# Getting a grip on myotonic dystrophy type 1

# Getting a grip on myotonic dystrophy type 1

This research project was financially supported
by a grant from the Prinses Beatrix Spierfonds.



Lay-out: Jos Hendrix
Cover photo: Wat Rong Khun, Chiang Rai, Thailand
Production: Universitaire Pers Maastricht
ISBN 978 94 6159 231 6

# Getting a grip on myotonic dystrophy type 1

**PROEFSCHRIFT**

ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
op gezag van de Rector Magnificus, Prof. dr. L.L.G. Soete
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op vrijdag 21 juni 2013 om 10.00 uur

door

**Mieke Catharina Elisabeth Hermans**

UNIVERSITAIRE PERS MAASTRICHT

**Promotor**

Prof. dr. Y.M. Pinto (Academisch Medisch Centrum, Amsterdam)

**Co-promotores**

Dr. C.G. Faber
Dr. I.S.J. Merkies

**Beoordelingscommissie**

Prof. dr. R.J. van Oostenbrugge, voorzitter
Dr. L.R.C. Dekker (Catharina Ziekenhuis, Eindhoven)
Prof. dr. B.G.M. van Engelen (UMC St. Radboud, Nijmegen)
Dr. A.J. van der Kooi (Academisch Medisch Centrum, Amsterdam)

# Table of contents

PART I

# Introduction

Chapter 1.1

# General introduction

## Myotonic dystrophy type 1

Myotonic dystrophy type 1 (DM1; OMIM #160900) is the most common type of muscular dystrophy in adults with a prevalence of about 5-20 per 100 000 in Western-Europe but higher prevalences have been found in certain populations due to founder effect and genetic isolation.[1] It was identified as a distinct disorder in 1909 by Steinert in Germany and by Batten and Gibb in the UK, but it took almost a century to delineate its main clinical features.[2] The most noticeable symptoms are myotonia, i.e. inability to relax the voluntary muscles after activity, and progressive weakness of facial and distal muscles. Along with these neuromuscular features, multiple systemic manifestations are seen (table 1.1).

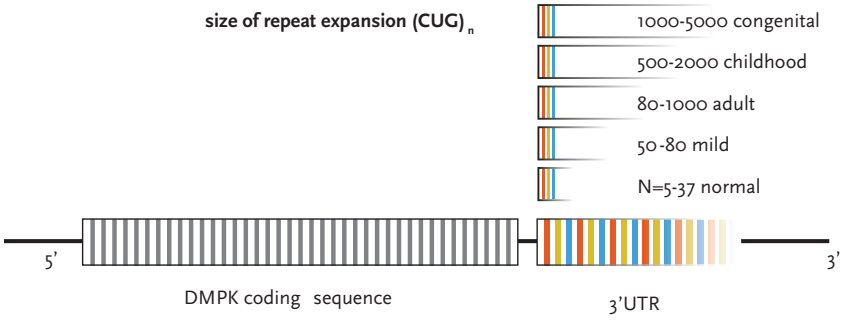
**Table 1.1.** Systemic involvement in myotonic dystrophy type 1

| System | Principal involvement |
|---|---|
| Cardiovascular | Conduction disorders<br>Cardiomyopathy<br>Low arterial blood pressure<br>Vasomotor symptoms |
| Lungs | Aspiration<br>Pneumonia<br>Alveolar hypoventilation |
| Smooth muscle | Gastro-intestinal tract (pharynx, oesophagus, stomach, colon, anal sphincter)<br>Urinary bladder<br>Uterus<br>Gall bladder |
| Eye | Cataract<br>Retinal degeneration<br>Ptosis |
| Brain | Variable mental retardation (congenital and childhood-onset type)<br>Hypersomnia<br>Apathy<br>Increased ventricular size (congenital type)<br>Increased focal hyperintense lesions on T2-MRI brain imaging |
| Endocrine | Testicular atrophy<br>Insuline resistance |
| Skin | Early balding<br>Pilomatrixomata |
| Skeletal | Cranial hyperostosis<br>Jaw and palate abnormalities<br>Talipes (congenital and childhood-onset type) |
| Other | Anesthetic complications |

## Genetics

A single mutation in the 3'untranslated region of the dystrophia myotonica protein kinase (DMPK) gene on chromosome 19q13.3 was identified in 1992.[3-5] A repetitive trinucleotide segment (CTG) is abnormally expanded, forming an unstable region. Unaffected individuals have between 5 and 37 copies of the trinucleotide sequence. Repeat lengths of 38-50 are considered premutation alleles, whereas DM1 patients have at least 50 repeats. Apparent new mutations result from healthy individuals with an increased repeat number that is insufficient to cause symptoms, but are unstable during germline transmission and can result in disease in the offspring. A larger repeat expansion leads to an earlier onset and more severe phenotype (figure 1.1).[6] This causes anticipation, i.e. an earlier age of onset and increased severity in subsequent generations.[7] However, analysis of CTG repeats from lymphocytes may be of limited value in individual patients because of the large overlap between expansion sizes seen in different phenotypes. Other genotype-phenotype correlations are unclear, probably because of differing levels of instability of mutant alleles in various somatic tissues.[8]

**Figure 1.1.** DMPK pre-mRNA with relationship between CUG repeat size and phenotype




## The clinical picture

Patients with DM1 can present with 4 different phenotypes: congenital type; childhood-onset type; adult-onset (classical) type, and late-onset (mild) type.[6]

**Late-onset type** has its onset after 50 years marked by cataract, mild muscle weakness and myotonia. Disease course is generally benign, and life expectancy is probably within the normal range.

**Adult-onset type** is the most prevalent form and manifests between 10 and 60 years. It is characterized by myotonia and muscle weakness. Myotonia is experienced predominantly in the hands and the tongue. Repeated contractions may diminish myotonia (warm-up phenomenon). The pattern

of muscle involvement includes facial weakness, involvement of anterior neck muscles, and distal weakness of the limbs. Weakness and wasting of facial, levator palpebrae and masticatory muscles lead to ptosis and a typical myopathic appearance (figure 1.2). As the disease progresses more general muscle weakness occurs. Axonal neuropathy is usually subclinical, but may contribute to muscle weakness in DM1.[9] Patients at an advanced state of muscle weakness are mostly able to maintain a limited mobility within the house, but half of them become partly or completely wheelchair bound.[10] Multi-organ symptoms, such as cataracts, cardiac involvement, sleep related breathing disorders, daytime sleepiness, apathy and avoidant personality features, early frontal balding in men and gastro-intestinal complaints develop at some time in their illness and may be of considerably greater importance to patients than the muscular aspects. Median survival is 59-60 years for adult-onset type patients.[10] Progressive respiratory failure is the leading cause of mortality, followed by death from cardiac causes in 20-30%.[10-12]

**Childhood-onset type** presents between 1 and 12 years of age and is characterized by dysarthria, learning difficulties, psychosocial problems and sometimes delayed motor development.[13] Swallowing difficulties may be present and articulation is often poor, due to weakness of the bulbar musculature. Intelligence is commonly in the lower normal range (IQ> 70-100) or mild mental retardation (IQ 50-70).[14, 15] Attention deficit problems seem to be a frequent problem in the population of children with DM1. Muscular weakness gradually progresses with age and patients finally exhibit the multi-system features of adult-onset type disease. Survival is lower in childhood-onset phenotype than in the adult-onset type of the disease, with a mean age at death of 44.7 years.[11]

**Congenital type** is characterized by hypotonia, bilateral facial weakness, respiratory deficiency, feeding and swallowing difficulties, talipes and other contractures in the neonatal period. Mortality during the neonatal period has been estimated at 25% of patients.[16] Patients who survive infancy are mentally retarded with total IQ ranging from 40-69 (median 54) and require special needs schooling.[17] Children with congenital type DM1 are able to walk. Progressive muscle weakness and myotonia become present over the age of 10 years. Median survival is 35 years for the congenital type. A child with congenital DM1 inherits the mutation from the mother, with very few exceptions.

**Cardiac involvement**

Involvement of the heart is one of the main systemic features of DM1 affecting a majority of patients. It was first recognized by Griffith in 1911, who noted extreme bradycardia in a DM1 patient. However, few patients had cardiac symptoms and it was not until the routine implementation of electrocardiography (ECG) that cardiac involvement was found to be frequent.[1]

**Figure 1.2.** Variation of facial appearance in DM1

**Legend to figure 1.2.** Left: mother with adult-onset type and daughter with childhood-onset type DM1. Both show weakness and atrophy of the facial muscles. Reprinted from Ned Tijdschr Geneeskd. 2005 Oct 8;149(41):2316. Right: typical inverted V-shaped upper lip in congenital type DM1.

Cardiac tissue is affected by interstitial fibrosis and fatty infiltration.[18] This degenerative process results in progressive failure of the conduction system. Cardiac involvement initially manifests as asymptomatic ECG abnormalities, commonly prolongation of the PR interval and QRS duration. Intracardiac electrophysiological studies revealed abnormalities in all areas of the conduction system, most commonly in the His-Purkinje system.[19] Arrhythmias can occur, including progressive heart block, atrial tachycardia, flutter, or fibrillation, and ventricular tachycardia or fibrillation.[20] Less commonly, myocardial involvement leads to left ventricular systolic dysfunction and clinically overt heart failure. Involvement of the heart is a major prognostic factor, since it is responsible for one third of deaths in patients with DM1.[10, 11] In those with cardiac death, the rate of sudden death (84.6%) seems disproportionately greater than the rate of progressive heart failure (15.4%) when compared to other more standard heart failure populations.[21] Progression to complete atrioventricular block or development of ventricular tachyarrhythmia is believed to be the cause of sudden death in these patients. However, it remains a challenge to identify patients who may benefit from primary prevention with implantable pacemakers or cardioverter-defibrillators.

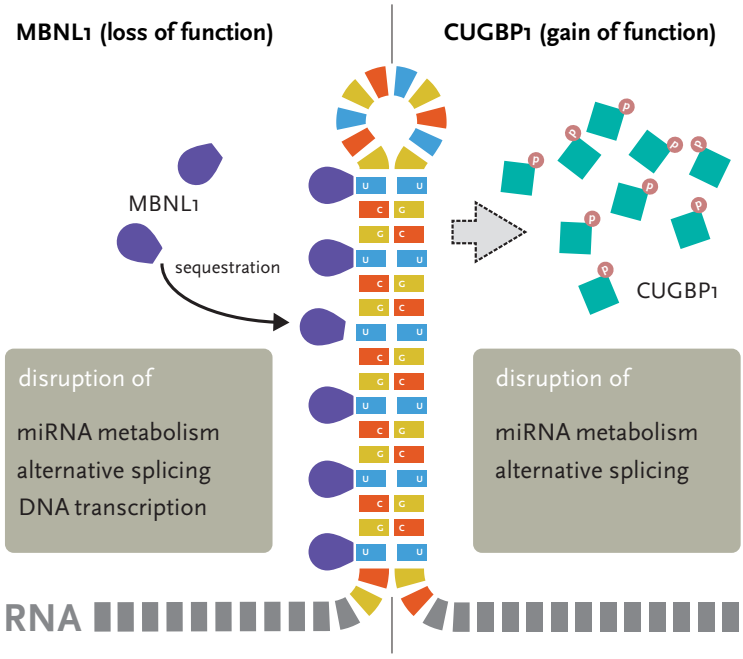
## Pathogenic mechanisms

Unlike most dominant inherited disorders, the expanded mutation in the DMPK gene does not produce dysfunctional proteins since the CTG triplet repeat lies outside the gene's open reading frame, but results in non-coding RNA transcripts. The underlying molecular mechanisms have not been completely

unravelled, but several hypotheses have been proposed and tested.
Expansion of the CTG repeat reduces the expression of DMPK itself.[22] Mutant transcripts accumulate in the nucleus,[23, 24] and presumably result in a depletion of mRNA in the cytoplasm and reduction of DMPK protein levels. Deficiency of the protein kinase may play a role in cardiac features since knockout mice show atrioventricular conduction delay and progression of conduction block with age, similar to the human phenotype.[25-27] However, histopathologic cardiac evaluation showed no degeneration of conduction fibres or myocardium in these mice.[28] Furthermore, myopathy is mild and other multisystem clinical features are absent in knockout mice.[29, 30] In conclusion, mouse DMPK knockout models failed to reproduce the major features of DM1.
Another proposed mechanism is a decrease in expression of neighbouring genes through a change in chromatin structure caused by the repeat expansion. SIX homeobox 5 (SIX5) is located immediately downstream[31] and is expressed in various tissues, including muscle, heart and eye lens.[32] SIX5-/- and SIX5+/- mice developed cataracts, but no myopathy or myotonia.[33-35] Heterozygous mice showed subtle intraventricular conduction delay and some enlargement of the left ventricle.[35] Another neighbouring gene located upstream is dystrophia myotonica-containing WD repeat (DMWD). It is highly expressed in testis and brain and proposed to play a part in mental retardation and male infertility in DM1, but there is no model to prove this hypothesis.[36]
There is strong evidence that the mutant RNA transcribed from the expanded allele plays a major role in myotonic dystrophy. In 1998, a second type of myotonic dystrophy (DM2) was identified resulting from a CCTG expansion within intron 1 of the zinc finger 9 (ZNF9) gene on chromosome 3.[37] DM2 patients have features resembling those in adult-onset DM1. The fact that repeat sequences localized on two different genomic loci with no apparent functional relationship cause similar clinical symptoms, suggests a common pathogenic mechanism involving RNA gain-of-function. RNA transcripts of the mutant allele accumulate in nuclear foci and interfere with *trans*-acting RNA-binding proteins CUG-binding protein 1 (CUGBP1) and muscleblind-like 1 (MBNL1) activity (figure 1.3). In DM1, MBNL1 is sequestered in ribonuclear foci in the nucleus resulting in decreased activity levels, while nuclear and cytoplasmic CUGBP1 levels are upregulated by hyperphosphorylation via different signalling pathways.[38] CUGBP1 and MBNL1 function as splice factors and altered activities of these regulatory proteins deregulate alternative splicing of a subset of developmentally regulated genes in multiple tissues, leading to inappropriate expression of embryonic splicing isoforms in adult tissues.[39] For example, CLCN1 chloride channel misplicing in skeletal muscles leads to a non-functional channel, which results in myotonia,[40] whereas abnormal splicing of the insulin receptor (INSR) might contribute to insulin resistance[41].

Loss of function of MBNL1 (and other members of the MBNL protein family) and CUGBP-1 up-regulation deregulates not only alternative splicing, but also transcription, miRNA metabolism and translation efficiency (figure 1.3).[42] Sequestration of transcription factors and other nuclear factors may contribute to deregulation of gene expression.

**Figure 1.3.** Molecular pathogenesis of DM1




**Legend to figure 1.3.** The pathogenic consequences of an expanded number of CUG repeats in the DMPK RNA include the sequestration of muscleblind-like protein 1 (MBNL1) into RNA foci and hyperphosphorylation and up-regulation of CUG triplet repeat RNA-binding protein 1 (CUGBP1), which leads to deregulation of global splicing events and other pathways in nuclei and cytoplasm of cells.

Transgenic mouse models have recreated many molecular and phenotypic disease features, pointing to an RNA-mediated disease mechanism. Overexpression of untranslated CUG repeats in the skeletal muscle of $HSA^{LR}$ (long repeat length) transgenic mice showed myotonia, histologically defined myopathy, MBNL1 sequestration in nuclear foci and spliceopathy, but no muscle weakness or wasting.[43] $MBNL1^{-/-}$ knockout mice develop myopathy, cataracts and RNA splicing abnormalities.[44] Neither mouse model was sufficient to recreate all muscle abnormalities. Induced CUGBP1 overexpression in adult

skeletal muscle reproduces molecular, histopathologic and functional changes typical of DM1, suggesting that CUGBP1 has a major role in DM1 skeletal muscle pathogenesis.[45-47] A heart-specific mouse model expressing expanded CUG RNA exhibited systolic and diastolic dysfunction, PR prolongation, QRS widening and arrhythmias,[48] which could be reproduced by overexpression of CUGBP1 in hearts of adult animals.[49]

The underlying molecular mechanisms of myotonic dystrophy are likely to be exceedingly complex as the mutation can affect gene expression in multiple ways. Misregulation of alternative splicing plays a central role in the development of important DM1 symptoms, but major disease symptoms, such as muscle wasting and cardiac arrhythmias, cannot be readily explained by any of the splicing changes identified to date. Several mechanisms may have independent as well as overlapping downstream effects, leading to multiple symptoms of DM1.

## Therapeutic strategies

At present, treatment consists of symptomatic therapy and supportive care by a multidisciplinary team. Many professionals may be involved, including a neurologist and cardiologist, physical therapist, occupational therapist, speech therapist, specialist in rehabilitation medicine, pulmonologist, ophthalmologist, social worker and psychologist. Moderate intensity strength training or exercise programs can be recommended in all patients without an increased risk of damage to the muscles but there is insufficient evidence to conclude that it offers benefit.[50] Pharmacological interventions aimed at improving muscle strength and function (creatine, recombinant human insulin-like growth factor I and dehydroepiandrosterone) have been used with little success.[51-53] Mexiletine is used occasionally for treatment of myotonia.[54] Psychostimulants (e.g. modafinil, dexamphetamine or methylphenidate) are routinely applied in clinical practice to address excessive daytime sleepiness.[55] Although management of symptoms can help diminish complaints, improve daily life, and prolong survival, it fails to slow or halt disease progression.

Advances in understanding of the pathogenesis have led to experimental approaches with therapeutic potential aimed at reversing symptoms. Approaches targeting the mutant RNA transcript with the use of antisense oligonucleotides (AON) hold great prospect for therapeutic intervention in DM1. These AONs have been shown to reduce transcripts from the mutant DMPK allele in animal models, leading to a decrease in mutant RNA, as well as diminished ribonuclear foci and a reversal of selected splicing deficits.[56, 57]

Alternative approaches targeting downstream changes include up-regulation of MBNL activity,[58] reduction of CUGBP1 protein levels,[59] and reversal of specific aberrant splicing events.[60]

Recommendations for DM1 management are based more on expert consensus and clinical experience than on evidence from randomized controlled trials. Future trials evaluating the efficacy of interventions in a clinical setting require appropriate outcome measures in order to accurately detect clinically meaningful changes.

