What is muscular dystrophy?
Forty years of progressive ignorance

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ABSTRACT This lecture traces recent advances in knowledge of the muscular dystrophies, as well as their increasing complexity. They are described through the eyes of the author from his first exposure to and complete ignorance of the disease in the late 1950s, through the advent of modern techniques, to the molecular genetic revolution, with the recognition of individual genes and proteins for disorders within the muscular dystrophy umbrella.

There initially seemed to be a logical sequence of linked membrane proteins from dystrophin in Duchenne and Becker dystrophy, through the dystrophin-associated glycoproteins (sarcoglycans) in some of the limb girdle muscular dystrophies (LGMD), to the extracellular matrix protein merosin (α-2 laminin) in congenital muscular dystrophy (CMD). The first spoke in the wheel came with the discovery of a calcium activated protease enzyme, calpain 3, in one form of LGMD, and subsequently another novel non-membrane protein, dysferlin, in another. There are currently at least eight distinct genetic forms of LGMD alone, and another eight separate genetic entities in the CMD group. This has highlighted our ignorance of the pathogenesis of the muscular dystrophies in relation to a diverse array of protein deficiencies.

To compound things further, the X-linked and dominant forms of Emery-Dreifuss muscular dystrophy have recently been linked to emerin and lamin A/C, respectively, two proteins of the nuclear membrane, opening up yet another new ballpark of discovery.

The fundamental descriptions of muscular dystrophy date back to the writings of the London physician Edward Meryon in the 1850s, Duchenne de Boulogne in the 1860s, William Gowers in the 1870s, and Wilhelm Erb in the 1880s (see Ref 1 for historical background). It may be difficult for the current generation of clinicians to perceive a muscle world in the late 1950s without serum creatine kinase, where haematoxylin, eosin, and a few other routine histological stains on paraffin embedded biopsies, riddled with artefact, were the order of the day, and neurologists were content to divide all neuromuscular cases into myopathic (primary muscle disorders) or neuropathic (neurogenic disorders). Electromyography (EMG) was just being established as a diagnostic tool. The average age of diagnosis of 'pseudohypertrophic' muscular dystrophy (Duchenne dystrophy) was around five years, and efforts were made to classify different forms of muscular dystrophy on the basis of clinical phenotype and pattern of inheritance.

Differential diagnosis

Milder forms of spinal muscular atrophy resembling LGMD were being recognised by EMG and histology and separated from the dystrophies. The advent of new techniques, such as histochemistry and electron microscopy, revolutionised the diagnosis of neuromuscular disorders. They helped to define a whole new family of so-called congenital myopathies (basically hereditary disorders with structural changes in the muscle), and also to identify, at a tissue level, metabolic disorders such as the glycogenoses and the mitochondrial and lipid storage disorders.

The discovery by Ebashi's group in 1959 of serum creatine kinase as a much more sensitive, and muscle specific, index of muscle dystrophy and other degenerative muscle disorders than the earlier transaminases and aldolase, provided a quantum leap in the screening of children with suspected muscular dystrophy. It also saved many a hapless Duchenne boy an unnecessary liver biopsy to investigate his elevated 'liver' enzymes, instead of a muscle biopsy investigating his elevated muscle enzymes.

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Until the late 1950s, muscular dystrophy had traditionally been defined as a genetically determined, primary, progressive degenerative disorder of voluntary muscle. The seminal work of Walton and Nattrass in 1954 had tried to classify the different forms of muscular dystrophy on the basis of their clinical phenotype and mode of inheritance. This is fine for Duchenne dystrophy, with its relentlessly progressive clinical course and classical dystrophic picture on muscle biopsy, and the same applies to the milder phenocopy of Becker muscular dystrophy. But what about the cases of Becker dystrophy, already diagnosed in the premolecular era and more recently confirmed as Becker dystrophy by deletions in the Duchenne gene, presenting with no
apparent weakness, but with cramps on severe exercise, a very high creatine kinase and an overtly dystrophic muscle biopsy? Molecular diagnosis has now expanded the phenotype further, with the recognition of isolated cardiomyopathy as a manifestation of Becker dystrophy, without skeletal muscle weakness, histological abnormality or dystrophin deficiency.

