Am. J. Hum. Genet. 74:793–804, 2004

REVIEW ARTICLE
Myotonic Dystrophy: RNA Pathogenesis Comes into Focus

Laura P. W. Ranum[1,2] and John W. Day[1,3]

[1]Institute of Human Genetics, [2]Department of Genetics, Cell Biology, and Development, and [3]Department of Neurology, University of Minnesota, Minneapolis

Myotonic dystrophy (DM)—the most common form of muscular dystrophy in adults, affecting 1/8,000 individuals—is a dominantly inherited disorder with a peculiar and rare pattern of multisystemic clinical features affecting skeletal muscle, the heart, the eye, and the endocrine system. Two genetic loci have been associated with the DM phenotype: *DM1,* on chromosome 19, and *DM2,* on chromosome 3. In 1992, the mutation responsible for DM1 was identified as a CTG expansion located in the 3′ untranslated region of the dystrophia myotonica-protein kinase gene (*DMPK*). How this untranslated CTG expansion causes myotonic dystrophy type 1(DM1) has been controversial. The recent discovery that myotonic dystrophy type 2 (DM2) is caused by an untranslated CCTG expansion, along with other discoveries on DM1 pathogenesis, indicate that the clinical features common to both diseases are caused by a gain-of-function RNA mechanism in which the CUG and CCUG repeats alter cellular function, including alternative splicing of various genes. We discuss the pathogenic mechanisms that have been proposed for the myotonic dystrophies, the clinical and molecular features of DM1 and DM2, and the characterization of murine and cell-culture models that have been generated to better understand these diseases.

Introduction

Steinert (1909) and Batten and Gibb (1909) identified myotonic dystrophy (DM [MIM 160900 and MIM 602668]) as a multisystemic disorder that is now recognized as one of the most common forms of muscular dystrophy in adults. In addition to muscular dystrophy and myotonia (involuntary persistence of muscle contraction), DM causes a consistent constellation of seemingly unrelated and rare clinical features, including: cardiac conduction defects, posterior subcapsular iridescent cataracts, and a peculiar and specific set of endocrine changes (Harper 2001). The 90 years between the identification of myotonic dystrophy as a clinical disorder and the identification of the first gene were important for the development of a detailed description of the clinical features, inheritance, and epidemiology of myotonic dystrophy. Clinical neurologists and geneticists identified non-Mendelian features of myotonic dystrophy type 1 (DM1) inheritance, including variable penetrance, anticipation (a tendency for the disease to worsen in subsequent generations), and a maternal transmission bias for congenital forms (Harper 2001), all of which preceded the identification of the CTG expansion responsible for DM1. The genetic cause of DM1 was identified in 1992 as a (CTG)*n* repeat in the 3′-UTR of a protein kinase gene (Brook et al. 1992; Buxton et al. 1992; Fu et al. 1992; Harley et al. 1992; Mahadevan et al. 1992).

In 1995, several reports described families with dominantly inherited multisystemic myotonic disorders that were genetically distinct from DM1 (Ricker et al. 1994; Rowland 1994; Thornton et al. 1994). Ricker and colleagues (1994) most thoroughly evaluated this new disorder and showed that the genetic cause in these families was distinct from the known causes of myotonia congenita, paramyotonia, and DM1, demonstrating the existence of a novel genetic disorder. Because affected family members often came to medical attention because of pelvic girdle weakness, he referred to the disorder as "proximal myotonic myopathy" (PROMM). A broader phenotype was described as "proximal myotonic dystrophy" in 1997 (Udd et al. 1997). Subsequently, we reported a large Minnesota kindred with a second form of myotonic dystrophy (DM2) that closely mimics the phenotype of adult-onset DM1, and we localized the disease gene to a 10-cM region on chromosome 3q (Ranum et al. 1998; Day et al. 1999). Ricker and colleagues then reported that the gene for PROMM in many families also mapped to the *DM2* locus (Ricker et al. 1999). The International Myotonic Dystrophy Consortium (IDMC 2000) and the Online Mendelian Inheritance in Man (OMIM) database updated the clinical and scientific nomenclature so that the chromosome 19 locus is now referred to as "myotonic dystrophy type

Received October 15, 2003; accepted for publication February 12, 2004; electronically published April 2, 2004.

Address for correspondence and reprints: Laura P. W. Ranum, Ph.D., MMC 206, 420 Delaware Street S.E., Minneapolis, MN 55455. E-mail: ranum001@umn.edu

0002-9297/2004/7405-0003$15.00

1" (*DM1*) and the locus on chromosome 3 to which DM2 and many PROMM families map is the *DM2* locus. The diseases are collectively referred to as "the myotonic dystrophies" (DM), and each individual genetic disorder is referred to by the same name as the locus name (DM1, DM2), whereas "PROMM" can be used to describe the clinical presentation but is not restricted to any specific genetic cause or locus.

Clinical Features

Muscle Dysfunction

The multisystemic nature of DM results in varied symptoms in affected individuals (table 1), although almost all affected individuals have clinically identifiable muscle involvement at the time of diagnosis. Muscle dysfunction is the most common presenting complaint in both DM1 and DM2 (Moxley 1996; Day et al. 1999, 2003; Ricker 1999; Thornton 1999; Harper 2001), with symptomatic weakness, pain and myotonia. Myotonia is involuntary muscle contraction and delayed relaxation due to muscle hyperexcitability.

There are similarities and differences in the muscle features of DM1 and DM2. Both diseases result in early clinically detectable weakness of neck flexors and lateral deep finger flexors (Moxley 1996; Day et al. 1999; Mathieu et al. 1999; Ricker 1999; Thornton 1999; Harper 2001; Day et al. 2003), although initial symptoms in DM2 often involve proximal lower extremity musculature, whereas DM1 is more apt to be associated with severe muscle atrophy and symptomatic finger flexor weakness. DM2 is not associated with severely atrophic facial and forearm muscles. Electrical myotonia is detectable by electromyography in almost all individuals with DM1 or DM2; although clinical symptoms of myotonia are not typically severe in either disease, patients with DM1 are often more symptomatic than those with DM2.

The histological features of DM muscle are distinct, though not pathognomonic, with dystrophic features accompanied by atrophic fibers, scattered severely atrophic fibers with pyknotic myonuclei, and a marked proliferation of fibers with central nuclei. There is one report of differences in fiber-type involvement in the two diseases, with more type 1 involvement in DM1 and more type 2 involvement in DM2 (Vihola et al. 2003).

Multisystemic Features

DM1 and DM2 have similar disease-specific effects on the eye, heart, and endocrine systems. Ocular involvement results in almost all adults with DM1 or DM2 having posterior subcapsular cataracts, with distinctive red and green iridescent opacities on slitlamp examination. Some adults are unaffected by these cataracts, but other subjects with DM1 or DM2 may require lens removal as early as the 2nd decade of life (Harper 2001; Day et al. 2003). Cardiac involvement leads to conduction defects, arrhythmias, and sudden death in both DM1 and DM2 (Nguyen et al. 1988; Colleran et al. 1997; Merino et al. 1998; Philips et al. 1998; Day et al. 2003) but does not correlate with severity of skeletal muscle involvement or repeat length (Lazarus et al. 1999). Endocrine abnormalities in both DM1 and DM2 result in hyperinsulinemia, hyperglycemia, and insulin insensitivity, with type 2 diabetes occurring in each disorder (Harper 2001; Day et al. 2003). Testicular failure is also common in both disorders, with associated hypotestosteronism, elevated follicle-stimulating hormone (FSH) levels, and oligospermia. Other serological abnormalities in both disorders include reduced levels of immunoglobulin G and M (Harper 2001; Day et al. 2003).

