Investigating the pathology of Emery–Dreifuss muscular dystrophy

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Abstract

EDMD (Emery–Dreifuss muscular dystrophy) is caused by mutations in either the gene encoding for lamin A/C (LMNA) located at 1q21.2-q21.3 or emerin (EMD) located at Xq28. Autosomal dominant EDMD caused by LMNA mutations is more common than the X-linked form and often more severe, with an earlier onset. At the histological and histochemical levels, both X-linked and autosomal dominant EDMD appear similar. However, individuals with the same genetic disorder often show remarkable differences in clinical severity, a finding generally attributed to the genetic background. The clinical and pathological findings in EDMD patients found to have mutations in more than one gene are also discussed. There is now much interest in the phenotype of several animal models for EDMD which should lead to an increased insight into the pathogenesis of this disorder, particularly that relating to the heart phenotype.

Introduction

EDMD (Emery–Dreifuss muscular dystrophy) is caused by mutations in either the gene encoding for lamin A/C (LMNA) located at 1q21.2-q21.3 or emerin (EMD) located at Xq28. To date, mutations in the LMNA gene have been associated with at least ten different human diseases including AD-EDMD (autosomal dominant EDMD) [1], dilated cardiomyopathy with conduction system defects and LGMD1B (limb-girdle muscular dystrophy 1B) with atrioventricular conduction disturbances [2], whereas mutations in emerin cause X-EDMD (X-linked EDMD) [3]. It has been shown recently that mutations in the genes encoding nesprins 1 and 2 can also cause EDMD [4].

AD-EDMD is more common than X-EDMD and is often more severe, with earlier onset, even in very early childhood. Both forms of EDMD are similar in that they each present with muscle weakness and early contractures of the elbow, the Achilles tendons and the spinal extensor muscles. The pattern of weakness is humero-peroneal in X-EDMD and scapulo-humero-peroneal in AD-EDMD. Wasting of the upper arms and lower legs is generally a characteristic of both forms. These features precede the appearance of what ultimately are the most deleterious aspects of the disease, namely the cardiac conduction defects that can lead to complete heart block. Most patients develop conduction defects before the third decade of life, with abnormalities being generally more severe in AD-EDMD.

The lamins belong to the intermediate filament protein family and characteristically possess a central α-helical coiled-coil rod domain, flanked by non-helical N-terminal ‘head’ and C-terminal ‘tail’ domains [5]. Nuclear lamins form homo- and hetero-polymers and are also able to associate with other nuclear membrane proteins, in so doing forming a network that underlies the nuclear membrane (for reviews, see [6,7]). In mammalian cells lamins are divided into A and B (B1 and B2) types. A-type lamins are expressed in terminally differentiated cells, whereas B-types are constitutively expressed in most tissues.

Lamins A (72 kDa) and C (67 kDa) are derived from the alternative splicing of the LMNA gene and share a common N-terminal domain, but have unique C-termini. Experimental evidence suggests that the incorporation of lamin A is dependent upon lamin B [8]. The incorporation of lamin C is facilitated by lamin A, but the presence of lamin A appears not to be essential, at least in mice, since genetically engineered mice that express only lamin C (and not A) are essentially normal [9]. Most of the so-called laminopathies are caused by missense mutations in lamin A/C spread throughout the entire length of the gene, the only exception being those associated with lipodystrophy which are clustered in exon 8. AD-EDMD is characterized by marked clinical variability, even between patients with the same mutation.

