Genetics and Molecular Pathogenesis of the Myotonic Dystrophies

John W. Day, MD, PhD, and Laura P.W. Ranum, PhD*

Address
*Department of Neurology, Institute of Human Genetics, MMC 206, University of Minnesota School of Medicine, 420 Delaware Street SE, Minneapolis, MN 55455, USA.
E-mail: johnday@umn.edu

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Pathogenic repeat expansions were initially identified as causing either a loss of gene product, such as in fragile X mental retardation, or an expansion of a polyglutamine region of a protein, as was first shown in spinobulbar muscular atrophy (Kennedy's disease). The pathogenic effect of the repeat expansion in myotonic dystrophy type 1, however, has been controversial because it does not encode a protein but nonetheless results in a highly penetrant dominant disease. Clinical and molecular characterization of myotonic dystrophy types 1 and 2 have now demonstrated a novel disease mechanism involving pathogenic effects of repeat expansions that are expressed in RNA but are not translated into protein.

Introduction

Myotonic dystrophy (DM) is a complex multisystemic disorder that was initially identified almost 100 years ago [1•]. A mutation was identified on chromosome 19 in 1992 for the first genetically identified form of the disease (DM1) [2,3]. In 1994, a similar disorder was identified [4,5] and referred to as either proximal myotonic myopathy (PROMM) [4,6], proximal myotonic dystrophy (PDM) [7], or myotonic dystrophy type 2 (DM2) [8,9], which led to a revised nomenclature [10]. DM1 now refers to the chromosome 19 form of the disease and DM2 refers to the new genetically distinct disorder.

Clinical Features of Myotonic Dystrophy

The clinical features of the two diseases are shown in Table 1, which compares the findings in 234 genetically confirmed cases of DM2 to the recognized DM1 phenotype.

The skeletal muscle features in both genetic forms of myotonic dystrophy include progressive weakness, stereotyped changes on biopsy [1•,11••,12,13], and myotonia. At onset, both forms of the disease affect neck flexors and distal upper extremity muscles (specifically deep flexors of the thumb and deep flexors of the lateral digits more than medial digits) [14]. Although DM1 patients often complain of finger weakness, DM2 patients often come to medical attention because of hip girdle muscle weakness, which led to the name PROMM [15], despite the fact that the finger flexor weakness is often an earlier finding on clinical examination [11••]. In later stages of both diseases, diffuse weakness can become marked, although bulbar and ventilatory weakness is more notable in DM1 than in DM2 [1•,11••]. DM1 also results in greater muscle atrophy than does DM2, which is occasionally associated with the hypertrophy typical of myotonia congenita [11••]. Although electrical myotonia is seen in almost all adults with both forms of DM (Table 1) [11••], myotonia is notably absent in infants with congenital onset DM1; in neither form of the disease is the myotonia as severe as occurs in chloride channelopathies [1•].

In addition to skeletal muscle involvement, both forms of DM affect the heart, eye, endocrine system, and central nervous system. Atrioventricular and intraventricular conduction abnormalities, as well as sudden death, occur in both diseases [11••,16,17]. The cataracts that are common in both diseases are unusual and indistinguishable between DM1 and DM2, with iridescent posterior subcapsular opacities on slit lamp examination [1•,11••]. 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 [1•,11••,18•,19•]. Mental retardation is seen in early-onset DM1 patients [1•] but has not been associated with DM2. Both DM1 and DM2 patients develop central nervous system white matter abnormalities [20]. Central hypersomnia is a recognized feature of DM1 that has not yet been specifically investigated in DM2, although daytime sleepiness has been reported [9].