Table 1

Clinical Features of the Myotonic Dystrophies

Feature	Finding for[a]	
	DM1	DM2
Myotonia	+	+
Facial weakness	+	+
Proximal weakness	+	+
Distal weakness	+	+
Sternocleidomastoid atrophy	+	+
Iridescent cataracts	+	+
Cardiac arrhythmias	+	+
Testicular failure	+	+
Hyperinsulinemia	+	+
Hypogammaglobulinemia	+	+
Elevated creatine kinase	+	+
Retardation/congenital abnormalities	+	−

[a] + = present; − = absent.

Congenital Myotonic Dystrophy

A primary difference between DM1 and DM2 is that DM1 can result in a severe congenital form that has not been reported for DM2. The congenital form of DM1 involves marked developmental abnormalities that can be present to a lesser degree in patients with DM1 who become symptomatic during adulthood; the hallmark developmental features, including craniofacial skeletal abnormalities, characteristic dysmorphic features, and mental retardation, have not been reported in DM2.

CNS Involvement

CNS involvement in DM1 involves both (1) developmental abnormalities with retardation and (2) degenerative changes, exemplified by abnormal white matter changes on MRI scanning, memory deficits, and personality abnormalities (Rubinsztein et al. 1997; Bun-

gener et al. 1998; Delaporte 1998; Ogata et al. 1998; Ono et al. 1998; Wilson et al. 1999). The CNS effects of DM2 mirror the degenerative changes of DM1, including the MRI abnormalities (Hund et al. 1997), but developmental abnormalities and retardation are not recognized features of DM2 (Day et al. 2003).

DM1 (CTG)*n* Repeat Expansion and Instability

In 1992, the chromosome 19 form of DM was shown to be caused by an expanded CTG repeat located in the 3′ UTR of the *myotonia-dystrophica protein kinase* (*DMPK* [MIM 605377]) gene (Brook et al. 1992; Buxton et al. 1992; Fu et al. 1992; Harley et al. 1992; Mahadevan et al. 1992) and the promoter region of the immediately adjacent homeodomain gene *SIX5* (MIM 600963) (Boucher et al. 1995). DM1 was the first autosomal dominant disease found to be caused by an untranslated trinucleotide repeat expansion, in which the mutation is transcribed into RNA but not translated into protein. The CTG expansion in *DM1* can vary from 50 to >4,000 repeats in affected individuals. Both intergenerational and somatic instability are observed with increases in repeat size on the order of ~50–80 repeats per year (Monckton et al. 1995; Martorell et al. 1998). For DM1, there are reasonably good correlations of repeat size and age at onset for CTGs <400 repeats (Hamshere et al. 1999).

The *DM1* CTG Expansion: Proposed Mechanisms of Pathogenesis

Haploinsufficiency of DMPK

Because most dominant disorders are caused by the expression of an abnormal protein with an altered function, it has not been clear how the multisystemic clinical features of dominantly inherited DM1 could be caused by a trinucleotide repeat that did not affect the protein coding portion of a gene (Tapscott 2000). Initially, the location of the mutation at the 3′ end of a kinase gene suggested that alterations in DMPK expression might cause the multisystemic clinical features of the disease. Most of the early expression studies were consistent with this hypothesis, indicating that DMPK mRNA and protein levels were reduced in patient muscle and cell culture (Fu et al. 1992; Hoffmann-Radvanyi et al. 1993; Novelli et al. 1993). However, *DMPK* knockout mice generated to test this hypothesis did not have the typical multisystemic features of the disease. Initial reports of these mice showed only a very mild, late-onset myopathy that is not typical of DM1 (Jansen et al. 1996; Reddy et al. 1996). More recently, both hemizygous and homozygous *DMPK* knockout mice have been reported to have cardiac conduction abnormalities. When considered together, these results suggested that DMPK may contribute to the cardiac features of DM1, but haploinsufficiency of DMPK does not cause the multisystemic clinical features of DM1. The fact that there are no reported cases of DM1 caused by point mutations in the *DMPK* gene further suggests that the multisystemic features of DM1 are not caused simply by DMPK haploinsufficiency.

Haploinsufficiency of SIX5 *and Neighboring Genes*

A second proposed mechanism is that the expanded repeat affects the expression of multiple genes in the region. Support for this hypothesis comes from the observation that the CTG expansion is a strong nucleosome-binding site that could potentially alter chromatin structure and have regional effects on the expression of multiple genes (Wang et al. 1994; Otten and Tapscott 1995). The expansion overlaps not only the 3′ end of *DMPK,* but the 5′ promoter region of the neighboring gene *SIX5. SIX5* has a strong resemblance to the fruit fly gene *sine oculis* (needed for eye development) and to a family of mouse genes that regulate distal limb muscle development. Because cataracts and distal muscle wasting are common in DM1, haploinsufficiency of SIX5 was suggested as a possible contributor to DM1 pathogenesis (Shaw et al. 1993; Boucher et al. 1995; Jansen et al. 1995). In addition to *DMPK* and *SIX5,* other neighboring genes suggested to be involved in aspects of DM1 pathogenesis included the *DMWD* gene immediately upstream of *DMPK,* which is expressed in the testis and is proposed to play a role in male infertility, and *FCGRT* (MIM 601437), an IgG receptor gene located 4 Mb from the CTG expansion, was proposed to underlie the low IgG levels in DM (Junghans et al. 2001). 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.

Expression studies have focused on examining the transcript levels of *DMPK, DMWD,* and *SIX5* in both individuals with DM1 and normal individuals. In the case of *DMPK,* an equal amount of unprocessed transcripts are produced from both the normal and the mutant *DMPK* genes (Krahe et al. 1995), indicating that there is no interference with *DMPK* transcription caused by the CTG expansion. Studies involving *SIX5* and *DMWD* have given conflicting results. Thornton and colleagues (1997) used an allele-specific RT-PCR assay and found that the expression of the CTG repeat expansion-linked *SIX5* allele was reduced—in some cases approaching inactivation—compared with the other *SIX5* allele. This reduction was seen with pre-mRNA, as well as with processed mRNA. Klesert and colleagues (1997) demonstrated that a DNase I hypersensitive site,

shown by Otten and Tapscott (1995) to be eliminated by the CTG repeat expansion, contains an enhancer sequence for the *SIX5* gene but not for the *DMPK* gene, and that expansion of the CTG reduces the transcription of the *SIX5* allele located *cis* to the expansion. Data generated by Hamshere and colleagues (1997), indicated that the cytoplasmic expression of both *SIX5* and *DMWD* was the same in both normal and DM1 cells.

The most compelling support for the involvement of neighboring genes in DM pathogenesis came from the fact that *Six5* knockout mice develop cataracts (Klesert et al. 2000; Sarkar et al. 2000). Although cataracts are one of the clinical features of DM, the cataracts in the *Six5* knockouts do not have the distinctive iridescent opacities or the posterior location that is associated with myotonic dystrophy in humans.

RNA Pathogenesis

A third hypothesized mechanism is that the enlarged CUG-containing transcripts that accumulate as foci in the nuclei of both cultured cells and biopsied tissue (Taneja et al. 1995; Davis et al. 1997) exert a *trans*-dominant effect that disrupts splicing and possibly other cellular functions (Timchenko et al. 1996*a*; Philips et al. 1998; Lu et al. 1999; Miller et al. 2000; Savkur et al. 2001). Strong experimental support for the involvement of an RNA mechanism in skeletal muscle pathology came from a mouse model developed by Mankodi and colleagues (2000). Initial attempts to develop animal models to study the effects of the expression of elongated CUG expansions in transgenic mice were hampered by infertility of the mice, which prevented successful breeding (Monckton et al. 1998). In 2000, Mankodi and colleagues avoided this problem by developing a mouse model in which the CTG expansion was inserted into the 3′ end of the human skeletal actin gene, a gene not thought to be involved in DM1 but the expression of which is limited to skeletal muscle (Mankodi et al. 2000). This mouse model, which expressed an mRNA with a CUG repeat tract of ~250 repeats was the first to develop the myotonia and the myopathic features characteristic of DM1 (Mankodi et al. 2000). However, because the expression of the CUG-containing transcripts in this model was limited to skeletal muscle, the role of the CUG expansion in the multisystemic features of DM was not addressed.