# References


1. Harper PS. Myotonic dystrophy. London: Saunders; 2001
2. Steinert H. Myopathologische beitrage. 1. Ueber das klinische und anatomische bild des muskelschwunds der myotoniker. *Dtsch Z Nervenheilkunde*. 1909;37:58-104
3. Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, Aburatani H, Hunter K, Stanton VP, Thirion JP, Hudson T, et al. 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*. 1992;69:385
4. Fu YH, Pizzuti A, Fenwick RG, Jr., King J, Rajnarayan S, Dunne PW, Dubel J, Nasser GA, Ashizawa T, de Jong P, et al. An unstable triplet repeat in a gene related to myotonic muscular dystrophy. *Science*. 1992;255:1256-1258
5. Mahadevan M, Tsilfidis C, Sabourin L, Shutler G, Amemiya C, Jansen G, Neville C, Narang M, Barcelo J, O'Hoy K, et al. Myotonic dystrophy mutation: An unstable ctg repeat in the 3' untranslated region of the gene. *Science*. 1992;255:1253-1255
6. Harley HG, Rundle SA, MacMillan JC, Myring J, Brook JD, Crow S, Reardon W, Fenton I, Shaw DJ, Harper PS. Size of the unstable ctg repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. *Am J Hum Genet*. 1993;52:1164-1174
7. Howeler CJ, Busch HF, Geraedts JP, Niermeijer MF, Staal A. Anticipation in myotonic dystrophy: Fact or fiction? *Brain*. 1989;112:779-797
8. Morales F, Couto JM, Higham CF, Hogg G, Cuenca P, Braida C, Wilson RH, Adam B, del Valle G, Brian R, Sittenfeld M, Ashizawa T, Wilcox A, Wilcox DE, Monckton DG. Somatic instability of the expanded ctg triplet repeat in myotonic dystrophy type 1 is a heritable quantitative trait and modifier of disease severity. *Hum Mol Genet*. 2012;21:3558-3567
9. Hermans MC, Faber CG, Vanhoutte EK, Bakkers M, De Baets MH, de Die-Smulders CE, Merkies IS. Peripheral neuropathy in myotonic dystrophy type 1. *J Peripher Nerv Syst*. 2011;16:24-29
10. de Die-Smulders CE, Howeler CJ, Thijs C, Mirandolle JF, Anten HB, Smeets HJ, Chandler KE, Geraedts JP. Age and causes of death in adult-onset myotonic dystrophy. *Brain*. 1998;121:1557-1563
11. Mathieu J, Allard P, Potvin L, Prevost C, Begin P. A 10-year study of mortality in a cohort of patients with myotonic dystrophy. *Neurology*. 1999;52:1658-1662
12. Groh WJ, Groh MR, Saha C, Kincaid JC, Simmons Z, Ciafaloni E, Pourmand R, Otten RF, Bhakta D, Nair GV, Marashdeh MM, Zipes DP, Pascuzzi RM. Electrocardiographic abnormalities and sudden death in myotonic dystrophy type 1. *N Engl J Med*. 2008;358:2688-2697
13. De Die-Smulders CE. Congenital and childhood-onset myotonic dystrophy. In: Harper PS, Van Engelen B, Eymard B, Wilcox D, eds. Myotonic dystrophy. Present management, future therapy. New York: Oxford University Press; 2004:162-175
14. Steyaert J, Umans S, Willekens D, Legius E, Pijkels E, de Die-Smulders C, Van den Berghe H, Fryns JP. A study of the cognitive and psychological profile in 16 children with congenital or juvenile myotonic dystrophy. *Clin Genet*. 1997;52:135-141
15. Angeard N, Gargiulo M, Jacquette A, Radvanyi H, Eymard B, Heron D. Cognitive profile in childhood myotonic dystrophy type 1: Is there a global impairment? *Neuromuscul Disord*. 2007;17:451-458
16. Campbell C, Sherlock R, Jacob P, Blayney M. Congenital myotonic dystrophy: Assisted ventilation duration and outcome. *Pediatrics*. 2004;113:811-816
17. Echenne B, Rideau A, Roubertie A, Sebire G, Rivier F, Lemieux B. Myotonic dystrophy type i in childhood long-term evolution in patients surviving the neonatal period. *Eur J Paediatr Neurol*. 2008;12:210-223
18. Nguyen HH, Wolfe JT, 3rd, Holmes DR, Jr., Edwards WD. Pathology of the cardiac conduction system in myotonic dystrophy: A study of 12 cases. *J Am Coll Cardiol*. 1988;11:662-671
19. Lazarus A, Varin J, Ounnoughene Z, Radvanyi H, Junien C, Coste J, Laforet P, Eymard B, Becane HM, Weber S, Duboc D. Relationships among electrophysiological findings and clinical status, heart function, and extent of DNA mutation in myotonic dystrophy. *Circulation*. 1999;99:1041-1046
20. Phillips MF, Harper PS. Cardiac disease in myotonic dystrophy. *Cardiovasc Res*. 1997;33:13-22

21. Bhakta D, Groh MR, Shen C, Pascuzzi RM, Groh WJ. Increased mortality with left ventricular systolic dysfunction and heart failure in adults with myotonic dystrophy type 1. *Am Heart J*. 2010;160:1137-1141, 1141 e1131
22. Carango P, Noble JE, Marks HG, Funanage VL. Absence of myotonic dystrophy protein kinase (dmpk) mrna as a result of a triplet repeat expansion in myotonic dystrophy. *Genomics*. 1993;18:340-348
23. Taneja KL, McCurrach M, Schalling M, Housman D, Singer RH. Foci of trinucleotide repeat transcripts in nuclei of myotonic dystrophy cells and tissues. *J Cell Biol*. 1995;128:995-1002
24. Davis BM, McCurrach ME, Taneja KL, Singer RH, Housman DE. 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 U S A*. 1997;94:7388-7393
25. Saba S, Vanderbrink BA, Luciano B, Aronovitz MJ, Berul CI, Reddy S, Housman D, Mendelsohn ME, Estes NA, 3rd, Wang PJ. Localization of the sites of conduction abnormalities in a mouse model of myotonic dystrophy. *J Cardiovasc Electrophysiol*. 1999;10:1214-1220
26. Berul CI, Maguire CT, Aronovitz MJ, Greenwood J, Miller C, Gehrmann J, Housman D, Mendelsohn ME, Reddy S. Dmpk dosage alterations result in atrioventricular conduction abnormalities in a mouse myotonic dystrophy model. *J Clin Invest*. 1999;103:R1-7
27. Berul CI, Maguire CT, Gehrmann J, Reddy S. Progressive atrioventricular conduction block in a mouse myotonic dystrophy model. *J Interv Card Electrophysiol*. 2000;4:351-358
28. Groh WJ. A transgenic model of myotonic dystrophy: Will the mouse roar? *J Cardiovasc Electrophysiol*. 1999;10:1221-1223
29. 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. Mice lacking the myotonic dystrophy protein kinase develop a late onset progressive myopathy. *Nat Genet*. 1996;13:325-335
30. Jansen G, Groenen PJ, Bachner D, Jap PH, Coerwinkel M, Oerlemans F, van den Broek W, Gohlsch B, Pette D, Plomp JJ, Molenaar PC, Nederhoff MG, van Echteld CJ, Dekker M, Berns A, Hameister H, Wieringa B. Abnormal myotonic dystrophy protein kinase levels produce only mild myopathy in mice. *Nat Genet*. 1996;13:316-324
31. Klesert TR, Otten AD, Bird TD, Tapscott SJ. Trinucleotide repeat expansion at the myotonic dystrophy locus reduces expression of dmahp. *Nat Genet*. 1997;16:402-406
32. Heath SK, Carne S, Hoyle C, Johnson KJ, Wells DJ. Characterisation of expression of mdmahp, a homeodomain-encoding gene at the murine dm locus. *Hum Mol Genet*. 1997;6:651-657
33. Klesert TR, Cho DH, Clark JI, Maylie J, Adelman J, Snider L, Yuen EC, Soriano P, Tapscott SJ. Mice deficient in six5 develop cataracts: Implications for myotonic dystrophy. *Nat Genet*. 2000;25:105-109
34. Sarkar PS, Appukuttan B, Han J, Ito Y, Ai C, Tsai W, Chai Y, Stout JT, Reddy S. Heterozygous loss of six5 in mice is sufficient to cause ocular cataracts. *Nat Genet*. 2000;25:110-114
35. Wakimoto H, Maguire CT, Sherwood MC, Vargas MM, Sarkar PS, Han J, Reddy S, Berul CI. Characterization of cardiac conduction system abnormalities in mice with targeted disruption of six5 gene. *J Interv Card Electrophysiol*. 2002;7:127-135
36. Jansen G, Bachner D, Coerwinkel M, Wormskamp N, Hameister H, Wieringa B. Structural organization and developmental expression pattern of the mouse wd-repeat gene dmr-n9 immediately upstream of the myotonic dystrophy locus. *Hum Mol Genet*. 1995;4:843-852
37. Liquori CL, Ricker K, Moseley ML, Jacobsen JF, Kress W, Naylor SL, Day JW, Ranum LP. Myotonic dystrophy type 2 caused by a cctg expansion in intron 1 of znf9. *Science*. 2001;293:864-867
38. Kuyumcu-Martinez NM, Wang GS, Cooper TA. Increased steady-state levels of cugbp1 in myotonic dystrophy 1 are due to pkc-mediated hyperphosphorylation. *Mol Cell*. 2007;28:68-78
39. Ranum LP, Day JW. Myotonic dystrophy: Rna pathogenesis comes into focus. *Am J Hum Genet*. 2004;74:793-804
40. Lueck JD, Lungu C, Mankodi A, Osborne RJ, Welle SL, Dirksen RT, Thornton CA. Chloride channelopathy in myotonic dystrophy resulting from loss of posttranscriptional regulation for clcn1. *Am J Physiol Cell Physiol*. 2007;292:C1291-1297
41. Savkur RS, Philips AV, Cooper TA. Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. *Nat Genet*. 2001;29:40-47

42. Sicot G, Gourdon G, Gomes-Pereira M. Myotonic dystrophy, when simple repeats reveal complex pathogenic entities: New findings and future challenges. *Hum Mol Genet*. 2011;20:R116-123
43. Mankodi A, Logigian E, Callahan L, McClain C, White R, Henderson D, Krym M, Thornton CA. Myotonic dystrophy in transgenic mice expressing an expanded cug repeat. *Science*. 2000;289:1769-1773
44. Kanadia RN, Johnstone KA, Mankodi A, Lungu C, Thornton CA, Esson D, Timmers AM, Hauswirth WW, Swanson MS. A muscleblind knockout model for myotonic dystrophy. *Science*. 2003;302:1978-1980
45. Ward AJ, Rimer M, Killian JM, Dowling JJ, Cooper TA. Cugbp1 overexpression in mouse skeletal muscle reproduces features of myotonic dystrophy type 1. *Hum Mol Genet*. 2010;19:3614-3622
46. Ho TH, Bundman D, Armstrong DL, Cooper TA. Transgenic mice expressing cug-bp1 reproduce splicing mis-regulation observed in myotonic dystrophy. *Hum Mol Genet*. 2005;14:1539-1547
47. Timchenko NA, Patel R, Iakova P, Cai ZJ, Quan L, Timchenko LT. Overexpression of cug triplet repeat-binding protein, cugbp1, in mice inhibits myogenesis. *J Biol Chem*. 2004;279:13129-13139
48. Wang GS, Kearney DL, De Biasi M, Taffet G, Cooper TA. Elevation of rna-binding protein cugbp1 is an early event in an inducible heart-specific mouse model of myotonic dystrophy. *J Clin Invest*. 2007;117:2802-2811
49. Koshelev M, Sarma S, Price RE, Wehrens XH, Cooper TA. Heart-specific overexpression of cugbp1 reproduces functional and molecular abnormalities of myotonic dystrophy type 1. *Hum Mol Genet*. 2010;19:1066-1075
50. Voet NB, van der Kooi EL, Riphagen, II, Lindeman E, van Engelen BG, Geurts A. Strength training and aerobic exercise training for muscle disease. *Cochrane Database Syst Rev*. 2010:CD003907
51. Vlachopapadopoulou E, Zachwieja JJ, Gertner JM, Manzione D, Bier DM, Matthews DE, Slonim AE. Metabolic and clinical response to recombinant human insulin-like growth factor i in myotonic dystrophy--a clinical research center study. *J Clin Endocrinol Metab*. 1995;80:3715-3723
52. Walter MC, Reilich P, Lochmuller H, Kohnen R, Schlotter B, Hautmann H, Dunkl E, Pongratz D, Muller-Felber W. Creatine monohydrate in myotonic dystrophy: A double-blind, placebo-controlled clinical study. *J Neurol*. 2002;249:1717-1722
53. Penisson-Besnier I, Devillers M, Porcher R, Orlikowski D, Doppler V, Desnuelle C, Ferrer X, Bes MC, Bouhour F, Tranchant C, Lagrange E, Vershueren A, Uzenot D, Cintas P, Sole G, Hogrel JY, Laforet P, Vial C, Vila AL, Sacconi S, Pouget J, Eymard B, Chevret S, Annane D. Dehydroepiandrosterone for myotonic dystrophy type 1. *Neurology*. 2008;71:407-412
54. Logigian EL, Martens WB, Moxley RTt, McDermott MP, Dilek N, Wiegner AW, Pearson AT, Barbieri CA, Annis CL, Thornton CA, Moxley RT, 3rd. Mexiletine is an effective antimyotonia treatment in myotonic dystrophy type 1. *Neurology*. 2010;74:1441-1448
55. Hilton-Jones D, Bowler M, Lochmueller H, Longman C, Petty R, Roberts M, Rogers M, Turner C, Wilcox D. Modafinil for excessive daytime sleepiness in myotonic dystrophy type 1--the patients' perspective. *Neuromuscul Disord*. 2012;22:597-603
56. Wheeler TM, Sobczak K, Lueck JD, Osborne RJ, Lin X, Dirksen RT, Thornton CA. Reversal of rna dominance by displacement of protein sequestered on triplet repeat rna. *Science*. 2009;325:336-339
57. Mulders SA, van den Broek WJ, Wheeler TM, Croes HJ, van Kuik-Romeijn P, de Kimpe SJ, Furling D, Platenburg GJ, Gourdon G, Thornton CA, Wieringa B, Wansink DG. Triplet-repeat oligonucleotide-mediated reversal of rna toxicity in myotonic dystrophy. *Proc Natl Acad Sci U S A*. 2009;106:13915-13920
58. Kanadia RN, Shin J, Yuan Y, Beattie SG, Wheeler TM, Thornton CA, Swanson MS. Reversal of rna missplicing and myotonia after muscleblind overexpression in a mouse poly(cug) model for myotonic dystrophy. *Proc Natl Acad Sci U S A*. 2006;103:11748-11753
59. Wang GS, Kuyumcu-Martinez MN, Sarma S, Mathur N, Wehrens XH, Cooper TA. Pkc inhibition ameliorates the cardiac phenotype in a mouse model of myotonic dystrophy type 1. *J Clin Invest*. 2009;119:3797-3806
60. Wheeler TM, Lueck JD, Swanson MS, Dirksen RT, Thornton CA. Correction of clc-1 splicing eliminates chloride channelopathy and myotonia in mouse models of myotonic dystrophy. *J Clin Invest*. 2007;117:3952-3957

Chapter 1.2

# Outline of the thesis

This thesis focuses on cardiac involvement and clinical measurement in patients with myotonic dystrophy type 1 (DM1).

In the first part of this thesis, we address the risk of sudden cardiac death, the presence of cardiomyopathy and its pathogenic mechanism. Objectives were to:

- provide a pragmatic approach for cardiac evaluation in hereditary muscular dystrophies based on literature review and consensus guidelines (**chapter 2**)
- determine the occurrence rate of sudden death in DM1 and identify patients at risk (**chapter 3**)
- determine the presence and nature of cardiomyopathy in DM1 using cardiovascular magnetic resonance imaging (**chapter 4**)
- identify differential gene expression patterns in DM1 hearts (**chapter 5**)

In the second part of the thesis, we show the importance of modern clinimetric methods and construct outcome measures for future DM1 studies. Objectives were to:

- perform a systematic literature review on the outcome measures used in DM1 clinical trials (**chapter 6**)
- introduce Rasch analysis to develop disease-specific interval scales (**chapter 7**)
- develop an activity and participation scale using the Rasch Model (**chapter 8 and 9**)
- develop a fatigue and daytime sleepiness scale using the Rasch Model (**chapter 10**)

The findings and conclusions described in this thesis and directions for future research are discussed in **chapter 11**.

PART II

# Cardiac disease in myotonic dystrophy type 1

Chapter 2

# Hereditary muscular dystrophies and the heart

*Mieke C.E. Hermans[a], Yigal M. Pinto[b], Ingemar S.J. Merkies[a,c], Christine E.M. de Die-Smulders[d], Harry J.G.M. Crijns[e], Catharina G. Faber[a]*

[a]Department of Neurology, Maastricht University Medical Centre, Maastricht
[b]Department of Cardiology, Academic Medical Centre Amsterdam, Amsterdam
[c]Department of Neurology, Spaarne Hospital, Hoofddorp
[d]Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht
[e]Department of Cardiology, Maastricht University Medical Centre, Maastricht

*Neuromuscul Disord 2010 Aug;20(8):479-92*

## Summary

Cardiac disease is a common clinical manifestation of neuromuscular disorders, particularly of muscular dystrophies. Heart muscle cells as well as specialized conducting myocardial fibres may be affected by the dystrophic process. The incidence and nature of cardiac involvement vary with different types of muscular dystrophies. Some mainly lead to myocardial disease, resulting in cardiomyopathy and heart failure, while others particularly affect the conduction system, leading to arrhythmias and sudden death. As prognosis of muscular dystrophy patients may be directly related to cardiac status, surveillance and timely management of cardiac complications are important. However, recognition of cardiac involvement requires active investigation and remains challenging since typical signs and symptoms of cardiac dysfunction may not be present and progression is unpredictable. In this review, we present a comprehensive overview of hereditary muscular dystrophies associated with cardiac disease to provide an efficient strategy for expertise management of these diseases.

## Introduction

Muscular dystrophies form a heterogeneous group of inherited disorders that are clinically characterized by progressive skeletal muscle wasting and weakness. Clinical diagnosis is based on distribution and severity of muscular involvement, mode of inheritance and concomitant symptoms. Genetic testing can confirm the diagnosis if the gene defect is known (table 2.1).
Cardiac disease is a common clinical manifestation of neuromuscular disorders, particularly of muscular dystrophies. Cardiac involvement is not necessarily related to the degree of skeletal myopathy and may even be the presenting or predominant symptom. Cardiac death can result from progressive heart failure, due to ventricular dysfunction, or from sudden death, presumably caused by heart block or malignant arrhythmias. As quality of life and survival of muscular dystrophy patients have improved as a result of advances in medical management, heart failure and arrhythmias contribute to a larger extent to mortality.
This review serves as a comprehensive overview of the extensive literature on hereditary muscular dystrophies associated with cardiac disease. Molecular pathology, clinical aspects, cardiac findings and the mechanism and risk of cardiac death of these disorders are summarized to provide insight into the appropriate clinical approach and therapeutic options.

**Search strategy and selection criteria**
A literature search was performed to find individual studies and reviews published on cardiac involvement in muscular dystrophies. Muscular dystrophies and myopathies of interest were identified using PubMed and OMIM database. Subsequently, a more extensive PubMed search was performed using the specific type of muscular dystrophy in combination with any of the following keywords: aetiology, diagnosis, heart disease, cardiomyopathy, arrhythmias, (sudden) death, prognosis, treatment. Furthermore, the bibliographies of all review articles published in the past 10 years regarding the cardiac involvement in muscular dystrophies were checked.

**Table 2.1.** Classification of muscular dystrophies[1]

| | Gene/protein; chromosome |
|---|---|
| **Dystrophinopathy** | |
| Duchene muscular dystrophy | Dystrophin; Xp21.2 |
| Becker muscular dystrophy | Dystrophin; Xp21.2 |
| X-linked dilated cardiomyopathy | Dystrophin; Xp21.2 |
| **Emery Dreifuss muscular dystrophy (EDMD)** | |
| X-linked EDMD (EDMD1) | Emerin; Xq28 |
| Autosomal dominant EDMD (EDMD2) | Lamin A/C; 1q21.2 |
| **Limb girdle muscular dystrophy (LGMD)** | |
| Autosomal dominant LGMD (type 1) | |
| - LGMD 1A | Myotilin; 5q31 |
| - LGMD 1B | Lamin A/C; 1q21.2 |
| - LGMD 1C | Caveolin-3; 3p25 |
| - LGMD 1D | 7q |
| - LGMD 1E | 6q23 |
| - LGMD 1F | 7q32 |
| - LGMD 1G | 4q21 |
| Autosomal recessive LGMD (type 2) | |
| - LGMD 2A | Calpain-3 ;15q15 |
| - LGMD 2B | Dysferlin; 2p13.2 |
| - LGMD 2C | γ-Sarcoglycan; 13q12 |
| - LGMD 2D | α-Sarcoglycan; 17q21 |
| - LGMD 2E | β-Sarcoglycan; 4q12 |
| - LGMD 2F | δ-Sarcoglycan; 5q33 |
| - LGMD 2G | Telethonin; 17q12 |
| - LGMD 2H | TRIM32; 9q31-q33 |
| - LGMD 2I | Fukutin-related protein; 19q13.3 |
| - LGMD 2J | Titin; 2q31 |
| - LGMD 2K | POMT1; 9q34.1 |
| - LGMD 2L | Anoctamin 5; 11p14 |
| - LGMD 2M | Fukutin; 9q31 |
| **Myotonic dystrophy (DM)** | |
| DM type 1 (Steinert's disease) | DMPK; 19q13.2-q13.3 |
| DM type 2 (proximal myotonic myopathy: PROMM) | ZNF9; 3q13.3-q24 |
| **Congenital muscular dystrophy (MDC)** | |
| Non-syndromic MDC | |
| - MDC 1A (merosin deficient) | Laminin α2; 6q22-23 |
| - MDC 1B | 1q42 |
| - MDC 1C | Fukutin-related protein; 19q13.3 |
| - Ullrich MDC | Collagen type 6A1-A3; 21q22.3 and 2q37.3 |

| | Gene/protein; chromosome |
|---|---|
| Syndromic MDC | |
| - Fukuyama MDC | Fukitin; 9q31 |
| - Muscle-eye-brain disease (Santavuori) | POMGnT1; 1p32-p34 |
| - Walker Warburg syndromes | POMT1; 9q34.1 - POMT2; 14q24.3 |
| - MDC 1D | LARGE; 22q12.3-13.1 |
| **Facioscapulohumeral muscular dystrophy (FSHD)** | 4q35 |
| **Myofibrillar myopathies (MFM)** | |
| Desmin-related MFM | Desmin; 2q35 |
| αB-crystallinopathy | αB-crystallin; 11q22.3-q23.1 |
| Filamin C-related MFM | Filamin C; 7q32 |
| Myotilin-related MFM | Myotilin; 5q31 |
| Rigid spine syndrome | SEPN1; 1p36-p35 |
| ZASP-related MFM | ZASP; 10q22.2-q23.3 |
| BAG3-related MFM | BAG3: 10q25.2-q26.2 |
| Other | |
| **Other muscular dystrophies** | |
| Distal myopathies | |
| - Welander | 2p13 |
| - Myioshi | Dysferlin; 2p13.1 |
| - Finnish type | Titin; 2q31 |
| - Nonaka (hereditary inclusion body myositis 2) | GNE; 9p12-p11 |
| - Gowers Laing | MYH7; 14q11 |
| - Distal dystrophy with rimmed vacuoles | 19p13.3 |
| X-linked vacuolar cardiomyopathy and myopathy (Danon disease) | LAMP-2; Xq24 |
| Barth syndrome | TAZ (Tafazzin); Xp28 |
| McLeod syndrome | XK membrane transport protein; Xp21.1 |
| Reducing body myopathy (RBM) | FHL1; Xq26-q27.2 |
| X-linked scapuloperoneal myopathy | FHL1; Xq26-q27.2 |
| X-linked myopathy with postural muscle atrophy (XMPMA) | FHL1; Xq26-q27.2 |
| Early-onset myopathy with fatal cardiomyopathy | Titin; 2q31 |
| Various phenotypes | MYH7; 14q12 |

