RNA pathogenesis of the myotonic dystrophies

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Abstract

Myotonic dystrophy (dystrophia myotonica, DM) is the most common form of muscular dystrophy in adults. The presence of two genetic forms of this complex multisystemic disease (DM1 and DM2) was unrecognized until the genetic cause of DM1 was identified in 1992. The fact that the DM1 mutation is an untranslated CTG expansion led to extended controversy about the molecular pathophysiology of this disease. When the DM2 mutation was identified in 2001 as being a similarly untranslated CCTG expansion, the molecular and clinical parallels between DM1 and DM2 substantiated the role of a novel mechanism in generating the unusual constellation of clinical features seen in these diseases: the repeat expansions expressed at the RNA level alter RNA processing, at least in part by interfering with alternative splicing of other genes. For example, in both DM1 and DM2, altered splicing of chloride channel and insulin receptor transcripts leads to myotonia and insulin resistance, respectively. Although other mechanisms may underlie the differences between DM1 and DM2, the pathogenic effects of the RNA mechanism are now clear, which will facilitate development of appropriate treatments.

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1. Introduction—unstable repeat disorders

A recurring genetic feature of neurodegenerative disorders has been the presence of unstable repeats. Sometimes referred to as microsatellite repeats, or short-tandem repeats, these trinucleotide, tetranucleotide (or longer) elements are repeated a variable number of times, and occur throughout the normal human genome. Each repeat is typically polymorphic in the general population. Repeats can become unstable during DNA replication, resulting in mitotic or meiotic expansions or contractions that often correspond to the tendency of DNA in specific repeats to form unusual secondary structures, such as hairpins

Depending on the specific repeat motif, and its genetic location, expanded repeats can sometimes become pathogenic.

In disease states, the number of repeats exceeds the normal range, sometimes dramatically, leading to various possible pathogenic mechanisms. Pathogenic repeat mutations were initially identified in 1991 in fragile X mental retardation (FMR) \cite{2} and spinobulbar muscular atrophy (SBMA, or Kennedy’s disease) \cite{3}. In FMR, a non-coding CGG repeat expansion at the 5' end of the \textit{FMR1} gene reduces FMR1 protein production. SBMA, alternatively, is caused by a CAG repeat expansion that encodes an abnormally long polyglutamine region within the androgen receptor. Thus, the first two identified repeat expansion diseases revealed two different pathogenic mechanisms—loss of gene product in FMR, and a pathogenic gain-of-function in the polyglutamine disorder, SBMA. Discovery of related pathogenic repeat expansion disorders followed, such as the loss of function produced by a GAA repeat
expansion in recessively inherited Friedreich’s Ataxia [4], and other polyglutamine expansions in dominantly inherited degenerative disorders including Huntington’s disease and spinocerebellar ataxia types 1, 2 and 3 [5–7]. Since initially all identified pathogenic expansions were trinucleotide expansions, the disorders were often referred to as trinucleotide repeat disorders. However, subsequently pathogenic tetranucleotide [8] and pentanucleotide [9] repeat expansions were identified, so that this category of diseases is now more accurately and generically referred to as the unstable repeat expansion disorders.

To date, three repeat expansion disorders of muscle have been identified: myotonic dystrophy type 1 (DM1), myotonic dystrophy type 2 (DM2), and oculopharyngeal muscular dystrophy (OPMD). Facioscapulohumeral dystrophy (FSHD) also involves repetitive genetic elements, but they are complex repeats that are deleted rather than expanded, implying a different pathogenic process. The short GAG repeat expansion in OPMD results in a slight increase in polyalanine residues that confers a toxic effect similar to polyglutamine expansions on the gene product, a polyadenosine-binding protein [10,11]. The repeat expansions in the two forms of myotonic dystrophy did not fit into either of the previously identified pathogenic mechanisms because they are dominantly inherited disorders in which the repeat expansion does not encode any portion of a protein. A review of the clinical and genetic features of the myotonic dystrophies helps to reveal what has now been recognized as a novel disease mechanism involving a pathogenic effect of RNA.