Additive Model of DM1 Pathogenesis

Subsequently, an additive model was proposed in which each of the above mechanisms contributes to DM1 pathogenesis (Groenen and Wieringa 1998; Tapscott 2000; Filippova et al. 2001; Larkin and Fardaei 2001), 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. An inconsistency with the additive model of DM1 pathogenesis was that the genetic locus for DM2 maps to a different chromosome, with no known conservation between the two chromosomal regions (Ranum et al. 1998).

DM2 Caused by CCTG Expansion in Intron 1 of *ZNF9*

Given the confusion in understanding the molecular pathogenesis of DM1, the identification of a second human mutation that causes the multisystemic effects of DM and a determination of what is common to these diseases at the molecular level, provided an independent means of evaluating the pathogenic pathway(s) of DM. In 2001, we demonstrated that DM2 is also caused by a transcribed but untranslated repeat expansion—but this time a CCTG repeat expansion located in intron 1 of the *zinc finger protein 9* (*ZNF9*) gene (MIM 116955). Although DM2 is generally a milder disease than DM1, the *DM2* CCTG expansions can be much larger than *DM1* CTG expansions, with alleles ranging in size from ~75 to 11,000 CCTG repeats (mean ~5,000 CCTGs). The smallest pathogenic size is not yet clear, because somatic instability has resulted in individuals with uncommon shorter expansions also having larger allele sizes in lymphocyte DNA (Liquori et al. 2001).

The *DM2* CCTG expansion mutations show both somatic and intergenerational instability. In peripheral blood samples, the degree of somatic instability is so extreme that one in five *DM2* expansions is not detectable by Southern analysis because of size heterogeneity. This causes a diagnostic challenge for DM2 not previously encountered with DM1 or any of the other reported microsatellite expansion disorders (Liquori et al. 2001; Day et al. 2003). Although anticipation has been reported in DM2/PROMM families, on the basis of clinical criteria (Schneider et al. 2000; Day et al. 2003), the expected trend of longer repeat expansions in patients with earlier ages at onset was not observed (Schneider et al. 2000; Day et al. 2003), with the somatic instability of the repeat clearly complicating this analysis (Liquori et al. 2001).

The *DM2* repeat tract contains the complex repeat motif $(TG)_n$ $(TCTG)_n$ $(CCTG)_n$. The CCTG portion of the repeat tract is interrupted on normal alleles, but as in other expansion disorders these interruptions are lost on affected alleles. Haplotype analysis of 228 control chromosomes identified a potential premutation allele with an uninterrupted $(CCTG)_{20}$ on a haplotype that was identical to the most common affected haplotype. Similar to some of the other microsatellite expansion disorders (Chung et al. 1993; Kunst and Warren 1994; Pulst et al. 1996; Gunter et al. 1998), the loss of se-

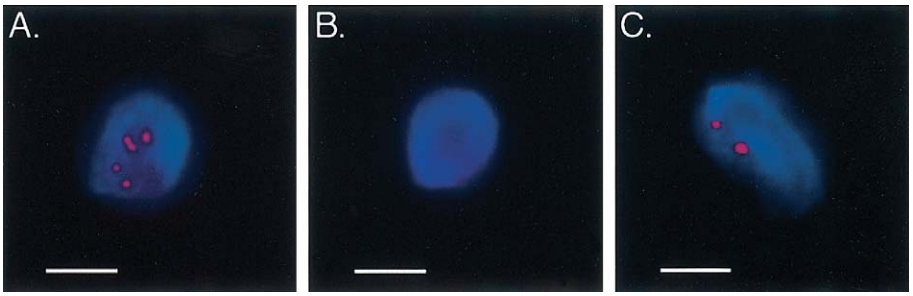

Figure 1 RNA in situ hybridization of the expansion. *A*, In situ hybridization of CAGG probe to DM2 muscle. *B*, In situ hybridization of CAGG probe to normal muscle. *C*, In situ hybridization of CAG probe to DM1 muscle. The scale bar is 5 μM. (Reprinted from Science 293:864–867.)

quence interruptions within the CCTG portion of the *DM2* repeat tract may predispose alleles to further expansion (Liquori et al. 2003).

Our 2001 discovery of the *DM2* CCTG expansion allowed for further analysis of the genetic heterogeneity of DM. It now appears that most if not all families with dominant multisystemic myotonic disorders have either *DM1* or *DM2* expansions, with no convincing examples that would suggest the existence of a third mutation that causes a similar disorder—that is, DM3 (Day et al. 2003). Families previously reported to have a form of DM or PROMM not linked to either the *DM1* or *DM2* loci were subsequently shown to carry the *DM2* CCTG expansion (Day et al. 2003). Linkage disequilibrium and haplotype analysis suggest that single founder mutations led to the CTG and CCTG expansions responsible for both DM1 and DM2 (Imbert et al. 1993; Neville et al. 1994; Chakraborty et al. 1996; Bachinski et al. 2003; Liquori et al. 2003).

DM2: Evidence that RNA Pathogenesis Causes Multisystemic Clinical Features

The location of the CCTG expansion within intron 1 of *ZNF9* is similar to the *DM1* CTG expansion, in that both repeats are transcribed into RNA but do not alter the protein coding portion of a gene (Liquori et al. 2001). The normal function of ZNF9 as a nucleic acid–binding protein (Pellizzoni et al. 1997, 1998; Shimizu et al. 2003) appears unrelated to any of the proteins encoded in the *DM1* region of chromosome 19. Similarly, genes in the *DM2* region (*KIAA1160, Rab 11B, glycoprotein IX, FLJ11631,* and *FLJ12057*) bear no obvious relationship to genes near the *DM1* locus (*DMPK, SIX5, DMWD,* and *FCGRT*). Even if the *DM2* expansion alters the regulation of *ZNF9* and other genes in the *DM2* region, it would be unlikely that alterations in the regulation of different sets of proteins at the *DM1* and *DM2* loci would result in diseases with such strikingly similar multisystemic features.

The first suggestion that CUG-containing transcripts were involved in DM1 pathogenesis came from FISH experiments demonstrating that CUG-containing nuclear RNA foci accumulate in DM1 cells (Taneja et al. 1995). Evidence that DM1 and DM2 share a common pathogenic RNA mechanism comes from experiments showing that similar CCUG-containing RNA foci are found in DM2 muscle (fig. 1) (Liquori et al. 2001). These results demonstrate that the CCTG expansion is expressed at the RNA level, but additional experiments are needed to determine if the RNA foci contain the entire unprocessed ZNF9 transcript or if the transcript is normally processed but the intron or the repeat tract alone resists degradation forming the RNA foci. Additional evidence that RNA foci containing the *DM1* and *DM2* repeat motifs behave in a similar manner is that several forms of the RNA-binding protein muscleblind (MBNL, MBLL, and MBXL) colocalize to the repeat-containing foci in both diseases (Mankodi et al. 2001; Fardaei et al. 2002).