## Dystrophinopathies

Dystrophinopathies refer to the disorders Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD) and X-linked dilated cardiomyopathy (XLCM). The incidence of DMD is estimated at 1 in 3500 male new-borns with a prevalence of 6 in 100 000 males.[2] DMD is characterized by weakness of leg, pelvic and shoulder girdle muscles starting in early childhood. The introduction of home nocturnal ventilation has raised the mean age of death from 19 to at least 25 years.[3]

BMD is a milder variant of DMD with a better prognosis. Incidence of BMD is approximately 1 in 18 450 males and prevalence is 2.4 per 100 000 in the general population.[2, 4] First symptoms usually appear between the age of 3 and 21 years with a mean age of onset at 11 years. Age at death is 21-89 years with an average age of about 45 years.[5-8]

XLCM is a rare disorder with rapidly progressive cardiomyopathy but almost no skeletal muscle impairment. The distinction between XLCM and a BMD patient with mild skeletal muscle weakness can be unclear. Therefore, it is argued to consider these entities of dystrophinopathy as part of a continuum of disease expression.[9]

The dystrophin gene codes for a large protein (dystrophin) that is part of a multimeric protein complex called the dystrophin-glycoprotein complex. In DMD patients, mutations disrupt the reading frame, while in BMD the reading frame is maintained.[10] Absence of the dystrophin protein (DMD) and reduced levels or abnormal configuration of dystrophin (BMD) leads to membrane fragility making muscle cells susceptible to damage from contraction.[11] Secondary increase in free radicals and activation of calcium-dependent proteases are thought to further contribute to muscle degeneration. As muscle disease advances, muscle repair and regeneration cannot adequately compensate for damage, leading to degeneration and necrosis of skeletal myofibers and cardiomyocytes and gradual replacement by fibrofatty tissue.[12]

### Cardiac involvement in Duchenne muscular dystrophy

In a large series of DMD patients, the evolution of cardiac involvement has been investigated.[13-15] Conduction defects, dilated and hypertrophic cardiomyopathy are first evident on ECG and echocardiography after 10 years of age. The incidence increases with age, being present in all patients over 18 years of age. Most patients develop dilated cardiomyopathy, sometimes preceded by localized hypertrophy and isolated conduction defects.[16] Characteristic ECG abnormalities are summarized in table 2.2. Rhythm abnormalities include sinus arrhythmia, atrial flutter, frequent premature atrial and ventricular beats. Ventricular arrhythmias occur frequently in DMD patients with impaired

ventricular function and may be an additional marker of deteriorating myocardial function.[17, 18]
Progressive heart disease due to congestive heart failure or sudden death is the cause of death in 10-20% of DMD patients, but the percentage of primary cardiac death is expected to rise now that more successful management of respiratory complications reduces mortality due to pulmonary causes.[19] Indeed, ventricular dysfunction appears to be a powerful predictor of mortality.[20] Clinical studies demonstrated a beneficial effect of ACE inhibitors on onset and progression of ventricular dysfunction and survival.[21, 22] ACE inhibition also improved skeletal muscle regeneration in the *mdx* mouse model for DMD.[23, 24]



**Cardiac involvement in Becker muscular dystrophy**
In BMD, the heart becomes invariably affected and cardiac disease can even be more pronounced than skeletal muscle weakness. ECG and echocardiographic abnormalities can be found in 17-74% of patients (table 2.2).[25-28] In a longitudinal study of 68 BMD patients, the occurrence of clinically apparent cardiomyopathy increased significantly with age, from 44% in patients under 20 years of age to 82% in patients older than 40 years during a mean follow-up period of 8 years.[26] In a series on 27 BMD patients, the incidence of ECG abnormalities progressed from 44% to 71% and dilated cardiomyopathy from 15% to 33% of patients over a 13-year period.[8]
As the level of physical activity is higher and respiratory function is better than in DMD, patients with BMD survive long enough to develop cardiac complications. Death from congestive heart failure and arrhythmias is estimated to occur in up to 50% of cases.[29] BMD has a high heart transplantation rate in the first year after diagnosis of cardiomyopathy.[30] The potential cardiac benefit of early treatment with afterload-reducing therapy has hardly been investigated in BMD patients. A retrospective observational study of 31 males with DMD or BMD reported normalized left ventricular size and function in 3 out of 4 treated BMD patients, indicating ventricular remodelling.[31] Although the timing of cardiomyopathy development remains unpredictable, mutations in specific regions of the dystrophin gene predispose BMD patients to early onset DCM.[32] These patients may benefit from early medical therapy or heart transplantation.

**Cardiac involvement in female carriers**
The prevalence of cardiac abnormalities in DMD and BMD carriers differs between studies, possibly because definitions vary widely. A 10-year follow-up study of 152 DMD and 45 BMD carriers reported clinical features of heart disease, such as hypertrophy, arrhythmias or dilated cardiomyopathy, in 40%.[33] The percentage of clinical cardiac involvement increased significantly with age, from 15% in carriers aged under 16 years to 45% in carriers 16 years

and older. By contrast, a retrospective study suggested that clinically relevant cardiac abnormalities are unlikely in female carriers under 16 years.[34] In a cross-sectional study of 85 DMD and 44 BMD carriers aged 18-58 years, left ventricular dilatation (18%) and dilated cardiomyopathy (8%) were found on echocardiography.[35] Dilated cardiomyopathy was found only in DMD carriers, whereas left ventricular dilatation was present in a proportion of both DMD and BMD carriers. ECG abnormalities were found in 47% of this population.[36] Another series of 56 adult carriers revealed no such ECG abnormalities, but found ventricular dilatation or hypertrophy in 14% and dilated cardiomyopathy in 7% of females.[37] Nevertheless, severe heart failure may develop in some women and require heart transplantation or result in premature (sudden) death.[33, 38, 39] Cardiac examination is recommended every 5 years in order to start timely therapy.[29] However, the benefit of early treatment awaits confirmation since a recent limited population-based study argues that it is unclear whether carriers of DMD and BMD have a reduced life expectancy or higher risk of cardiac death.[40]

**X-linked dilated cardiomyopathy (XLCM)**
XLCM is a primary myocardial dystrophinopathy, presenting as congestive heart failure in teenage males. The disease course is rapidly progressive resulting in cardiac death within 1-2 years. Manifesting female carriers have later onset, usually during the fifth decade, and slow progression of heart failure.[9, 41] Most reported XLCM families have mutations in the 5' end of the dystrophin gene, resulting in absence of the M-isoform in heart and skeletal muscle.[42] Skeletal muscle, however, escapes the dystrophic changes by maintaining dystrophin synthesis by exon skipping or alternative splicing that the heart is not able to put in place.[42]

The reported cardiac abnormalities and mortality data of patients with dystrophin mutations are summarized in table 2.2.

## Emery-Dreifuss muscular dystrophy

Emery-Dreifuss muscular dystrophy (EDMD) is characterized by early contractures of elbows, Achilles tendons (toe walking) and posterior cervical muscles (rigid spine), and muscle weakness in a humero-peroneal distribution. Joint contractures occur in early childhood, often before there is any significant weakness.

**Table 2.2.** Cardiac abnormalities and prognosis in dystrophinopathies

| Disease | ECG | Echo | Cardiac death | Age at death |
|---|---|---|---|---|
| DMD | - tall R waves or R/S ratio >1 in lead V1 or V2<br>- Q waves in lateral leads<br>- complete or incomplete LBBB or complete RBBB<br>- tachyarrhythmias | - myocardial hypertrophy<br>- dilated cardiomyopathy | 10-20%[29] | mean 19 years in non- ventilated patients, mean 25 years in ventilated patients[3] |
| BMD | - tall R waves or R/S ratio >1 in lead V1<br>- Q waves in lateral leads<br>- complete or incomplete LBBB or complete RBBB<br>- tachyarrhythmias | - myocardial hypertrophy<br>- dilated cardiomyopathy | 50%[29] | mean 45 years, range 21-89 years[5-8] |
| Carriers | - tall R waves or R/S ratio >1 in lead V1 or V2<br>- Q waves in lateral leads<br>- incomplete LBBB and complete RBBB | - myocardial hypertrophy<br>- left ventricular dilatation<br>- dilated cardiomyopathy | no major cause of death[40] | unknown |
| XLCM | | - dilated cardiomyopathy | ~100% | mean 22 years, range 15-52 years[9, 41, 42] |

X-linked recessive EDMD (EDMD1) has a prevalence of 1 in 100 000 males. It can result from various mutations in the STA gene.[43] The protein product (emerin) is a component of the inner nuclear membrane. It is a member of a protein complex that links filamentous actin in the cytoskeleton to the nuclear lamina and/or chromatin.[44, 45] Heterogeneous mutations have been reported and almost always result in complete absence of emerin in muscle.[46]
Autosomal dominant EDMD (EDMD2) and a rare autosomal recessive form of EDMD are caused by a mutation in the lamin A/C gene.[47, 48] Prevalences are unknown. Lamin A/C codes for alternatively spliced lamins A and C. Interestingly, lamin A/C mutations can cause several other phenotypes, including limb-girdle muscular dystrophy type 1B (LGMD1B), autosomal dominant dilated cardiomyopathy with conduction defects (CMD1A), autosomal recessive EDMD, autosomal recessive axonal neuropathy, familial partial lipodystrophy, mandibuloacral dysplasia and progeria syndromes, all referred to as laminopathies. Hundreds of mutations are found along the lamin A/C gene, without phenotype-specific hotspots.[49] De novo mutations are frequent in the LMNA gene.[50]

The EDMD phenotypes caused by emerin or lamin A/C mutations are clinically indistinguishable, suggesting a close functional relationship between the two proteins.[51] In the absence of an informative family history, emerin immunodetection studies help to distinguish between EDMD1 and EDMD2.[52] Recently, it has been shown that mutations in genes encoding nesprins 1 and 2 (proteins binding both emerin and lamins A/C) can also cause EDMD.[53] Some have suggested that muscle cells lacking emerin or lamin A/C may be particularly sensitive to mechanic stress during contraction due to loss of structural integrity of the nuclear membrane.[54] An alternative hypothesis suggests that these nuclear envelope proteins may have a role in chromatin organization and regulation of gene expression. As a result, lamin A/C and emerin mutations may cause defective cellular signaling in response to mechanical stimulation, impaired activation of anti-apoptotic genes and abnormal apoptosis defective muscle differentiation and regeneration.[55, 56]

**Cardiac involvement in EDMD1**

In EDMD1, cardiac disease often presents as conduction disturbances (table 2.3).[57] Ventricular myocardium may become involved leading to mild ventricular dilatation and low-normal systolic function.[58, 59] Eventually atrial paralysis will occur, with loss of electrical and mechanical activity of the atria. Under microscopy, marked loss of atrial myocardium and its replacement by fibrous and fat tissue have been observed, as well as various degrees of interstitial fibrosis in the ventricular myocardium. However, no specific degeneration of the conduction system was found.[60]

Sudden death is by far the most common cause of death and can be highly unpredictable.[61] Therefore, routine ECG and yearly Holter monitoring are indicated and pacemaker implantation should be considered if sinus node or AV node disease develops.[29] Pacemakers are often needed by 30 years of age (range 14-44).[62-64] Once patients are successfully paced, the incidence of sudden death appears low.[64] However, atrial arrhythmias and atrial standstill can cause disabling embolic stroke.[63] Heart failure and ventricular arrhythmias seem to occur only in a minority of patient, but the risk may increase as patients with a pacemaker may survive longer.[59, 63]

**Cardiac involvement in carriers**

Female carriers of an emerin mutation have no muscular symptoms, though some may be at risk of cardiac arrhythmias,[46, 59, 64] and sudden death.[60] Reports may have been biased by cases of autosomal dominant disease. Systemic studies on the natural history of cardiac involvement in EDMD1 carriers are not available, but it seems appropriate to offer ECG surveillance.

**Cardiac involvement in EDMD2 and other laminopathies**

The laminopathies EDMD2 and LGMD1B have an overlapping cardiac phenotype. LGMD1B differs from the EDMD2 phenotype by later onset of muscular weakness, the absence of significant early contractures and the predominance of pelvic girdle weakness with late involvement of humeral muscles and sparing of the peroneal and tibial muscles.[65] CMD1A is characterized by progressive conduction-system disease and dilated cardiomyopathy without muscular symptoms.[66] It is interesting to note that the same mutation within a family can lead to either EDMD2, CMD1A or LGMD1B phenotypes.[67, 68]

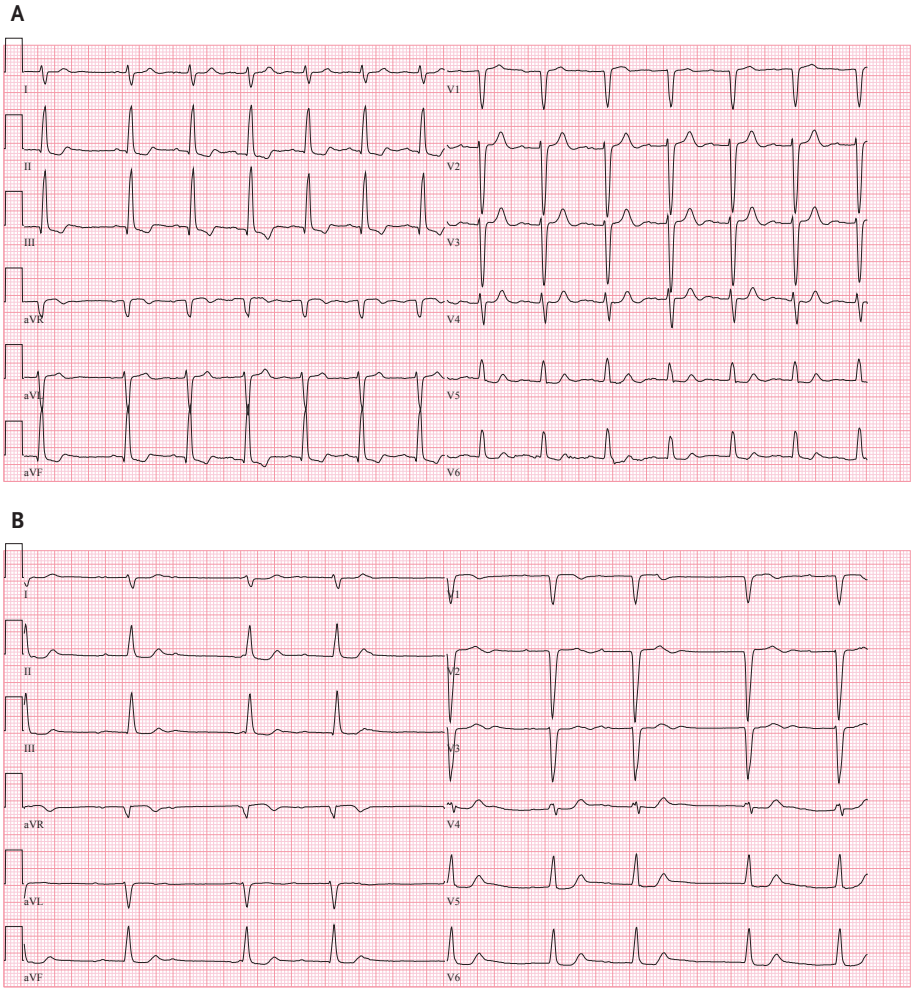
A review from the literature of 299 patients with lamin A/C mutation causing LGMD1B, EDMD2 or CMD1A, reported conduction disease as well as cardiac failure.[69] Dysrhythmias (sinus bradycardia, atrioventricular conduction block, or atrial arrhythmias) were present in 71% of patients increasing with age to 92% after the age of 30 (figure 2.1). Heart failure was reported at a later age but less frequently then dysrhythmias. Patients died at a mean age of 46 years. The incidence of sudden death was very high and more prevalent than death due to heart failure (46% versus 12%, respectively). The risk of sudden death was equal in lamin A/C mutation carriers with a neuromuscular phenotype or isolated cardiac phenotype. However, it appeared that sudden death was not reduced by pacemaker therapy, suggesting a ventricular arrhythmogenic cause of death.[67, 69]

**Table 2.3.** Cardiac abnormalities and prognosis in Emery-Dreifuss muscular dystrophy

| Disease | ECG | Echo | Cardiac death | Age at death |
|---|---|---|---|---|
| EDMD1 | - low P wave amplitude<br>- atrioventicular block<br>- atrial fibrillation<br>- atrial standstill with junctional escape rhythm | - atrial dilatation<br>- ventricular dilatation<br>- left ventricular dysfunction | high risk of sudden death in non-paced patients | sudden death usually before 50 years (range 25-59)[61, 64] |
| Carrier | - low P wave amplitude<br>- atrioventicular block<br>- atrial fibrillation | - atrial dilatation | probably no major cause of death | unknown |
| EDMD2* | - low P wave amplitude<br>- atrioventricular block with initially narrow QRS<br>- atrial arrhythmias | - dilated cardiomyopathy | 43% sudden death<br>11% heart failure[69] | mean 45 years[69] |

* based on cases with LMNA mutations leading to emery-dreifuss or limb-girdle muscular dystrophy

**Figure 2.1.** Electrocardiographic changes in a patient with limb girdle muscular dystrophy type 1B




**Legend to figure 2.1.** A: ECG showing sinus rhythm with low amplitude P wave and PR prolongation at the age of 28. QRS duration is already increased. B: at 32 years of age the ECG showed sinusbradycardia with grade II AV-block.

A prospective study on 19 patients with a lamin A/C mutation receiving an implantable cardioverter-defibrillator (ICD), showed effective treatment of possibly lethal tachyarrhythmias.[70] It is not yet clear which clinical factors predict increased risk of sudden death and can guide therapy.
A recent report suggests that competitive sport activity is possibly an important risk factor,[71] but this awaits larger studies to more definitively establish

clinical parameters of risk in this group. A summary of the reported cardiac abnormalities in EDMD is presented in table 2.3.



## Limb-girdle muscular dystrophy

Limb-girdle muscular dystrophies (LGMD) are a clinically and genetically heterogeneous group of muscular dystrophies in which the pelvic and shoulder girdle musculature are predominantly involved.[72] Currently, 7 autosomal dominant (LGMD1A to LGMD1G) and 13 autosomal recessive forms (LGMD2A to LGMD2M) are recognized.[73] The disease course of autosomal dominant LGMD usually is relatively mild.[74] Age of onset ranges from childhood to the fourth decade. The clinical picture of autosomal recessive LGMD is more severe and closely resembles that of the dystrophinopathies.[74] Distinction between the different types of LGMD is difficult on clinical criteria alone and requires protein analysis in muscle biopsy and genetic studies. Estimated prevalence of all forms of limb-girdle muscular dystrophy ranges from 1 in 23 000 to 1 in 150 000.[2, 74]

Protein defects in LGMD occur in several pathways concerned with skeletal and/or cardiac muscle function. These include proteins associated with the dystrophin-glycoprotein complex, nuclear lamina or sarcomere. Furthermore, disruption of proteins that mediate sarcolemmal repair or other cell-signalling pathways and defective enzymes can result in the limb-girdle dystrophic phenotype.[75] Although the primary defect in many limb-girdle dystrophies is known, the exact functional role of the affected proteins and the precise pathogenic mechanism leading is largely unknown. Moreover, mutations within the same gene can give rise to distinct phenotypes without limb-girdle syndrome (i.e. allelic heterogeneity).

**Cardiac involvement in LGMD1**

Cardiac involvement is common in LGMD1B and has been described above. To date, LGMD1A (myotilinopathy) and LGMD1C (caveolinopathy) have not been associated with cardiac problems.[76, 77] However, cardiomyopathy has been reported in allelic disorders with mutations in myotilin (myofibrillar myopathies) and caveolin-3 (autosomal dominant rippling muscle disease), which may overlap with LGMD1A and C, respectively. LGMD1E has been reported in only one family to date.[78] The phenotype is similar to LGMD1B. Dilated cardiomyopathy with conduction defects (table 2.4) and/or adult-onset limb-girdle muscular dystrophy were present in affected members. Sudden death was observed despite pacemaker therapy. Reports on LGMD1D, 1F and 1G are very rare and so far no cardiac abnormalities have been reported in these diseases.

