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REGULAR REVIEW

# Recent advances in understanding muscular dystrophy

K M D Bushby

The isolation of the causative genes in the muscular dystrophies, and from these the identification of the defective protein products, is at last giving an insight into the molecular basis of these conditions. These advances have come about through the techniques of reverse genetics, or positional cloning.[1] All the muscular dystrophies have consistently eluded attempts to use standard biochemical techniques to identify the underlying defects in affected muscle. Most advances have been made in Duchenne and Becker muscular dystrophy, for which the gene and protein product have been known for some time.[2 3] The gene for myotonic dystrophy has been isolated very recently,[4–6] and linkage analysis has identified chromosomal localisations for facioscapulohumeral dystrophy on 4q,[7] a form of recessive limb-girdle on 15q,[8] a form of dominant limb-girdle on 5q,[9] and Emergy-Dreifuss muscular dystrophy at Xq28.[10]

The gene involved in Duchenne and Becker muscular dystrophy is the largest yet identified, comprising over 2·5 megabases of genomic DNA with at least 75 exons and some very large introns.[2 11] This encodes a 14 kilobases mRNA, from which a previously unknown muscle protein, dystrophin, is translated. The predicted molecular weight of dystrophin is approximately 427 kilodaltons and it comprises about 0·001% of total muscle protein.[3] Dystrophin is also present in brain, where it uses a different promotor and exists as a different isoform.[12] It appears to be localised to neurons.[13] A protein with significant homology to dystrophin encoded by a gene on chromosome 6 has also been described.[14]

Study of the sequence of dystrophin suggests the presence of four district domains.[15] The N-terminal region has homology to α-actinin and appears to have a site of interaction with F-actin.[16] The second domain is thought to contain 24 or 25 triple helical repeats,[17 18] which may confer elasticity on the molecule.[17] There follows a sequence relatively rich in cysteine residues and a C-terminus domain that is highly conserved between species.[19] Dystrophin has been shown by electron microscopy to be located at the internal cytoplasmic face of the plasma membrane[20] where it is associated with integral membrane glycoproteins[21] via its C-terminus domain. The structure of this glycoprotein complex has recently been described and it has been shown that dystrophin deficient fibres also show a reduction in some components of this complex.[22 23] The structure and functional characteristics of the glycoprotein complex associated with dystrophin suggests that the function of dystrophin may be to link the sarcolemma membrane skeleton through a transmembrane complex to an extracellular glycoprotein which binds laminin.[22] It has been shown that dystrophin deficient fibres are more sensitive to hypo-osmotic shock than controls,[24] which supports the theory that dystrophin deficiency leads to mechanical weakening of the plasma membrane. An influx of calcium has been observed in dystrophin negative fibres, which may, directly or indirectly, stimulate protease activity,[25] and myotubes negative for dystrophin have been shown to have abnormal calcium ion channels.[26]

It is now known that most mutations in the dystrophin gene are deletions, which can be identified in approximately 65% of patients with Duchenne muscular dystrophy and up to 85% in Becker muscular dystrophy.[27 28] The high frequency of deletions and in particular the tendency for deletions to cluster in two particular regions of the gene has not yet fully been explained.[29 30] In those families where a patient has been shown to have a deletion DNA analysis offers a specific diagnostic test and a means of reliable and quick prenatal diagnosis. The demonstration of DNA abnormalities in patients without deletions is much more difficult in such a large gene, and only a handful of such mutations (point mutations and mutations in the promotor region) have been described.[31 32] Techniques such as the use of dosage analysis for deletion detection in females, based on both Southern blotting and polymerase chain reaction (PCR) analysis, pulsed field gel electrophoresis, and most recently lymphocyte RNA PCR[33] have led to direct carrier detection being possible in some cases. However, in many cases carrier risk still has to be assessed as a probability calculation based on the results of creatine kinase testing and intragenic polymorphic markers. The high mutation rate in Duchenne muscular dystrophy has long been recognised, with approximately one third of cases estimated to result from a new mutation. The use of techniques that can directly detect a mutation in a boy and his mother has led to the recognition that germline mosaicism exists at a significant level in the Duchenne muscular dystrophy gene, with the risk of another affected son to a mother of an isolated case of Duchenne muscular dystrophy being around 20% with the at-risk

**Department of Human Genetics, 19/20 Claremont Place, Newcastle upon Tyne NE2 4AA**

Correspondence to:
Dr Bushby.

haplotype even when a deletion has been identified in the boy and shown to be somatically absent in the mother.[34]