Although the additive model of DM1 suggested that CUG repeats in RNA cause the myotonia and muscular dystrophy of DM1, the causes of other DM features—including cardiac conduction defects and cataracts—had been ascribed to haploinsufficiency of genes in the *DM1* region. The clinical and molecular parallels between DM1 and DM2 suggest a simpler model of DM pathogenesis (figs. 2 and 3), in which the clinical features common to both diseases—including myotonia, muscular dystrophy, cataracts, cardiac arrythmias, insulin insensitivity and diabetes, hypogammaglobulinemia, and testicular failure—are caused by the pathogenic effects of RNA containing the CUG and CCUG expansions (Day et al. 1999; Liquori et al. 2001)

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

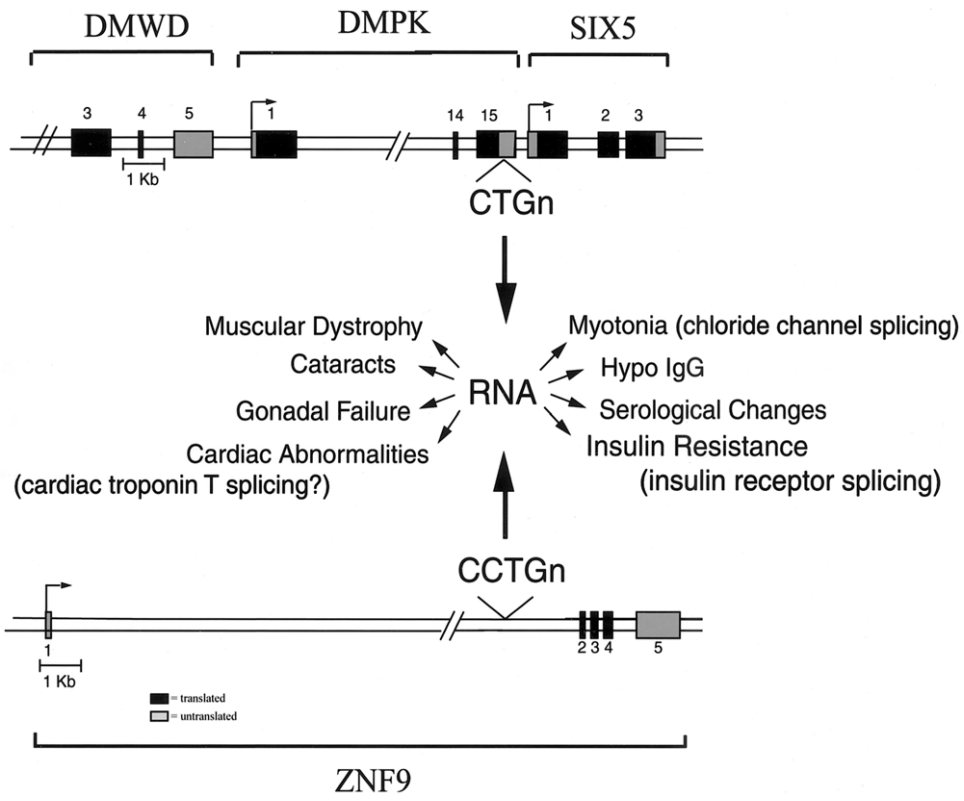

Figure 2 RNA model of DM pathogenesis. The unusual and rare multisystemic clinical parallels between DM1 and DM2 suggest a similar pathogenic mechanism. The discovery that *DM2* mapped to chromosome 3 and not the *DM1* region of chromosome 19 make it unlikely that regional effects of gene expression play a major role in causing the common clinical features of the disease because regional disregulation would affect different sets of genes. The discovery that a CCTG repeat expansion located on chromosome 3 which is expressed at the RNA but not the protein level causes DM2, and the observation that both CUG and CCUG repeat–containing foci accumulate in affected muscle nuclei suggests that a gain-of-function RNA mechanism underlies the clinical features common to both diseases. Specific changes in pre-mRNA splicing have been that have been associated with several genes, including the insulin receptor, the chloride channel, and cardiac troponin T, are correlated with insulin resistance, myotonia, and possibly cardiac abnormalities. (Adapted from Curr Opin Genet Dev 12:266–271.)

congenital form or mental retardation that can occur in DM1 (Liquori et al. 2001). Downstream differences of the CUG vs. CCUG expansions could be responsible for the clinical distinctions between these diseases as could differences in temporal or spatial levels of transcripts containing the expanded repeats. A possible mechanism for congenital DM1 has been suggested by Fillipova and colleagues, who showed that methylation at the *DM1* locus in congenital cases can increase expression of *DMPK,* resulting in higher levels of CUG-containing transcripts and the more severe congenital phenotype (Filippova et al. 2001). Alternatively, the differences between DM1 and DM2 could involve locus-specific genes such as *DMPK*, *SIX5,* or *DMWD* for DM1, and *ZNF9* for DM2.

Dominant RNA Mechanism: Specific Targets of Abnormal Pre-mRNA Splicing

Evidence that a gain-of-function alteration at the RNA level plays a role in DM1 pathogenesis includes (i) the observation that an expanded repeat in the 3′ UTR of *DMPK* mRNA inhibits myoblast differentiation (Amack et al. 1999); (ii) transgenic models in which >250 CTG repeats expressed at the RNA level cause myotonia and muscular dystrophy (Mankodi et al. 2000; Seznec et al.

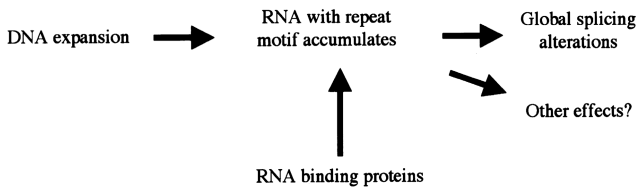

Figure 3 Schematic diagram of steps involved in RNA gain-of-function mechanism. CTG and CCTG expansions at the DM1 or DM2 loci result in the accumulation of CUG or CCUG repeat–containing transcripts as nuclear RNA foci. RNA-binding proteins, including CUG-BP and the muscleblind family of proteins bind to or are disregulated by the repeat-containing RNA transcripts resulting in specific *trans*-alterations in pre-mRNA splicing.

2001); (iii) CUG- and CCUG-containing transcripts that accumulate as RNA foci alter the regulation or localization of CUG-binding proteins, including CUG-BP (Timchenko et al. 1996*a*) and three different forms of muscleblind (Miller et al. 2000; Fardaei et al. 2002); (iv) altered CUG- and CCUG-induced splicing changes that are directly related to phenotypic features of DM. A recent report using cell-culture models suggests that CUG RNA expansions bind and sequester transcription factors in DM1 and may disrupt gene regulation including the chloride channel ClC-1 (Ebralidze et al. 2004), but the role of altered gene transcription in DM pathogenesis is not clear and will need further investigation.

The currently identified splicing alterations are described in further detail below.

Cardiac Troponin T (cTNT)

Initial efforts to understand how the *DM1* CUG expansion expressed at the RNA level could mediate a *trans*-dominant effect focused on the identification of RNA-binding proteins that could bind to CUG repeat motifs. The first of these proteins to be isolated and described in detail was CUG-binding protein (CUG-BP) (Timchenko et al. 1996*a*, 1996*b*). In a landmark discovery in 1998, Phillips and colleagues (1998) reported that CUG-BP, a conserved heterogeneous nuclear ribonucleoprotein, could mediate a *trans*-dominant effect of excess CUG repeat–containing RNA by disregulating the alternative splicing of cardiac troponin T (cTNT). CUG-BP was shown to bind to intronic CUG repeat sequences in human cardiac troponin T (cTNT) pre-mRNA, which are normally used as splicing signals. The presence of excess numbers of CUG repeats in transfection studies and in DM1 cardiac and skeletal muscle cultures cause the preferential inclusion of exon 5, which is a splice form produced during the early development of heart and skeletal muscle but not in adult heart. This typical splicing pattern of cTNT is disrupted in DM1 striated muscle and in transfected cells expressing either CUG-BP protein or elongated CUG-containing transcripts. This was the first demonstrated splicing target of CUG-BP and the first demonstration that the presence of elongated CUG repeat motifs lead to *trans* alterations in gene splicing (Philips et al. 1998).