**Table 2.4.** Cardiac involvement and prognosis in limb girdle muscular dystrophy

| Disease | ECG | Echo | Cardiac death | Age at death |
|---|---|---|---|---|
| LGMD1B* | - low P wave amplitude<br>- atrioventricular block with initially narrow QRS<br>- atrial arrhythmias | - dilated cardiomyopathy | 43% sudden death<br>11% heart failure[69] | mean 45 years[69] |
| LGMD1E | - atrioventicular block<br>- complete RBBB<br>- ventricular tachycardia | - dilated cardiomyopathy | considerable risk<br>33% sudden death[78] | mean 47 years[78] |
| LGMD2C-F | - tall R waves in lead V1 or V2<br>- Q waves in lateral leads<br>- incomplete RBBB | - ventricular dilatation<br>- left ventricular dysfunction | - impact on prognosis unclear<br>- cardiomyopathy can be fatal[89, 90] | third decade[99, 100] |
| LGMD2I | - dysmorphic notched P waves<br>- Q waves in lateral leads<br>- complete or incomplete RBBB or incomplete LBBB | - dilated cardiomyopathy | considerable risk | range 16-67[94, 95] |

* based on cases with LMNA mutations leading to emery-dreifuss or limb-girdle muscular dystrophy

**Cardiac involvement in Sarcoglycanopathies (LGMD 2C, 2D, 2E, and 2F)**

Sarcoglycanopathies comprise four subtypes of autosomal recessive LGMD that are caused by mutations in the transmembrane proteins of the sarcoglycan complex. This complex is integrated in the muscle membrane as a part of the dystrophin-glycoprotein complex.[79] A wide range of mutations in any of the α-sarcoglycan (LGMD2D), β-sarcoglycan (LGMD2E), γ-sarcoglycan (LGMD2C) or δ-sarcoglycan genes (LGMD2F) destabilize the whole sarcoglycan complex,[80] resulting in an inability to protect the membrane against contraction-induced degeneration. The estimated prevalence of sarcoglycanopathies is approximately 1 in 178 000.[81] LGM2D is the most frequent sarcoglycanopathy, followed by LGMD2E and LGMD2C, while LGMD2F is most uncommon.[81-83] Disease severity is related to the percentage of residual sarcoglycan protein. Patients present at a mean age of 8 years with pelvic muscle weakness and early scapular winging.[84, 85] Individuals with partial deficiency will generally exhibit symptoms between adolescence and early adulthood.[86]

In small series, mild ECG and/or echocardiographic abnormalities occurred in 20-30% in any type of sarcoglycanopathy (table 2.4).[87, 88] The risk of significant cardiac involvement appears low in LGMD2D patients, while severe dilated cardiomyopathy and lethal ventricular arrhythmias may be an important feature in LGMD2E patients with severe DMD-like dystrophy.[89, 90] However, a

clear genotype-phenotype correlation and long-term follow-up to determine the relative impact on prognosis has not yet been established. Therefore, it is argued to investigate patients with sarcoglycanopathy for cardiomyopathy as in dystrophinopathies.[29] Respiratory involvement is also common and can be a cause of death.[88]



**Cardiac involvement in LGMD2I**

LGMD2I is caused by mutations in the fukutin-related protein gene (FKRP).[91] FKRP mutations were initially found in a severe form of congenital muscular dystrophy (MDC1C) and have been reported in some cases of muscle-eye-brain disease en Walker-Warburg syndrome.[92] Mutant FKRP directly or indirectly disturbs glycosylation of the transmembrane protein α-dystroglycan in muscle cells. Appropriate glycosylation is necessary for α-dystroglycan to bind components of the extracellular matrix, including laminin-2.[93] In LGMD2I patients, the 826C>A mutation is almost invariably found in one or two alleles. Homozygosity for this missense mutation is associated with milder muscular dystrophy.[94] However, phenotypic variability is remarkable between affected relatives and ranges from severe muscle weakness to isolated exertional myoglobinuria or muscle cramps.

Cardiac involvement has been reported in 29-62% of LGMD2I patients with the use of different definitions.[94-98] Left ventricular wall motion abnormalities and dilated cardiomyopathy start as early as the teen years. A substantial proportion of patients develop symptomatic cardiac failure over time, starting at a mean age of 38 years (range 18-58).[95] Cardiac magnetic resonance imaging displays early functional and morphological ventricular abnormalities, including ventricular wall fibrosis.[98] Significant arrhythmias or sudden death have not been reported. Cardiac abnormalities are not always associated with early and severe muscle weakness.[95, 98] A summary of the reported cardiac abnormalities in LGMD2I is presented in table 2.4. LGMD2I patients are also at risk of respiratory impairment and may require nocturnal ventilation.[94, 95]

**LGMD2 without cardiac involvement**

There is no clear evidence for cardiac involvement in LGMD2A (calpainopathy) and LGMD2B (dysferlinopathy).[97] Limb-girdle type 2G, 2H, 2J, 2K 2L and 2M have only rarely been described and have thus far not been associated with cardiac abnormalities. Heterozygous family members are asymptomatic.

## Myotonic dystrophy

Myotonic dystrophy is characterized by autosomal dominant inheritance, progressive muscle weakness and wasting, myotonia and multisystem

complications. There are two distinct forms recognized: myotonic dystrophy type 1 and myotonic dystrophy type 2.

**Myotonic dystrophy type 1**

Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy in adults. It has a prevalence of 2.1-14.3 per 100 000 worldwide, but higher prevalences have been found in certain populations due to founder effect and genetic isolation.[101] Muscle involvement consists of myotonia and weakness of facial, sternocleidomastoid and distal muscles. Additionally, DM1 patients usually have serious systemic manifestations including cataracts, gastro-intestinal problems, central nervous system involvement and cardiac abnormalities.

An earlier age of onset and increased severity of clinical symptoms has been observed in subsequent generations.[102] Based on age at onset and symptoms, four clinical categories can be distinguished: congenital type, childhood-onset type, adult-onset (classical) type and late-onset (mild) type.[103] Life expectancy is greatly reduced in DM1 patients, particularly in those with early onset of the disease and proximal muscular involvement.[104] Median survival was 59-60 years for adult-type patients and 35 years for the congenital type.[105] Progressive respiratory failure is the leading cause of mortality, followed by death from cardiac causes in 20-30%.[104-106]

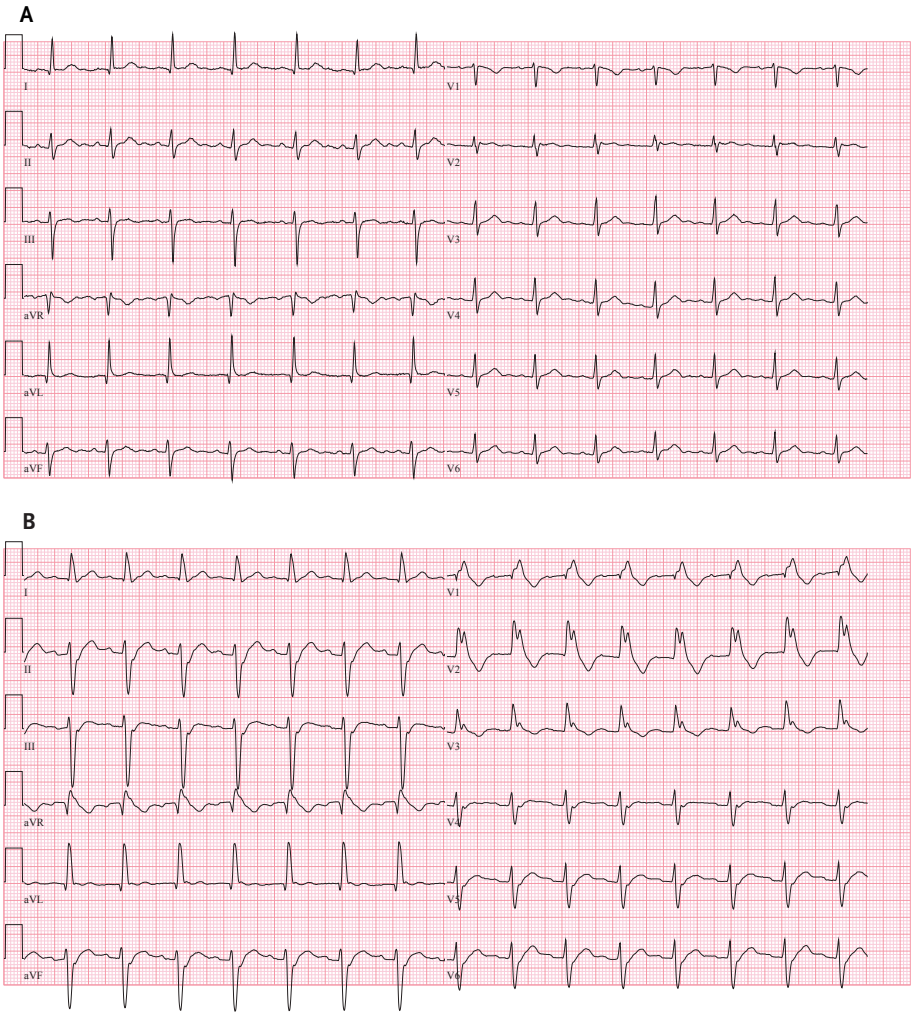
DM1 results from an expansion of a repetitive trinucleotide segment in the 3'untranslated region of the myotonic dystrophy protein kinase (DMPK) gene.[107] The normal DMPK allele contains 5-35 CTG repeats, whereas it is expanded to hundreds or even thousands of copies in DM1 patients. The degree of the expansion correlates broadly with severity of the phenotype and is inversely correlated with age at onset.[103] As a rule, the repeat expands in subsequent generations, explaining anticipation.

The molecular pathway is not completely unravelled. Reduction of DMPK protein levels through depletion of mRNA in the cytoplasm may play a role in the cardiac disease.[108] Furthermore, expression levels of neighbouring genes may be decreased through a change in chromatin structure.[109] However, this does not seem to explain the wide range of clinical manifestations. The dominant mechanism most likely involves toxicity of the mutant DMPK mRNA accumulating in nuclear foci, resulting in abnormal RNA-processing of additional transcripts.[110] However, muscle wasting cannot be readily explained by any of the splicing changes identified to date. Unlike some other forms of muscular dystrophy, muscle wasting in DM1 is characterized by muscle atrophy instead of extensive necrosis, suggesting defects in muscle protein metabolism. Furthermore, impaired proliferative capacity of DM1 satellite cells has been shown *in vitro*, which may involve a mechanism of premature senescence.[111]

## Cardiac involvement in myotonic dystrophy type 1

Cardiac involvement is one of the main systemic features of DM1 and a major prognostic factor. Serious cardiac abnormalities can occur as early as the second decade of life in patients with the congenital/childhood type.[112] The reported cardiac abnormalities in DM1 are summarized in table 2.5. Conduction deficits occur in approximately 65% of adult patients.[113] In most patients, conduction disease progresses gradually (figure 2.2), but the clinical course cannot be predicted in individual patients.[114, 115]

**Figure 2.2.** Progressive cardiac conduction changes on the electrocardiograms of a patient with adult type myotonic dystrophy type 1




**Legend to figure 2.2.** A: normal sinus rhythm was seen at 25 years of age. B: At the age of 37, the patient had right bundle branch block and left axis deviation with a long PR interval.

Evaluation of pacemaker recordings show frequent occurrence of asymptomatic paroxysmal episodes of atrial and ventricular arrhythmias.[116] His-Purkinje conduction delay is the most frequent electrophysiological abnormality.[117] Pathological investigation show focal replacement fibrosis of conduction tissue and diffuse interstitial fibrosis throughout the myocardium.[118] Mild to moderate ventricular dysfunction and subtle structural changes can be found on echocardiograms and magnetic resonance imaging.[119, 120] However, the prevalence of congestive heart failure is low and estimated at 2-7%.[120, 121] There is no consensus from literature whether or not CTG repeat size has value as a prognostic indicator of cardiac disease.
Sudden death is well documented in DM1. A large prospective trial demonstrated that an electrocardiogram showing severe abnormalities (defined as rhythm other than sinus, PR interval >240 ms, QRS duration >120 ms or second- or third degree atrioventricular block) is an independent risk factor of sudden death.[106] Until recently, sudden death was thought to be primarily the result of conduction blocks. However, reports of sudden death in patients with implanted pacemakers, as well as sudden death closely related to documented spontaneous ventricular tachycardia, suggest that ventricular tachyarrhythmias are possibly a more frequent cause of death than previously thought.[106, 122] Ventricular tachyarrhythmias are often inducible by electrophysiological studies, but their predictive value remains to be determined.[117] An ECG is an appropriate screening test and should be made yearly from diagnosis. In some patients cause of syncope may be difficult to establish and tilt table testing, repeated Holter monitoring or an implantable loop recorder may be indicated. Holter monitoring is recommended in patients with ECG abnormalities to detect asymptomatic conduction blocks and arrhythmias.[29] It is important to address sudden death and the occurrence of ventricular arrhythmias should be assessed with the highest priority. Moreover, the effectiveness of prophylactic ICD implantation should be investigated in patients with an increased risk of sudden death and relatively good neuromuscular prognosis.

**Myotonic dystrophy type 2**
Myotonic dystrophy type 2 (DM2) is a recently delineated autosomal dominant disorder with features resembling those in adult-onset DM1. The clinical course of DM2 is usually more favourable compared to DM1. To date, most reported DM2 families are of German-Eastern European descent, suggesting that there may be a single founder mutation.[123] Patients present typically in adult life and exhibit predominantly proximal lower limb weakness or weakness of deep finger flexors.[124, 125] An earlier age of onset is present in offspring of affected parents, but no congenital cases or mental retardation have been noted in DM2. A review of 209 DM2 patients reported that first symptoms occurred between 13

and 67 years (median age at onset 48 years).[124] Facial muscle weakness is less severe, myotonia is usually less apparent and muscle atrophy is milder than in DM1 patients. Muscle pain is a major complaint of DM2 patients. Furthermore, systemic involvement, like cataract, cardiac involvement and endocrine dysfunction may also be present. Contrary to DM1, respiratory problems or failure do not occur, influencing prognosis to a large extent.[124, 125]
The mutation underlying DM2 is an unstable expanded CCTG-repeat.[126] Expanded allele sizes ranged from 75 to 11 000 repeats. The clinical and molecular similarities that exist between the different myotonic dystrophies suggest a common pathogenic mechanism involving repeat containing RNA transcripts.[127]

**Cardiac involvement in myotonic dystrophy type 2**
Cardiac problems are considered to be a less frequent complication in DM2 than in DM1 (table 2.5). Conduction abnormalities were found in 20-36% of patients.[124, 125, 128] Furthermore, a recent study of 38 DM2 patients, aged 57 ± 15.2 years, reported (paroxysmal) atrial fibrillation in 6, mild left ventricular dysfunction in 4 and congestive heart failure in 2 patients.[128] Sporadic cases of cardiomyopathy with ventricular arrhythmias and sudden death in DM2 patients have also been reported.[129-131] Life expectancy may be reduced in single cases, mainly if there is severe cardiac involvement. Although the risk for such events appears low, experts have recommended annual ECG and more extensive evaluation if symptoms are reported.[132]

**Table 2.5.** Cardiac abnormalities and prognosis in myotonic dystrophy

| Disease | ECG | Echo | Cardiac death | Age at death |
|---|---|---|---|---|
| Myotonic dystrophy type 1 | - atrioventricular block<br>- complete or incomplete RBBB and LBBB<br>- atrial and ventricular arrhythmias | - left ventricular dilatation and dysfunction | 20-30%[104, 105] | mean 54 years[104, 105] |
| Myotonic dystrophy type 2 | - atrioventricular block<br>- complete or incomplete RBBB and LBBB<br>- atrial and ventricular arrhythmias | - left ventricular dysfunction | probably no major cause of death[133] | unknown |

## Congenital muscular dystrophy

Congenital muscular dystrophy (MDC) describes a number of inherited disorders in which muscle weakness is present at birth or within the first 6 months of life. MDC has either a slowly progressive or non-progressive course. Morbidity and mortality rates depend on the type of congenital muscular dystrophy. Some children die in infancy, whereas others can live into adulthood with only minimal disability. Nearly all forms are inherited in an autosomal-recessive manner. Structural brain defects, with or without mental retardation, are additional features of syndromic MDC. Approximately 60% of all forms is defined as classic MDC (without mental retardation). In northern-east Italy, the overall incidence of MDC has been estimated at 1 in 21 500 with a prevalence of 1 in 150 000.[134]
Syndromic congenital muscular dystrophy (e.g. Fukuyama MDC, muscle-eye-brain-disease, Walker-Warburg-syndrome and MDC1D) and the non-syndromic subtype MDC1C are associated with mutations in proteins that are presumed to be glycosyltransferases.[93] Reduced glycosylation of α-dystroglycan abolishes its interaction with extracellular matrix receptors, such as laminin-2. Disorders sharing this pathogenic mechanism are referred to as dystroglycanopathies which also applies to LGMD2I, LGMD2K and LGMD2M (see above).
MDC1A, commonly known as merosin-deficient MDC, accounts for about 30-40% of MDC cases in European countries, but only 6% in Japan. Affected individuals have a primary deficiency of the α2 chain of laminin-2 (formerly named merosin) due to mutations in the LAMA2 gene.[135] Laminin-2 interacts with dystroglycan which is in turn associated, intracellularly, with dystrophin. LAMA2 defects can also cause partial merosin-deficient MDC. However, some MDC cases with partial merosin deficiency are unlinked to LAMA2. These include MDC1B, linked to chromosome 1q42, and several MDC subtypes with unknown gene defects.[135]

**Cardiac involvement in dystroglycanopathies**
Fukuyama MDC is caused by the gene encoding the fukutin protein and is rarely reported outside Japan. Affected infants are floppy and exhibit motor developmental delay and severe mental retardation.[136] In most patients, systolic left ventricular dysfunction develops after 10 years of age, resulting in heart failure. Death from congestive heart failure or respiratory problems occurs by the age of 20.[137] Myocardial fibrosis of the left ventricular free walls has been observed at autopsy.
MDC1C is caused by a mutation in the gene encoding the fukutin-related protein (FKRP). Mutations in this gene may also cause LGMD2I, which usually has a milder phenotype.[94] MDC1C is characterized by weakness and hypotonia from

birth, hypertrophy of lower limb muscles and inability to achieve ambulation. Progressive respiratory muscle weakness leading to ventilatory insufficiency is a constant feature in the second decade of life. Mildly impaired left ventricular function can also be found.[138]

**Cardiac involvement in merosin-deficient MDC (MDC1A)**

Occasional reports suggest that cardiac involvement may be present in MDC1A. Reduced left ventricular systolic function in a few patients has been addressed.[139] Echocardiography in 6 MDC1A patients showed reduced ejection fraction in 3 subjects.[140] Nevertheless, no clinical significant cardiomyopathy has been reported so far. Recurrent aspiration and respiratory insufficiency appear important determinants of prognosis. Data on life expectancy are lacking, but death during the first decade has been observed in patients with MDC1A.[141] A summary of the reported cardiac abnormalities in the abovementioned congenital muscular dystrophies is presented in table 2.6. Rare MDC subtypes with unknown gene defects are possibly accompanied by cardiac involvement.

**Table 2.6.** Cardiac abnormalities and prognosis in congenital muscular dystrophy

| Disease | ECG | Echo | Cardiac death | Age at death |
|---|---|---|---|---|
| Fukuyama MDC | - tall R waves or R/S ratio >1 in lead V1<br>- deep narrow Q waves in lead V6 | - dilated cardiomyopathy | ~50%[142] | Mean 17 years (range 3-27)[142] |
| MDC-1C | | - left ventricular dysfunction | no major cause of death | 2nd decade[138] |
| MDC-1A | | - left ventricular dysfunction | no major cause of death | Some in first decade[141] |

## Facioscapulohumeral muscular dystrophy

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant myopathy with normal life span and a prevalence of 1 in 20 000. Shoulder girdle weakness is usually the presenting clinical symptom. It is often asymmetrical with relative sparing of the deltoid muscles. Facial muscle involvement, often mild and asymmetrical, is frequently present in the majority of cases at the time of diagnosis. Involvement of abdominal, foot extensor and pelvic muscles occur at a later stage of the disease. Symptoms start on average between 16 and 20 years of age, but may vary from first to sixth decade.[143]

The majority of cases are linked to the subtelomeric region of chromosome 4. A deletion of an integral number of repeats produces a shortened DNA fragment.[144] Severity of disease and age at onset correlate with the residual fragment size, which remains constant in successive generations.[143]

**Cardiac involvement in facioscapulohumeral muscular dystrophy**
Clinically significant cardiac disease is uncommon in patients with FSHD. Studies reported to date indicate that patients may have arrhythmias (0-12%), particularly supraventricular paroxysmal tachycardia, and minor ECG abnormalities.[145-148] Severe cardiomyopathy is rare and usually unrelated to the FSHD. Earlier reports of atrial standstill in patients with facioscapulohumeral type of muscular dystrophy most probably represent a case of misdiagnosed EDMD1.[148]

## Myofibrillar myopathies

Myofibrillar myopathies (MFM) refer to a group of genetically distinct disorders that are frequently associated with peripheral neuropathy and involvement of the heart muscle (table 2.7). Clinically, MFM presents in adult life with slowly progressive weakness of distal and proximal muscles. Diagnosis is mainly based on muscle histology, showing morphologic changes resulting from disintegration of the sarcomeric Z-disk and myofibrils, followed by accumulation of myofibrillar degradation products and ectopic expression of multiple proteins.[149] Several mutations have been identified (table 2.1), however, in most cases the disease-associated protein or gene defect has not been elucidated.
Desminopathies manifest with various phenotypes depending on the type of inheritance and the location of mutations. Cardiac involvement may precede, coincide with or succeed skeletal myopathy. Conduction blocks and arrhythmias requiring a pacemaker are frequent features of desmin-related MFM, attributed to the fact that desmin is a major component of Purkinje fibres.[150] Mutations in α-B crystallin (i.e. chaperone for desmin) can cause phenotypic features of desminopathy. Some patients with α-B crystallinopathy develop hypertrophic cardiomyopathy.[151, 152] Mutation in BAG3 causes severe childhood muscular dystrophy with cardiomyopathy and severe respiratory insufficiency.[153] Cardiomyopathy is considered rare in myotilin-related MFM and very rare in ZASP-related MFM and filamin-C-related MFM.

