however changed with the onset of the genetic ‘era’, when genetic defects underlying the various clinical types became known. A genetic classification was made, and was further enriched by our knowledge of the gene products, the actual proteins which are defective. This has lead to a genetic classification encompassing all aspects of the dystrophies - i.e. the clinical phenotype, inheritance pattern, gene mutations and the defective protein. This is briefly shown in Table 1. this classification is yet to be completed as we are yet to learn much more about the actual protein. We now know dystrophinopathies, sarcoglycanopathies and lamininopathies, and we are likely to get some more named after the protein involved. The gene loci for two important member of the dystrophy family, facioscapulohumeral muscular dystrophy (FSHMD) and distal myopathy have

**Table 1: Genetic classification of the muscular dystrophies**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Inheritance</th>
<th>Gene mutation</th>
<th>Protein</th>
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<tbody>
<tr>
<td>A. X-linked dystrophies</td>
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<tr>
<td>Duchenne/Becker</td>
<td>XR</td>
<td>Xp21</td>
<td>Dystrophin</td>
</tr>
<tr>
<td>Emery-Dreifuss</td>
<td>XR</td>
<td>Xq28</td>
<td>Emerin</td>
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<tr>
<td>B. Limb-girdle muscular dystrophies (LGMD)</td>
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<td></td>
</tr>
<tr>
<td>LGMD 1A</td>
<td>AD</td>
<td>5q22-34</td>
<td></td>
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<tr>
<td>LGMD 1B</td>
<td>AD</td>
<td>1q11-1</td>
<td></td>
</tr>
<tr>
<td>LGMD 1C</td>
<td>AD</td>
<td>3q25</td>
<td>Calveolin-3</td>
</tr>
<tr>
<td>LGMD 2A</td>
<td>AR</td>
<td>15q15</td>
<td>Calpain-3</td>
</tr>
<tr>
<td>LGMD 2B</td>
<td>AR</td>
<td>2p12</td>
<td>Dysferin</td>
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<tr>
<td>LGMD 2C</td>
<td>AR</td>
<td>13q12</td>
<td>γ-sarcoglycan</td>
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<tr>
<td>LGMD 2D</td>
<td>AR</td>
<td>17q12</td>
<td>α-sarcoglycan</td>
</tr>
<tr>
<td>LGMD 2E</td>
<td>AR</td>
<td>4q12</td>
<td>β-sarcoglycan</td>
</tr>
<tr>
<td>LGMD 2F</td>
<td>AR</td>
<td>5q33</td>
<td>δ-sarcoglycan</td>
</tr>
<tr>
<td>LGMD 2G</td>
<td>AR</td>
<td>17q11</td>
<td></td>
</tr>
<tr>
<td>C. Congenital muscular dystrophies (CMD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a) (With CNS involvement) Fukuyama CMD</td>
<td>AR</td>
<td>9q31-33</td>
<td>Fukutin</td>
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<tr>
<td>Walker-Warbarg CMD</td>
<td>AR</td>
<td>9q31-33</td>
<td></td>
</tr>
<tr>
<td>Muscle-eye-brain CMD</td>
<td>AR</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>b) (Without CNS involvement)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Merosin-deficient classic type</td>
<td>AR</td>
<td>6q2</td>
<td>Laminin-A2 (mersin)</td>
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<tr>
<td>Merosin-positive classic type</td>
<td>AR</td>
<td></td>
<td></td>
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<tr>
<td>Integrin-deficient CMD</td>
<td>AR</td>
<td>12q13</td>
<td>Integrin-A7</td>
</tr>
<tr>
<td>D. Distal dystrophies</td>
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<td></td>
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<tr>
<td>Late adult onset 1A (Wellander)</td>
<td>AD</td>
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<tr>
<td>Late adult onset 1B (Markesbery)</td>
<td>AD</td>
<td>2p</td>
<td></td>
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<tr>
<td>Early adult onset 1A (Nonaka)</td>
<td>AR</td>
<td>9p1-1q1</td>
<td></td>
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<tr>
<td>Early adult onset 1B (Miyoshi)</td>
<td>AR</td>
<td>2q12-14</td>
<td>Dysferin</td>
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<tr>
<td>Early adult onset 1C (Laing)</td>
<td>AD</td>
<td>14</td>
<td></td>
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<tr>
<td>E. Other dystrophies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facioscapulohumeral</td>
<td>AD</td>
<td>4q35</td>
<td></td>
</tr>
<tr>
<td>Oculopharyngeal</td>
<td>AD</td>
<td>14q11</td>
<td>Poly(A) binding protein-2</td>
</tr>
<tr>
<td>Scapuloperoneal dystrophy</td>
<td>AD</td>
<td>12</td>
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</table>