2. Clinical aspects of the myotonic dystrophies

2.1. History of DM

Almost 100 years ago Steinert [12], and Batten and Gibb [13] independently described the disorder now known as myotonic dystrophy, distinguishing it from Thomsen’s previously identified non-dystrophic myotonias [14]. The initial description of the disease focused on skeletal muscle, with myotonia similar to Thomsen’s disease, but weakness and atrophy that distinguished the new disorder. Further clinical evaluation revealed the disease to have a complex multisystemic phenotype, with involvement of eyes, heart, endocrine systems and central nervous system that clearly distinguished this disorder from the non-dystrophic myotonias, but also from the non-myotonic muscular dystrophies [15]. Genetic heterogeneity in myotonic dystrophy was unsuspected until genetic testing was developed in 1992 for the chromosome 19 mutation that causes DM1 [16,17]. Shortly after genetic testing became available, a second multisystemic myotonic disorder was identified [18,19], and referred to as either proximal myotonic myopathy (PROMM) [18,20], proximal myotonic dystrophy (PDM) [21], or myotonic dystrophy type 2 (DM2) [22,23] in attempt to emphasize differences or similarities with the Chromosome 19 form of the disease. The substantial clinical overlap of these diseases led to the adoption of a revised nomenclature [24], with DM1 referring to the Chromosome 19 form of the disease, and DM2 referring to the new form of the disease. The clinical features of the two diseases are shown in Table 1, comparing the findings in 234 genetically confirmed cases of DM2 to the classically defined DM1 phenotype.

2.2. Time course of DM

Individual patients with DM1 are often identified as having congenital, juvenile or adult-onset disease based on age of symptom onset [15]. However, due to ascertainment biases (e.g. family and patient awareness of deficits, and medical provider awareness of myotonic dystrophy), affected individuals of all ages have a wide range of static developmental abnormalities and superimposed degenerative features at the time they come to medical attention, which complicates the description of a typical phenotype based solely on age of symptom onset.

2.2.1. Neonatal onset

Patients with congenital DM1, as described by Harper [25], demonstrate an almost pure form of the developmental
deficits associated with DM. The pregnancies of these infants are complicated by hyperhydramnios, and the children are born with severe neurological, neuromuscular and musculoskeletal abnormalities. They have craniofacial abnormalities, including tapered chin, high-arched palate and prominent brow, and may be born with arthrogryposis and talipes in addition to other orthopedic abnormalities [15]. Central nervous system abnormalities common in these individuals most notably include mental retardation and global cerebral atrophy. Myotonia is absent from affected infants, and some common degenerative features, including cataracts and central hypersomnia, occur during adulthood in these patients rather than in infancy or childhood. At present, no definite congenital form of DM2 has been reported [26].

2.2.2. Juvenile onset
Cases of DM1 that come to medical attention during childhood typically manifest developmental abnormalities that are less severe than seen in congenital onset cases [27], though this is in part influenced by the ascertainment biases mentioned previously. These patients have cognitive deficits and learning abnormalities [28] that again distinguish them from DM2, in which such a juvenile form of the disease has not been distinguished. As in the congenital cases, degenerative features often develop as these children affected by DM1 reach adulthood.

2.2.3. Adult-onset
DM1 patients come to medical attention during adulthood for several reasons: because they have congenitally affected children, because other affected family members are identified, or because they develop symptoms of the degenerative disease (e.g. weakness, myotonia, cataracts, or gonadal failure). On examination, although some adult onset patients have no recognizable developmental defects, others manifest clear indicators of developmental abnormalities, including the craniofacial changes and a highly arched palate that belie the in utero effects of their disease. The degenerative features of the disease occur in all adult onset cases over time, but can progress at different rates, with some middle-aged individuals succumbing to the disease over several years and others having a relatively static course for decades [29,30]. DM2 typically comes to medical attention in adulthood, though myotonia, myalgias and cataracts can occur within the first two decades of life.

Individuals with DM2 manifest only the degenerative features of myotonic dystrophy. In DM2, as in DM1, the disease can follow faster or slower courses even within one family: in a family we have studied, fraternal twins showed markedly different disease courses, with one dying secondary to diffuse weakness and cardiac involvement when he was 53 years old although his brother was very active and vigorous with no symptomatic weakness, and only mild weakness of neck and finger flexors on exam at 65 years of age. Though there may well be disease-specific differences between the degenerative features of DM1 and DM2, these will only become clear by comparing DM2 patients with DM1 individuals who do not manifest any significant developmental components of the disease.

2.3. DM effects on skeletal muscle

The skeletal muscle features of myotonic dystrophy in both genetic forms include progressive weakness, dystrophic changes on biopsy, and myotonia. Muscle pain can be a significant feature of both disorders [15,26].