The so-called limb girdle muscular dystrophies were well established on clinical grounds, with progressive weakness of variable severity resembling Duchenne or Becker dystrophy, and a dystrophic biopsy, but with a clearly autosomal recessive inheritance. Well-defined large pedigrees were documented in geographical isolates in Switzerland, North Africa and Amish communities in Indiana.

Other forms of dystrophy raise other questions of identity. Facioscapulohumeral dystrophy, which selectively involves facial and shoulder girdle muscles, may be relatively static or may show marked progression with spread to the trunk and lower limbs. However, the histology only occasionally shows an overtly dystrophic picture, and the most common change is a well-preserved muscle with focal atrophic fibres. Some biopsies show a remarkable inflammatory cell response. Creatine kinase is usually normal. Is this really a dystrophy?

Emery-Dreifuss muscular dystrophy raises similar problems of definition. The clinical picture is fairly consistent and distinct, with a tendency to contractures of the spinal extensor muscles, the elbow flexors and the tendo Achilles as the main presenting features, with focal wasting of some muscles rather than weakness. Cardiac involvement is a consistent feature. Creatine kinase is usually moderately elevated, and biopsy shows focal degenerative changes. The condition is X-linked but very different from Duchenne/Becker dystrophy. A similar phenotype with a dominant inheritance has also been recognised.

Congenital muscular dystrophy provided a similar dilemma and heated debate in earlier years. Here was a condition that was often fairly static and non-progressive – in fact, some cases might even improve with time – and creatine kinase might be normal, yet the muscle biopsy was overtly dystrophic, often with marked degeneration and proliferation of fat and connective tissue. This condition was further compounded by the association of mental retardation and structural changes in the brain in some variants with the same dystrophic muscle changes, also with a clear implication of central nervous system involvement.

Impact of molecular genetics

Looking back over the past few years, of special interest is the symbiotic relationship that has evolved between the clinician and the molecular geneticist. The clinician initially has a key role in identifying the clinical phenotype of the disease under study and selecting potentially informative families for the geneticist to study. Once the gene has been cloned, mutations identified in relation to the disease and the relevant protein characterised, this new information in turn becomes available as a confirmatory diagnostic tool for the clinical diagnosis, and also provides the basis for genetic counselling and prenatal diagnosis.

The whole process has been greatly enhanced by advances in technology and, in the case of autosomal recessive disorders, by studying large consanguineous families and looking for homozygosity by familial descent of the gene with closely linked markers. When a locus is established, there can be rapid screening for candidate genes and candidate proteins in the chromosome region. It has also led to the recognition of cases with unusual phenotype due to mutations in a particular gene, such as cases of partial merosin-deficient cases of CMD presenting in adult life.

Genetic and biochemical advances have brought new insight into many of the muscular dystrophies, and helped to clarify some of the fundamental issues of designation and nomenclature. However, in their wake has come further chaos and confusion, opening up a series of totally new questions.

The limb girdle muscular dystrophies (Table 1)

The first major development after the discovery of dystrophin was the discovery of a protein by Kay Davies' group with about 85% homology to dystrophin, but encoded by an autosomal recessive gene on chromosome 6. It was initially called dystrophin related protein (DRP), but was subsequently renamed utrophin (u' for ubiquitous, not an Americanisation of 'eu-' to contrast with 'dys-','trophin). It is not normally expressed on the muscle membrane but at the neuromuscular junction. There was high hope that this protein might provide the answer to the autosomal forms of muscular dystrophy such as LGMD. But this was not to be, and utrophin still remains a protein in search of a disease – or possibly, more recently, in search of a cure, with efforts to upregulate it as potential compensation for the deficient dystrophin.

The next major development was the discovery in the USA (Campbell) and in Japan (Ozawa) of a group of proteins linked to dystrophin and spanning the muscle membrane;

| LGMD    | Gene location | Protein          |
|---------|---------------|------------------|
| LGMD2A  | 15q15-q21     | calpain 3        |
| LGMD2B  | 2pter-p12     | dysferlin        |
| LGMD2C  | 3q12          | γ-sarcoglycan    |
| LGMD2D  | 17q12-q21     | α-sarcoglycan    |
| LGMD2E  | 4q12          | β-sarcoglycan    |
| LGMD2F  | 5q33-q34      | δ-sarcoglycan    |
| LGMD2G  | 17q11-q12     | telethonin       |
| LGMD2H  | 9q31-q33      | ?                |

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these were named dystrophin associated proteins (DAGs) or glycoproteins. They were further characterised by their molecular size: thus, the 50 kD, 43 kD and 35 kD DAGs soon became colloquially referred to as 50DAG, 43DAG and 35DAG, respectively. In view of the secondary reduction of these glycoproteins in Duchenne dystrophy, it was suggested that they might play a pivotal role in the pathogenesis of muscular dystrophy, either as a secondary process in Duchenne dystrophy or as a primary deficit in dystrophies still to be identified.