Genetics of DM1

The DM1 mutation was identified in 1992 as a (CTG)*n* repeat in the 3'-untranslated region of the dystrophia myotonica protein kinase gene (*DMPK*) [2,3,21–23]. This location of the DM1 mutation meant that DM1 was the first dominantly inherited disease found to be caused by an untranslated repeat expansion. In 1995, the DM1

Table 1. Comparison of clinical features in myotonic dystrophy types 1 and 2

Clinical feature	DM2	DM1
Skeletal muscle features (*n* = 234 for DM2)		
Myotonia on EMG	90	+++
Weakness		
Any weakness on exam	82	+++
Neck flexors	75	+++
Thumb or deep finger flexors	55	+++
Hip flexors	64	+
Deep knee bend	54	+
Multisystemic features (*n* = 234)		
Cardiac: conduction defect on ECG	20	++
Cataracts: history or bedside examination	60	++
Additional laboratory findings (*n* given for each test)		
Elevated creatine count (90)	90	++
Low IgG (20)	65	++
Low IgM (20)	11	+/-
Low testosterone (22)	29	++
High FSH (26)	65	++
Insulin insensitivity (16)	75	++

+—Well recognized and common late in the course of disease.
++—Common among all patients.
+++—Expected in all patients.
+/-—Recognized but not common.
DM—myotonic dystrophy; ECG—electrocardiogram; EMG—electromyogram; FSH—follicle-stimulating hormone.

mutation was also found to be in the promoter region of the immediately adjacent homeodomain gene *SIX5* [24]. The CTG expansion in DM1 patients can vary from 80 to more than 4000 repeats in affected individuals, with clinically unaffected individuals having 50 to 100 CTG repeats. Intergenerational and somatic instability are observed in which repeat size can increase by approximately 50 to 80 repeats per year [25]. There is a rough correlation of DM1 repeat size and age of onset for CTGs less than 400 repeats [26].

Genetics of DM2

We began studying DM2 in 1992 as an independent approach to defining the underlying pathogenesis of the myotonic dystrophies. We linked the DM2 mutation to chromosome 3q21 in 1998 [8,9], and subsequently showed that DM2 is caused by an untranslated CCTG repeat expansion in intron 1 of the zinc finger protein 9 (*ZNF9*) gene [27••]. The DM2 repeat tract contains the complex motif $(TG)_n(TCTG)_n(CCTG)_n$; the TG, TCTG, and CCTG tracts are all 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 the interruptions are lost on affected alleles (and in an unaffected individual who possibly carries a premutation) [28]. DM2 CCTG expansions can be much larger than the DM1 CTG expansions, with alleles ranging in size from approximately 75 to 11,000 CCTG repeats (mean of 5000 CCTGs). The smallest pathogenic size is not clear because uncommon shorter expansions are found in individuals with multiple allele sizes in lymphocyte DNA [11••,27••]. 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 [29], indicate that larger repeats do not result in increasingly severe pathogenic effects.

On Southern analysis of DNA from peripheral blood samples, 20% of DM2 expansions are not detectable because the mutation size heterogeneity caused by somatic instability results in a broad smear without any definable bands. Consequently, DM2 molecular diagnosis requires a polymerase chain reaction–based assay of the repeat that is not necessary for diagnosis of DM1 or other repeat expansion disorders [11••]. Although intergenerational decreases in age of onset have been reported in DM2 families based on clinical criteria [11••,30], the expected trend of longer repeat expansions in patients with earlier disease was not observed, although the somatic instability of the repeat clearly complicated this analysis [11••].

The Common Denominator in Pathogenesis of Myotonic Dystrophy

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. ZNF9 is a nucleic acid binding protein [31,32] with effects that are unrelated to any of the proteins encoded in the DM1 region on chromosome 19. Similarly, genes in the DM2 region (*KIAA1160, Rab 11B, glycoprotein IX, FLJ11631*, and *FLJ12057*) bear no obvious relationship to the genes at the DM1 locus (*DMPK, SIX5, DMWD*, and *FCGRT*). It is hard to imagine how dysregulation of the different sets of genes at the DM1 and DM2 loci would result in diseases with such strikingly similar multisystemic features. The molecular and clinical parallels between DM1 and DM2 thus 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 (Fig. 1) [11••].