2.3.1. DM1 patterns of weakness

The pattern of muscle weakness in DM1 classically involves the facial muscles (ptosis without ophthalmoplegia, weakness of eye and mouth closure), muscles of mastication (temporalis), neck flexors and distal upper extremity muscles (specifically deep flexors of the thumbs and deep flexors of the lateral fingers more than medial fingers) [31]. As the disease progresses, involvement of the triceps and ankle dorsiflexors typically, though not invariably, precede involvement of shoulder and hip girdle musculature. In later stages of the disease, diffuse profound weakness can develop, involving bulbar, ventilatory, and pelvic floor, as well as hip and shoulder girdle muscles [15]. In addition to the weakness, DM1 is associated with loss of bulk in the involved muscles, with volar forearm atrophy being an early and frequent component of the disease.

2.3.2. DM2 patterns of weakness

Patients with DM2 who come to medical attention because of weakness typically present with problems associated with hip girdle muscles, for example, climbing stairs or arising from the floor [32]. However, on manual muscle testing the initial sites of weakness are often similar to DM1, involving neck flexors and deep flexors of the thumb and lateral digits [23,26]. This distinction led to differences in the initial descriptions of the disease; although symptomatic patients ascertained individually in a neuromuscular clinic consistently noted hip girdle weakness (and were described as having PROMM [18,20]), those identified through genetic studies of oligosymptomatic or asymptomatic family members had detectable isolated neck flexor and finger flexor weakness on manual motor testing (and were described as having DM2 [22,23]). Some families in whom the proximal muscle involvement was more clearly dystrophic were initially thought to have a variant condition, and were identified as having proximal myotonic dystrophy (PDM). As is discussed below, each of these phenotypes, PROMM, PDM and DM2 are now known to be caused by the same DM2 mutation. As DM2 progresses, all proximal and distal limb muscles may be affected. Although dyspnea and dysphagia have been reported by individual patients, the significance of ventilatory and bulbar muscle involvement in DM2 has not been determined.
2.3.3. Myotonia

Though myotonia is more symptomatic in DM1 than DM2, neither disease is associated with the severe myotonia sometimes seen in chloride channelopathies [15,26]; treatment of myotonia is not usually beneficial in either disease. Grip and percussion myotonia are more evident in DM1, though are present in 75% of 234 DM2 subjects we studied ranging in age from 21 to 78 years. Electrical myotonia is seen in almost all adults with both forms of DM (90% of 234 individuals with DM2, though detection of myotonia is almost 100% if at least 5 muscles are thoroughly investigated [26]). Clinical and electrical myotonia are absent in infants with congenital onset DM1, and have not been thoroughly studied in children with either disease. In both diseases myotonia is not the only abnormal spontaneous activity seen on EMG, with fibrillation potentials being commonplace and often predominant, which can sometimes lead to clinical misdiagnoses of inflammatory myopathy or denervation. Although the possibility of denervation in DM has been postulated to explain fibrillations and histological features, no definite evidence of denervation or motor neuronopathy has ever been established, indicating that these features are likely secondary to direct effects on skeletal muscle. EMG recordings of small, polyphasic short-duration motor unit potentials indicative of myopathy are seen in both forms of DM but are more common in DM1.

2.3.4. Muscle histology

The characteristic histological features of muscle in DM1 and DM2 (Fig. 1) are very similar, and sufficiently stereotypic that a diagnosis of DM can be suggested based on muscle biopsy alone [15,26,33]. In both diseases, affected muscles show a markedly increased variation in fiber diameter that commonly ranges from less than 10 μ to greater than 100 μ. The severely atrophic fibers have pyknotic nuclei with minimal remaining contractile elements, and are similar in appearance to the severely atrophic fibers in neurogenic atrophy. Muscle from patients with both diseases can show ring fibers and central nuclear chains. ATPase staining in DM1 has classically shown atrophy of type 1 fibers, which is not typically seen in DM2; myosin immunostaining in DM2 has shown that the nuclear bag fibers stain positively for fast myosin, indicating that they are principally derived from type 2 fibers [33,34]. Basophilic regenerating fibers, splitting fibers, fibrosis and adipose deposition occur in both diseases to a variable degree depending on the extent of muscle involvement. Sarcoplasmic masses, an infrequent feature of DM1, have not been reported in DM2.