The breakthrough came soon with the discovery of a 50DAG deficiency in the well-characterised recessive dystrophy in North Africa (also named Maghrebian, meaning west for geographical location, and severe childhood autosomal recessive muscular dystrophy (SCARMID)). The defect was thought to be primary, comparable to dystrophin in Duchenne muscular dystrophy, so a special name was coined for it: ‘adhalin’, from the Arabic for muscle (adhal), to honour the first Moroccan, Arab-speaking child in whose muscle the deficiency was discovered. However, the celebrations were short-lived when the gene for the disease was localised to chromosome 13, whereas the 50DAG protein was located on chromosome 17. Thus, the deficit was secondary to a primary deficit of another protein, which later turned out to be the 35DAG. Cases were subsequently discovered with a primary deficiency of the 50DAG and disease gene location on chromosome 17.

All change!

Just when the dust seemed to be settling on this interesting group of muscular dystrophies, and clinicians were becoming comfortable with the nomenclature, the biochemists renamed the dystrophin-associated glycoproteins ‘sarcoglycans’, categorising them as α-, β- and γ-sarcoglycan in descending order of molecular size. An additional protein, comparable to γ-sarcoglycan but encoded by a separate gene, was named δ-sarcoglycan.

The geneticists then decided it was time to get their house in order. As is customary among molecular geneticists, they agreed on a nomenclature dependent on priority of discovery, seemingly completely out of tune with the biochemical classification:

- **LGMD1**: the rare dominantly inherited cases
- **LGMD2**: the recessive group.

This meant that the first recessive LGMD to have a gene location identified on chromosome 15, the mild, late-onset Reunion Island dystrophy, was named **LGMD2A**. Unexpectedly, it also proved to be the first aberrant muscular dystrophy due to deficiency of a calcium-activated protease enzyme, calpain 3. Those following were named:

- **LGMD2B**: located on chromosome 2 and not yet characterised at the time
- **LGMD2C**: the original secondary adhalin deficiency, with primary deficiency of 35DAG (or γ-sarcoglycan).

- **LGMD2D**: primary deficiency of 50DAG (or α-sarcoglycan) on chromosome 17.
- **LGMD2E**: deficiency of β-sarcoglycan on chromosome 4.
- **LGMD2F**: an additional 35 kD glycoprotein (δ-sarcoglycan) deficiency on chromosome 5.
- **LGMD2G** and **LGMD2H**: additional loci subsequently identified on chromosomes 17q and 9q, respectively.

It is to be hoped that some logical and more common sense resolution will be found for this totally chaotic nomenclature.

The congenital muscular dystrophies (Table 2)

The CMDs have also seen an exponential expansion with the application of molecular genetic techniques. Credit for the first description of the clinical and pathological features goes to Frederick Batten’s classical paper in 1903 (he is more revered for his description in the same year of the neurodegenerative disorder with the cherry red spot which bears his name). Yet, as recently as the 1970s some leading myologists were still questioning the existence of congenital dystrophy as an entity.

Much of the initiative for the recent advances in CMD has come from the multidisciplinary collaborative studies organised through the weekend workshops of the European Neuromuscular Centre (ENMC) in the Netherlands. The first workshop, convened in 1993, concentrated on trying to define individual clinical syndromes. It was attended by most of the main players on the world stage who had described syndromes of CMD with associated brain malformation and mental retardation. They included Fukuyama (Japan; Fukuyama CMD), Santavuori (Finland; muscle-eye-brain disease), and Dobyns (USA; Walker-Warburg syndrome). Also present were clinicians with an interest in the classical form of congenital dystrophy without associated brain malformation or mental retardation. A consensus was reached that these individual disorders were sufficiently distinct to be considered as potentially separate and non-allelic disorders, and it was decided to set up separate genetic studies on each of them.

