Original articles

Dysferlin and muscular dystrophy

K. M. D. Bushby

Reader and Consultant in Clinical Genetics, Department of Human Genetics, U.K.

————————

Abstract

The limb-girdle muscular dystrophies are a highly heterogeneous group of muscle disorders with many different genetic causes now known. Amongst the causes of LGMD, the dysferlin gene stands out as novel for several reasons. It is the first known example of a C2 domain containing protein involved in a muscular dystrophy, mutations in the gene can be involved in a variable phenotype, and a naturally occurring mouse model for dysferlin deficiency has recently been identified. This article reviews the progress made in understanding this form of limb-girdle muscular dystrophy to date.

Key words : Muscular dystrophy ; LGMD2B ; Miyoshi myopathy ; diagnosis.

Introduction

Dysferlin was identified through a positional cloning strategy aimed at determining the gene involved in two forms of muscular dystrophy — a form of limb-girdle muscular dystrophy known as limb-girdle muscular dystrophy 2B or LGMD2B, and a form of distal muscular dystrophy known as Miyoshi myopathy (MM) (Bashir *et al.*, 1998 ; Liu *et al.*, 1998). Linkage of both of these diseases to the same region of chromosome 2p13 led to the first suspicion that these two apparently distinct muscle disorders might be allelic (Bashir *et al.*, 1994 ; Bejaoui *et al.*, 1995), and following the identification of the dysferlin gene, it has become clear that even the same dysferlin mutations may cause a variable phenotype, with the chief distinguishing features between the two conditions being the predominant involvement of either the proximal (in LGMD2B) or distal (in Miyoshi myopathy) musculature (Weiler *et al.*, 1999). Other features of the two disorders are more similar — they both tend to have onset in the late teens with previously good muscle performance, and both are characterised by an extremely high level of serum creatine kinase (Mahjneh *et al.*, 1996 ; Linssen *et al.*, 1997). Dysferlin was named according to the only sequence homology detected at the time of its cloning — to a *c elegans* protein FER-1 which is a spermatogenesis factor involved in the fusion of membranous organelles. Mutations in the fer-l gene cause infertility (Achanzar and Ward, 1997). Since the cloning of human dysferlin, however, it has become apparent that dysferlin is a member of a gene family of which there are at least two additional human members. These genes share a number of characteristics, most notably the presence of multiple C2 domains and a C terminus transmembrane domain. Mutations in the second human fer-l like gene, otoferlin, cause autosomal recessive non-syndromic deafness (Yasunaga *et al.*, 1999), while the third fer-1 like gene (fer-lL3), is a widely expressed gene for which a mutant phenotype has not yet been described (Belt Davis *et al.,* 2000 ; Britton *et al.* submitted). Murine dysferlin is highly homologous to human dysferlin (Vafiadaki *et al.* submitted), and the SJL mouse has been identified as a naturally occurring model for dysferlin deficiency (Bittner *et al.*, 1999). This mouse develops a progressive muscle weakness with dystrophic muscle pathology. Like in human dysferlin deficiency, the dystrophic changes in skeletal muscle in the SJL mouse are accompanied by prominent inflammatory changes.

The dysferlin gene

The human dysferlin gene is located on chromosome 2p13. It has 55 exons, ranging in size from 30-461 bp (Aoki *et al.*, submitted). The sizes of all the introns are not yet known, but can be very large. The total genomic distance covered by the dysferlin gene is at least 150kb. The open reading frame of 6243bp encodes a 7kb cDNA of 2080 amino acids. Northern analysis indicates that this 7kb transcript is most highly expressed in skeletal muscle, heart and placenta. A different transcript of approximately 4kb is detected in all regions of the brain, though not in spinal cord. In brain tissue, the full length 7kb transcript is found in addition to the 4kb transcript only in the cerebellum and medulla (Bashir *et al.*, 1998 ; Liu *et al.*, 1998).

Dysferlin expression in adult muscle and human development

A monoclonal antibody to dysferlin (raised towards an epitope within amino acids 1999-2016 encoded by exon 53, just before the predicted transmembrane domain) detects a protein of around 230kD, close to the 237kD predicted from the amino acid sequence (Anderson *et al.*, 1999). The protein is the same size in all tissues with strongest expression in skeletal muscle, heart and kidney. There was no evidence of a protein product corresponding to the smaller transcript detected on Northern analysis in brain tissue.

As predicted from the sequence analysis, immunolocalisation of dysferlin confirmed its position at the muscle fibre membrane, probably internally (Anderson *et al.*, 1999). Using a polyclonal dysferlin antibody, dysferlin was detected in micosomal, nuclear and mitochondrial fractions following muscle cell fractionation, but these results were not confirmed by immunocytochemistry (Matsuda *et al.*, 1999). Although many membrane proteins are involved in muscular dystrophy, notably the components of the dystrophin associated complex (Ozawa *et al.*, 1998) there is however no evidence that dysferlin is associated with this complex. Loss or reduction of a member of the dystrophin-associated complex is typically associated with a secondary reduction in the other complex members (Passos-Bueno *et al.*, 1999), while the members of the sarcoglycan complex are consistently normal in dysferlinopathy.