Dystrophin analysis, using antibodies to different parts of the dystrophin molecule, can be used both on muscle sections and western blots in diagnosis. Abnormalities of dystrophin seem to be specific to the Xp21 dystrophies, and so the finding of abnormal dystrophin can confirm the diagnosis even in the absence of a cDNA deletion.[35 36] The use of a combination of genetic and dystrophin analyses to distinguish Becker muscular dystrophy accurately from the often clinically similar conditions of spinal muscular atrophy and limb-girdle muscular dystrophy has allowed the prevalence of this condition to be reassessed, and it has been shown to be much commoner than had previously been thought,[37] the prevalence being similar to that of Duchenne muscular dystrophy and the cumulative birth incidence approximately one third. Some isolated cases of females with limb-girdle muscle weakness have been shown by dystrophin analysis to be manifesting carriers of dystrophin mutations[38] a finding of great significance in counselling these women. Dystrophin abnormalities do not seem to be so easily detectable in female carriers without symptoms.[39]

Studies of the gene and protein abnormalities in large numbers of patients are beginning to unravel the complexity of the relationship between genotype and phenotype in the Xp21 dystrophies. A range of clinical severity, from the classical Duchenne muscular dystrophy patients at one end to the relatively mild Becker muscular dystrophy patients at the other, with patients of 'intermediate' severity between, is now known to exist as a result of different mutations in this gene.[40] The ability to look directly for the molecular defect has also led to the recognition that severe muscle cramps may, at least for many years, be the only manifestation of Xp21 dystrophy.[41] Occasional asymptomatic cases have been recognised with dystrophin gene deletions.[42] The clinical range of severity is now known to be a reflection of a similar range of gene and protein defects. Deletions that disrupt the reading frame of the gene[43] (with the expected result that no recognisable protein could be produced beyond the position of the mutation), are often associated with undetectable dystrophin, and these patients are generally very severely affected. However, dystrophin is detected in some patients with out of frame deletions, and the size of this dystrophin implies that the deletion behaves as if 'in frame', that is recognisable dystrophin is produced right through to the C-terminus of the protein,[44 45] and the size of the protein is as predicted from the loss of the deleted exons.

Preliminary results suggest that the presence in muscle fibres of even a small amount of dystrophin may confer some functional advantage on these boys (LVB Nicholson, personal communication). At the other end of the clinical spectrum, patients with Becker muscular dystrophy are most commonly found to have deletions which do not disrupt the translational reading frame of the gene, so that a dystrophin molecule is produced which is internally deleted, but with both ends present and intact. Even very large deletions, providing they are in frame can be associated with a mild phenotype,[46] but it appears that deletions 45–47 and 45–48 of the dystrophin gene do tend to be associated with a relatively consistently mild clinical course.[28] Across the range of Xp21 dystrophy, increasing abundance of dystrophin does seem to be associated with a milder clinical course, but there is no one value for abundance which reliably predicts a particular phenotype.

It is likely that alternative splicing mechanisms around deletions at the RNA level may be more widespread in the dystrophin gene than has yet been described,[47] and this may account for apparent anomalies in phenotype-genotype correlations. Interactions with the dystrophin-associated glycoproteins or other genes may yet account for some of the unexplained variability in the dystrophin gene.

With an understanding of the molecular basis of the Xp21 dystrophies, work on possible therapeutic measures can now be more rationally planned and assessed. Animal models with dystrophin abnormalities have been identified.[48 49] Myoblast transfer and gene therapy using a 'mini-gene' construct have both shown some promising results in animal work,[50 51] and dystrophin transcripts have been identified in patients after myoblast transfer,[52] but widespread use of either technique in patients is still generally held to be a long way off.

The mutation responsible for myotonic dystrophy has recently been described.[4–6] There is variable amplification of a trinucleotide (CTG) repeat at the 3′ end of a gene, with an increase in the number of repeats associated with the disease phenotype. The number of repeats increases with the transmission of the mutation to successive generations. This mechanism explains the phenomenon of anticipation seen in families with myotonic dystrophy, where the severity of the disease is often seen to increase through successive generations. However, as amplification of the repeat may occur whether the mutation is passed through the male or female line, this mechanism is not enough alone to explain why congenital myotonic dystrophy is almost always maternally inherited. Women with myotonic dystrophy have around a 10% chance of having a child with congenital myotonic dystrophy, a risk which increases to 40% after the birth of one congenitally affected child, and which also rises with the severity of the disease in the mother.[53]

The gene has homology with genes encoding cyclic-AMP-dependent protein kinase, and its protein product has been designated myotonin protein kinase.[54] A 3 kilobase transcript is expressed at high levels in heart and lower levels in skeletal muscle and brain.[55]

Linkage analysis in the other muscular dystrophies should also ultimately lead to the identification of the genes and proteins involved, and no doubt to new ideas of how normal and abnormal genes and proteins interact in muscle. In the meantime, the work so far achieved has resulted in significant improvements in diagnosis and counselling for patients and their families.

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