Mechanisms of RNA Toxicity

After RNA inclusions were identified in DM1 muscle nuclei [33], investigators tried to identify RNA binding proteins that might be dysregulated by the CUG repeat motifs [34,35]. Recent suggestions that ribonuclear inclusions in DM1 and DM2 sequester transcription factors [36] have not yet been confirmed in tissues from affected patients. Direct evidence that the repeat expansions in RNA

are responsible for DM pathogenesis includes 1) a CTG expansion in the 3' UTR of *DMPK* mRNA inhibits myoblast differentiation [37]; 2) transgenic models with CTG expansions expressed at the RNA level cause myotonia and muscular dystrophy [38••,39]; 3) CUG- and CCUG-containing transcripts accumulate as RNA foci [27••,33,40,41]; 4) CUG-containing transcripts alter the regulation or localization of RNA binding proteins, including CUG-BP [42] and muscleblind (MBNL1, MBLL and MBXL) [41,43]; and 5) altered RNA binding protein activity [44] caused by the CUG and CCUG RNA expansions results in abnormal splicing and function of several gene products, including cardiac troponin T (cTNT), the insulin receptor (IR), and the muscle chloride channel (ClC-1).

In a landmark discovery [45], increased CUG-BP activity in DM1 muscle was shown to alter splicing of cTNT by binding intronic CUG-containing splicing signals in pre-mRNA. In adult DM1 cardiac and skeletal muscle, cTNT transcripts abnormally include exon 5, resulting in a fetal isoform of the protein. This was the first demonstration that elongated CUG repeat expansions in RNA lead to trans alterations in gene splicing. The cTNT discovery was followed by the demonstration that IR alternative splicing is also aberrantly regulated in DM1 skeletal muscle. Exon 11 is preferentially excluded from IR in DM1 muscle, which results in predominant expression of the insulin-insensitive splice form, IR-A [18•]. These results have now also been duplicated in DM2 [19•], further demonstrating the common pathogenic mechanism responsible for both diseases, and supporting a model in which altered activity of RNA-binding proteins leads to abnormal splicing of the IR, insulin resistance, and diabetes in DM1 and DM2. Another gene now shown to be abnormally spliced is the muscle chloride channel ClC-1, resulting in the myotonia; abnormal isoforms and overall reduction in ClC-1 protein in the sarcolemma have been demonstrated in transgenic mice as well as in muscle from DM1 and DM2 patients [46••,47••]. Splicing alterations of the microtubule-associated tau mRNA have been observed in central nervous system tissue from DM1 patients [48] and in a murine model [39], which may underlie various central nervous system alterations in DM1 and DM2. Also, altered splicing of myotubularin-related 1 (*MTMR1*) gene has 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 atrophy of congenital DM1 [49].

Role of Muscleblind in Splicing Changes

The role of different RNA binding proteins (CUG-BP and MBNL) in DM pathogenesis has been unclear. Although increased CUG-BP activity in DM1 results in a trans dominant effect on gene splicing, CUG-BP does not co-localize with the ribonuclear inclusions. In contrast, the muscleblind RNA binding proteins do co-localize with the ribonuclear inclusions, but were not initially associated with any specific molecular pathogenic effects. Direct evidence of muscleblind involvement in disease pathogenesis comes from the recently developed MBNL1 knockout mice, which model the myotonia, myopathy, cataracts, and RNA splicing abnormalities of DM1 and DM2 [50••]. More recently, MBNL was shown to have a direct effect on gene splicing that is opposite to that of CUG-BP [44]. Interestingly, overexpression of CUG-BP causes many of 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, whereas 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 physiologic features of the myotonic dystrophies.