| CMD | Gene location | Protein |
|-----|---------------|---------|
| CMD (M–) | 6q2 | merosin (α2-laminin) |
| CMD (M+) | ? | ? |
| CMD (M+) (RSS) | 1q | ? |
| CMD (M+) (RSS) | ? | ? |
| CMD (M+) | 12q13 | integrin α7 |
| Fukuyama CMD | 9q31-q33 | Fukutin |
| Muscle-eye-brain | 1q | ? |
| Walker-Warburg | ? | ? |
The first major breakthrough came later the same year with the location of the gene for Fukuyama CMD on chromosome 9q. This involved a remarkable piece of serendipity on the part of the Japanese clinicians. Having assembled some twenty potentially informative consanguineous or multiplex families for a systematic genome-wide search for the gene, they noted that a single affected individual in a consanguineous family also had xeroderma pigmentosus. They questioned whether the concurrence of two rare autosomal recessive disorders in the same individual might reflect a close linkage between the two genes. They found the Fukuyama locus on 9q at the same site as xeroderma pigmentosus, with genetic concordance in all their available families. This, in turn, provided a tremendous boost for the collaborative programme already set up by the consortium to study families with classical CMD, and the Fukuyama locus could rapidly be excluded.

The next advance followed soon after with the discovery by Tomé and his colleagues in Paris that merosin (α-2 laminin), one of the extracellular matrix proteins he was screening by immunocytochemical techniques, was deficient in the muscle biopsies of about half their cases of classical CMD. This was confirmed in a large cohort of patients by our group in London. Further genetic studies showed that the locus for the merosin-deficient CMD on chromosome 6q was also the locus for merosin, thus confirming a primary deficiency.

This new development now provided a sieve for separating the merosin-deficient and merosin-positive muscle biopsies of classical CMD. Clinical analysis showed that the merosin-deficient group were, by and large, more severely affected and rarely achieved independent ambulation. Also, the striking white matter changes in the brain, already well documented in some cases on T2-weighted magnetic resonance imaging (MRI), were consistently present in the merosin-deficient but not the merosin-positive cases.

The merosin-deficient group then expanded further with the recognition of late onset, and even adult, cases with a partial merosin deficiency which still linked to the merosin locus on 6q, and also had the classical white matter changes on MRI. Other cases with merosin deficiency, however, were not linked to 6q and did not show the brain imaging changes. The merosin deficiency is therefore thought to be secondary to another protein deficiency which now awaits discovery.

The ENMC consortium also tried to identify individual clinical syndromes within the somewhat heterogeneous merosin-positive group. One of these was a syndrome comprising CMD with rigid spine syndrome and early respiratory failure. A gene locus on chromosome 1p35-36 has been identified for this syndrome. A few more families have been found to link to this locus, whereas others with a similar clinical phenotype do not. This opens the way for yet more genetic heterogeneity, a hallmark of so many of these muscle disorders.

There have recently also been further advances on the CMD with brain malformation. The gene for Fukuyama muscular dystrophy has been characterised and involves a unique mutation with a retrotransposon insertion. The novel protein it encodes has been named fukutin. I consulted my Japanese colleagues on this choice of name because it might occasion some embarrassment among their English speaking colleagues, and expressed surprise that it had not been named fukuyamin, seemingly an obvious choice. They explained that fukuyamin was too close in sound to 'ukuyamin', a Japanese term of condolence when someone has lost a relative, which they considered inappropriate in the circumstances.

The location and function of fukutin still await resolution. It is thought to be a secreted protein, probably in the extracellular matrix. A gene locus has been found on chromosome 1q for muscle-eye-brain disease, thus confirming its distinction from both Fukuyama CMD and Walker-Warburg syndrome (which is not at this locus).

What's in a name?

These advances in molecular genetics have brought out a new trend in the nomenclature of the muscular dystrophies, with a move away from eponymous or descriptive terms to the more scientific biochemical terminology. Thus, it was suggested early on to drop the time-honoured designations of Duchenne and Becker dystrophy, and we started referring to dystrophinopathy. Fair enough, but at the end of the day the patient needs to know what disease he has and the implications in relation to its course and prognosis. The biochemical nomenclature has now extended further and sarcoglycanopathies have become part of the vernacular.