Protein analysis by immunoblotting in human fetal limb tissue during development showed a clear band of the same size as in adult tissue from the earliest time it was investigated (carnegie stage 15- 16, embryonic age 5-6 weeks) (Anderson *et al.*, 1999). RNA tissue in situ analysis during human development confirmed this early expression with low-level ubiquitous expression of dysferlin from Carnegie stage 19. The highest expression was seen in the skeletal muscles of the upper and lower limbs, with no significant difference between expression in the proximal and distal muscles at this stage (Keers *et al.*, unpublished data).

Sequence analysis of dysferlin and its relationship to the other members of the fer-1 like family

It is now clear that dysferlin is a member of a protein family defined by the similarity of these proteins to the c elegans protein FER-1. FER-1 is a protein expressed only in primary spermatocytes, and mutations in the fer-l gene block the fusion of spermatic vesicles, leading to the production of abnormal and infertile sperm (Achanzar and Ward, 1997). FER-1 and the FER-1 like proteins share several structural features, including a C terminus transmembrane domain, with the majority of the protein cytoplasmic. The other striking structural feature of this group of proteins is the presence of multiple C2 domains (Vafiadaki *et al.*, submitted, Britton *et al.* submitted). The members of the FER-1 like family are highly homologous sharing amino acid similarity within and outside the C2 domains (Rizo and Sudhof, 1999). C2 domains are protein motifs often involved in protein protein interactions and also linked with membrane trafficking and vesicle transport.

Otoferlin, the second human far-1-like protein (fer-1L2) is much smaller than dysferlin, and contains only three C2 domains. Expression of otoferlin predominates in the inner ear though a broader expression pattern is detected by RT-PCR. Mutations in otoferlin have been described in a form of non-syndromic prelingual deafness (DFBN9, described in a Lebanese population) (Yasunaga *et al.*, 1999). The third human fer-1-like protein, Fer-lL3 or myoferlin, is the most similar of the human proteins to DYSF, with six C2 domains (Belt Davis *et al.*, 2000 ; Britton *et al.* submitted). As yet, no mutant phenotypes have been described in association with mutations in this gene, which by RT-PCR is expressed predominantly in the eye, salivary gland and oesophagus. This contrasts with the abundant expression of FER-1L3 protein in cardiac and skeletal muscle, with upregulation in the muscles of the mdx mouse, an animal model of Xp21 muscular dystrophy. These findings have led to the suggestion that FER1-L3 might be able to act as a modifier gene in muscular dystrophy and particularly in the context of the phenotypic variability seen in dysferlinopathy, might act to modify this phenotype as well (Belt Davis *et al.*, 2000).

The phenotype of dysferlinopathy

At least two recognisable human diseases are caused by dysferlin mutations. LGMD2B or limb-girdle muscular dystrophy type 2B was defined through linkage to chromosome 2p in several large families (Bashir *et al.*, 1994 ; Passos Bueno *et al.*, 1995). Detailed clinical analysis of these families shows a proximal muscular weakness at onset, involving predominantly the lower limbs. Onset is typically in the late teens or early twenties. Extremely high serum creatine kinase levels re found in active disease, but fall as the disease progresses. These patients have a very slowly progressive disease, with confinement to a wheelchair very late in adult life if at all. Although initial symptoms always relate to proximal lower limb weakness, muscle imaging is able in some patients to detect asymptomatic distal lower limb muscle involvement. In the later stages of the disease, muscle imaging reveals much more widespread muscle involvement. Nonetheless, a very clearly identifi-

able pattern of muscle involvement is present in these patients, distinct from the other forms of limb-girdle muscular dystrophy and contributing to a characteristic gait (Mahjneh *et al.*, 1996 ; Mahjneh *et al.* in press).

Miyoshi myopathy was first described as a distal muscle disease, with onset typically in the gastrocnemius muscles, leading to the inability to stand on tiptoe being frequently reported as an early symptom. Like LGMD2B, onset of MM tends to be within a fairly restricted period between the late teens and early twenties. Again similar to LGMD2B, a hallmark of symptomatic MM is the massive elevation of serum creatine kinase, though limited information about presymptomatic cases does indicate that elevation of CK may not be marked in the presymptomatic phase (Linssen *et al.*, 1997).

