Available online at www.sciencedirect.com

SciVerse ScienceDirect

Neuromuscular Disorders 23 (2013) 377–387

www.elsevier.com/locate/nmd

Review

Muscular dystrophy in dysferlin-deficient mouse models

Mark A. Hornsey [a], Steven H. Laval [a,*], Rita Barresi [b], Hanns Lochmüller [a], Kate Bushby [a]

[a] Newcastle University, Institute of Genetic Medicine, International Centre for Life, Central Parkway, Newcastle-upon-Tyne NE1 3BZ, United Kingdom
[b] NCG Diagnostic and Advisory Service for Rare Neuromuscular Diseases, Muscle Immunoanalysis Unit, Dental Hospital, Newcastle-upon-Tyne, United Kingdom

Received 5 November 2012; received in revised form 9 January 2013; accepted 5 February 2013

Abstract

Mutations in the dysferlin gene result in the development of a range of early adult-onset, progressive muscular dystrophies, collectively known as the dysferlinopathies. There is currently no effective treatment for these disorders. Several spontaneous and engineered alleles at the mouse dysferlin locus have been isolated and these dysferlin-deficient mouse strains are providing valuable insights into the role dysferlin plays in skeletal muscle physiology, heart function, and the regulation of the innate immune system. In addition, mouse models of dysferlinopathy are now widely used to test novel therapeutic strategies. Each dysferlin-deficient mouse strain has been characterised to varying degrees using a variety of histological and functional assays, occasionally producing results inconsistent with other strains. Here, we review each mouse model and physiological changes in various systems which accompany their muscle disease with emphasis on the how the disease process develops in different mouse models of dysferlinopathy. This review highlights the urgent requirement for standardised assays and outcome measures that will unify and coordinate research efforts throughout the field, procedures that are necessary if potential therapies are to be tested efficiently and effectively.

Keywords: Dysferlin; Muscular dystrophy; Mouse models

1. Review

Mutations in the dysferlin gene, *DYSF*, result in the development of a number of progressive muscular dystrophies known collectively as the dysferlinopathies [1–3], which generally present with a characteristic pattern of muscle involvement. Affected individuals often have a history of excellent athletic performance, before experiencing their first symptoms mainly around early adulthood. Patients have high serum creatine kinase (CK) levels, on average more than forty times the norm, and present with distinct patterns of weakness with predominantly proximal or distal onset, that defines the disorder as either, limb girdle muscular dystrophy type 2B (LGMD 2B), Miyoshi myopathy or distal myopathy with anterior tibial onset [1,4–7]. As the disease progresses, it increasingly involves both the proximal and distal musculature, and therefore these diagnostic categories are generally viewed as one disorder with a wide spectrum of clinical onset [8–12].

Sequence analysis of the dysferlin gene reveals no obvious mutational hotspots and no obvious genotype–phenotype correlation [13]. Affected individuals have a markedly reduced or complete loss of the dysferlin protein in skeletal muscle, which shows classic signs of progressive dystrophy [14,15]. Biopsies from dysferlinopathy patients have a higher percentage of immature fibres than other muscular dystrophy controls [16], together with a very prominent mononuclear cell infiltrate in muscle, and consequently, the disease in these patients is often misdiagnosed as polymyositis [11,17,18]. Disease progression is relatively slow when compared with other muscular dystrophies; nevertheless, patients are eventually confined to a wheelchair. There is currently no effective treatment.

Dysferlin is a member of the "ferlin" family as it shares homology with the *Caenorhabditis elegans* gene *fer-1* [1].

* Corresponding author.
E-mail address: Steven.Laval@newcastle.ac.uk (S.H. Laval).

0960-8966/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.nmd.2013.02.004

FER-1 is involved in calcium-dependent, vesicle fusion within the developing spermatid and mutation of the gene results in infertility [19]. Dysferlin has seven Ca^{2+}-binding motifs known as C2 domains that have significant homology to similar domains found in protein kinase C and the synaptotagmin family. C2 domains bind proteins and phospholipids in a Ca^{2+} dependent manner and the synaptotagmins are known regulators of vesicle fusion [20–26]. Several groups are now defining dysferlin's protein structure [27,28] and elucidating its phospholipid binding specificities [23,29]. Dysferlin is a type II transmembrane protein that localises to the periphery of muscle fibres [30]. A large body of evidence suggests that dysferlin is an important regulator of vesicle fusion at the sarcolemma, and has an essential role in muscle membrane repair [31–36]. However, dysferlin functions clearly extend beyond sarcolemma repair with possible roles in vesicle trafficking, endocytosis, membrane receptor recycling, membrane turnover [37,38], muscle regeneration and T-tubule formation [16,39], focal adhesion [40] and ATP-dependent intercellular signalling [41]. Dysferlin is also expressed in cells of the innate immune system, and there is evidence that it modulates macrophage function [42], regulates cytokine release [16] and controls complement activation [43–45]. There is also significant expression found within endothelial cells, syncytiotrophoblasts of the human placenta and podocytes of the kidney [46–50].