**Table 2.7.** Cardiac involvement in myofibrillar myopathies

| Disease | Genetics | Cardiac abnormalities | Morbidity/mortality |
|---|---|---|---|
| Desmin-related MFM[150] | - AD (80%), AR (6%), sporadic (14%) | - atrioventricular block<br>- ventricular tachycardia<br>- dilated or restrictive cardiomyopathy | - heart disease in >60%<br>- severity is mutation dependent<br>- onset 2nd to 4th decade in AD, childhood to 20's in AR |
| α-B crystallinopathy [151, 152] | - AD | - hypertrophic cardiomyopathy | - in cases with Arg120Gly missense mutation, but not truncating mutations |
| BAG3-related MFM[153] | - 3 dominant cases | - restrictive or hypertrophic cardiomyopathy | - cardiac involvement in all cases<br>- onset in teens<br>- severe respiratory involvement |
| MFM with ARVD (ARVD7)[154] | - swedish family<br>- AD<br>- similar locus to ZASP | - ventricular tachycardia<br>- atrial flutter<br>- right ventricular dilatation | - heart disease in 25%<br>- onset 20-60 years |

AD=autosomal dominant; AR=autosomal recessive

## Other muscular dystrophies

In distal myopathies, cardiomyopathy is not a consistent feature. Mild-moderate cardiomyopathy was present in 1 of the 3 affected members with late-onset autosomal dominant distal myopathy, which has recently been identified as a **ZASPopathy**.[155]

A few rare X-linked myopathies are associated with cardiomyopathy. **X-linked vacuolar cardiomyopathy and myopathy (Danon disease)** is caused by mutations in the gene encoding lysosome-associated membrane protein-2 (LAMP-2) at Xq24.[156] Muscle biopsies are characterized by autophagic vacuoles with sarcolemmal features.[157] Cardiac disease is the dominant clinical feature and most important prognostic factor. Skeletal muscle involvement is usually mild and is observed in 90% of male patients and 33% of female relatives. Mild mental retardation was found in 70% of males and 6% of females. A study of 20 men and 18 women with genetically confirmed Danon disease, reported cardiomyopathy in all patients.[158] Men were severely affected before the age of 20 years. The most frequent findings were hypertrophic cardiomyopathy with impaired left ventricular functions and Wolff-Parkinson-White syndrome. Most affected women developed predominantly dilated cardiomyopathy in adulthood. All deceased patients died from cardiac failure at young age.

The mean age at death was 19 years in male patients and 40 years in females. Heart transplantation may be the only effective therapeutic option in patients with Danon disease.[158]

**Barth syndrome**, an X-linked disorder in infancy that is characterized by cardiomyopathy, neutropenia, skeletal myopathy, and growth delay, is caused by mutations in the taffazin gene at Xq28 that result in cardiolipin deficiency and abnormal mitochondria.[159] Female carriers appear to be healthy. In a study of 34 males, 90% had dilated cardiomyopathy, 53% had prominent LV trabeculations and ventricular arrhythmias were frequent in adolescents.[159]

**McLeod syndrome** is a late-onset X-linked disorder caused by mutations of XK, a gene of unknown function, and characterized by movement disorders (chorea), cognitive impairment, areflexia, myopathy and acantocytosis. A few cases of manifesting female mutation carriers have been reported.[160] About 65% of individuals develop cardiac manifestations including dilated cardiomyopathy, atrial fibrillation, and tachyarrhythmia.[160]

Four-and-a-half LIM domain gene (FHL1) mutations have been identified as the causative gene for **reducing body myopathy (RBM)**, X-linked scapuloperoneal myopathy (SPM) and **X-linked myopathy with postural muscle atrophy (XMPMA)**. It should be considered in the differential diagnosis of patients X-linked scapuloperoneal muscle involvement (e.g. EDMD). FHL1 is highly expressed in skeletal and cardiac muscle. Hypertrophic cardiomyopathy was observed in 4 out of 9 XMPMA patients, and dilated cardiomyopathy in 1 out of 11 RBM patients.[161, 162] Respiratory failure appeared to be the major prognostic factor.

An **early-onset myopathy with fatal cardiomyopathy** due to homozygous C-terminal TTN deletions has been described as the only titinopathy involving both heart and skeletal muscle.[163]

**Mutations in the beta-cardiac myosin heavy chain gene (MYH7)** may cause various phenotypes ranging from pure peripheral muscle disease to isolated cardiomyopathies. However, MYH7 mutations combining symptomatic distal myopathy and cardiac involvement have been reported.[164, 165]

## Management of muscular dystrophies

### General aspects

Muscular dystrophies are associated with progressive physical disabilities and medical complications. Although curative treatment is not yet available, quality of life and survival of muscular dystrophy patients have improved as a result of advances in medical management based on multidisciplinary care.[166] In general, current symptomatic treatment is designed to maintain and support muscle strength, promote mobility, prevent contractures and maintain

optimal physical and emotional health. In ambulant DMD patients, the use of corticosteroids (0.75 mg/kg/day) is now generally recommended since it has been demonstrated to improve muscle strength and function in 6 months to 2 years.[167] Registries have suggested possible cardiac benefit from corticosteroid therapy,[168] whereas some experimental study suggests no effect.[169]
The number of DMD patients surviving into adulthood is increasing, because of such as enhanced use of ventilatory support and management of spinal deformities. Despite these treatment regimens, progression of muscular dystrophies cannot be prevented.



**Cardiac management**
Cardiomyopathy is the most prominent cardiac feature in dystrophinopathies, sarcoglycanopathies, and diseases associated with mutation in the fukutin-related protein. Most cardiomyopathies in muscular dystrophy patients are of the dilated type. Cardiac evaluation generally includes a history and physical examination, electrocardiogram and transthoracic echocardiogram at diagnosis. Periodic Holter monitoring may be considered for patients with demonstrated cardiac dysfunction. Standard heart failure treatment, including ACE inhibitors or beta-adrenoreceptor blockers, is strongly recommended.[19] These drugs should be started after the first abnormal echocardiography and may even be considered before abnormalities are found. A pacemaker is indicated in case of bradycardia or atrioventricular blocks, whereas symptomatic ventricular arrhythmias may require ICD implantation. Cardiac transplantation may be considered in motivated, ambulant patients with end-stage heart failure and relatively good neuromuscular prognosis. Hypertrophic cardiomyopathy is uncommon in muscular dystrophies, but has been typically described in males with Danon disease, α-B crystallinopathy and XMPMA. Myocardial hypertrophy may be present on echocardiograms among DMD and BMD patients or carriers. Other muscular dystrophies, mainly represented by laminopathies, Emery-Dreifuss muscular dystrophy and myotonic dystrophy, are particularly associated with arrhythmias. These patients should undergo regular risk stratification for sudden death, which is most often caused by complete heart block or ventricular arrhythmias, but may also be a result of atherosclerotic or thrombo-embolic events. Medication known to adversely affect cardiac conduction (e.g. beta-blockers, class Ic antiarrhythmic agents, amiodarone, and tricyclic antidepressants) should be avoided. Early detection and treatment of conduction defects in muscular dystrophy patients is essential, since implantation of a pacemaker can be lifesaving. However, in lamin A/C carriers and DM1 patients, there is established evidence that pacemakers do not decrease the rates of sudden death, suggesting that lethal tachyarrhythmias contribute to mortality.[69, 106] Myocardial degeneration, cardiac conduction-

system abnormalities and increased ventricular loading as a result of respiratory or cardiac failure may all be involved in the development of malignant ventricular arrhythmias. Pacemaker recordings or implantable loop recording devices provide continuous monitoring of heart rate and rhythm and may help diagnose these arrhythmias. The safety and efficacy of antiarrhythmic drugs is controversial as their negative inotropic and pro-arrhythmic actions are enhanced in patients with cardiac dysfunction. Therefore, the implantation of an ICD, rather than a pacemaker, may be considered for lamin A/C carriers and DM1 patients.

Recommendations for cardiac investigations and follow-up of muscular dystrophy patients are based on present consensus guidelines, since published evidence is quite limited.[19, 29] An algorithm with a more detailed pragmatic approach based on present consensus guidelines is presented in figure 2.3. Furthermore, treatment of atrial fibrillation is based on preventing embolism and cardioverting or controlling the tachyarrhythmia. Complete cardiac evaluation should be undertaken before scoliosis surgery or other major surgical procedures and monitoring should continue in the postoperative period.

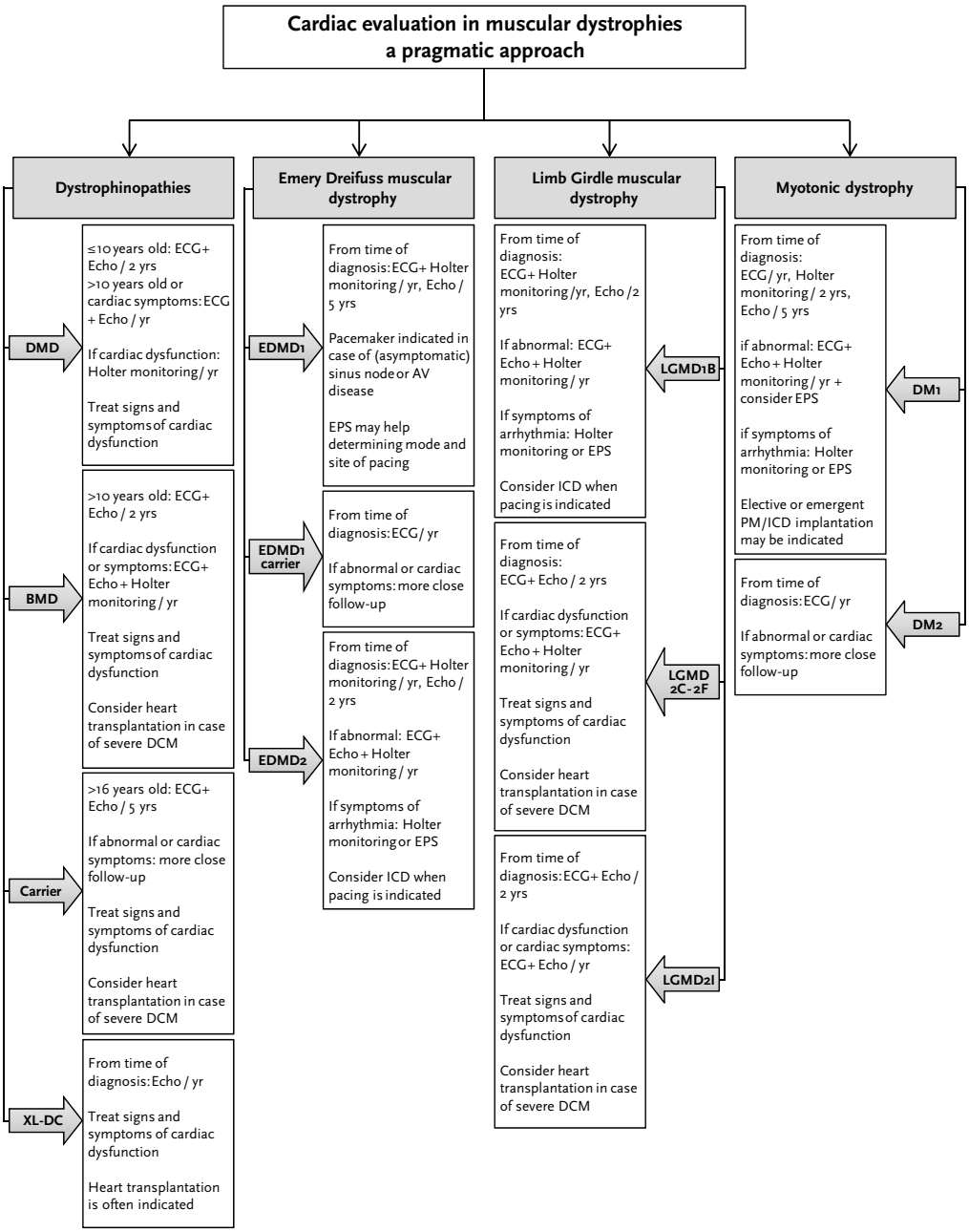
**Future perspectives**

Muscular dystrophies are caused by mutations in genes encoding proteins that are indispensable for normal muscle function. In the absence of either component of the dystrophin-glycoprotein complex, membrane tears may simply develop as a result of mechanical stress, leading to muscle degeneration and cardiomyopathy. Mutations in nuclear envelope proteins can also cause muscular dystrophy and cardiomyopathy. These proteins are implicated in maintaining nuclear integrity of muscle as well as regulation of muscle- and heart-specific gene expression. Furthermore, a novel RNA-mediated disease mechanism is likely the cause of clinical features in DM and may primarily affect cardiac function. Advances in the understanding of molecular genetics and the pathogenesis of muscular dystrophies have raised expectations of more effective therapy.

In the past several years, DMD has been an important target for new therapeutic approaches. These include gene replacement therapy (adeno-associated virus vector and naked plasmid delivery), gene repair strategies (exon skipping and read-through), cell-based therapy (myoblast or stem cell transplantation) and drug therapy to compensate the dystrophic muscle.[170] Several therapeutic concepts have moved from the laboratory setting to clinical trials, in order to proof their effect and to test safety and tolerability.

The ultimate therapeutic target in DM is reversal of RNA toxicity. Methodologies based on neutralization or elimination of toxic RNA (antisense oligonucleotides and short interfering RNA) and interruption of the pathways that lead to

**Figure 2.3.** Pragmatic approach for cardiac evaluation in muscular dystrophies based on literature review and consensus guidelines[19, 29]



abnormal splicing (skipping of abnormally included exons, down-regulation of CUGBP1 activity, and up-regulation of MBNL1 activity) have shown promise in preclinical models.[171] Less specific treatments that prevent or counteract muscle degeneration, reduce inflammation or stimulate muscle metabolism or regeneration would be feasible for all dystrophic patients.[170, 171] As our understanding of pathogenesis continues to improve, more specific treatment that targets the disease mechanism will begin to replace the supportive care currently available.

## Conclusion

Surveillance and timely management of cardiac complications are important since long-term prognosis may be directly related to cardiac status. With increased quality of life and prolonged survival of muscular dystrophy patients, heart failure and arrhythmias contribute to a larger extent to premature death. Recognition of cardiac involvement requires active investigation, since the typical signs and symptoms of cardiac dysfunction may not be present, possibly secondary to the patient's muscular impairment and limited mobility. Although the benefits of pre-symptomatic diagnosis or treatment of cardiac involvement may not always have been established, patients should be made aware of the risk of developing cardiomyopathy and offered appropriate care for the cardiac aspects of their neuromuscular disorder. Periodic examination is therefore advised and initiated as soon as the neuromuscular diagnosis is ascertained. Prospective studies are mandatory to evaluate current strategies and to adjust guidelines for surveillance, heart failure treatment and device implantation.

## References

1. Online Mendelian Inheritance in Man (OMIM®). Available at www.ncbi.nlm.nih.gov/omim
2. Emery AE. Population frequencies of inherited neuromuscular diseases--a world survey. *Neuromuscul Disord*. 1991;1:19-29
3. Eagle M, Baudouin SV, Chandler C, Giddings DR, Bullock R, Bushby K. Survival in duchenne muscular dystrophy: Improvements in life expectancy since 1967 and the impact of home nocturnal ventilation. *Neuromuscul Disord*. 2002;12:926-929
4. Bushby KM, Thambyayah M, Gardner-Medwin D. Prevalence and incidence of becker muscular dystrophy. *Lancet*. 1991;337:1022-1024
5. Emery AE, Skinner R. Clinical studies in benign (becker type) x-linked muscular dystrophy. *Clin Genet*. 1976;10:189-201
6. Bradley WG, Jones MZ, Mussini JM, Fawcett PR. Becker-type muscular dystrophy. *Muscle Nerve*. 1978;1:111-132
7. Bushby KM, Gardner-Medwin D. The clinical, genetic and dystrophin characteristics of becker muscular dystrophy. I. Natural history. *J Neurol*. 1993;240:98-104
8. Hoogerwaard EM, de Voogt WG, Wilde AA, van der Wouw PA, Bakker E, van Ommen GJ, de Visser M. Evolution of cardiac abnormalities in becker muscular dystrophy over a 13-year period. *J Neurol*. 1997;244:657-663
9. Towbin JA, Hejtmancik JF, Brink P, Gelb B, Zhu XM, Chamberlain JS, McCabe ER, Swift M. X-linked dilated cardiomyopathy. Molecular genetic evidence of linkage to the duchenne muscular dystrophy (dystrophin) gene at the xp21 locus. *Circulation*. 1993;87:1854-1865
10. Monaco AP, Bertelson CJ, Liechti-Gallati S, Moser H, Kunkel LM. An explanation for the phenotypic differences between patients bearing partial deletions of the dmd locus. *Genomics*. 1988;2:90-95
11. Petrof BJ, Shrager JB, Stedman HH, Kelly AM, Sweeney HL. Dystrophin protects the sarcolemma from stresses developed during muscle contraction. *Proc Natl Acad Sci U S A*. 1993;90:3710-3714
12. Wallace GQ, McNally EM. Mechanisms of muscle degeneration, regeneration, and repair in the muscular dystrophies. *Annu Rev Physiol*. 2008
13. Perloff JK. Cardiac rhythm and conduction in duchenne's muscular dystrophy: A prospective study of 20 patients. *J Am Coll Cardiol*. 1984;3:1263-1268
14. Ishikawa K. Cardiac involvement in progressive muscular dystrophy of the duchenne type. *Jpn Heart J*. 1997;38:163-180
15. Sanyal SK, Johnson WW. Cardiac conduction abnormalities in children with duchenne's progressive muscular dystrophy: Electrocardiographic features and morphologic correlates. *Circulation*. 1982;66:853-863
16. Nigro G, Comi LI, Politano L, Bain RJ. The incidence and evolution of cardiomyopathy in duchenne muscular dystrophy. *Int J Cardiol*. 1990;26:271-277
17. Yanagisawa A, Miyagawa M, Yotsukura M, Tsuya T, Shirato C, Ishihara T, Aoyagi T, Ishikawa K. The prevalence and prognostic significance of arrhythmias in duchenne type muscular dystrophy. *Am Heart J*. 1992;124:1244-1250
18. Chenard AA, Becane HM, Tertrain F, de Kermadec JM, Weiss YA. Ventricular arrhythmia in duchenne muscular dystrophy: Prevalence, significance and prognosis. *Neuromuscul Disord*. 1993;3:201-206
19. American Academy of Pediatrics. Cardiovascular health supervision for individuals affected by duchenne or becker muscular dystrophy. *Pediatrics*. 2005;116:1569-1573
20. Corrado G, Lissoni A, Beretta S, Terenghi L, Tadeo G, Foglia-Manzillo G, Tagliagambe LM, Spata M, Santarone M. Prognostic value of electrocardiograms, ventricular late potentials, ventricular arrhythmias, and left ventricular systolic dysfunction in patients with duchenne muscular dystrophy. *Am J Cardiol*. 2002;89:838-841
21. Duboc D, Meune C, Lerebours G, Devaux JY, Vaksmann G, Becane HM. Effect of perindopril on the onset and progression of left ventricular dysfunction in duchenne muscular dystrophy. *J Am Coll Cardiol*. 2005;45:855-857
22. Duboc D, Meune C, Pierre B, Wahbi K, Eymard B, Toutain A, Berard C, Vaksmann G, Weber S, Becane HM. Perindopril preventive treatment on mortality in duchenne muscular dystrophy: 10 years' follow-up. *Am Heart J*. 2007;154:596-602