Following the identification of linkage to chromosome 2p13 in both LGMD2B and MM, some families were described in whom both phenotypes were present in the presence of the same haplotype (Weiler *et al.*, 1996 ; Ilarioshkin *et al.*, 1996, 1997). It has now been confirmed that the same mutation may result in a different presentation in different family members. The phenotype associated with dysferlin mutations may therefore be a "limb-girdle syndrome" or posterior distal myopathy — in addition, dysferlin mutations have been described in a distal myopathy with anterior compartment involvement (Liu *et al.*, 1998). Various unusual modes of presentation have been reported, such as a subacute, maybe painful swelling of the calf muscle (Argov *et al.*, in press). Biopsy findings in patients with either a proximal or distal onset may include a prominent inflammatory component, and this in conjunction with the massively elevated CK leads to a common area of misdiagnosis being polymyositis.

A review of the spectrum of mutations so far reported in dysferlin indicates that mutations are mainly single base substitutions, producing either missense or nonsense changes (Harrison *et al.*, submitted). There is no correlation so far apparent between mutation type or position and phenotype. No recurrent mutations have yet been reported. There is however a founder mutation present at an exceptionally high frequency in the Libyan Jewish population — the 1624 del G mutation is found in as many as 10% of the Libyan Jewish population studied. In the homozygous state, this mutation may be responsible for either a proximal or a distal presentation. A thorough clinical study of this population shows that the progression of the disease is slow and irrespective of the mode of onset (Argov *et al.* in press). This contrasts to the situation in the Canadian Aboriginal population where a Pro791Arg mutation can also be responsible for a variable phenotype, but where a faster progression is correlated with a proximal onset (Weiler *et al.*,

1999). Just as the type and position of mutation do not correlate with disease type, protein analysis also appears to be unable to distinguish between proximal and distal onset dysferlinopathy. Where samples have been available from families with the same mutation but a variable presentation, the protein profile in the muscles studied has been the same (Wiler *et al.*, 1999 ; Anderson *et al.*, 1999). However, as multiple muscle biopsies are rarely available from these patients, it has so far been impossible to address expression patterns of dysferlin in these differentially affected muscles. The cause for the variable phenotype in dysferlinopathy therefore remains unexplained. Possible explanations include the operation of modifier genes, possibly acting through a direct or indirect interaction with dysferlin.

A dysferlin mutation has been described in the SJL mouse, defining it as a naturally occurring model for human dysferlinopathy. The SJL mouse develops muscle weakness, (as detected by suspending the mice by their tails) from around three weeks of age. This weakness is accompanied by histological changes in muscle consistent with muscular dystrophy, also detectable from three weeks of age, though frankly dystrophic changes, accompanied by inflammatory foci are not fully developed until around seven months. The SJL mouse is a highly inbred strain, and the muscular dystrophy is inherited as an autosomal recessive trait mapping to mouse chromosome 6, in an area of synteny with human chromosome 2p13 (Bittner *et al.*, 1999). The SJL mice have a 171bp cDNA deletion in dysferlin, the result of a splice site mutation which removes exon 45 and most of the C2E domain, which is highly conserved between human and mouse (and indeed between the other members of the fer-l like family) (Vafiadaki *et al.*, submitted). The mouse dysferlin gene is > 90% homologous to human dysferlin, though its northern expression pattern is somewhat different, with apparently four transcripts produced. The 7kb transcript which is present in most human tissues is present only in mouse skeletal muscle and heart, while the 4kb transcript detected only in human brain is present in most mouse tissues. Additional transcripts of 2kb and 1.35kb are also present in most mouse tissues tested. By contrast, as in the human, antibody studies show only a single protein product close to the predicted size of 237kb, suggesting these additional transcripts are not translated.

The SJL mouse has been extensively used in the past as an experimental model for induced autoimmune diseases including experimental autoimmune encephalitis and inflammatory muscle disease. It is also widely used by a stock mouse for transgenic experiments. Clearly the interpretation of all such experiments needs to be treated with caution given the primary genetic muscle disease

now known to be present in these mice and in the future its main usefulness will be as an extra model for understanding dysferlinopathy.

Conclusions

Dysferlinopathy, despite its variable presentation (with regard to proximal versus distal muscle involvement) has some highly reproducible features phenotypically. These include late teenage or early adult onset, massive elevation of serum creatine kinase levels and usually relatively slow progression. Suspicion of the diagnosis can be confirmed by examination of dysferlin in a muscle biopsy section, followed by confirmatory mutational analysis.

BIBLIOGRAPHY

ACHANZAR W. E., WARD S. A nematode gene required for sperm vesicle fusion. *Cell Science*, 1997, **110** : 1073-1081.

ANDERSON L. V. B., DAVISON K., MOSS J. A. *et al*. Dysferlin is a plasma membrane protein and is expressed early in human development. *Hum. Mol. Gen.*, 1999, **8** : 855-861.

BASHIR R., BRITTON S., STRACHAN T. *et al*. A gene related to Caenorhabditis elegans spermatogeneisis factor fer-l is mutated in limb-girdle muscular dystrophy 2B. *Nature Genetics*, 1998, **20** : 37-42.