2.4. DM effects on the heart

The most common cardiac features of DM1 include atrioventricular and intraventricular conduction abnormalities, atrial fibrillation and ventricular arrhythmias [35]. The cardiac effects can lead to potentially lethal arrhythmias that are not always predicted by presence of conduction defects [36]. Sudden death is not common in DM1, but does occur, necessitating use of pacemakers and implantable defibrillators in some patients. DM2 has very similar effects on the heart, with recognized conduction defects, atrial fibrillation, ventricular arrhythmias and sudden death [26], with sudden death occasionally
occurring in young subjects without symptomatic skeletal muscle involvement [37]. The prevalence of these abnormalities in DM2 is still being investigated, but all have been reported to occur (Table 1). The occurrence of cardiomyopathy in DM1 has been ascribed to secondary effects of arrhythmia and ventilatory insufficiency, but the occurrence of cardiomyopathy in DM2 patients with no ventilatory insufficiency has re-opened the question of possible direct myopathic effects of DM1 and DM2 mutations on the myocardium.

2.5. Multisystemic effects of DM

DM1 and DM2 have similar but not identical effects on non-muscle systems. The cataracts in these two diseases are unusual and indistinguishable, in that they are iridescent, multi-colored posterior subcapsular opacities on slit lamp examination [15,26]. In both forms of DM the cataracts occur at similar ages, with onset in the second decade or later in patients with either congenital or adult onset forms of DM1. Similarly, cataracts in DM2 are identifiable by slit lamp examination in the second decade of life, and can require surgical correction as early as the middle of the third decade [26].

Central nervous system involvement represents one of the principle differences between DM1 and DM2, though it remains unclear whether this indicates a specific pathophysiological distinction or is simply a manifestation of the other primary difference between the two diseases—the lack of developmental abnormalities in DM2. Mental retardation, a recognized feature of congenital and juvenile onset DM1 [15], has not been definitively and causally associated with DM2 (although retarded DM2 individuals have been reported, these occurrences may be either incidental or an infrequent consequence of the disease [23]). In addition to mental retardation, DM1 subjects develop central nervous system white matter abnormalities that are also seen in DM2 individuals, suggesting a commonality of this degenerative feature in both diseases [38]. Cognitive and cerebral blood flow abnormalities have also been reported in DM2 [39]. Although neither form of DM has been associated with clinically evident dementia, longitudinal studies are notably missing in both diseases that would clarify the possible neuropsychological consequences of the observed white matter changes. Central hypersomnia, another recognized CNS effect of DM1, appears to be a degenerative disorder, occurring in adulthood [40–43]; hypersomnia has not yet been definitively studied in DM2 though increased daytime sleepiness has been reported by DM2 patients [23]. Lastly, episodic hyperhydrosis has been reported in both DM1 and DM2, though whether this has a peripheral or central etiology remains unclear for either disease [23,44].

Various other features are common to both diseases, including testicular failure (both hypotestosteronism and oligospermia), hypogammaglobulinemia (serum levels of both IgG and IgM are reduced), and insulin resistance [15,26,45,46]. Gastrointestinal involvement in DM1 is common, sometimes leading to profound intestinal hypokinesis and pseudo-obstruction; although DM2 patients report gastrointestinal symptoms, it remains to be determined whether these are disease-specific effects. This constellation of unusual features can allow the clinical identification of patients and families phenotypically prior to the availability of genetic testing. Identification of families with myotonia, muscular dystrophy, iridescent cataracts, cardiac conduction abnormalities, insulin resistance, testicular failure and hypogammaglobulinemia will be useful in identifying additional novel forms of myotonic dystrophy.

Hypoventilation and difficulty protecting the airway are recognized post-anesthetic complication of DM1, but have not been seen in DM2, in which ventilatory and bulbar musculature is relatively spared. Another recognized feature of DM1, pilomatrixoma, has not been reported in DM2, although a second dermatologic feature, frontal balding, is a recognized feature of both disorders.

3. Genetics of the myotonic dystrophies

3.1. Identification of DM1 mutation

Prior to identification of the DM1 mutation clinicians had already identified non-Mendelian features in DM1 inheritance, including anticipation (a tendency for the disease to worsen in subsequent generations) and a maternal transmission bias for congenital forms [15,47]. The genetic cause was identified in 1992 as a (CTG)n repeat in the 3’-untranslated region of the dystrophia myotonica protein kinase gene DMPK [16,17,48–50], making DM1 the first dominantly inherited disease found to be caused by an untranslated repeat expansion; the mutation is transcribed into RNA but not translated into protein. In 1995, the DM1 mutation was also found to be in the promoter region of the immediately adjacent homeodomain gene SIX5 [51]. The CTG expansion in DM1 can vary from 80 to more than 4000 repeats in affected individuals, with clinically unaffected individuals having repeats of 50–100 CTGs. Somatic instability has been reported in different tissues, and in individuals over time, with repeat sizes increasing ~50–80 repeats per year [52,53]. Intergenerational instability can be dramatic, with expansions of several thousand repeats occurring in a single generation, most commonly secondary to maternal transmission. There is a rough correlation between DM1 repeat size and age of onset for CTGs <400 repeats [54], but poor correlation between repeat length and disease severity for longer repeats.
3.2. Proposed mechanisms of DM1 pathogenesis