23. Cohn RD, van Erp C, Habashi JP, Soleimani AA, Klein EC, Lisi MT, Gamradt M, ap Rhys CM, Holm TM, Loeys BL, Ramirez F, Judge DP, Ward CW, Dietz HC. Angiotensin ii type 1 receptor blockade attenuates tgf-beta-induced failure of muscle regeneration in multiple myopathic states. *Nat Med*. 2007;13:204-210
24. Chamberlain JS. Ace inhibitor bulks up muscle. *Nat Med*. 2007;13:125-126
25. Steare SE, Dubowitz V, Benatar A. Subclinical cardiomyopathy in becker muscular dystrophy. *Br Heart J*. 1992;68:304-308
26. Nigro G, Comi LI, Politano L, Limongelli FM, Nigro V, De Rimini ML, Giugliano MA, Petretta VR, Passamano L, Restucci B, et al. Evaluation of the cardiomyopathy in becker muscular dystrophy. *Muscle Nerve*. 1995;18:283-291
27. Saito M, Kawai H, Akaike M, Adachi K, Nishida Y, Saito S. Cardiac dysfunction with becker muscular dystrophy. *Am Heart J*. 1996;132:642-647
28. Melacini P, Fanin M, Danieli GA, Villanova C, Martinello F, Miorin M, Freda MP, Miorelli M, Mostacciuolo ML, Fasoli G, Angelini C, Dalla Volta S. Myocardial involvement is very frequent among patients affected with subclinical becker's muscular dystrophy. *Circulation*. 1996;94:3168-3175
29. Bushby K, Muntoni F, Bourke JP. 107th enmc international workshop: The management of cardiac involvement in muscular dystrophy and myotonic dystrophy. 7th-9th june 2002, naarden, the netherlands. *Neuromuscul Disord*. 2003;13:166-172
30. Connuck DM, Sleeper LA, Colan SD, Cox GF, Towbin JA, Lowe AM, Wilkinson JD, Orav EJ, Cuniberti L, Salbert BA, Lipshultz SE. Characteristics and outcomes of cardiomyopathy in children with duchenne or becker muscular dystrophy: A comparative study from the pediatric cardiomyopathy registry. *Am Heart J*. 2008;155:998-1005
31. Jefferies JL, Eidem BW, Belmont JW, Craigen WJ, Ware SM, Fernbach SD, Neish SR, Smith EO, Towbin JA. Genetic predictors and remodeling of dilated cardiomyopathy in muscular dystrophy. *Circulation*. 2005;112:2799-2804
32. Kaspar RW, Allen HD, Ray WC, Alvarez CE, Kissel JT, Pestronk A, Weiss RB, Flanigan KM, Mendell JR, Montanaro F. Analysis of dystrophin deletion mutations predicts age of cardiomyopathy onset in becker muscular dystrophy. *Circ Cardiovasc Genet*. 2009;2:544-551
33. Politano L, Nigro V, Nigro G, Petretta VR, Passamano L, Papparella S, Di Somma S, Comi LI. Development of cardiomyopathy in female carriers of duchenne and becker muscular dystrophies. *JAMA*. 1996;275:1335-1338
34. Nolan MA, Jones OD, Pedersen RL, Johnston HM. Cardiac assessment in childhood carriers of duchenne and becker muscular dystrophies. *Neuromuscul Disord*. 2003;13:129-132
35. Hoogerwaard EM, Bakker E, Ippel PF, Oosterwijk JC, Majoor-Krakauer DF, Leschot NJ, Van Essen AJ, Brunner HG, van der Wouw PA, Wilde AA, de Visser M. Signs and symptoms of duchenne muscular dystrophy and becker muscular dystrophy among carriers in the netherlands: A cohort study. *Lancet*. 1999;353:2116-2119
36. Hoogerwaard EM, van der Wouw PA, Wilde AA, Bakker E, Ippel PF, Oosterwijk JC, Majoor-Krakauer DF, van Essen AJ, Leschot NJ, de Visser M. Cardiac involvement in carriers of duchenne and becker muscular dystrophy. *Neuromuscul Disord*. 1999;9:347-351
37. Grain L, Cortina-Borja M, Forfar C, Hilton-Jones D, Hopkin J, Burch M. Cardiac abnormalities and skeletal muscle weakness in carriers of duchenne and becker muscular dystrophies and controls. *Neuromuscul Disord*. 2001;11:186-191
38. Melacini P, Fanin M, Angelini A, Pegoraro E, Livi U, Danieli GA, Hoffman EP, Thiene G, Dalla Volta S, Angelini C. Cardiac transplantation in a duchenne muscular dystrophy carrier. *Neuromuscul Disord*. 1998;8:585-590
39. Davies JE, Winokur TS, Aaron MF, Benza RL, Foley BA, Holman WL. Cardiomyopathy in a carrier of duchenne's muscular dystrophy. *J Heart Lung Transplant*. 2001;20:781-784
40. Holloway SM, Wilcox DE, Wilcox A, Dean JC, Berg JN, Goudie DR, Denvir MA, Porteous ME. Life expectancy and death from cardiomyopathy amongst carriers of duchenne and becker muscular dystrophy in scotland. *Heart*. 2008;94:633-636
41. Berko BA, Swift M. X-linked dilated cardiomyopathy. *N Engl J Med*. 1987;316:1186-1191
42. Ferlini A, Sewry C, Melis MA, Mateddu A, Muntoni F. X-linked dilated cardiomyopathy and the dystrophin gene. *Neuromuscul Disord*. 1999;9:339-346
43. Bione S, Maestrini E, Rivella S, Mancini M, Regis S, Romeo G, Toniolo D. Identification of a novel x-linked gene responsible for emery-dreifuss muscular dystrophy. *Nat Genet*. 1994;8:323-327

44. Manilal S, Nguyen TM, Sewry CA, Morris GE. The emery-dreifuss muscular dystrophy protein, emerin, is a nuclear membrane protein. *Hum Mol Genet*. 1996;5:801-808
45. Nagano A, Koga R, Ogawa M, Kurano Y, Kawada J, Okada R, Hayashi YK, Tsukahara T, Arahata K. Emerin deficiency at the nuclear membrane in patients with emery-dreifuss muscular dystrophy. *Nat Genet*. 1996;12:254-259
46. Manilal S, Recan D, Sewry CA, Hoeltzenbein M, Llense S, Leturcq F, Deburgrave N, Barbot J, Man N, Muntoni F, Wehnert M, Kaplan J, Morris GE. Mutations in emery-dreifuss muscular dystrophy and their effects on emerin protein expression. *Hum Mol Genet*. 1998;7:855-864
47. Bonne G, Di Barletta MR, Varnous S, Becane HM, Hammouda EH, Merlini L, Muntoni F, Greenberg CR, Gary F, Urtizberea JA, Duboc D, Fardeau M, Toniolo D, Schwartz K. Mutations in the gene encoding lamin a/c cause autosomal dominant emery-dreifuss muscular dystrophy. *Nat Genet*. 1999;21:285-288
48. Raffaele Di Barletta M, Ricci E, Galluzzi G, Tonali P, Mora M, Morandi L, Romorini A, Voit T, Orstavik KH, Merlini L, Trevisan C, Biancalana V, Housmanowa-Petrusewicz I, Bione S, Ricotti R, Schwartz K, Bonne G, Toniolo D. Different mutations in the lmna gene cause autosomal dominant and autosomal recessive emery-dreifuss muscular dystrophy. *Am J Hum Genet*. 2000;66:1407-1412
49. Benedetti S, Merlini L. Laminopathies: From the heart of the cell to the clinics. *Curr Opin Neurol*. 2004;17:553-560
50. Bonne G, Mercuri E, Muchir A, Urtizberea A, Becane HM, Recan D, Merlini L, Wehnert M, Boor R, Reuner U, Vorgerd M, Wicklein EM, Eymard B, Duboc D, Penisson-Besnier I, Cuisset JM, Ferrer X, Desguerre I, Lacombe D, Bushby K, Pollitt C, Toniolo D, Fardeau M, Schwartz K, Muntoni F. Clinical and molecular genetic spectrum of autosomal dominant emery-dreifuss muscular dystrophy due to mutations of the lamin a/c gene. *Ann Neurol*. 2000;48:170-180
51. Manilal S, Sewry CA, Pereboev A, Man N, Gobbi P, Hawkes S, Love DR, Morris GE. Distribution of emerin and lamins in the heart and implications for emery-dreifuss muscular dystrophy. *Hum Mol Genet*. 1999;8:353-359
52. Bonne G, Leturcq F, Récan-Budiartha D, Ben Yaou R. Updated 21 november 2007. Emery-dreifuss muscular dystrophy. In: *Genereviews* at genetests: Medical genetics information resource (database online). Copyright, university of washington, seattle. 1997-2009. Available at www.genetests.org
53. Zhang Q, Bethmann C, Worth NF, Davies JD, Wasner C, Feuer A, Ragnauth CD, Yi Q, Mellad JA, Warren DT, Wheeler MA, Ellis JA, Skepper JN, Vorgerd M, Schlotter-Weigel B, Weissberg PL, Roberts RG, Wehnert M, Shanahan CM. Nesprin-1 and -2 are involved in the pathogenesis of emery dreifuss muscular dystrophy and are critical for nuclear envelope integrity. *Hum Mol Genet*. 2007;16:2816-2833
54. Tsuchiya Y, Hase A, Ogawa M, Yorifuji H, Arahata K. Distinct regions specify the nuclear membrane targeting of emerin, the responsible protein for emery-dreifuss muscular dystrophy. *Eur J Biochem*. 1999;259:859-865
55. Lammerding J, Hsiao J, Schulze PC, Kozlov S, Stewart CL, Lee RT. Abnormal nuclear shape and impaired mechanotransduction in emerin-deficient cells. *J Cell Biol*. 2005;170:781-791
56. Bakay M, Wang Z, Melcon G, Schiltz L, Xuan J, Zhao P, Sartorelli V, Seo J, Pegoraro E, Angelini C, Shneiderman B, Escolar D, Chen YW, Winokur ST, Pachman LM, Fan C, Mandler R, Nevo Y, Gordon E, Zhu Y, Dong Y, Wang Y, Hoffman EP. Nuclear envelope dystrophies show a transcriptional fingerprint suggesting disruption of rb-myod pathways in muscle regeneration. *Brain*. 2006;129:996-1013
57. Waters DD, Nutter DO, Hopkins LC, Dorney ER. Cardiac features of an unusual x-linked humeroperoneal neuromuscular disease. *N Engl J Med*. 1975;293:1017-1022
58. Voit T, Krogmann O, Lenard HG, Neuen-Jacob E, Wechsler W, Goebel HH, Rahlf G, Lindinger A, Nienaber C. Emery-dreifuss muscular dystrophy: Disease spectrum and differential diagnosis. *Neuropediatrics*. 1988;19:62-71
59. Bialer MG, McDaniel NL, Kelly TE. Progression of cardiac disease in emery-dreifuss muscular dystrophy. *Clin Cardiol*. 1991;14:411-416
60. Fishbein MC, Siegel RJ, Thompson CE, Hopkins LC. Sudden death of a carrier of x-linked emery-dreifuss muscular dystrophy. *Ann Intern Med*. 1993;119:900-905
61. Merlini L, Granata C, Dominici P, Bonfiglioli S. Emery-dreifuss muscular dystrophy: Report of five cases in a family and review of the literature. *Muscle Nerve*. 1986;9:481-485
62. Funakoshi M, Tsuchiya Y, Arahata K. Emerin and cardiomyopathy in emery-dreifuss muscular dystrophy. *Neuromuscul Disord*. 1999;9:108-114

63. Boriani G, Gallina M, Merlini L, Bonne G, Toniolo D, Amati S, Biffi M, Martignani C, Frabetti L, Bonvicini M, Rapezzi C, Branzi A. Clinical relevance of atrial fibrillation/flutter, stroke, pacemaker implant, and heart failure in emery-dreifuss muscular dystrophy: A long-term longitudinal study. *Stroke*. 2003;34:901-908
64. Sakata K, Shimizu M, Ino H, Yamaguchi M, Terai H, Fujino N, Hayashi K, Kaneda T, Inoue M, Oda Y, Fujita T, Kaku B, Kanaya H, Mabuchi H. High incidence of sudden cardiac death with conduction disturbances and atrial cardiomyopathy caused by a nonsense mutation in the sta gene. *Circulation*. 2005;111:3352-3358
65. van der Kooi AJ, Ledderhof TM, de Voogt WG, Res CJ, Bouwsma G, Troost D, Busch HF, Becker AE, de Visser M. A newly recognized autosomal dominant limb girdle muscular dystrophy with cardiac involvement. *Ann Neurol*. 1996;39:636-642
66. Fatkin D, MacRae C, Sasaki T, Wolff MR, Porcu M, Frenneaux M, Atherton J, Vidaillet HJ, Jr., Spudich S, De Girolami U, Seidman JG, Seidman C, Muntoni F, Muehle G, Johnson W, McDonough B. Missense mutations in the rod domain of the lamin a/c gene as causes of dilated cardiomyopathy and conduction-system disease. *N Engl J Med*. 1999;341:1715-1724
67. Becane HM, Bonne G, Varnous S, Muchir A, Ortega V, Hammouda EH, Urtizberea JA, Lavergne T, Fardeau M, Eymard B, Weber S, Schwartz K, Duboc D. High incidence of sudden death with conduction system and myocardial disease due to lamins a and c gene mutation. *Pacing Clin Electrophysiol*. 2000;23:1661-1666
68. Brodsky GL, Muntoni F, Miocic S, Sinagra G, Sewry C, Mestroni L. Lamin a/c gene mutation associated with dilated cardiomyopathy with variable skeletal muscle involvement. *Circulation*. 2000;101:473-476
69. van Berlo JH, de Voogt WG, van der Kooi AJ, van Tintelen JP, Bonne G, Yaou RB, Duboc D, Rossenbacker T, Heidbuchel H, de Visser M, Crijns HJ, Pinto YM. Meta-analysis of clinical characteristics of 299 carriers of lmna gene mutations: Do lamin a/c mutations portend a high risk of sudden death? *J Mol Med*. 2005;83:79-83
70. Meune C, Van Berlo JH, Anselme F, Bonne G, Pinto YM, Duboc D. Primary prevention of sudden death in patients with lamin a/c gene mutations. *N Engl J Med*. 2006;354:209-210
71. Pasotti M, Klersy C, Pilotto A, Marziliano N, Rapezzi C, Serio A, Mannarino S, Gambarin F, Favalli V, Grasso M, Agozzino M, Campana C, Gavazzi A, Febo O, Marini M, Landolina M, Mortara A, Piccolo G, Vigano M, Tavazzi L, Arbustini E. Long-term outcome and risk stratification in dilated cardiolaminopathies. *J Am Coll Cardiol*. 2008;52:1250-1260
72. Bushby K. The limb-girdle muscular dystrophies. In: Emery AE, ed. Diagnostic criteria for neuromuscular disorders. London: Royal Society of Medicine Press; 1997:17-22
73. Harley HG, Rundle SA, Reardon W, Myring J, Crow S, Brook JD, Harper PS, Shaw DJ. Unstable DNA sequence in myotonic dystrophy. *Lancet*. 1992;339:1125-1128
74. van der Kooi AJ, Barth PG, Busch HF, de Haan R, Ginjaar HB, van Essen AJ, van Hooff LJ, Howeler CJ, Jennekens FG, Jongen P, Oosterhuis HJ, Padberg GW, Spaans F, Wintzen AR, Wokke JH, Bakker E, van Ommen GJ, Bolhuis PA, de Visser M. The clinical spectrum of limb girdle muscular dystrophy. A survey in the netherlands. *Brain*. 1996;119 ( Pt 5):1471-1480
75. Laval SH, Bushby KM. Limb-girdle muscular dystrophies--from genetics to molecular pathology. *Neuropathol Appl Neurobiol*. 2004;30:91-105
76. Minetti C, Sotgia F, Bruno C, Scartezzini P, Broda P, Bado M, Masetti E, Mazzocco M, Egeo A, Donati MA, Volonte D, Galbiati F, Cordone G, Bricarelli FD, Lisanti MP, Zara F. Mutations in the caveolin-3 gene cause autosomal dominant limb-girdle muscular dystrophy. *Nat Genet*. 1998;18:365-368
77. Hauser MA, Conde CB, Kowaljow V, Zeppa G, Taratuto AL, Torian UM, Vance J, Pericak-Vance MA, Speer MC, Rosa AL. Myotilin mutation found in second pedigree with lgmd1a. *Am J Hum Genet*. 2002;71:1428-1432
78. Messina DN, Speer MC, Pericak-Vance MA, McNally EM. Linkage of familial dilated cardiomyopathy with conduction defect and muscular dystrophy to chromosome 6q23. *Am J Hum Genet*. 1997;61:909-917
79. Crosbie RH, Lim LE, Moore SA, Hirano M, Hays AP, Maybaum SW, Collin H, Dovico SA, Stolle CA, Fardeau M, Tome FM, Campbell KP. Molecular and genetic characterization of sarcospan: Insights into sarcoglycan-sarcospan interactions. *Hum Mol Genet*. 2000;9:2019-2027
80. Holt KH, Campbell KP. Assembly of the sarcogly81. Fanin M, Duggan DJ, Mostacciuolo ML, Martinello F, Freda MP, Soraru G, Trevisan CP, Hoffman EP, Angelini C. Genetic epidemiology of muscular dystrophies resulting from sarcoglycan gene mutations. *J Med Genet*. 1997;34:973-977

82. Duggan DJ, Manchester D, Stears KP, Mathews DJ, Hart C, Hoffman EP. Mutations in the delta-sarcoglycan gene are a rare cause of autosomal recessive limb-girdle muscular dystrophy (lgmd2). *Neurogenetics*. 1997;1:49-58
83. Vainzof M, Passos-Bueno MR, Pavanello RC, Marie SK, Oliveira AS, Zatz M. Sarcoglycanopathies are responsible for 68% of severe autosomal recessive limb-girdle muscular dystrophy in the brazilian population. *J Neurol Sci*. 1999;164:44-49
84. Eymard B, Romero NB, Leturcq F, Piccolo F, Carrie A, Jeanpierre M, Collin H, Deburgrave N, Azibi K, Chaouch M, Merlini L, Themar-Noel C, Penisson I, Mayer M, Tanguy O, Campbell KP, Kaplan JC, Tome FM, Fardeau M. Primary adhalinopathy (alpha-sarcoglycanopathy): Clinical, pathologic, and genetic correlation in 20 patients with autosomal recessive muscular dystrophy. *Neurology*. 1997;48:1227-1234
85. Barresi R, Confalonieri V, Lanfossi M, Di Blasi C, Torchiana E, Mantegazza R, Jarre L, Nardocci N, Boffi P, Tezzon F, Pini A, Cornelio F, Mora M, Morandi L. Concomitant deficiency of beta- and gamma-sarcoglycans in 20 alpha-sarcoglycan (adhalin)-deficient patients: Immunohistochemical analysis and clinical aspects. *Acta Neuropathol*. 1997;94:28-35
86. Angelini C, Fanin M, Freda MP, Duggan DJ, Siciliano G, Hoffman EP. The clinical spectrum of sarcoglycanopathies. *Neurology*. 1999;52:176-179
87. Melacini P, Fanin M, Duggan DJ, Freda MP, Berardinelli A, Danieli GA, Barchitta A, Hoffman EP, Dalla Volta S, Angelini C. Heart involvement in muscular dystrophies due to sarcoglycan gene mutations. *Muscle Nerve*. 1999;22:473-479
88. Politano L, Nigro V, Passamano L, Petretta V, Comi LI, Papparella S, Nigro G, Rambaldi PF, Raia P, Pini A, Mora M, Giugliano MA, Esposito MG, Nigro G. Evaluation of cardiac and respiratory involvement in sarcoglycanopathies. *Neuromuscul Disord*. 2001;11:178-185
89. Barresi R, Di Blasi C, Negri T, Brugnoni R, Vitali A, Felisari G, Salandi A, Daniel S, Cornelio F, Morandi L, Mora M. Disruption of heart sarcoglycan complex and severe cardiomyopathy caused by beta sarcoglycan mutations. *J Med Genet*. 2000;37:102-107
90. Fanin M, Melacini P, Boito C, Pegoraro E, Angelini C. Lgmd2e patients risk developing dilated cardiomyopathy. *Neuromuscul Disord*. 2003;13:303-309
91. Brockington M, Yuva Y, Prandini P, Brown SC, Torelli S, Benson MA, Herrmann R, Anderson LV, Bashir R, Burgunder JM, Fallet S, Romero N, Fardeau M, Straub V, Storey G, Pollitt C, Richard I, Sewry CA, Bushby K, Voit T, Blake DJ, Muntoni F. Mutations in the fukutin-related protein gene (fkrp) identify limb girdle muscular dystrophy 2i as a milder allelic variant of congenital muscular dystrophy mdc1c. *Hum Mol Genet*. 2001;10:2851-2859
92. Beltran-Valero de Bernabe D, Voit T, Longman C, Steinbrecher A, Straub V, Yuva Y, Herrmann R, Sperner J, Korenke C, Diesen C, Dobyns WB, Brunner HG, van Bokhoven H, Brockington M, Muntoni F. Mutations in the fkrp gene can cause muscle-eye-brain disease and walker-warburg syndrome. *J Med Genet*. 2004;41:e61
93. Muntoni F, Torelli S, Brockington M. Muscular dystrophies due to glycosylation defects. *Neurotherapeutics*. 2008;5:627-632
94. Mercuri E, Brockington M, Straub V, Quijano-Roy S, Yuva Y, Herrmann R, Brown SC, Torelli S, Dubowitz V, Blake DJ, Romero NB, Estournet B, Sewry CA, Guicheney P, Voit T, Muntoni F. Phenotypic spectrum associated with mutations in the fukutin-related protein gene. *Ann Neurol*. 2003;53:537-542
95. Poppe M, Bourke J, Eagle M, Frosk P, Wrogemann K, Greenberg C, Muntoni F, Voit T, Straub V, Hilton-Jones D, Shirodaria C, Bushby K. Cardiac and respiratory failure in limb-girdle muscular dystrophy 2i. *Ann Neurol*. 2004;56:738-741
96. Boito CA, Melacini P, Vianello A, Prandini P, Gavassini BF, Bagattin A, Siciliano G, Angelini C, Pegoraro E. Clinical and molecular characterization of patients with limb-girdle muscular dystrophy type 2i. *Arch Neurol*. 2005;62:1894-1899
97. Sveen ML, Thune JJ, Kober L, Vissing J. Cardiac involvement in patients with limb-girdle muscular dystrophy type 2 and becker muscular dystrophy. *Arch Neurol*. 2008;65:1196-1201
98. Wahbi K, Meune C, Hamouda el H, Stojkovic T, Laforet P, Becane HM, Eymard B, Duboc D. Cardiac assessment of limb-girdle muscular dystrophy 2i patients: An echography, holter ecg and magnetic resonance imaging study. *Neuromuscul Disord*. 2008;18:650-655
99. Beckmann JS, Brown RH, Muntoni F, Urtizberea A, Bonnemann C, Bushby KM. 66th/67th enmc sponsored international workshop: The limb-girdle muscular dystrophies, 26-28 march 1999, naarden, the netherlands. *Neuromuscul Disord*. 1999;9:436-445
100. Fayssoil A. Cardiac diseases in sarcoglycanopathies. *Int J Cardiol*. 2009