BASHIR R, STRACHAN T., KEERS S. *et al*. A gene for autosomal recessive limb-girdle muscular dystrophy maps to chromosome 2p. *Hum. Mol. Gen.*, 1994, **6** : 455-457 (1994).

BEJAOUI K, HIRABAYASHI K, HENTATI F. *et al*. Linkage of Miyoshi myopathy (distal autosomal recessive muscular dystrophy) locus to chromosome 2p12-14. *Neurology*, 1995, **45** : 768-772.

BELT DAVIS D., DELMONTE A. J., LY C. T. & MCNALLY E. M. Myoferlin, a candidate gene and potential modifier of muscular dystrophy. *Hum. Mol. Gen.*, 2000, **9** : 217-226.

BITTNER R. E., ANDERSON L. V. B., BURKHARDT E. *et al*. Dysferlin deletion in SJL mice (SJL-Dysf) defines a natural model for limb girdle muscular dystrophy 2B. *Nature Genetics*, 1999, **23** : 141-142.

ILLARIOSHKIN, S. N., IVANOVA-SMOLENSKAYA, I. A., TANAKA H. *et al*. Clinical and molecular analysis of a large family with three distinct phenotypes of progressive muscular dystrophy. *Brain*, 1996, **119** : 1895-1909.

ILLARIOSHKIN S. N., IVANOVA-SMOLENSKAYA I. A., TANAKA H., POLESHCHUK V. V., MARKOVA E. D., TSUJI S. Refined genetic location of the chromosome 2p-linked progressive muscular dystrophy gene. *Genomics*, 1997, **42** : 345-348.

LINSSEN W. H. J. P., NOTERMANS N. C., VAN DER GRAAF Y. *et al*. Miyoshi-type distal muscular dystrophy. Clinical spectrum in 24 Dutch patients. *Brain*, 1997, **120** : 1989-1996.

LIU J., AOKI M., ILLA I. *et al*. Dysferlin, a novel skeletal muscle gene is mutated in Miyoshi myopathy and limb girdle muscular dystrophy. *Nature Genetics*, 1998, **20**(1) : 31-36.

MAHJNEH I., PASSOS-BUENO M. R., ZATZ M. *et al*. The phenotype of chromosome 2p-linked limbgirdle muscular dystrophy. *Neuromusc. Disord.*, 1996, **6** : 483-490.

MATSUDA C., AOKI M., HAYASHI Y. K., HO M. F., ARAHATA K., BROWN R. H. Dysferlin is a surface membrane-associated protein that is absent in Miyoshi myopathy, *Neurology*, 1999, **53** : 1119-1122.

OZAWA E., NOGUCHI S., MIZUNO Y., HAGIWARA Y., YOSHIDA M. From dystrophinopathy to sarcoglycanopathy, evolution of a concept of muscular dystrophy. *Muscle & Nerve*, 1998, **21** : 421-438.

PASSOS-BUENO M. R., BASHIR R., MOREIRA E. S. *et al*. Confirmation of the 2p locus for the mild autosomal recessive limb-girdle muscular dystrophy gene (LGMD2B) in three families allows refinement of the candidate region. *Genomics*, 1995, **27** : 192-195.

PASSOS-BUENO M. R, VAINZOF M., MOREIRA E. S., ZATZ M. Seven autosomal recessive limb-girdle muscular dystrophies in the Brazilian population : from LGMD2A to LGMD2G. *Am. J. Med. Genet.*, 1999, **82** : 392-398.

RIZO J., SUDHOF T. C. C2-domains, structure and function of a universal Ca2+-binding domain. *J. Biol. Chem.*, 1999, **273** : 15879-15882.

WEILER T., BASHIR R., ANDERSON L. V. B. *et al*. Identical mutation in patients with limb-girdle muscular dystrophy typle 2B or Miyoshi myopathy suggests a role for modifier gene(s). *Hum. Mol. Gen.*, 1999, **8** : 871-877.

WEILER T., GREENBERG C. R., NYLEN E. *et al*. Limb-girdle muscular dystrophy and Miyoshi myopathy in an aboriginal Canadian kindred pam to LGMD2B and segregate with the same haplotype. *Am. J. Hum. Genet.*, 1996, **59** : 872-878.

YASUNAGA S., GRATI M., COHEN-SALMON M. *et al*. A mutation in OTOF, encoding otoferlin, a FER-1-like protein, causes DFNB9, a nonsyndromic form of deafness. *Nature Genetics*, 1999, **21** : 363-369.

K. M. D. BUSHBY,
Reader and Consultant in Clinical Genetics,
Department of Human Genetics,
19/20 Claremont Place,
Newcastle upon Tyne, NE2 4AA, UK.