3.2.1. Haploinsufficiency of DMPK

How the multisystemic features of DM1 could be caused by a non-coding repeat has been enigmatic, because most dominant disorders are caused by the altered function of a mutant protein product [55]. Early expression studies were consistent with the hypothesis that the mutation interfered with DMPK production, in that mRNA and protein levels were reduced in patient muscle and cell culture [56–58]. However, DMPK knockout mice showed only a very mild, late-onset myopathy without the multisystemic features of the disease [59,60], though subsequent reports suggested cardiac conduction abnormalities also occur in these mice [61]. The fact that no DMPK point mutations have been associated with a DM phenotype further suggests that the multisystemic features of DM1 are not simply caused by DMPK haploinsufficiency.

3.2.2. Haploinsufficiency of SIX5 and neighboring genes

A second proposed mechanism has been that the mutation interferes with expression of multiple genes in the DM1 region, possibly through regional effects produced by repeat-induced alterations in chromatin structure [62,63]. In addition to DMPK and the neighboring homeodomain gene SIX5, other regional genes suggested to be involved in DM1 pathogenesis have included myotonic dystrophy gene with WD repeats, DMWD [64], which is prominently expressed in the testis and brain [65], and the gene encoding the Immunoglobulin G Fc Fragment Receptor and Transporter FCGRT, located 4 Mb from the CTG expansion [66]. In this model, the multisystemic features of DM1 would be explained by haploinsufficiency of a number of neighboring genes, with expression level and hence disease severity, dependent on repeat length. In support of this possibility, SIX5 knockout mice develop cataracts [67,68], but without the posterior subcapsular location or the distinctive iridescent opacities that are characteristic of cataracts in DM patients.

3.2.3. RNA pathogenesis

A third hypothesized mechanism is that the enlarged CUG-containing transcripts accumulate as intranuclear foci and disrupt cellular function [45,69–74]. Direct support for this model came from a transgenic mouse model [75], in which the CTG expansion was inserted into the 3' end of the human skeletal actin gene, a gene not directly involved in DM1 but which is expressed only in skeletal muscle. This mouse model expressed an mRNA with a CUG repeat tract of ~250 repeats, and caused the myotonia and myopathic features characteristic of DM1, but because CUG-containing transgene expression was limited to skeletal muscle the role of the CUG expansion in the multisystemic features of DM was not addressed.

3.2.4. Additive model of DM1 pathogenesis

Subsequently, an additive model was proposed in which each of the above mechanisms contributes to DM1 pathogenesis [55,76–78], with some aspects of the disease caused by haploinsufficiency of DMPK, SIX5 and other neighboring genes, and other clinical features resulting from effects of the CUG expansion in RNA [22,23].

3.3. Genetics of DM2

We began studying DM2 in 1992 as an independent approach to defining the underlying pathogenesis of the myotonic dystrophies. In 1998, we linked the DM2 mutation to a 3 cM region at 3q21 [22,23], and subsequently, in 2001, we demonstrated that DM2 is caused by a transcribed but untranslated CCTG repeat expansion located in intron 1 of the zinc finger protein 9 (ZNF9) gene [8]. The DM2 repeat tract contains the complex motif (TG)n,(TCTG)n,(CCTG)n; the TG, TCTG and CCTG tracts are all independently polymorphic in the general population, but only the CCTG portion expands in affected individuals. The CCTG portion of the repeat tract is usually interrupted on normal alleles, but as in some of the other expansion disorders these interruptions are lost on affected alleles (and in an allele of an unaffected individual who possibly carries a pre-mutation [79]). Although DM2 is generally a milder disease than DM1, the DM2 CCTG expansions can be much larger than the DM1 CTG expansions with alleles ranging in size from ~75 to 11,000 CCTG repeats (mean ~5000 CCTGs). The smallest pathogenic size is not clear because uncommon shorter expansions are found in individuals with multiple allele sizes in lymphocyte DNA [8,26]. The lack of correlation between repeat size and disease severity, and the recent observation that individuals homozygous for large DM2 repeats do not have a more severe disease [80], indicate that the pathogenic effects of the mutation are saturable; larger repeats do not appear to result in increasingly severe pathogenic effects.