101. Harper PS. Myotonic dystrophy. London: Saunders; 2001
102. Howeler CJ, Busch HF, Geraedts JP, Niermeijer MF, Staal A. Anticipation in myotonic dystrophy: Fact or fiction? *Brain*. 1989;112:779-797
103. Harley HG, Rundle SA, MacMillan JC, Myring J, Brook JD, Crow S, Reardon W, Fenton I, Shaw DJ, Harper PS. Size of the unstable ctg repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. *Am J Hum Genet*. 1993;52:1164-1174
104. Mathieu J, Allard P, Potvin L, Prevost C, Begin P. A 10-year study of mortality in a cohort of patients with myotonic dystrophy. *Neurology*. 1999;52:1658-1662
105. de Die-Smulders CE, Howeler CJ, Thijs C, Mirandolle JF, Anten HB, Smeets HJ, Chandler KE, Geraedts JP. Age and causes of death in adult-onset myotonic dystrophy. *Brain*. 1998;121:1557-1563
106. Groh WJ, Groh MR, Saha C, Kincaid JC, Simmons Z, Ciafaloni E, Pourmand R, Otten RF, Bhakta D, Nair GV, Marashdeh MM, Zipes DP, Pascuzzi RM. Electrocardiographic abnormalities and sudden death in myotonic dystrophy type 1. *N Engl J Med*. 2008;358:2688-2697
107. Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, Aburatani H, Hunter K, Stanton VP, Thirion JP, Hudson T, et al. 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*. 1992;69:385
108. Berul CI, Maguire CT, Gehrmann J, Reddy S. Progressive atrioventricular conduction block in a mouse myotonic dystrophy model. *J Interv Card Electrophysiol*. 2000;4:351-358
109. Klesert TR, Otten AD, Bird TD, Tapscott SJ. Trinucleotide repeat expansion at the myotonic dystrophy locus reduces expression of dmahp. *Nat Genet*. 1997;16:402-406
110. Ranum LP, Day JW. Myotonic dystrophy: Rna pathogenesis comes into focus. *Am J Hum Genet*. 2004;74:793-804
111. Bigot A, Klein AF, Gasnier E, Jacquemin V, Ravassard P, Butler-Browne G, Mouly V, Furling D. Large ctg repeats trigger p16-dependent premature senescence in myotonic dystrophy type 1 muscle precursor cells. *Am J Pathol*. 2009;174:1435-1442
112. Bassez G, Lazarus A, Desguerre I, Varin J, Laforet P, Becane HM, Meune C, Arne-Bes MC, Ounnoughene Z, Radvanyi H, Eymard B, Duboc D. Severe cardiac arrhythmias in young patients with myotonic dystrophy type 1. *Neurology*. 2004;63:1939-1941
113. Phillips MF, Harper PS. Cardiac disease in myotonic dystrophy. *Cardiovasc Res*. 1997;33:13-22
114. Hawley RJ, Milner MR, Gottdiener JS, Cohen A. Myotonic heart disease: A clinical follow-up. *Neurology*. 1991;41:259-262
115. Fragola PV, Luzi M, Calo L, Antonini G, Borzi M, Frongillo D, Cannata D. Cardiac involvement in myotonic dystrophy. *Am J Cardiol*. 1994;74:1070-1072
116. Lazarus A, Varin J, Babuty D, Anselme F, Coste J, Duboc D. Long-term follow-up of arrhythmias in patients with myotonic dystrophy treated by pacing: A multicenter diagnostic pacemaker study. *J Am Coll Cardiol*. 2002;40:1645-1652
117. Lazarus A, Varin J, Ounnoughene Z, Radvanyi H, Junien C, Coste J, Laforet P, Eymard B, Becane HM, Weber S, Duboc D. Relationships among electrophysiological findings and clinical status, heart function, and extent of DNA mutation in myotonic dystrophy. *Circulation*. 1999;99:1041-1046
118. Nguyen HH, Wolfe JT, 3rd, Holmes DR, Jr., Edwards WD. Pathology of the cardiac conduction system in myotonic dystrophy: A study of 12 cases. *J Am Coll Cardiol*. 1988;11:662-671
119. De Ambroggi L, Raisaro A, Marchiano V, Radice S, Meola G. Cardiac involvement in patients with myotonic dystrophy: Characteristic features of magnetic resonance imaging. *Eur Heart J*. 1995;16:1007-1010
120. Bhakta D, Lowe MR, Groh WJ. Prevalence of structural cardiac abnormalities in patients with myotonic dystrophy type i. *Am Heart J*. 2004;147:224-227
121. Orndahl G, Thulesius O, Enestroem S, Dehlin O. The heart in myotonic disease. *Acta Med Scand*. 1964;176:479-491
122. Hermans MC, Faber CG, Pinto YM. Sudden death in myotonic dystrophy. *N Engl J Med*. 2008;359:1626-1628; author reply 1328-1629
123. 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. 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*. 2003;73:835-848

124. Day JW, Ricker K, Jacobsen JF, Rasmussen LJ, Dick KA, Kress W, Schneider C, Koch MC, Beilman GJ, Harrison AR, Dalton JC, Ranum LP. Myotonic dystrophy type 2: Molecular, diagnostic and clinical spectrum. *Neurology*. 2003;60:657-664
125. Meola G, Moxley RT, 3rd. Myotonic dystrophy type 2 and related myotonic disorders. *J Neurol*. 2004;251:1173-1182
126. Liquori CL, Ricker K, Moseley ML, Jacobsen JF, Kress W, Naylor SL, Day JW, Ranum LP. Myotonic dystrophy type 2 caused by a cctg expansion in intron 1 of znf9. *Science*. 2001;293:864-867
127. Botta A, Vallo L, Rinaldi F, Bonifazi E, Amati F, Biancolella M, Gambardella S, Mancinelli E, Angelini C, Meola G, Novelli G. Gene expression analysis in myotonic dystrophy: Indications for a common molecular pathogenic pathway in dm1 and dm2. *Gene Expr*. 2007;13:339-351
128. Wahbi K, Meune C, Becane HM, Laforet P, Bassez G, Lazarus A, Radvanyi-Hoffman H, Eymard B, Duboc D. Left ventricular dysfunction and cardiac arrhythmias are frequent in type 2 myotonic dystrophy: A case control study. *Neuromuscul Disord*. 2009
129. von zur Muhlen F, Klass C, Kreuzer H, Mall G, Giese A, Reimers CD. Cardiac involvement in proximal myotonic myopathy. *Heart*. 1998;79:619-621
130. Schoser BG, Ricker K, Schneider-Gold C, Hengstenberg C, Durre J, Bultmann B, Kress W, Day JW, Ranum LP. Sudden cardiac death in myotonic dystrophy type 2. *Neurology*. 2004;63:2402-2404
131. Schenk S, Loscher S, Mickley F, Hartmann A. Female patient with proximal myotonic myopathy and ventricular tachycardia. *Z Kardiol*. 2005;94:754-760
132. Udd B, Meola G, Krahe R, Thornton C, Ranum LP, Bassez G, Kress W, Schoser B, Moxley R. 140th enmc international workshop: Myotonic dystrophy dm2/promm and other myotonic dystrophies with guidelines on management. *Neuromuscul Disord*. 2006;16:403-413
133. Finsterer J. Myotonic dystrophy type 2. *Eur J Neurol*. 2002;9:441-447
134. Mostacciuolo ML, Miorin M, Martinello F, Angelini C, Perini P, Trevisan CP. Genetic epidemiology of congenital muscular dystrophy in a sample from north-east italy. *Hum Genet*. 1996;97:277-279
135. Allamand V, Guicheney P. Merosin-deficient congenital muscular dystrophy, autosomal recessive (mdc1a, mim#156225, lama2 gene coding for alpha2 chain of laminin). *Eur J Hum Genet*. 2002;10:91-94
136. Toda T, Kobayashi K, Kondo-Iida E, Sasaki J, Nakamura Y. The fukuyama congenital muscular dystrophy story. *Neuromuscul Disord*. 2000;10:153-159
137. Nakanishi T, Sakauchi M, Kaneda Y, Tomimatsu H, Saito K, Nakazawa M, Osawa M. Cardiac involvement in fukuyama-type congenital muscular dystrophy. *Pediatrics*. 2006;117:e1187-1192
138. Brockington M, Blake DJ, Prandini P, Brown SC, Torelli S, Benson MA, Ponting CP, Estournet B, Romero NB, Mercuri E, Voit T, Sewry CA, Guicheney P, Muntoni F. Mutations in the fukutin-related protein gene (fkrp) cause a form of congenital muscular dystrophy with secondary laminin alpha2 deficiency and abnormal glycosylation of alpha-dystroglycan. *Am J Hum Genet*. 2001;69:1198-1209
139. Dubowitz V. 41st enmc international workshop on congenital muscular dystrophy 8-10 march 1996, naarden, the netherlands. *Neuromuscul Disord*. 1996;6:295-306
140. Spyrou N, Philpot J, Foale R, Camici PG, Muntoni F. Evidence of left ventricular dysfunction in children with merosin-deficient congenital muscular dystrophy. *Am Heart J*. 1998;136:474-476
141. Fardeau M, Tome FM, Helbling-Leclerc A, Evangelista T, Ottolini A, Chevallay M, Barois A, Estournet B, Harpey JP, Faure S, Guicheney P, Hillaire D. [congenital muscular dystrophy with merosin deficiency: Clinical, histopathological, immunocytochemical and genetic analysis]. *Rev Neurol (Paris)*. 1996;152:11-19
142. Mukoyama M, Hizawa K, Kagawa N, Takahashi K. [the life spans, cause of death and pathological findings of fukuyama type congenital muscular dystrophy--analysis of 24 autopsy cases]. *Rinsho Shinkeigaku*. 1993;33:1154-1156
143. Lunt PW. 44th enmc international workshop: Facioscapulohumeral muscular dystrophy: Molecular studies 19-21 july 1996, naarden, the netherlands. *Neuromuscul Disord*. 1998;8:126-130
144. van Deutekom JC, Wijmenga C, van Tienhoven EA, Gruter AM, Hewitt JE, Padberg GW, van Ommen GJ, Hofker MH, Frants RR. Fshd associated DNA rearrangements are due to deletions of integral copies of a 3.2 kb tandemly repeated unit. *Hum Mol Genet*. 1993;2:2037-2042
145. Stevenson WG, Perloff JK, Weiss JN, Anderson TL. Facioscapulohumeral muscular dystrophy: Evidence for selective, genetic electrophysiologic cardiac involvement. *J Am Coll Cardiol*. 1990;15:292-299
146. de Visser M, de Voogt WG, la Riviere GV. The heart in becker muscular dystrophy, facioscapulohumeral dystrophy, and bethlem myopathy. *Muscle Nerve*. 1992;15:591-596

147. Laforet P, de Toma C, Eymard B, Becane HM, Jeanpierre M, Fardeau M, Duboc D. Cardiac involvement in genetically confirmed facioscapulohumeral muscular dystrophy. *Neurology*. 1998;51:1454-1456
148. Trevisan CP, Pastorello E, Armani M, Angelini C, Nante G, Tomelleri G, Tonin P, Mongini T, Palmucci L, Galluzzi G, Tupler RG, Barchitta A. Facioscapulohumeral muscular dystrophy and occurrence of heart arrhythmia. *Eur Neurol*. 2006;56:1-5
149. Selcen D. Myofibrillar myopathies. *Curr Opin Neurol*. 2008;21:585-589
150. Goldfarb LG, Dalakas MC. Tragedy in a heartbeat: Malfunctioning desmin causes skeletal and cardiac muscle disease. *J Clin Invest*. 2009;119:1806-1813
151. Vicart P, Caron A, Guicheney P, Li Z, Prevost MC, Faure A, Chateau D, Chapon F, Tome F, Dupret JM, Paulin D, Fardeau M. A missense mutation in the alphab-crystallin chaperone gene causes a desmin-related myopathy. *Nat Genet*. 1998;20:92-95
152. Selcen D, Engel AG. Myofibrillar myopathy caused by novel dominant negative alpha b-crystallin mutations. *Ann Neurol*. 2003;54:804-810
153. Selcen D, Muntoni F, Burton BK, Pegoraro E, Sewry C, Bite AV, Engel AG. Mutation in bag3 causes severe dominant childhood muscular dystrophy. *Ann Neurol*. 2009;65:83-89
154. Melberg A, Oldfors A, Blomstrom-Lundqvist C, Stalberg E, Carlsson B, Larrson E, Lidell C, Eeg-Olofsson KE, Wikstrom G, Henriksson G, Dahl N. Autosomal dominant myofibrillar myopathy with arrhythmogenic right ventricular cardiomyopathy linked to chromosome 10q. *Ann Neurol*. 1999;46:684-692
155. Griggs R, Vihola A, Hackman P, Talvinen K, Haravuori H, Faulkner G, Eymard B, Richard I, Selcen D, Engel A, Carpen O, Udd B. Zaspopathy in a large classic late-onset distal myopathy family. *Brain*. 2007;130:1477-1484
156. Nishino I, Fu J, Tanji K, Yamada T, Shimojo S, Koori T, Mora M, Riggs JE, Oh SJ, Koga Y, Sue CM, Yamamoto A, Murakami N, Shanske S, Byrne E, Bonilla E, Nonaka I, DiMauro S, Hirano M. Primary lamp-2 deficiency causes x-linked vacuolar cardiomyopathy and myopathy (danon disease). *Nature*. 2000;406:906-910
157. Murakami N, Goto Y, Itoh M, Katsumi Y, Wada T, Ozawa E, Nonaka I. Sarcolemmal indentation in cardiomyopathy with mental retardation and vacuolar myopathy. *Neuromuscul Disord*. 1995;5:149-155
158. Sugie K, Yamamoto A, Murayama K, Oh SJ, Takahashi M, Mora M, Riggs JE, Colomer J, Iturriaga C, Meloni A, Lamperti C, Saitoh S, Byrne E, DiMauro S, Nonaka I, Hirano M, Nishino I. Clinicopathological features of genetically confirmed danon disease. *Neurology*. 2002;58:1773-1778
159. Spencer CT, Bryant RM, Day J, Gonzalez IL, Colan SD, Thompson WR, Berthy J, Redfearn SP, Byrne BJ. Cardiac and clinical phenotype in barth syndrome. *Pediatrics*. 2006;118:e337-346
160. Danek A, Rubio JP, Rampoldi L, Ho M, Dobson-Stone C, Tison F, Symmans WA, Oechsner M, Kalckreuth W, Watt JM, Corbett AJ, Hamdalla HH, Marshall AG, Sutton I, Dotti MT, Malandrini A, Walker RH, Daniels G, Monaco AP. Mcleod neuroacanthocytosis: Genotype and phenotype. *Ann Neurol*. 2001;50:755-764
161. Windpassinger C, Schoser B, Straub V, Hochmeister S, Noor A, Lohberger B, Farra N, Petek E, Schwarzbraun T, Ofner L, Loscher WN, Wagner K, Lochmuller H, Vincent JB, Quasthoff S. An x-linked myopathy with postural muscle atrophy and generalized hypertrophy, termed xmpma, is caused by mutations in fhl1. *Am J Hum Genet*. 2008;82:88-99
162. Schessl J, Taratuto AL, Sewry C, Battini R, Chin SS, Maiti B, Dubrovsky AL, Erro MG, Espada G, Robertella M, Saccoliti M, Olmos P, Bridges LR, Standring P, Hu Y, Zou Y, Swoboda KJ, Scavina M, Goebel HH, Mitchell CA, Flanigan KM, Muntoni F, Bonnemann CG. Clinical, histological and genetic characterization of reducing body myopathy caused by mutations in fhl1. *Brain*. 2009;132:452-464
163. Carmignac V, Salih MA, Quijano-Roy S, Marchand S, Al Rayess MM, Mukhtar MM, Urtizberea JA, Labeit S, Guicheney P, Leturcq F, Gautel M, Fardeau M, Campbell KP, Richard I, Estournet B, Ferreiro A. C-terminal titin deletions cause a novel early-onset myopathy with fatal cardiomyopathy. *Ann Neurol*. 2007;61:340-351
164. Overeem S, Schelhaas HJ, Blijham PJ, Grootscholten MI, ter Laak HJ, Timmermans J, van den Wijngaard A, Zwarts MJ. Symptomatic distal myopathy with cardiomyopathy due to a myh7 mutation. *Neuromuscul Disord*. 2007;17:490-493
165. Uro-Coste E, Arne-Bes MC, Pellissier JF, Richard P, Levade T, Heitz F, Figarella-Branger D, Delisle MB. Striking phenotypic variability in two familial cases of myosin storage myopathy with a myh7 leu1793pro mutation. *Neuromuscul Disord*. 2009;19:163-166
166. Manzur AY, Kinali M, Muntoni F. Update on the management of duchenne muscular dystrophy. *Arch Dis Child*. 2008;93:986-990
167. Manzur AY, Kuntzer T, Pike M, Swan A. Glucocorticoid corticosteroids for duchenne muscular dystrophy. *Cochrane Database Syst Rev*. 2008:CD003725

168. Silversides CK, Webb GD, Harris VA, Biggar DW. Effects of deflazacort on left ventricular function in patients with duchenne muscular dystrophy. *Am J Cardiol*. 2003;91:769-772
169. Bauer R, Straub V, Blain A, Bushby K, MacGowan GA. Contrasting effects of steroids and angiotensin-converting-enzyme inhibitors in a mouse model of dystrophin-deficient cardiomyopathy. *Eur J Heart Fail*. 2009;11:463-471
170. Cossu G, Sampaolesi M. New therapies for duchenne muscular dystrophy: Challenges, prospects and clinical trials. *Trends Mol Med*. 2007;13:520-526
171. Wheeler TM. Myotonic dystrophy: Therapeutic strategies for the future. *Neurotherapeutics*. 2008;5:592-600

Chapter 3

# Sudden death in myotonic dystrophy type 1

*Mieke C.E. Hermans[a], Yigal M. Pinto[b], Laura Corsel[c], Christine E.M. de Die-Smulders[d], Menko-Jan de Boer[e], Arthur A.M. Wilde[b], Catharina G. Faber[a]*

[a]Department of Neurology, Maastricht University Medical Centre
[b]Department of Cardiology, Academic Medical Centre, Amsterdam
[c]Adelante Rehabilitation, Hoensbroek
[d]Department of Clinical Genetics, University Hospital Maastricht
[e]Department of Cardiology, Isala Clinics, Zwolle

*Adapted from N Engl J Med. 2008 Oct;359(15):1626-8*

## Summary

Cardiac involvement is one of the main systemic features of myotonic dystrophy type 1 (DM1) and a major determinant of prognosis. We aimed to determine the occurrence rate of sudden death in a large cohort of DM1 patients and attempted to identify risk factors. A long-term follow-up of 412 DM1 patients revealed that 178 patients died, of which 40 were sudden deaths. Importantly, sudden death was not prevented by pacemakers. A case-control comparison of available electrocardiograms showed that QRS duration was significantly prolonged in patients with documented sudden death. In sporadic cases, insertion of implantable cardioverter-defibrillators (ICD) in subjects with DM1 resulted in appropriate therapy for ventricular arrhythmias. We recommend that patients with myotonic dystrophy type 1 are systematically screened to identify those with the combination of an increased risk of sudden death and a relatively good neuromuscular prognosis. An ICD might be considered in subjects who need a pacemaker and have a wide QRS complex, but the potential benefit of prophylactic ICD therapy should be evaluated in a prospective trial.