*Probably same condition as Miyoshi distal dystrophy; † Probably same condition as LGMD-2B*
been identified in recent years. However, the gene products are yet to be found. It is unlikely that these would be structural muscle membrane proteins. As such, these conditions though mentioned in the classification proposed, would not be discussed in the present review.

**The Dystrophinopathies**

The clinical features of DMD/BMD are well known. It is unusual for DMD to start in the neonatal period, with difficulty in walking becoming apparent by 2-3 years of age. Progress is relentless and lower limbs seem to be more affected. The patient is wheelchair bound by the start of teens and usually die of respiratory muscle weakness and its consequences by the end of the second decade of life. Although cardiomyopathy is profound, it rarely is clinically apparent probably due to the wheelchair-bound life the patient leads. Defects of GI smooth muscles resulting in acute gastric dilatation, intestinal pseudo-obstruction with sudden episodic vomiting are well known. Rarely severe constipation results; degeneration of smooth muscles leads to these clinical problems.

The average IQ is low, which is however non-progressive and neuropathological correlation is not known.

In BMD the clinical picture starts late and progresses much slowly and so most live upto 30 years of age. A few patients are known who have a phenotype which is midway between DMD and BMD and are known as ‘outliers’. These boys are recognised by three years of age because of relative preservation of strength of neck flexion whereas DMD patients lack this ability throughout their earlier life. These patients also retain the ability to climb stairs and walk even at 12 years, by the time most DMD patients are wheelchair-bound.

Clinical dystrophinopathy can also present with myalgias and myoglobinuria with persistent weakness. Rarely pure cardiomyopathy has been the sole presentation of dystrophinopathy.

Although striking elevation of CK is found in all dystrophinopathies, the definitive diagnosis now rests on a muscle biopsy with special staining with dystrophin antibodies and not finding any on the microscope. DNA analysis also may show the genotype variations of the largest gene in the body. Indeed DMD is a genetically heterogeneous disease although having the same phenotypic presentation. About 2/3 of DMD/BMD cases show large scale deletions, and a few show gene duplications. Deletions disrupting the open reading frame result in DMD with no or very little dystrophin synthesis. In BMD patients deletions maintain the translational reading frame producing a truncated dystrophin. The results in the difference in the phenotype of these two diseases. DNA analysis also is a major tool in prenatal diagnosis of this disorder and forms the basis of genetic counselling.

Absence of dystrophin leads to a reduction in all the components of the DGC which are synthesised but not properly integrated into the muscle membrane rendering it unstable. This results in repeated leaks of excess Ca$^{++}$ into cells starting a cascade of events leading to muscle necrosis. This has been shown to be true also in animal models.

Treatment of dystrophinopathies has been supportive with proper physiotherapy and timely surgical interventions as required to prevent contractures due to muscle fibrosis. Recent trials have also shown benefit with use of corticosteroids which significantly showed increase in muscle strength, function and overall well being and prolonged independent living with delay in the onset of wheelchair-bound stage. But prednisolone at the dose of 0.75 mg/kg on a daily basis produces significant side effects which reduces its merits. The mechanism of action seems to be by increasing muscle mass by decreasing necrosis-there is no effect on the dystrophin level in the muscle.