The DM2 CCTG expansion mutations show both somatic and intergenerational instability to a greater extent than is seen in DM1. In peripheral blood samples, the mutation size heterogeneity caused by somatic instability is so extreme that 20% of DM2 expansions are not detectable by Southern analysis because the DNA at the DM2 locus forms a broad smear without any definable bands. Consequently, DM2 molecular diagnosis depends on the use of a PCR-based assay of the repeat that is not necessary for diagnosis of DM1 or other repeat expansion disorders [26]. Although intergenerational decrease in age of onset has been reported in DM2 families [26,81], the inevitable ascertainment biases in these observations, the fact that longer repeat expansions have not been observed in patients with earlier onset of disease, and the dramatic somatic mosaicism of the DM2 expansion clearly complicate the interpretation of this clinical observation [26,52].
3.4. Implications from DM2 on DM pathogenesis

Both the DM2 CCTG expansion within intron 1 of ZNF9 and the DM1 CTG expansion in DMPK are transcribed into RNA but do not alter the protein coding portion of any gene [8]. The normal function of ZNF9 as a nucleic acid-binding protein [82,83] appears unrelated to any of the proteins encoded in the DM1 region of chromosome 19. Similarly, the other genes in the DM2 region (KIAA1160, Rab 11B, glycoprotein IX, FLJ11631, and FLJ12057) bear no obvious relationship to genes at the DM1 locus (DMPK, SIX5, DMWD, FCGR7). Even if the DM2 expansion alters the regulation of ZNF9 or other genes in the DM2 region, dysregulation of these different sets of proteins at the DM1 and DM2 loci would not be expected to result in diseases with such strikingly similar multisystemic features. Although the additive model of DM1 suggested that CUG repeats in RNA cause myotonia and muscular dystrophy, the other DM features, including effects on the heart, eye, endocrine system and immunoglobulin levels were ascribed to haploinsufficiency of genes in the DM1 region. The molecular and clinical parallels between DM1 and DM2 strongly indicate that the clinical features common to both diseases including myotonia, muscular dystrophy, cataracts, cardiac arrhythmias, insulin insensitivity and diabetes, hypogammaglobulinemia, and testicular failure are caused by the pathogenic effects of RNA containing the CUG and CCUG expansions [23,26].

4. Molecular pathogenesis of the myotonic dystrophies

4.1. Mechanisms of RNA toxicity

Efforts to understand how the DM1 CUG expansion expressed at the RNA level could mediate a dominant effect on other genes not localized to the DM1 locus (a so-called ‘trans-dominant’ effect) focused on the identification of RNA-binding proteins that might bind to the CUG repeat motifs. Recent suggestions that ribonuclear inclusions in DM1 and DM2 sequester DNA transcription factors [84] have not yet been confirmed in tissues from affected patients. Direct evidence that RNA containing the repeat expansion is responsible for DM pathogenesis includes: (1) a CTG expansion in the 3′-UTR of DMPK mRNA inhibits myoblast differentiation [85]; (2) transgenic models with CTG expansions expressed at the RNA level cause myotonia and muscular dystrophy [75,86]; (3) transcripts containing CUG and CCUG expansions accumulate as RNA foci [8,69,87,88]; (4) transcripts containing CUG and CCUG expansions alter the regulation or localization of RNA-binding proteins, including CUG-BP [71] and three different forms of muscleblind (MBNL1, MBLL and MBXL) [74,87–89]; (5) altered RNA-binding protein activity caused by the CUG and CCUG RNA expansions results in altered splicing and the abnormal function of several genes, detailed below, that are related to DM pathophysiology including Cardiac Troponin T, Insulin Receptor, Chloride Channel, Tau Protein and Myotubularin [45,72,90,91].

4.2. Gene splicing abnormalities in the myotonic dystrophies

4.2.1. Cardiac troponin T

A landmark discovery in 1998 [72] CUG-BP was found to be up-regulated in DM1 muscle, and the increased CUG-BP activity was shown to alter splicing of cardiac troponin T (cTNT) by binding to intronic CUG containing sequences that serve as splicing signals in cTNT pre-mRNA. In cardiac and skeletal muscle from adult DM1 subjects, cTNT transcripts contain exon 5, resulting in a splice form normally seen in fetal tissue. This was the first demonstrated splicing target of CUG-BP and the first demonstration that the presence of elongated CUG repeat expansions in RNA leads to trans-dominant alterations in gene splicing.