Insulin Receptor (IR)

A classic clinical feature in both DM1 and DM2 patients is insulin resistance; the reduced insulin response in skeletal muscle predisposes DM patients to diabetes. Savkur et al. (2001) demonstrated that alternative splicing of the insulin receptor (IR) pre-mRNA is aberrantly regulated in DM1 skeletal muscle tissue, resulting in predominant expression of the insulin insensitive nonmuscle splice form lacking exon 11. Steady state levels of CUG-BP are increased in skeletal muscle of DM1 patients and disregulation of CUG-BP is thought to mediate an IR alternative splicing switch by the binding to an intronic element upstream of the alternatively spliced exon 11. These results support a model in which increased expression of a splicing regulator contributes to insulin resistance in DM1 by affecting IR alternative splicing (Savkur et al. 2001).

Muscle-Specific Chloride Channel

A classic feature of both DM1 and DM2 is myotonia, in which voluntary muscle contraction is followed by involuntary repetitive firing of action potentials that delay a patient's ability to relax muscle (Harper 2001). Using skeletal muscle from a transgenic mouse model of DM1, Mankodi and colleagues (2000, 2002) showed that expression of expanded CUG repeats in skeletal muscle reduces the transmembrane chloride conductance to levels consistent with those expected to cause myotonia. Additional studies determined that aberrant splicing of Clc-1, the main chloride channel in muscle, resulting in loss of Clc-1 protein from the surface membrane (Mankodi et al. 2002). Similar splicing alterations were observed in skeletal muscle from both DM1 and DM2 patients (Charlet et al. 2002; Mankodi et al. 2002). Charlet and colleagues (2002) demonstrated that CUG-BP, which is elevated in DM1 skeletal muscle, binds to the ClC-1 pre-mRNA, and overexpression of CUG-BP in transfected cells reproduces the aberrant pattern of ClC-1 splicing. Both groups propose that disruptions in alternative splicing regulation of ClC-1 causes a channelopathy and membrane hyperexcitability, leading to the classic DM feature of myotonia (Charlet et al. 2002; Mankodi et al. 2002).

Tau and Myotubularin

Splicing alterations in the microtubule-associated tau pre-mRNA have been observed in CNS tissue from patients with DM1 (Sergeant et al. 2001) and in a murine model described by Seznec and colleagues (2001), which may underlie various CNS alterations in both diseases. Also, muscle-specific changes in alternative splicing of the myotubularin-related 1 (*MTMR1*) gene are observed in congenital DM1 muscle cells in culture and in skeletal muscle samples from patients with congenital-onset DM1. These results suggest that MTMR1 plays a role in muscle formation and represents a novel target for abnormal mRNA splicing in myotonic dystrophy that may be responsible for the profound muscle atrophy of congenital DM1 (Buj-Bello et al. 2002).

Are RNA Foci Pathogenic or Protective?

Neurodegenerative diseases, such as Alzheimer disease and those caused by polyglutamine expansions, result in

protein aggregates or plaques and tangles that accumulate in affected tissues and have led investigators to debate their role in disease pathogenesis (Hsiao et al. 1995; Klement et al. 1998). Similar discussions in the DM field revolve around the role of RNA foci in disease pathogenesis. CUG-BP, the first RNA-binding protein with a demonstrated pathogenic effect, has been shown to bind to single-stranded CUG repeats. In contrast with the muscleblind family of proteins, CUG-BP does not colocalize with the nuclear RNA foci in DM1 and DM2. Although it appeared possible that both groups of proteins play a role in the pathogenic effects of DM, colocalization of a protein to the ribonuclear inclusions did not appear to be necessary to cause a pathogenic effect.

Direct evidence supporting the hypothesis that sequestration of muscleblind in CUG and CCUG containing RNA foci plays a central role in disease pathogenesis comes from a recently developed muscleblind knockout model of myotonic dystrophy. Kanadia and colleagues showed that disruption of the mouse *Mbnl1* gene leads to muscle, eye, and RNA-splicing abnormalities that are characteristic of DM1 and DM2 in humans (Kanadia et al. 2003).

Data now indicate that overexpression of CUG-BP and depletion of Mbnl1 both can cause specific alternative-splicing changes. Previous studies have reported that expression of elongated CUG-containing transcripts result in increased levels of CUG-BP through an unknown mechanism. In contrast, the sequestration of Mbnl1 in CUG and CCUG RNA foci has been proposed as a mechanism that results in Mbnl1 depletion. It now appears that both of these proteins play a role in affecting alternative splicing and that, in the case of ClC1, changes through antagonistic effects in which CUG-BP promotes the inclusion of exons that are normally favored during fetal development, whereas the normal function of Mbnl1 is to favor splice forms expressed in adults. These data predict that overexpression of CUG-BP or depletion of Mbnl1 would both result in the splicing alterations and that changes in the regulation of either these proteins can cause downstream splicing changes that have now been shown to be characteristic of the myotonic dystrophies.

Other Noncoding Disorders

Although the striking clinical and molecular parallels between DM1 and DM2 demonstrate that RNA pathogenesis plays a much broader role in the multisystemic features of myotonic dystrophy than previously suspected, noncoding microsatellite expansions for *SCA8* (Koob et al. 1999) and *SCA10* (Matsuura et al. 2000) were identified before the *DM2* expansion. The pathogenic mechanism of the *DM1* and *DM2* expansions are evident only because they cause such a distinctive phenotype. Molecular parallels between the SCA8 CUG repeat and the *DM1* and *DM2* repeats suggest the possibility that the known toxic properties of transcripts with elongated CUGs underlie the cerebellar degeneration in SCA8 (Ranum and Day 2002). Although, in SCA10, it is possible that the enormous ATTCT expansion in an intron of a gene may cause disease through haploinsufficiency, the dominant inheritance pattern and the fact the expression of the SCA10 transcript does not appear to be reduced in affected individuals (Matsuura et al. 2000) make this potential mechanism less likely. Parallels between SCA10 and the RNA mechanism involved in DM1, DM2, and possibly SCA8 suggest a gain-of-function mechanism at the RNA level could be involved. Unlike SCA8, the gene that harbors the mutation for SCA10 is ubiquitously expressed, indicating that, if a toxic RNA mechanism is involved, secondary proteins that interact with the AUUCU repeat motif may confer organ-specific pathogenicity (Matsuura et al. 2000). FMR premutation alleles that have recently been reported to be associated with a tremor-ataxia syndrome may involve a similar RNA mechanism (Brunberg et al. 2002; Aziz et al. 2003; Jacquemont et al. 2003).

Conclusions

The clinical and molecular parallels of DM1 and DM2 demonstrate the multisystemic effects of CUG and CCUG expansions. A detailed description of *trans*-dominant splicing alterations of genes relevant to the DM phenotype is emerging, providing a convincing model of how these RNA expansions cause myotonic dystrophy. In other microsatellite-expansion diseases, including SCA8 and SCA10, transcribed noncoding expansions cause very different CNS phenotypes, suggesting that dominant RNA mechanisms may be a general cause of disease.