Where do we go from here? Merosinopathy has surfaced for the merosin-deficient form of CMD involving the extracellular matrix laminin protein, merosin, which links with the sarcoglycans and thus with dystrophin. But the biochemists now tell us that merosin is no longer an acceptable designation, that it is the α-2 chain of laminin that is specifically involved – so now we have α-2 lamininopathy as a specific form of muscular dystrophy.

The chain of linked proteins, starting from dystrophin and passing to the sarcoglycans and then through the sarcolemmal membrane to the extracellular laminins, followed a logical and rational path, but now seems to have opened up an endless series of ramifications. Recently, Hayashi and colleagues in Japan found a deficiency of integrin α7 in seven of 117 biopsies screened with a label of unclassified congenital myopathy/CMD. Four of these also had merosin deficiency, and the integrin deficiency was considered to be secondary. The remaining three were considered to have a primary deficiency of integrin α7 – and, indeed, they all were found to have mutations in the integrin α7 gene. So have we now arrived at integrin α7-opathy? Integrin α7 is a laminin receptor in muscle. This new myopathy, based on a protein deficiency and a gene mutation rather than on a specific clinical phenotype or overtly dystrophic biopsy, is still a distant relative (on the
biochemical side of the family) of the dystrophinopathies. Is it a muscular dystrophy? I fear we are rapidly heading for a cul de sacopathy!

The above series of apparently related diseases covers a group of structural proteins and has opened links with the laminins and dystrophin in the extracellular matrix. Presumably this is not too far a cry from the collagens and Bethlem myopathy, an autosomal dominant disorder with mild weakness and associated contractures, linked to the α subunit of collagen VI.

Two further advances have recently compounded the complexity of the LGMDs. A predominantly distal form of muscular dystrophy, Miyoshi myopathy, with a high prevalence in Japan, was found to share the same locus with the proximal LGMD2B on chromosome 2p, and to be associated with the same unique gene related to the gene for the spermatogenesis factor fer-1 in Caenorhabditis elegans.

The protein encodes in muscle, and which is located at the sarcolemma, has been named dysferlin. Another novel and unique protein in relation to the LGMDs, named telethonin (in appreciation of the financial support from the Italian telethon), has been found to account for LGMD2G, and mutations have been found in the gene. It is the first sarcomeric protein to be associated with a dystrophy.

To add fuel to the fire, X-linked Emery-Dreifuss muscular dystrophy has a deficiency of a protein localised to the nuclear membrane, which has been named emerin. It is ubiquitous in nuclei, and its function is still not known. The well recognised dominantly inherited form of Emery-Dreifuss dystrophy has recently also been linked to a nuclear membrane protein, lamin A/C. It has been shown to be allelic, with a dominantly inherited LGMD with associated cardiac involvement, LGMD1B on chromosome 1q.

An alternative approach to nomenclature is by association or affiliation. Thus, the various ion channel disorders (channelopathies) have now subsumed the former periodic paralyses and myotonias and several other disorders, creating an ever-increasing empire. The mitochondrial myopathies and cytopathies have blossomed into a formidable array of disorders involving the mitochondrial genome, and there are already some of the well-established metabolic disorders affecting muscle such as the lipid storage myopathies and the glycogenoses. A movement towards the term ‘membranopathies’ to cover the whole family of disorders associated with structural proteins that span the sarcolemmal membrane could readily be envisaged, with a separate subsection for the nuclear membranopathies. This would incorporate a large proportion of the current muscular dystrophies. A section of enzymopathies could be considered, headed by calpain as a start. Inevitably, we will end up with a mixed bag of proteins whose location or function is still awaiting resolution. For convenience and simplicity, these could conveniently be grouped in a section of ‘odd-man-outopathies’, pending more appropriate placement.

One of the big challenges now is to try to understand how this impressive array of seemingly unrelated genes and proteins can produce a common denominator of muscular dystrophy, and to try to unravel the pathogenesis of the process in the different circumstances. It is not surprising that after some 40 years of research we are still asking what is muscular dystrophy from a clinical, histological, genetic and biochemical point of view. Perhaps it is axiomatic of all advances in medicine that the more we know, the more we find we do not know.

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For an update on the molecular genetics of any individual muscle disorders, see the gene tables in a current issue of Neuromuscular Disorders.

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