Emerin is a 34 kDa protein that has a hydrophobic C-terminus anchored in the nuclear membrane and an N-terminal tail projecting into the nucleoplasm [10]. Mutations associated with X-EDMD have been found throughout the gene, with no particular ‘hotspots’. A list of identified mutations can be found at the Emery–Dreifuss website (http://www.dmd.nl/nmdb/home.php?select_db=EMD). The majority of these result in an absence of immunostaining of emerin at the nuclear envelope which is particularly useful from a diagnostic viewpoint. Female carriers do not usually...
Mechanisms of disease

The lamins have been attributed to a wide range of functions that include making the nuclear membrane stiffer and more resilient to mechanical stress relative to the plasma membrane [11]. It is now recognized that an increasing number of these functions probably depend on the formation of complexes with other proteins such as LEM [LAP2 (lamina-associated polypeptide 2), emerin and Man1]-domain proteins (including emerin), nesprins and the SUN [Sad1 and UNC (uncoordinated)]-domain proteins. Indeed, many of these proteins depend on lamin A for their correct organization [12]. Emerin, which has been shown to bind to lamin A, may fulfill several roles through its interactions with barrier-to-autointegration factor, transcriptional repressors, an mRNA splicing regulator, nesrin, nuclear myosin I and F-actin (filamentous actin) [13]. Although emerin-deficient fibroblasts have less profound deficiencies in strain-induced gene regulation than those deficient in lamin A [6], the similarity of heart involvement in both AD-EDMD and X-EDMD suggests that these proteins have at least one functionally important pathway in common.

The pathogenetic mechanisms underlying the variability in the clinical phenotypes of EDMD are unclear, although one recent study which included patients with frameshift mutations suggested that late-onset phenotypes may arise through loss-of-function secondary to haploinsufficiency, whereas dominant-negative or toxic gain-of-function may explain the increased severity seen in those phenotypes with an early onset [14]. However, we have reported four clinically variable cases with mutations in the same codon of LMNA exon 11: one patient had congenital weakness and died in early childhood, two others displayed severe cardiac problems early (in one of these, cardiac signs preceded the onset of skeletal muscle weakness by many years), and the fourth had a mild and late-onset LGMD1B phenotype [15]. Overall, these findings suggest that the pathogenesis of these disorders may be strongly influenced by genetic modifiers.

Pathology of EDMD

At the histological and histochemical levels, both X-EDMD and AD-EDMD appear similar, with the degree of change relating to the involvement of the muscle that has been biopsied. Generally, quadriceps biopsies show little abnormal variation in fibre size (a common feature of pathological muscle), with only the occasional atrophic or hypertrophic fibre. Internal nuclei, which in rodent muscle is considered to be a marker of regeneration, may be present, although in this case, it remains to be shown whether this reflects previous cycles of regeneration. Necrotic fibres and an increase in either adipose or connective tissue are only very rarely observed. Small basophilic fibres that may be slightly granular in appearance and show aggregation of NADH-TR (NADH-tetrazolium reductase) stain may also be evident. These fibres may be regenerating fibres as they label positively for neonatal myosin and are strongly labelled by antibodies directed against desmin, another feature of regenerating fibres.

A two-fibre-type pattern is usually maintained with oxidative enzyme stains and ATPase, with a tendency for the type 1 fibres to be smaller, but not usually to the degree seen in some forms of congenital myopathy. A predominance of type 1 fibres may also occur. Structural changes such as cores can also occur. There are usually only a few fibres present that express neonatal myosin, suggesting that there is little ongoing muscle fibre regeneration. It is not clear whether this is a reflection of the lack of necrosis, which precedes regeneration, or whether there is some kind of malfunction in the regenerative process that could account for the muscle wasting. Indeed, recent work does suggest that lamin A/C and emerin are critical for skeletal muscle satellite cell differentiation [16]. Electron microscopy indicates that apoptosis may also be a contributing factor, as many nuclei in EDMD biopsies show an aggregation of chromatin and lack of attachment of chromatin to the nuclear membrane [17].

Immunohistochemical studies of emerin clearly show its absence from all nuclei in affected male patients with X-EDMD. In addition to muscle, the absence can also be demonstrated in skin, buccal cells and lymphocytes [18–20], and skin biopsy samples from carriers of the X-EDMD can be used to demonstrate that a proportion of nuclei are negative for emerin. In both X-EDMD and AD-EDMD, the immunolabelling of lamins in muscle biopsies shows no detectable difference from normal controls. As in all dominant conditions, only one allele is mutated and the normal allele produces a normal product. Emerin immunolabelling also appears normal with underlying LMNA mutations. No immunohistochemical differences in nuclear membrane proteins between internal and subsarcolemmal nuclei have as yet been identified.