Electronic-Database Information

The URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/

References

Amack JD, Paguio AP, Mahadevan MS (1999) *Cis* and *trans* effects of the myotonic dystrophy (DM) mutation in a cell culture model. Hum Mol Genet 8:1975–1984

Aziz M, Stathopulu E, Callias M, Taylor C, Turk J, Oostra B, Willemsen R, Patton M (2003) Clinical features of boys with fragile X premutations and intermediate alleles. Am J Med Genet 121:119–127

Bachinski LL, Udd B, Meola G, Sansone V, Bassez G, Eymard B, Thornton CA, Moxley RT, Harper PS, Rogers MT, Jurkat-Rott K, Lehmann-Horn F, Wieser T, Gamez J, Navarro

C, Bottani A, Kohler A, Shriver MD, Sallinen R, Wessman M, Zhang S, Wright FA, Krahe R (2003) Confirmation of the type 2 myotonic dystrophy (CCTG)*n* expansion mutation in patients with proximal myotonic myopathy/proximal myotonic dystrophy of different European origins: a single shared haplotype indicates an ancestral founder effect. Am J Hum Genet 73:835–848

Batten F, Gibb H (1909) Myotonia atrophica. Brain 32:187–205

Boucher CA, King SK, Carey N, Krahe R, Winchester CL, Rahman S, Creavin T, Meghji P, Bailey MES, Chartier FL, Brown SD, Siciliano MJ, Johnson KJ (1995) A novel homeodomain-encoding gene is associated with a large CpG island interrupted by the myotonic dystrophy unstable (CTG)*n* repeat. Hum Mol Genet 4:1919–1925

Brook JD, McCurrah ME, Harley HG, Buckler AJ, Church D, Aburatani H, Hunter K, Stanton VP, Thirion J-P, Hudson T, Sohn R, Zemelman B, Snell RG, Rundle SA, Crow S, Davies J, Shelbourne P, Buxton J, Jones C, Juvonen V, Johnson K, Harper PS, Shaw DJ, Housman DE (1992) Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member. Cell 68:799–808

Brunberg JA, Jacquemont S, Hagerman RJ, Berry-Kravis EM, Grigsby J, Leehey MA, Tassone F, Brown WT, Greco CM, Hagerman PJ (2002) Fragile X premutation carriers: characteristic MR imaging findings of adult male patients with progressive cerebellar and cognitive dysfunction. Am J Neuroradiol 23:1757–66

Buj-Bello A, Laugel V, Messaddeq N, Zahreddine H, Laporte J, Pellissier JF, Mandel JL (2002) The lipid phosphatase myotubularin is essential for skeletal muscle maintenance but not for myogenesis in mice. Proc Natl Acad Sci USA 99: 15060–15065

Bungener C, Jouvent R, Delaporte C (1998) Psychopathological and emotional deficits in myotonic dystrophy. J Neurol Neurosurg Psychiatry 65:353–356

Buxton J, Shelbourne P, Davies J, Jones C, Van Tongeren T, Aslanidis C, de Jong P, Jansen G, Anvret M, Riley B, Williamson R, Johnson K (1992) Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy. Nature 355:547–548

Chakraborty R, Stivers DN, Deka R, Yu LM, Shriver MD, Ferrell RE (1996) Segregation distortion of the CTG repeats at the myotonic dystrophy locus. Am J Hum Genet 59:109–118

Charlet BN, Savkur RS, Singh G, Philips AV, Grice EA, Cooper TA (2002) Loss of the muscle-specific chloride channel in type 1 myotonic dystrophy due to misregulated alternative splicing. Mol Cell 10:45–53

Chung M-y, Ranum LPW, Duvick LA, Servadio A, Zoghbi HY, Orr HT (1993) Evidence for a mechanism predisposing to intergenerational CAG repeat instability in spinocerebellar ataxia type 1. Nat Genet 5:254–258

Colleran JA, Hawley RJ, Pinnow EE, Kokkinos PF, Fletcher RD (1997) Value of the electrocardiogram in determining cardiac events and mortality in myotonic dystrophy. Am J Cardiol 80:1494–1497

Davis BM, McCurrach ME, Taneja KL, Singer RH, Housman DE (1997) Expansion of a CUG trinucleotide repeat in the 3′ untranslated region of myotonic dystrophy protein kinase transcripts results in nuclear retention of transcripts. Proc Natl Acad Sci USA 94:7388–7393

Day JW, Ricker K, Jacobsen JF, Rasmussen LJ, Dick KA, Kress W, Schneider C, Koch MC, Beilman GJ, Harrison AR, Dalton JC, Ranum LPW (2003) Myotonic dystrophy type 2: molecular, diagnostic and clinical spectrum. Neurology 60: 657–664

Day JW, Roelofs R, Leroy B, Pech I, Benzow K, Ranum LPW (1999) Clinical and genetic characteristics of a five-generation family with a novel form of myotonic dystrophy (DM2). Neuromuscul Disord 9:19–27

Delaporte C (1998) Personality patterns in patients with myotonic dystrophy. Arch Neurol 55:635–640

Ebralidze A, Wang Y, Petkova V, Ebralidse K, Junghans RP (2004) RNA leaching of transcription factors disrupts transcription in myotonic dystrophy. Science 303:383–387

Fardaei M, Rogers MT, Thorpe HM, Larkin K, Hamshere MG, Harper PS, Brook JD (2002) Three proteins, MBNL, MBLL and MBXL, co-localize in vivo with nuclear foci of expanded-repeat transcripts in DM1 and DM2 cells. Hum Mol Genet 11:805–814

Filippova GN, Thienes CP, Penn BH, Cho DH, Hu YJ, Moore JM, Klesert TR, Lobanenkov VV, Tapscott SJ (2001) CTCF-binding sites flank CTG/CAG repeats and form a methylation-sensitive insulator at the DM1 locus. Nat Genet 28: 335–343

Fu Y-H, Pizzuti A, Fenwick RGJ, King J, Rajnarayan S, Dunne PW, Dubel J, Nasser GA, Ashizawa T, De Jong P, Wieringa B, Korneluk R, Perryman MB, Epstein HF, Caskey CT (1992) An unstable triplet repeat in a gene related to myotonic muscular dystrophy. Science 255:1256–1258

Groenen P, Wieringa B (1998) Expanding complexity in myotonic dystrophy. Bioessays 20:901–912

Gunter C, Paradee W, Crawford DC, Meadows KA, Newman J, Kunst CB, Nelson DL, Schwartz C, Murray A, Macpherson JN, Sherman SL, Warren ST (1998) Re-examination of factors associated with expansion of CGG repeats using a single nucleotide polymorphism in FMR1. Hum Mol Genet 7:1935–1946

Hamshere MG, Newman EE, Alwazzan M, Athwal BS, Brook JD (1997) Transcriptional abnormality in myotonic dystrophy affects DMPK but not neighboring genes. Proc Natl Acad Sci USA 94:7394–7399

Hamshere MG, Harley H, Harper P, Brook JD, Brookfield JF (1999) Myotonic dystrophy: the correlation of (CTG) repeat length in leucocytes with age at onset is significant only for patients with small expansions. J Med Genet 36:59–61

Harley HG, Brook JD, Rundle SA, Crow S, Reardon W, Buckler AJ, Harper PS, Houseman DE, Shaw D (1992) Expansion of an unstable DNA region and phenotypic variation in myotonic dystrophy. Nature 355:545–546

Harper PS (2001) Myotonic dystrophy. Vol 37. W. B. Saunders, London

Hoffmann-Radvanyi H, Lavedan C, Rabes JP, Savoy D, Duros C, Johnson K, Junien C (1993) Myotonic dystrophy: absence of CTG enlarged transcript in congenital forms, and low expression of the normal allele. Hum Mol Genet 2:1263–1266

Hsiao KK, Borchelt DR, Olson K, Johannsdottir R, Kitt C, Yunis W, Xu S, Eckman C, Younkin S, Price D, Iadecola C, Clark HB, Carlson G (1995) Age-related CNS disorder and

early death in transgenic FVB/N mice overexpressing Alzheimer amyloid precursor proteins. Neuron 15:1203–1218

Hund E, Jansen O, Koch M, Ricker K, Fogel W, Niedermaier N, Otto M, Kuhn E, Meinck H (1997) Proximal myotonic myopathy with MRI white matter abnormalities of the brain. Neurology 48:33–37

IDMC (2000) New nomenclature and DNA testing guidelines for myotonic dystrophy type 1 (DM1). Neurology 54:1218–1221

Imbert G, Kretz C, Johnson K, Mandel JL (1993) Origin of the expansion mutation in myotonic dystrophy. Nat Genet 4:72–76

Jacquemont S, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, Greco C, Des Portes V, Jardini T, Levine R, Berry-Kravis E, Brown WT, Schaeffer S, Kissel J, Tassone F, Hagerman PJ (2003) Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. Am J Hum Genet 72:869–878