Studying animal models has provided tremendous insight into the pathological process behind many disorders, including muscular dystrophy, while providing a necessary platform for the testing of new therapies. Mice are highly complex organisms with a muscle structure very similar to humans. The human and mouse dysferlin genes share >90% amino acid sequence homology [51], and there are a number of dysferlin-deficient mice (naturally occurring and constructed) that are being used as models of dysferlinopathy [52]. Here we review the data on these models, the advantages and limitations of each for therapeutic testing, and their suitability in view of future research directions.

2. Spontaneous mutation – A/J mice

One of the most widely used mouse models of dysferlinopathy was discovered at the Jackson Laboratory. Mice on the A/J inbred background develop a progressive muscular dystrophy, the result of an ETn retrotransposon insertion within intron 4 of the dysferlin gene (Table 1). Histological dystrophic features appear at 4–5 months of age, with the proximal muscles more affected than distal ones. Evidence of compromised sarcolemma integrity was demonstrated by elevated serum CK, increased Evan's blue dye (EBD) uptake and ultra-structural abnormalities at the sarcolemma. Disease progression is slow, except in the abdominal muscles, which show a more rapid rate of muscle wasting [53]. There is one report of histological alterations at P1 in the hind limb muscle of A/J mice backcrossed onto the 129SvJ background. These alterations were "less evident" at 8 weeks of age, suggesting a possible delay in maturation [37].

3. Spontaneous mutation – SJL/J mice

The SJL/J mouse is another naturally occurring model of dysferlinopathy, and also develops a mild, progressive myopathy [14]. A splice-site mutation in the dysferlin gene results in a 171 bp in-frame RNA deletion that removes 57 amino acids and most of the C2–E domain [51]. Dysferlin expression is reduced to 15% of wild type levels and serum CK levels are elevated (Table 1). A progressive dystrophy develops with minimal involvement at 2 and 4 months (small numbers of centrally nucleated myofibres), more common necrotic fibres at 6 months, followed by progressively advanced dystrophic features, including fat deposition, appearing at 10 months [53–57]. Most degenerative fibres in the muscles of SJL/J mice were of the fast-twitch type [56], while selective loss of fast-twitch/type 2 fibres has been observed in patients [58]. SJL/J muscle has a prominent macrophage and $CD4^+$ infiltrate that is also noted in patient biopsies. Increasing cell infiltrates in both SJL/J and A/J mice were observed from 2 months to one year of age and consisted of approximately 60% macrophages ($Mac\text{-}1^+$ and/or $Mac\text{-}3^+$) and 30% T cells; $CD4^+$ cells more abundant than $CD8^+$ [54,56].

A study directly comparing the dystrophy in muscles of A/J and SJL/J confirmed the earlier onset in SJL/J mice compared with A/J and a more rapid rate of progression [56]. Only the diaphragm is spared with mild lesions appearing in the SJL/J mouse and none in the A/J. However, force measurement studies by a second group showed 50% functional deterioration of the A/J diaphragm at 10 and 36 weeks of age. Histology at 10 weeks appeared normal with pathological alterations only apparent in the older animals [59].

4. A/J and SJL/J mice on defined genetic backgrounds

A/J and SJL/J mice possess a number of features not typically observed in patients. A/J mice are poor breeders that exhibit a progressive loss of hearing, a high incidence of lung adenomas and are deficient in complement C5. SJL/J mice have a susceptibility to autoimmune diseases and a high incidence of reticulum cell sarcomas (see the JAX® database for comprehensive lists). Aggressive behaviour has been reported in SJL/J mice [53], however there is no evidence of similar traits in other dysferlin-deficient mice. Genetic background can have a profound effect on the observed phenotype of any given gene-targeted allele [60], and the susceptibilities noted above are possibly the result of unknown genetic modulators and not the dysferlin-deficiency itself. To help