Gene replacement therapy is an exciting new therapy but we are yet not sure whether it is in the near or distant future, as many obstacles need to be overcome on all aspects.
before anything fruitful is expected. Alternative experimental procedure of upgrading the expression of another muscle membrane protein called utrophin, which might act as a membrane protecting molecule in presence of dystrophin deficiency, is in the offing.23

**THE SACROGLYCANOPATHIES OR LIMB-GIRDLE MUSCULAR DYSTROPHY**

It would have been ideal if all LGMD phenotypes could be linked to the 5 sacroglycan proteins yet identified. There are other proteins identified whose exact location in the DGC is still not clear and they produce the clinical entity of LGMD. They are also included in this broad groups. Almost all of them have their genes detected and few also have their corresponding proteins identified. It is hoped that with minor changes, they will be under the broad heading of sacroglycanopathies in the near future. Table 1 gives the exact position as of now.

The clinical grouping of the LGMD is based on the fact that all phenotypes present with weakness in the proximal group of muscles of both limbs, sparing the face, extraocular and pharyngeal muscles. The degree of weakness varies from early-onset severe disorder to a much milder late onset form. The clinical pattern of the milder form itself shows considerable variations. Calf-hypertrophy is also not a constant feature. Some phenotypes show prominent calf-muscle contracture rather than pseudohypertrophy.

The proximal muscle weakness however is also associated with distal muscle weakness in some phenotypes and so exclusive involvement of proximal and exclusion of distal groups is not a feature of the LGMD as the name suggests.2

All sacroglycanopathies, or the LGMD’s, unlike dystrophinopathies, spare mentation, and cardiomyopathy is less common and not a regular feature.24 The CK is raised in all types of LGMD and is higher in the genotypes showing recessive inheritance but this feature does not help in the precise diagnosis. Muscle biopsy with staining with specific antibodies against the various proteins remains the only way to get to an exact diagnosis. This is a very difficult job as deficiency of one protein of the DGC also leads to at least some quantitative changes in other proteins and immunohistochemistry can be confusing.10

The DNA analysis is also cumbersome with so many genotypic and phenotypic variations as shown in Table 1.

The α-sacroglycan gene is the best studied, and other mutations with other sacroglycan genes are going on.25,26 The studies have also found out that one protein ‘calpain-3’ in one form of LGMD, is not a structural protein but an enzyme-a muscle specific ca-activated neutral protease. This is an unique feature but it is not known whether this enzyme has any role to play in the post-translational phase of the structural proteins of the DGC. All the sacroglycanopathies cause a ‘fragile - membrane’, resulting in muscular dystrophy, the details of which are however still incomplete. The sacroglycans, five of them, seem to be interdependent of each other forming a very important group in the DGC. Primary deficiency of one leads to a disorganisation of the whole complex, although relative sparing of some components of the DGC is seen depending on the primary defect.26 The final result of all this is however membrane instability and muscle membrane tears resulting in a two way traffic. CK escaping and excess Ca\(^{2+}\) entering the cell and leading to muscle necrosis and ultimate fibrosis.

The treatment of sacroglycanopathies is basically rehabilitation with no improvement with any medical therapy. Gene therapy is still in the conceptual stage but theoretically appears to have less hurdles than in dystrophinopathies as the genes are much smaller.27

**THE LAMININOPATHIES OR CONGENITAL MUSCULAR DYSTROPHIES**

This group of muscular dystrophies are as heterogenous phenotypically and genotypically as are the LGMD’s. The proteins are also not known in many forms of this group but of the three diseases, which have been extensively studied, there seems to be some problem with the ‘laminin’ part of the DGC. Laminin is the ultimate extracellular anchoring protein and any of its structures of function causes the muscular dystrophy.10

The unique feature of the CMD’s as a group is the early age of onset of the clinical picture and some CNS involvement, although this itself is considerably variable.2 It is rather premature to rush with a classification.