Jansen G, Bachner D, Coerwinkel M, Wormskamp N, Hameister H, Wieringa B (1995) Structural organization and developmental expression pattern of the mouse WD-repeat gene DMR-N9 immediately upstream of the myotonic dystrophy locus. Hum Mol Genet 4:843–852

Jansen G, Groenen PJTA, Bachner D, Jap PHK, Coerwinkel M, Oerlemans F, van den Broek W, Gohlsch B, Pette D, Plomp JJ, Molenaar PC, Nederhof MGJ, van Echted CJA, Dekker M, Berns A, Hameister H, Wieringa B (1996) Abnormal myotonic dystrophy protein kinase levels produce only mild myopathy in mice. Nat Genet 13:316–324

Junghans RP, Ebralidze A, Tiwari B (2001) Does (CUG)*n* repeat in DMPK mRNA "paint" chromosome 19 to suppress distant genes to create the diverse phenotype of myotonic dystrophy? A new hypothesis of long-range *cis* autosomal inactivation. Neurogenetics 3:59–67

Kanadia RN, Johnstone KA, Mankodi A, Lungu C, Thornton CA, Esson D, Timmers AM, Hauswirth WW, Swanson MS (2003) A muscleblind knockout model for myotonic dystrophy. Science 302:1978–1980

Klement IA, Skinner PJ, Kaytor MD, Yi H, Hersch SM, Clark HB, Zoghbi HY, Orr HT (1998) Ataxin-1 nuclear localization and aggregation: role in polyglutamine-induced disease in SCA1 transgenic mice. Cell 95:41–53

Klesert TR, Otten AD, Bird TD, Tapscott SJ (1997) Trinucleotide repeat expansion at the myotonic dystrophy locus reduces expression of DMAHP. Nat Genet 16:402–406

Klesert TR, Cho DH, Clark JI, Maylie J, Adelman J, Snider L, Yuen EC, Soriano P, Tapscott SJ (2000) Mice deficient in Six5 develop cataracts: implications for myotonic dystrophy. Nat Genet 25:105–109

Koob MD, Moseley ML, Schut LJ, Benzow KA, Bird TD, Day JW, Ranum LPW (1999) An untranslated CTG expansion causes a novel form of spinocerebellar ataxia (SCA8). Nat Genet 21:379–384

Krahe R, Ashizawa T, Abbruzzese C, Roeder E, Carango P, Giacanelli M, Funanage VL, Siciliano MJ (1995) Effect of myotonic dystrophy trinucleotide repeat expansion on DMPK transcription and processing. Genomics 28:1–14

Kunst CB, Warren ST (1994) Cryptic and polar variation of the fragile X repeat could result in predisposing normal alleles. Cell 77:853–861

Larkin K, Fardaei M (2001) Myotonic dystrophy-a multigene disorder. Brain Res Bull 56:389–395

Lazarus A, Varin J, Ounnoughene Z, Radvanyi H, Junien C, Coste J, Laforet P, Eymard B, Becane HM, Weber S, Duboc D (1999) Relationships among electrophysiological findings and clinical status, heart function, and extent of DNA mutation in myotonic dystrophy. Circulation 99:1041–1046

Liquori C, Ricker K, Moseley ML, Jacobsen JF, Kress W, Naylor S, Day JW, Ranum LPW (2001) Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. Science 293:864–867

Liquori CL, Ikeda Y, Weatherspoon M, Ricker K, Schoser BG, Dalton JC, Day JW, Ranum LPW (2003) Myotonic dystrophy type 2: human founder haplotype and evolutionary conservation of the repeat tract. Am J Hum Genet 73:849–862

Lu X, Timchenko NA, Timchenko LT (1999) Cardiac elav-type RNA-binding protein (ETR-3) binds to RNA CUG repeats expanded in myotonic dystrophy. Hum Mol Genet 8:53–60

Mahadevan M, Tsilfidis C, Sabourin L, Shutler G, Amemiya C, Jansen G, Neville C, Narang M, Barcelo J, O'Hoy K, Leblond S, Earle-MacDonald J, De Jong PJ, Wieringa B, Koneluk RG (1992) Myotonic dystrophy mutation: an unstable CTG repeat in the 3′ untranslated region of the gene. Science 255:1253–1255

Mankodi A, Logigian E, Callahan L, McClain C, White R, Henderson D, Krym M, Thornton CA (2000) Myotonic dystrophy in transgenic mice expressing an expanded CUG repeat. Science 289:1769–1773

Mankodi A, Takahashi MP, Jiang H, Beck CL, Bowers WJ, Moxley RT, Cannon SC, Thornton CA (2002) Expanded CUG repeats trigger aberrant splicing of ClC-1 chloride channel pre-mRNA and hyperexcitability of skeletal muscle in myotonic dystrophy. Mol Cell 10:35–44

Mankodi A, Urbinati CR, Yuan QP, Moxley RT, Sansone V, Krym M, Henderson D, Schalling M, Swanson MS, Thornton CA (2001) Muscleblind localizes to nuclear foci of aberrant RNA in myotonic dystrophy types 1 and 2. Hum Mol Genet 10:2165–2170

Martorell L, Monckton DG, Gamez J, Johnson KJ, Gich I, de Munain AL, Baiget M (1998) Progression of somatic CTG repeat length heterogeneity in the blood cells of myotonic dystrophy patients. Hum Mol Genet 7:307–312

Mathieu J, Allard P, Potvin L, Prevost C, Begin P (1999) A 10-year study of mortality in a cohort of patients with myotonic dystrophy. Neurology 52:1658–1662

Matsuura T, Yamagata T, Burgess DL, Rasmussen A, Grewal RP, Watase K, Khajavi M, McCall AE, Davis CF, Zu L, Achari M, Pulst SM, Alonso E, Noebels JL, Nelson DL, Zoghbi HY, Ashizawa T (2000) Large expansion of the ATTCT pentanucleotide repeat in spinocerebellar ataxia type 10. Nat Genet 26:191–194

Merino JL, Carmona JR, Fernandez-Lozano I, Peinado R, Basterra N, Sobrino JA (1998) Mechanisms of sustained ventricular tachycardia in myotonic dystrophy: implications for catheter ablation. Circulation 98:541–546

Miller JW, Urbinati CR, Teng-Umnuay P, Stenberg MG, Byrne BJ, Thornton CA, Swanson MS (2000) Recruitment of human muscleblind proteins to (CUG)(*n*) expansions associated with myotonic dystrophy. Embo J 19:4439–4448

Monckton DG, Ashizawa T, Siciliano MJ (1998) Murine models for myotonic dystrophy. In: Wells RD, Warren ST (eds) Genetic instabilities and hereditary neurological diseases. Academic Press, San Diego, pp 181–193

Monckton DG, Wong L-JC, Ashizawa T, Caskey CT (1995) Somatic mosaicism, germline expansions, germline reversions and intergenerational reductions in myotonic dystrophy males: small pool PCR analyses. Hum Mol Genet 4:1–8

Moxley R (1996) Proximal myotonic myopathy: mini-review of a recently delineated clinical disorder. Neuromuscul Disord 6:87–93

Neville C, Mahadevan M, Barcelo J, Korneluk RG (1994) High resolution genetic analysis suggests one ancestral predisposing haplotype for the origin of the myotonic dystrophy mutation. Hum Mol Genet 3:45–51

Nguyen HH, Wolfe JT 3rd, Holmes DR Jr, Edwards WD (1988) Pathology of the cardiac conduction system in myotonic dystrophy: a study of 12 cases. J Am Coll Cardiol 11:662–671

Novelli G, Gennarelli M, Zelano G, Pizzuti A, Fattorini C, Caskey CT, Dallapiccola B (1993) Failure in detecting mRNA transcripts from the mutated allele in myotonic dystrophy muscle. Biochem Mol Biol Int 29:291–297

Ogata A, Terae S, Fujita M, Tashiro K (1998) Anterior temporal white matter lesions in myotonic dystrophy with intellectual impairment: an MRI and neuropathological study. Neuroradiology 40:411–415