Differences Between DM1 and DM2

Although DM1 and DM2 phenotypes are strikingly similar, they are not identical. DM2 does not show a congenital form, with the attendant craniofacial and musculoskeletal abnormalities, and does not manifest the severe central nervous system involvement sometimes encountered in DM1 [11••]. 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*) and the genes for the various RNA binding proteins, or could be caused by differences in the downstream effects of CUG as opposed to CCUG expansions. Alternatively, the differences between DM1 and DM2 could involve locus-specific genes such as *DMPK, SIX5*, or *DMWD* for DM1, and *ZNF9* for DM2. A possible mechanism for congenital DM1 is the demonstrated methylation of a genetic insulator at the DM1 locus in congenital cases [51•], which results in higher DMPK expression in the more severe congenital phenotype. Further clinical and molecular comparisons of DM1 and DM2 are needed to clarify the differences between the two disorders, which will help determine whether there are distinct pathogenic mechanisms responsible for the phenotypic differences, or, alternatively, to what extent the clinical distinctions simply reflect disease-specific differences in severity of the CUG and CCUG repeat expansions in RNA.

Possibility of DM3

Linkage disequilibrium and haplotype analysis indicate that single founder mutations led to both the CTG expansion in DM1 [52,53] and the CCTG expansion in DM2 [28,55]. All previously reported families with dominant