4.2.2. Insulin receptor

A classic clinical feature in both DM1 and DM2 patients is insulin resistance [92], predisposing patients to diabetes. Alternative splicing of the insulin receptor (IR) pre-mRNA is aberrantly regulated in DM1 skeletal muscle in that exon 11 is preferentially excluded, which results in predominant expression of the insulin insensitive splice form, IR-A [45,93]. These results have been duplicated in DM2 [46], further demonstrating the common pathogenic mechanism responsible for both diseases, and supporting a model in which increased activity of CUG-BP leads to insulin resistance and diabetes in DM1 and DM2 by alternative splicing of IR.

4.2.3. Chloride channel

A classic feature of both DM1 and DM2 is clinical and electrical myotonia. Skeletal muscle from a transgenic mouse model of DM1 has reduced muscle transmembrane chloride conductance, aberrant splicing of the muscle chloride channel (CIC-1), and loss of CIC-1 protein from the surface membrane [94]; skeletal muscle from DM1 and DM2 subjects also have abnormal CIC-1 splicing and loss of protein [94], demonstrating the pathophysiological basis of this element in the complex DM phenotype. CUG-BP, which is elevated in DM1 skeletal muscle, binds to the CIC-1 pre-mRNA, resulting in the aberrant pattern of CIC-1 splicing [90]. Thus, the CIC-1 splicing abnormalities cause the chloride channelopathy that underlies myotonia in both DM1 and DM2, substantiating the trans-dominant pathogenic role of CUG and CCUG expansions in disease pathogenesis.

4.2.4. Tau and myotubularin

Splicing alterations of RNA encoding the microtubule-associated tau protein have been observed in CNS tissue from DM1 patients [91] and in a murine model [86], which may underlie various CNS alterations in DM1 and DM2.
Also, altered splicing of myotubularin-related 1 (MTMR1) transcripts have been reported in congenital DM1 muscle cells in culture and in skeletal muscle samples from congenital DM1 patients, suggesting a role for MTMR1 in myotonic dystrophy, possibly in the profound muscle hypotrophy of congenital DM1 [95].

4.3. Role of muscleblind and CUG-BP

A point of confusion in DM pathogenesis has involved the role of different RNA-binding proteins (CUG-BP and MBNL). Although CUG-BP is up-regulated in DM1 muscle, and increased CUG-BP results in a trans-dominant effect on gene splicing, CUG-BP does not co-localize with the ribonuclear inclusions and the mechanisms underlying its up-regulation are unknown. In contrast, the RNA-binding proteins in the muscleblind family do co-localize with the ribonuclear inclusions, but were not initially associated with any specific molecular pathogenic effects. Direct evidence supporting the role of muscleblind in disease pathogenesis comes from the recently developed MBNL1 knockout mouse model of myotonic dystrophy, which has the myotonia, myopathy, cataracts and RNA splicing abnormalities characteristic of DM1 and DM2 [96]. Furthermore, recent experiments in transfected cells have confirmed a direct effect of MBNL1 on gene splicing [97]. There appears to be a relationship between effects of CUG-BP and MBNL1, in that over-expression of CUG-BP causes the same specific alternative splicing changes that occur with depletion of MBNL1. In general, CUG-BP appears to promote splice forms normally involved in fetal development while MBNL1 preferentially leads to adult splice forms. These data predict that over-expression of CUG or CCUG repeat expansions, over-expression of CUG-BP, or depletion of MBNL1 would all result in a similar set of splicing alterations, the downstream effects of which would lead to characteristic molecular and physiological features of the myotonic dystrophies (Fig. 2).

4.4. Pathophysiological implications of the differences between DM1 and DM2

Although DM1 and DM2 phenotypes are strikingly similar, they are not identical. DM2 does not show