Ono S, Takahashi K, Jinnai K, Kanda F, Fukuoka Y, Kurisaki H, Mitake S, Inagaki T, Yamano T, Nagao K (1998) Loss of serotonin-containing neurons in the raphe of patients with myotonic dystrophy: a quantitative immunohistochemical study and relation to hypersomnia. Neurology 50:535–538

Otten AD, Tapscott SJ (1995) Triplet repeat expansion in myotonic dystrophy alters the adjacent chromatin structure. Proc Natl Acad Sci USA 92:5465–5469

Pellizzoni L, Lotti F, Maras B, Pierandrei-Amaldi P (1997) Cellular nucleic acid binding protein binds a conserved region of the 5′ UTR of *Xenopus laevis* ribosomal protein mRNAs. J Mol Biol 267:264–275

Pellizzoni L, Lotti F, Rutjes SA, Pierandrei-Amaldi P (1998) Involvement of the *Xenopus laevis* Ro60 autoantigen in the alternative interaction of La and CNBP proteins with the 5′ UTR of L4 ribosomal protein mRNA. J Mol Biol 281:593–608

Philips AV, Timchenko LT, Cooper TA (1998) Disruption of splicing regulated by a CUG-binding protein in myotonic dystrophy. Science 280:737–741

Pulst S-M, Nechiporuk A, Nechiporuk T, Gispert S, Chen X-N, Lopes-Cendes I, Pearlman S, Starkman S, Orozco-Diaz G, Lunkes A, DeJong P, Rouleau GA, Auburger G, Korenberg JR, Figueroa C, Sahba S (1996) Moderate expansion of a normally biallelic trinucleotide repeat in spinocerebellar ataxia type 2. Nat Genet 14:269–276

Ranum LPW, Rasmussen P, Benzow K, Koob M, Day J (1998) Genetic mapping of a second myotonic dystrophy locus. Nat Genet 19:196–198

Ranum LPW, Day JW (2002) Dominantly inherited, non-coding microsatellite expansion disorders. Curr Opin Genet Dev 12:266–271

Reddy S, Smith DB, Rich MM, Leferovich JM, Reilly P, Davis BM, Tran K, Rayburn H, Bronson R, Cros D, Balice-Gordon RJ, Housman D (1996) Mice lacking the myotonic dystrophy protein kinase develop a late onset progressive myopathy. Nat Genet 13:325–335

Ricker K (1999) Myotonic dystrophy and proximal myotonic myophathy. J Neurol 246:334–338

Ricker K, Grimm T, Koch MC, Schneider C, Kress W, Reimers CD, Schulte-Mattler W, Mueller-Myhsok B, Toyka KV, Mueller CR (1999) Linkage of proximal myotonic myopathy to chromosome 3q. Neurology 52:170–171

Ricker K, Koch MC, Lehmann-Horn F, Pongratz D, Otto M, Heine R, Moxley RT III (1994) Proximal myotonic myopathy: a new dominant disorder with myotonia, muscle weakness, and cataracts. Neurology 44:1448–1452

Rowland (1994) Thornton-Griggs-Moxley disease: myotonic dystrophy type 2. Ann Neurol 36:803–804

Rubinsztein JS, Rubinsztein DC, McKenna PJ, Goodburn S, Holland AJ (1997) Mild myotonic dystrophy is associated with memory impairment in the context of normal general intelligence. J Med Genet 34:229–233

Sarkar PS, Appukuttan B, Han J, Ito Y, Ai C, Tsai W, Chai Y, Stout JT, Reddy S (2000) Heterozygous loss of Six5 in mice is sufficient to cause ocular cataracts. Nat Genet 25:110–114

Savkur RS, Philips AV, Cooper TA (2001) Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. Nat Genet 29:40–47

Schneider C, Ziegler A, Ricker K, Grimm T, Kress W, Reimers CD, Meinck H, Reiners K, Toyka KV (2000) Proximal myotonic myopathy: evidence for anticipation in families with linkage to chromosome 3q. Neurology 55:383–388

Sergeant N, Sablonniere B, Schraen-Maschke S, Ghestem A, Maurage CA, Wattez A, Vermersch P, Delacourte A (2001) Dysregulation of human brain microtubule-associated tau mRNA maturation in myotonic dystrophy type 1. Hum Mol Genet 10:2143–2155

Seznec H, Agbulut O, Sergeant N, Savouret C, Ghestem A, Tabti N, Willer JC, Ourth L, Duros C, Brisson E, Fouquet C, Butler-Browne G, Delacourte A, Junien C, Gourdon G (2001) Mice transgenic for the human myotonic dystrophy region with expanded CTG repeats display muscular and brain abnormalities. Hum Mol Genet 10:2717–2726

Shaw DJ, McCurrach M, Rundle SA, Harley HG, Crow SR, Sohn R, Thirion J-P, Hamshere MG, Buckler AJ, Harper PS, Housman DE, Brook JD (1993) Genomic organisation and transcriptional units at the myotonic dystrophy locus. Genomics 18:673–679

Shimizu K, Chen W, Ashique AM, Moroi R, Li YP (2003) Molecular cloning, developmental expression, promoter analysis and functional characterization of the mouse CNBP gene. Gene 307:51–62

Steinert H (1909) Myopathologische Beitrage 1: Uber das klinischeund anatomische Bild des Muskelschwunds der Myotoniker. Dtsch Z Nervenheilkd 37:58–104

Taneja KL, McCurrach M, Schalling M, Housman D, Singer RH (1995) Foci of trinucleotide repeat transcripts in nuclei of myotonic dystrophy cells and tissues. J Cell Biol 128: 995–1002

Tapscott SJ (2000) Deconstructing myotonic dystrophy. Science 289:1701–1702

Thornton C (1999) The myotonic dystrophies. Semin Neurol 19:25–33

Thornton CA, Griggs RC, Moxley RT (1994) Myotonic dystrophy with no trinucleotide repeat expansion. Ann Neurol 35:269–272

Thornton CA, Wymer JP, Simmons Z, McClain C, Moxley RT 3rd (1997) Expansion of the myotonic dystrophy CTG repeat reduces expression of the flanking DMAHP gene. Nat Genet 16:407–409

Timchenko LT, Miller JW, Timchenko NA, DeVore DR, Datar KV, Lin L, Roberts R, Caskey CT, Swanson MS (1996*a*) Identification of a (CUG)*n* triplet repeat RNA-binding protein and its expression in myotonic dystrophy. Nucleic Acids Res 24:4407–4414

Timchenko LT, Timchenko NA, Caskey CT, Roberts R (1996b) Novel proteins with binding specificity for DNA CTG repeats and RNA CUG repeats: implications for myotonic dystrophy. Hum Mol Genet 5:115–1121

Udd B, Krahe R, Wallgren-Petterson C, Falck B, Kalimo H (1997) Proximal myotonic dystrophy—a family with autosomal dominant muscular dystrophy, cataracts, hearing loss and hypogonadism: heterogeneity of proximal myotonic syndromes? Neuromuscul Disord 7:217–228

Vihola A, Bassez G, Meola G, Zhang S, Haapasalo H, Paetau A, Mancinelli E, Rouche A, Hogrel JY, Laforet P, Maisonobe T, Pellissier JF, Krahe R, Eymard B, Udd B (2003) Histopathological differences of myotonic dystrophy type 1 (DM1) and PROMM/DM2. Neurology 60:1854–1857

Wang Y-H, Amirhaeri S, Kang S, Wells RD, Griffith JD (1994) Preferential nucleosome assembly at DNA triplet repeats from the myotonic dystrophy gene. Science 265:669–671

Wilson BA, Balleny H, Patterson K, Hodges JR (1999) Myotonic dystrophy and progressive cognitive decline: a common condition or two separate problems? Cortex 35:113–121