Immunolabelling of proteins associated with the sarcolemma is normal in both forms of EDMD with the exception of laminin β1 [17]. A selective reduction of laminin β1 labelling at the sarcolemma, but not the blood vessels or capillary network, may be evident in a proportion of cases. In contrast, the intensity of laminin α2 and laminin γ1 labelling is normal. The underlying reason for this remains unclear, but it should be remembered that this reduction is not specific for EDMD and may also be seen in cases of Bethlem myopathy, in patients with mutations in the FKRP (fukutin-related protein) and other neuromuscular disorders. Interestingly, it is an age-related phenomenon and has only been observed in adult and adolescent cases.

EDMD caused by mutations in more than one gene

Individuals with the same genetic disorder often show remarkable differences in clinical severity, a finding generally attributed to the genetic background. We identified two
patients with genetically proven EDMD, who followed an unusual disease course. The first patient was a member of a family with molecularly proven X-EDMD. However, the clinical features were unusually severe for this condition in that he presented at 2.5 years with severe proximal weakness and markedly elevated serum creatine kinase activity. The muscle biopsy resembled that of a severe limb-girdle type of muscular dystrophy with a wide variation in fibre size, a pronounced increase in connective tissue and necrosis [21]. Muscle weakness progressed rapidly, leading to loss of independent ambulation by the age of 12. In addition, the patient developed cardiac conduction system disease requiring pacing at the age of 11 and severe dilated cardiomyopathy in the early teens. Despite pacing, he had several syncopal episodes attributed to ventricular dysrhythmias. As the cardiac phenotype resembled that of patients with AD-EDMD, the lamin A/C gene was investigated which resulted in the identification of a de novo mutation. This case illustrates the importance of considering the possibility of a causative mutation in more than one gene, particularly in genes in which mutations are common, such as LMNA.

Another example was a patient with a cardioskeletal myopathy, similar to his mother who had died more than 20 years previously. A laminopathy was suspected, and a mutation in exon 11 of the LMNA gene was identified, although this mutation was not present in his mother, but was instead identified in his virtually asymptomatic father. Unusual accumulations of desmin observed in the cardiac muscle biopsy of the propositus prompted us to examine the desmin gene and we identified a desmin mutation, in addition to the LMNA mutation in the propositus. Linkages between the nuclear envelope and cytoskeleton of the cell are thought to exist, and it has been suggested that the disruption of these contributes to the EDMD phenotype [4]. Ultrastructural studies of the cardiomyocytes of lamin-null mice also show some disruption of desmin at the nuclear envelope, although a precise role for desmin in the pathogenesis is not supported by our recent work [22]. Overall, these patient studies suggest that separate mutations in related proteins that are believed to interact, or that represent different parts of a presumed functional pathway, may synergistically contribute to disease severity in AD-EDMD.

Patient biopsies provide a ‘snapshot’ of the disease process, but further investigations into the pathogenesis of this disorder, particularly the heart phenotype, will probably utilize several of the recently generated genetically engineered mouse strains. At least two mutant lines appear to be particularly promising in this regard. The first of these carries a missense H222P lamin A mutation originally identified in a family with a typical AD-EDMD [23]. Mice homozygous for this mutation display reduced locomotor activity with abnormal stiff walking posture. Their lifespan does not go beyond 9 months of age, and they develop cardiac chamber dilation and hypokinesia with conduction defects, together with skeletal muscle degeneration and fibrosis. The second line carries an N195K lamin A mutation that results in early death due to arrhythmia attributed by the authors to a disruption of cardiomyocyte internal organization and/or the expression of transcription factors essential to normal cardiac development, aging or function [24]. The most deleterious aspect of EDMD is the heart phenotype, and these mice should provide crucial insight into the processes that translate a disruption in the cardiomyocyte nuclear envelope to the observed pattern of disease progression.

References


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