![Fig. 2. Pathogenic model of DM1 and DM2. The model of RNA pathogenesis in DM1 and DM2 is due to the untranslated expansions in each disease. Both expansions are transcribed; the DMPK mRNA containing the CUG expansion is incorporated into the ribonuclear inclusions; the CCUG expansion from the DM2 transcript is incorporated into ribonuclear inclusions, though it remains unclear whether any other elements of the ZNF9 transcript are also contained within the inclusions. Muscleblind protein (MBNL) binds to the ribonuclear inclusions; CUG-BP is increased by unclear mechanisms. Decreased MBNL and increased CUG-BP activity alter splicing of transcripts involved in DM pathogenesis, e.g. transcripts encoding the chloride channel and insulin receptor. Although the genes responsible for some clinical features have not yet been identified (e.g. testicular failure and hypogammaglobulinemia), the occurrence of these abnormalities in both DM1 and DM2 indicates that they are likely to be caused by the toxic effects of repeat expansions in RNA, possibly involving the resultant decrease in MBNL and increase in CUG-BP.](image-url)
a congenital form, with the attendant craniofacial and musculoskeletal abnormalities, and does not manifest the severe central nervous system involvement sometimes encountered in DM1 [26]. The clinical distinctions between these diseases could result from differences in temporal or spatial expression patterns of the genes containing the expanded repeats (DMPK and ZNF9), or by differences in the interactions of CUG as opposed to CCUG expansions with the various RNA-binding proteins and downstream genes. Alternatively, the differences between DM1 and DM2 could involve other hypothesized mechanisms: altered effects of locus specific genes (such as DMPK, SIX5 or DMWD for DM1, and ZNF9 for DM2); specific effects of additional elements in the 3′-UTR of DMPK [98,99]; or other hypothesized effects [84]. A molecular modification that is potentially important in pathogenesis of developmental changes in DM1 is the demonstrated methylation of the DM1 locus in congenital cases [77,100]. The observed methylation of insulator sites adjacent to the DM1 CTG expansion may explain the increased DMPK expression in congenitally affected individuals [77,101]. Further comparisons of DM1 and DM2 are needed to clarify the clinical and molecular features of both disorders, which will help determine whether distinct pathogenic mechanisms are responsible for the phenotypic differences, or, alternatively, if the clinical distinctions simply reflect disease-specific differences in temporal and spatial expression of the transcripts containing DM1 and DM2 expansions, or intrinsic differences in pathogenicity of CUG compared to CCUG repeat expansions.

5. Possibility of DM3

Whether initially described as having PROMM, PDM or DM2, all families identified to date with the complete multisystemic myotonic dystrophy syndrome but without the DM1 mutation on chromosome 19, have been shown to carry the same DM2 mutation. The initial descriptions of several families initially thought to have different clinical disorders are now ascribed to inter-observer differences, and variable presentation of the single disease, DM2 [26]. Linkage disequilibrium and haplotype analysis indicate that an individual founder mutation led to the CTG expansion in DM1 [102–104], and that likewise the CCTG expansion in DM2 arose from a single founder [79,105].

A family recently suggested to have DM3 [106] has cataracts, electrical myotonia and myopathic histological changes, in addition to having several clinical features that have not been reported in DM1 or DM2 (spongiform encephalopathy, motor neuron degeneration, dementia). It will be interesting to determine whether the abnormal spontaneous EMG activity is an intrinsic feature of muscle, as is true of the myotonia seen in DM1 and DM2, or is secondary to the observed motor neuron loss in this family. It will also be important to determine whether ribonuclear inclusions or splicing abnormalities occur in this family in attempt to clarify whether the pathogenic pathway involved is similar to DM1 and DM2, or is more consistent with the molecular pathogenesis of the tauopathies. Complete clinical and genetic characterization of additional families recently reported as possibly having DM3 [107], will also be valuable in the refining our understanding of DM pathogenesis.

6. Conclusion

The recent clinical and genetic characterization of DM1 and DM2 have now substantiated a third pathogenic mechanism that results from elongated repeat tracts. In addition to the previous recognition that repeat expansions can cause pathogenic loss of gene expression in recessive disorders (e.g. Friedreich’s ataxia), or encode novel pathogenic protein products in dominantly inherited disorders (e.g. Huntington’s disease), we now realize CUG and CCUG repeat expansions in RNA cause the dominantly inherited myotonic dystrophies by altering processing of multiple transcripts: abnormal regulation of RNA-binding proteins results in abnormal splicing of the chloride channel, insulin receptor and other genes, which underlies the complex multisystemic phenotype of these disorders. Other molecular mechanisms may be involved in aspects of the myotonic dystrophy phenotype, such as the features that are seen in DM1 but not DM2, but identification of a primary pathophysiological process now provides a target against which to direct therapeutic interventions. Refined pharmacological approaches can be developed to correct some of the specific features of the disease (for example, myotonia, insulin resistance) while definitive genetic approaches are being developed to correct the underlying molecular abnormalities [108].

Note added in proof

A recent article demonstrates the location of DM1 ribonuclear inclusions in the central nervous system, and documents abnormal splicing in DM1 brain of the amyloid precursor protein, NMDA NR1 receptors, and tau protein, providing evidence that some CNS features of DM1 result from a trans-dominant alteration of splicing in neurons secondary to the CUG expansion [109].

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