On the known defects, laminin \(\alpha_2\) (formally called merosin) chain deficiency results in a phenotype where patients present in infancy - with hypotonia, delayed motor milestones and joint contracture around large joints. Proximal muscles are more affected while those of head and neck seem to be spared. Rarely patients have peripheral neuropathy also.24 The severity varies and death within few years of life to life upto the teens are reported, although locomotor defects are there from infancy. CNS involvement is a common feature of the lamininopathies and in \(\alpha_2\) chain deficiency, widespread hypomyelination in MRI scan is seen.29 Rarely patients are epileptic and mentally retarded and the hypomyelination seems to wane with time suggesting delay in myelination rather than a defect.30

CK elevation is not to that extent as in the dystrophinopathies or sacroglycanopathies and late in the course may drop to near normal. Muscle biopsy with immunostaining with antibody against laminin \(\alpha_2\) gives the specific diagnosis, although punch biopsy of the skin with absence of the protein in the basal lamina is a very good less invasive alternative means of diagnosis.31

It is an autosomal recessive disorder with the gene residing in chromosome 6. Laminin \(\alpha_2\) chain serves as the ligand for α-dystroglycan of the DGC as shown in Fig. 1. Its deficiency disrupts the extracellular part of the anchor of the muscle cytoskeleton.32 However its role in the cerebral
hypomyelination is unknown yet.

There is another lamininopathy due to laminin A chain deficiency, which is a major integrin receptor isof orm present in skeletal muscle acting as the integrin receptor for laminin A.\textsuperscript{33} Mutations of the gene in chromosome 12 results in a CMD presenting early in life with hypotonia and delayed milestones of development. Mental retardation is often coexisting but is not sure if it is a feature of the disease. CK elevation is mild and muscle biopsy with immunostaining is diagnostic. In any event this A7 deficiency disease is a rarity with only isolated cases reported.\textsuperscript{22} However it gives us further clues to the structural proteins of skeletal muscles.

The Fukuyama congenital muscular dystrophy has recently been shown to affect laminin A, staining of the DGC. Although Fukutin (the gene product) is a secretory protein and not a structural one,\textsuperscript{37} it is still grouped under the broad category of lamininopathies. How this secretory protein affects the structure is not yet known. It is possible that its action is extracellular where laminin A2 resides and also has something to do with neuronal migration resulting in the malformation of the gyri in the brain, and the CNS disturbances that result.\textsuperscript{36}

The Fukuyama type CMD usually presents with severe CNS defects, ocular abnormalities and muscular dystrophy and is seen only in Japan.\textsuperscript{37} The patient is perhaps just able to crawl and never learn to walk, they become bed-ridden by 10 years and usually die by 20 years of age. Severe mental retardation and intractable seizures accompany. High myopia and mottled retinal pigmentary changes with optic atrophy are also present.\textsuperscript{38}

MRI scans show polymicrogyria of cerebrum and cerebellum. Hydrocephalus, focal interhemispheric fusion and hypoplasia of the pyramidal tracts may also occur. Immunostaining shows partial deficiency of laminin - A chain in muscle biopsy.\textsuperscript{39} Genetic studies reveal it as an autosomal recessive disease with responsible gene residing in chromosome 9.\textsuperscript{40} The gene produces a secretory protein named fukutin, and the mutation of the gene has been traced to the Yayoi people who migrated from Korea and China to Japan centuries ago.\textsuperscript{36}

There are other rarer conditions related to the clinical features of the Fukuyama type CMD but since their pathogenesis is not known it is not possible to as yet to extend the test for lamininopathies.

Many other clinical phenotypes of CMD remain unclassified in the genetic and pathogenetic light. May be more would be revealed in the next 10 years of the new millennium.

REFERENCES