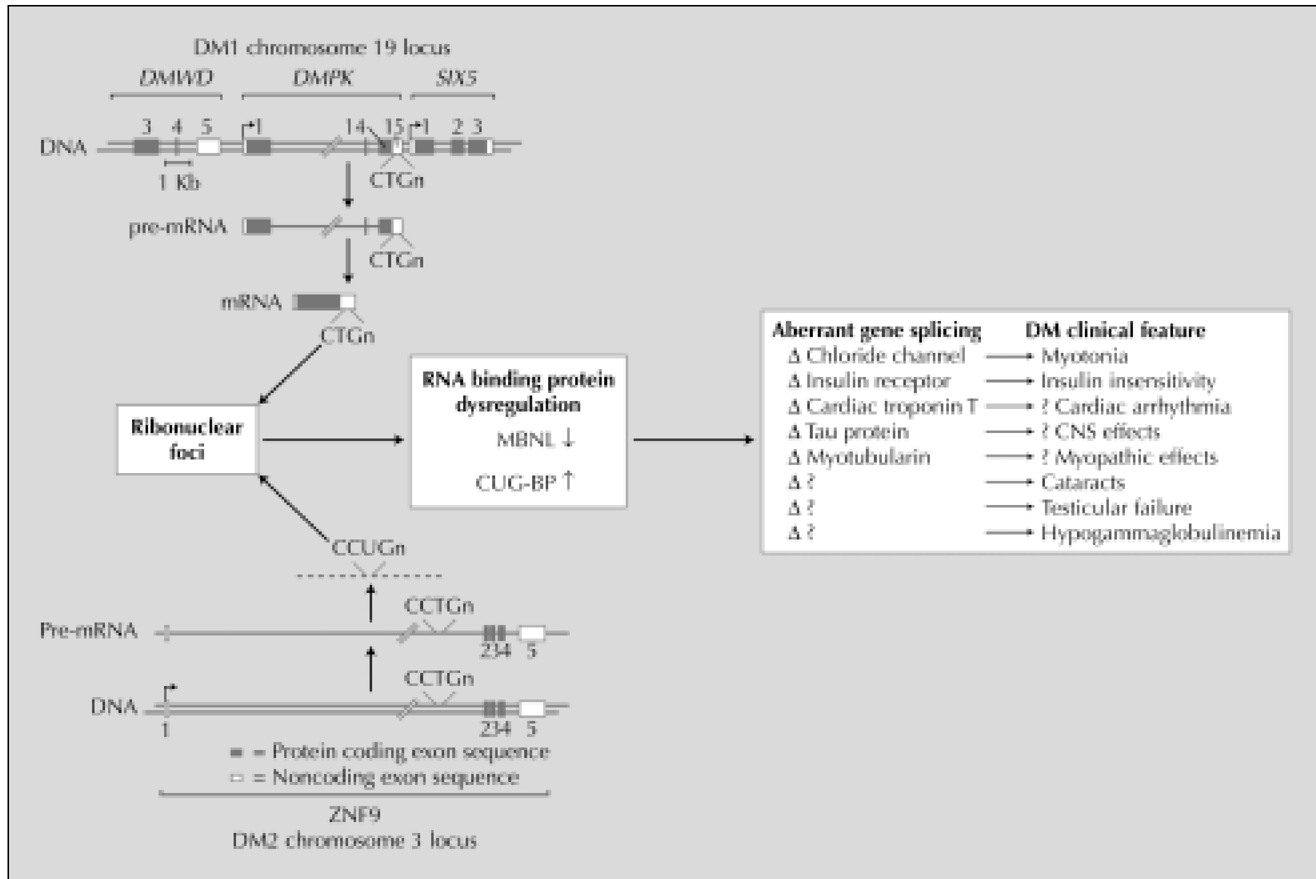

Figure 1. Pathogenic RNA model of myotonic dystrophy type 1 (DM1) and type 2 (DM2). The model of DM1 and DM2 reflects the pathogenic effects of the untranslated expansions in each disease. Genes encoding both expansions are transcribed. The DM1 dystrophia myotonica protein kinase (*DMPK*) mRNA containing the CUG expansion is incorporated into ribonuclear inclusions, as is RNA containing the CCUG expansion from the DM2 transcript (although it remains unclear how much of the zinc finger protein 9 [*ZNF9*] transcript is in the inclusions). Muscleblind protein (MBNL) binds to the ribonuclear inclusions. CUG-BP activity is increased by unclear mechanisms, which, along with the decrease in MBNL, alters splicing of transcripts involved in DM pathogenesis (*eg*, transcripts encoding the chloride channel and insulin receptor). Although the genes responsible for some clinical features have not yet been identified (*eg*, testicular failure and hypogammaglobulinemia), the occurrence of these abnormalities in both DM1 and DM2 indicates that they are likely to be caused by the same mechanism. (CNS—central nervous system.)

multisystemic myotonic disorders other than DM1, whether reported as having PROMM, PDM, or DM2, or previously reported not to be linked to the DM2 locus, have now been shown to carry DM2 expansions [11••].

A family recently suggested to have DM3 [55] has features typical of the myotonic dystrophies, including cataracts and electrical myotonia, but also has several clinical features that have not been reported in DM1 or DM2, including spongioform encephalopathy, motor neuron degeneration, and dementia. No investigations of ribonuclear inclusions or splicing abnormalities were reported, and it is unclear whether the spontaneous electrical activity is an intrinsic feature of muscle, as in DM1 and DM2 myotonia, or is secondary to the observed motor neuron disorder. Additional genetic and clinical testing will be needed to determine whether the pathogenic pathway involved in this family is related to DM1 and DM2, or is more consistent with the molecular pathogenesis of the tauopathies. Additional families recently reported as possibly having DM3 [56] have characteristic features of myotonic dystrophy, although complete clinical and genetic characterization is pending.

Conclusions

The molecular and clinical similarities of DM1 and DM2 have now substantiated a disease mechanism in which RNA containing CUG and CCUG repeat expansions alters processing of multiple transcripts, leading to abnormal splicing of the chloride channel, insulin receptor, and other genes that underlie the multisystemic phenotype characteristic of these diseases. Other molecular mechanisms may be involved in aspects of myotonic dystrophy, such as the features that are seen in DM1 but not DM2, but we now have a primary pathophysiologic target against which to direct possible therapeutic regimens. Pharmacologic approaches can be developed to correct specific features of the disease (*eg*, myotonia, insulin resistance), and genetic approaches to a definitive treatment are also being developed [57].

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