## Introduction

Myotonic dystrophy type 1 (DM1), also known as Steinert's disease, is an autosomal dominant inherited disorder, caused by an expansion of an unstable CTG repeat in the myotonic dystrophy protein kinase gene on chromosome 19q13.3.[1] The normal allele contains 5-35 repeats, but in DM1 it is expanded to hundreds or even thousands of copies. Patients can be classified into four clinical types based on age at onset and severity of neurological symptoms: that is congenital type, childhood-type, adult-onset (classical) type and late-onset (mild) type.[2] Most notably, the age of onset decreases and the severity of neurological symptoms increases with successive generations (i.e. anticipation). DM1 has a prevalence of 5-20 per 100 000 and is characterized by muscle weakness, myotonia and involvement of several organs including the heart.[3] The exact pathogenic mechanism most likely involves repeat-induced alterations in transcript levels of various genes, including cardiac specific ones.[4] Conduction disturbances appear to be the predominant cardiac manifestation and often necessitate pacemaker implantation.[5-7] Despite routine screening of the electrocardiogram (ECG) and implantation of cardiac pacemakers, DM1 patients still die at a young age, often suddenly,[8-10] suggesting that the prevention and treatment of cardiac complications can be improved.
In the present study, we have retrospectively collected data on 412 DM1 patients in order to determine the occurrence rate of sudden death and evaluated available ECGs to identify risk factors. Furthermore, we aimed to report the efficacy of implantable cardioverter-defibrillator (ICD) therapy in sporadic cases.

## Methods

The population for this study was selected from the genetic register of DM1 families in the region of Maastricht, the Netherlands. Since 1950, generations of neurologists and geneticists examined patients and their family members to set up a genetic register.[8] The diagnosis of myotonic dystrophy had been made by expert neuromuscular physicians and was confirmed by genetic tests when available (since the early 90's of the previous century). This resulted in a register containing information on disease type, results of genetic tests and major complications.
The population for this study consists of 412 patients from 81 families. Patients of all ages and disease types were included, except for 5 patients with a rare type of myotonic dystrophy combined with hereditary polyneuropathy.[11] Age at death was verified by information from family members, medical records and the registry office. Data on cause of death were obtained either from medical

records, in the case of death in hospital, or from the general practitioner, in the case of death at home. Sudden death was defined as instantaneous unexpected death due to natural causes without symptoms of illness in the preceding 24 hours.
In 2005, we collected data on standard 12-lead ECGs available from the last 20 years and obtained information from the Dutch Pacemaker Registration. ECG recordings were evaluated for conduction defects. Two independent researchers measured PR interval in lead II and QRS width in the lead showing the widest complex (MH, YP). QTc interval was corrected for differences in heart rate by a modification of Bazett's formula ($QTc = QT/\sqrt{RR}$). When more than one ECG was made, the last one available was analysed. ECGs after pacemaker implantation were excluded from the analysis. Since 2003, we implanted an ICD instead of a pacemaker in 3 patients with a potential high risk profile and evaluated the occurrence of appropriate discharges.

### Statistics

Results are presented as absolute values or means ± standard deviation. Clinical characteristics were compared with Mann-Whitney U tests or Pearson's chi-square tests. For each patient with documented sudden death and ECG available, two control patients were chosen through frequency matching on sex, age (in 10 year age groups) and type of DM1. All analyses were performed using SPSS software version 15.0. A *P*-value of <0.05 at a two-sided level was considered statistically significant.

## Results

Over the past 55 years, we have collected data on age and causes of death of 412 DM1 patients including 233 men and 179 women (table 3.1). A pacemaker had been implanted in 31 patients. At the end of data collection, 178 patients had died (43%) and the mode of death could be accurately determined in 111 cases (62%). The mean age at death was 53.9 ± 17.8 years. Sudden death occurred in 40 patients, including 9 patients carrying a DDD or VVI pacemaker (figure 3.1). Other common causes of death were pneumonia (n=35), postoperative complications (n=5), malignancies (n=16), and others (n=14).

**Figure 3.1.** Schematic illustration of the occurrence of sudden death in DM1 patients

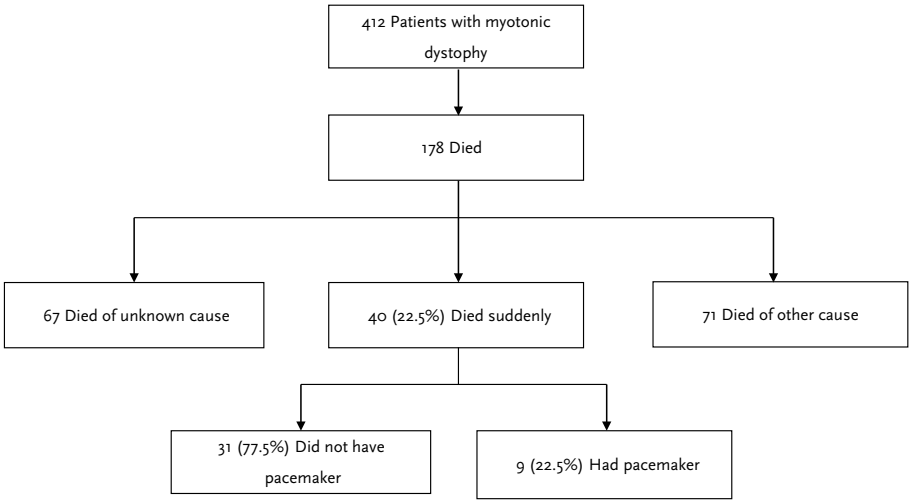





**Table 3.1.** Age and cause of death in DM1 patients

| | Congenital/ Childhood type | Adult type | Mild type | All patients (n=412) |
|---|---|---|---|---|
| No. of deceased patients | 35 | 107 | 36 | 178 |
| Age at death (years) | 34 | 55 | 71 | 54 |
| Sudden death | 9 | 24 | 7 | 40 |
| with pacemaker | 3 | 5 | 1 | 9 |
| without pacemaker | 6 | 19 | 6 | 31 |
| Other cause of death | 13 | 46 | 12 | 71 |
| with pacemaker | 1 | 8 | 1 | 10 |
| without pacemaker | 12 | 38 | 11 | 61 |
| Unknown cause of death | 13 | 37 | 17 | 67 |
| with pacemaker | 2 | 1 | 0 | 3 |
| without pacemaker | 11 | 36 | 17 | 64 |

The mean age at sudden death was 46.1 ± 13.5 years for the congenital/ childhood type, 55.3 ± 8.1 years for the adult type and 69.6 ± 7.1 years for the mild phenotype ($P$<0.001). Risk of sudden death increased with age (OR 1.04; 95% CI 1.01-1.06), but was independent of sex. Among patients with a known cause of death, the proportion of sudden deaths did not differ between pacemaker carriers and those without ($P$=0.26). Overall mortality was higher among pacemaker recipients ($P$=0.001).

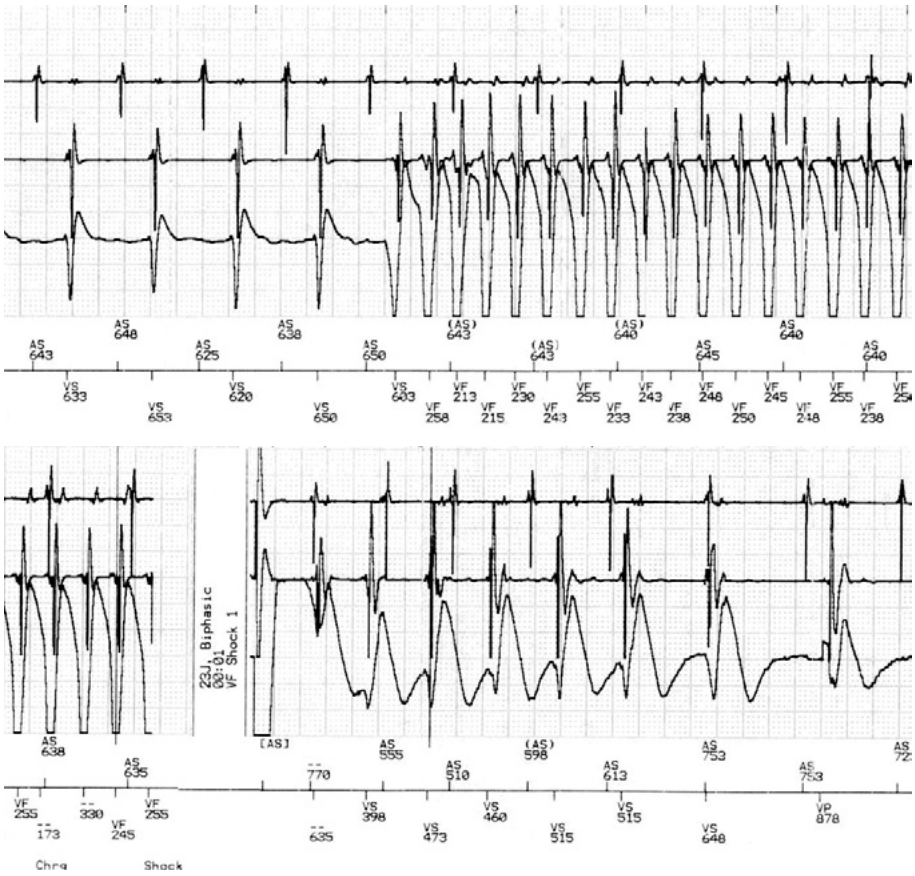
The median duration of cardiac follow-up was 7 years (interquartile range 2-10 years). ECGs were available from 117 patients, of which 33 ECGs belonged to deceased DM1 patients. Median time between the last ECG and time of death was 6.5 months. ECGs were available from 7 patients that died suddenly. These patients were classified as congenital/childhood type (n=3) or adult onset type (n=4). A case-control comparison corrected for age, sex and type of DM1, showed significantly wider QRS complexes in patients that died suddenly (*P*=0.003; table 3.2).

**Table 3.2.** ECG parameters in sudden deaths and matched controls

| | **Patients with sudden death n = 7** | **Controls n = 14** | **P-value** |
|---|---|---|---|
| PR-interval ± SD (ms) | 225.8 ± 31.8 | 201.2 ± 30.2 | 0.13 |
| QRS-duration ± SD (ms) | 133.0 ± 29.5 | 99.6 ± 9.3 | 0.003 |
| QTc-interval ± SD (ms) | 420.6 ± 20.1 | 417.4 ± 18.4 | 0.61 |

QRS duration was also significantly longer in patients that died suddenly compared to patients whose death was not sudden (*P*=0.02). The sample size was too small to test ECG indicators in a predictive model.
Evaluation of ICD implantations identified 3 DM1 patients who received an ICD since 2003. One patient is a 56 year old woman without specific cardiac complaints who received a DDDR pacemaker for progressive conduction delay (first degree AV block and a broad QRS complex). Fifteen months after implantation, an asymptomatic nocturnal rapid ventricular tachycardia (VT) was monitored by the pacemaker which prompted us to replace it by an ICD. Ten months later a syncopal episode occurred which turned out to be caused by a fast VT successfully terminated by a 23 Joule biphasic shock of the ICD (figure 3.2). The other 2 patients are young men, aged 28 and 33, with complaints of fatigue, dyspnoea on exertion and dizziness. One of them already had undergone right bundle branch ablation several years before, because of re-entry tachycardias. Over the years QRS width had increased and left ventricular function had worsened in both patients. It was decided to insert a prophylactic ICD with biventricular pacing mode. Both patients had an appropriate shock delivered by the ICD with restoration of sinus rhythm. The memory-stored rhythm detection mode recorded sustained VT in 1 patient and VT progressing to ventricular fibrillation (VF) in the other.

**Figure 3.2.** Intracardiac recordings of spontaneous sustained ventricular tachycardia as recorded from the implantable cardioverter-defibrillator (ICD) of a DM1 patient




**Legend to figure 3.2.** Reading down, the tracings show the recordings from the right atrium, right ventricular apex and a pseudosurface electrocardiograph derived from signals recorded between the shock coils and the ICD. Sinusrhythm is restored after a 23 J biphasic direct current shock.

## Discussion

Sudden death, most probably of cardiac origin, occurred in one third of all classified deaths and was associated with higher age and more severe cardiac involvement. In particular, QRS duration was prolonged in patients with documented sudden death compared to controls and deaths from other causes. As conduction disturbances are the predominant manifestation of cardiac disease and progress in time, the principal cause of sudden death in DM1 has traditionally been attributed to paroxysmal heart block.[12-14] Practice guidelines

therefore recommend pacemaker insertion in advanced or symptomatic conduction delay.[15] However, overall mortality among pacemaker carriers was high in our population. This may reflect more advanced disease in patients with a pacemaker, but also suggests that pacemakers fail to prolong life. More important is the fact that the rate of sudden death was not decreased by pacemakers. Earlier reports have also mentioned that patients with DM1 suddenly died despite carrying a pacemaker.[10, 16-19]

These observations suggest that sudden death in DM1 cannot be fully attributed to bradyarrhythmia and conduction block. Ventricular arrhythmias are increasingly recognized and thought to contribute to mortality in patients with DM1.[18, 20-22] Cardiac involvement in DM1 represents an ideal substrate for ventricular tachycardia and bundle branch re-entry tachycardia is the most likely mechanism, since the His-Purkinje system is often severely impaired.[23, 24]

Invasive electrophysiological studies (EPS) have been performed to assess the individual risk of life-threatening tachyarrhythmic events in DM1. Young age appeared to be a powerful predictor of inducible ventricular arrhythmias.[25] Unfortunately its value remains uncertain, since reported inducible ventricular arrhythmias are often non-specific. The value of non-invasive tests, such as signal-averaged electrocardiography, Holter recording and magnetic resonance imaging, in predicting arrhythmic risk is also still controversial.[26-29]

The incidence of potentially lethal tachyarrhythmias is considered not sufficiently high to justify defibrillator therapy routinely when permanent pacing is indicated.[30] We have started to employ prophylactic ICD therapy in patients with a potential high risk profile, comprising severe conduction disorders with a wide QRS complex. This proved to be appropriate therapy in the first 3 patients. Our observation of frequent sudden death in patients with pacemakers combined with examples of documented spontaneous sustained VT does not establish a direct relationship, but suggests that the role of ventricular arrhythmia in sudden death should not be underestimated. Continued research should result in better recognition of DM1 patients at risk of ventricular arrhythmias and sudden death for optimal primary prevention. This hopefully will deal with the unacceptable rates of sudden death in patients with DM1.

Limitations of our study lie in the fact that data were analysed retrospectively and we were unable to retrieve information on medical history and specific causes of death in a number of patients. ECG and echocardiography were often not available, since routine cardiac screening in DM1 patients was implemented only 20 years ago. Furthermore, patients often do not complain nor have cardiac symptoms and many refrain from seeing a doctor. Still, the cause of death could be determined in 62% of patients.

## Conclusion

Our systematic retrospective analysis demonstrates that sudden death is frequent in DM1 patients and is certainly not always prevented by pacemakers. Sudden death was associated with more severe cardiac involvement, evidenced more by QRS widening than by PR prolongation. Lethal ventricular tachyarrhythmias may be the responsible mechanism in particular cases. Although the sample size may be too small to draw far-reaching conclusions, we suggest that cardiac screening of DM1 patients should include careful monitoring of QRS duration. If a pacemaker is implanted in a DM1 patient with broadened QRS width, an implantable cardioverter-defibrillator might be considered. Further prospective studies, which are currently performed, should make clear how to select patients who could benefit from an ICD.

# References


1. Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, Aburatani H, Hunter K, Stanton VP, Thirion JP, Hudson T, et al. 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*. 1992;69:385
2. Harley HG, Rundle SA, MacMillan JC, Myring J, Brook JD, Crow S, Reardon W, Fenton I, Shaw DJ, Harper PS. Size of the unstable ctg repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. *Am J Hum Genet*. 1993;52:1164-1174
3. Harper PS. Myotonic dystrophy. London: Saunders; 2001
4. Ranum LP, Day JW. Myotonic dystrophy: Rna pathogenesis comes into focus. *Am J Hum Genet*. 2004;74:793-804
5. Church SC. The heart in myotonia atrophica. *Arch Intern Med*. 1967;119:176-181
6. Orndahl G, Thulesius O, Enestroem S, Dehlin O. The heart in myotonic disease. *Acta Med Scand*. 1964;176:479-491
7. Perloff JK, Stevenson WG, Roberts NK, Cabeen W, Weiss J. Cardiac involvement in myotonic muscular dystrophy (steinert's disease): A prospective study of 25 patients. *Am J Cardiol*. 1984;54:1074-1081
8. de Die-Smulders CE, Howeler CJ, Thijs C, Mirandolle JF, Anten HB, Smeets HJ, Chandler KE, Geraedts JP. Age and causes of death in adult-onset myotonic dystrophy. *Brain*. 1998;121:1557-1563
9. Mathieu J, Allard P, Potvin L, Prevost C, Begin P. A 10-year study of mortality in a cohort of patients with myotonic dystrophy. *Neurology*. 1999;52:1658-1662
10. Groh WJ, Groh MR, Saha C, Kincaid JC, Simmons Z, Ciafaloni E, Pourmand R, Otten RF, Bhakta D, Nair GV, Marashdeh MM, Zipes DP, Pascuzzi RM. Electrocardiographic abnormalities and sudden death in myotonic dystrophy type 1. *N Engl J Med*. 2008;358:2688-2697
11. Spaans F, Jennekens FG, Mirandolle JF, Bijlsma JB, de Gast GC. Myotonic dystrophy associated with hereditary motor and sensory neuropathy. *Brain*. 1986;109 ( Pt 6):1149-1168
12. Melacini P, Buja G, Fasoli G, Angelini C, Armani M, Scognamiglio R, Dalla Volta S. The natural history of cardiac involvement in myotonic dystrophy: An eight-year follow-up in 17 patients. *Clin Cardiol*. 1988;11:231-238
13. Fragola PV, Autore C, Magni G, Antonini G, Picelli A, Cannata D. The natural course of cardiac conduction disturbances in myotonic dystrophy. *Cardiology*. 1991;79:93-98
14. Hawley RJ, Milner MR, Gottdiener JS, Cohen A. Myotonic heart disease: A clinical follow-up. *Neurology*. 1991;41:259-262
15. Gregoratos G, Abrams J, Epstein AE, Freedman RA, Hayes DL, Hlatky MA, Kerber RE, Naccarelli GV, Schoenfeld MH, Silka MJ, Winters SL, Gibbons RJ, Antman EM, Alpert JS, Hiratzka LF, Faxon DP, Jacobs AK, Fuster V, Smith SC, Jr. Acc/aha/naspe 2002 guideline update for implantation of cardiac pacemakers and antiarrhythmia devices: Summary article: A report of the american college of cardiology/american heart association task force on practice guidelines (acc/aha/naspe committee to update the 1998 pacemaker guidelines). *Circulation*. 2002;106:2145-2161
16. Lazarus A, Varin J, Babuty D, Anselme F, Coste J, Duboc D. Long-term follow-up of arrhythmias in patients with myotonic dystrophy treated by pacing: A multicenter diagnostic pacemaker study. *J Am Coll Cardiol*. 2002;40:1645-1652
17. Motta J, Guilleminault C, Billingham M, Barry W, Mason J. Cardiac abnormalities in myotonic dystrophy. Electrophysiologic and histopathologic studies. *Am J Med*. 1979;67:467-473
18. Grigg LE, Chan W, Mond HG, Vohra JK, Downey WF. Ventricular tachycardia and sudden death in myotonic dystrophy: Clinical, electrophysiologic and pathologic features. *J Am Coll Cardiol*. 1985;6:254-256
19. Bharati S, Bump FT, Bauernfeind R, Lev M. Dystrophica myotonia. Correlative electrocardiographic, electrophysiologic, and conduction system study. *Chest*. 1984;86:444-450
20. Cannom DS, Wyman MG, Goldreyer BN. Clinical and induced ventricular tachycardia in a patient with myotonic dystrophy. *J Am Coll Cardiol*. 1984;4:625-628
21. Hiromasa S, Ikeda T, Kubota K, Hattori N, Coto H, Maldonado C, Kupersmith J. Ventricular tachycardia and sudden death in myotonic dystrophy. *Am Heart J*. 1988;115:914-915
22. Bassez G, Lazarus A, Desguerre I, Varin J, Laforet P, Becane HM, Meune C, Arne-Bes MC, Ounnoughene Z, Radvanyi H, Eymard B, Duboc D. Severe cardiac arrhythmias in young patients with myotonic dystrophy type 1. *Neurology*. 2004;63:1939-1941

23. Merino JL, Carmona JR, Fernandez-Lozano I, Peinado R, Basterra N, Sobrino JA. Mechanisms of sustained ventricular tachycardia in myotonic dystrophy: Implications for catheter ablation. *Circulation*. 1998;98:541-546
24. Merino JL, Peinado R, Sobrino JA. Sudden death in myotonic dystrophy: The potential role of bundle-branch reentry. *Circulation*. 2000;101:E73
25. Lazarus A, Varin J, Ounnoughene Z, Radvanyi H, Junien C, Coste J, Laforet P, Eymard B, Becane HM, Weber S, Duboc D. Relationships among electrophysiological findings and clinical status, heart function, and extent of DNA mutation in myotonic dystrophy. *Circulation*. 1999;99:1041-1046
26. Milner MR, Hawley RJ, Jachim M, Lindsay J, Jr., Fletcher RD. Ventricular late potentials in myotonic dystrophy. *Ann Intern Med*. 1991;115:607-613
27. Fragola PV, Calo L, Antonini G, Morino S, Luzi M, De Nardo D, Cannata D. Signal-averaged electrocardiography in myotonic dystrophy. *Int J Cardiol*. 1995;50:61-68
28. Vignaux O, Lazarus A, Varin J, Coste J, Carlier P, Argaud C, Laforet P, Weber S, Legmann P, Duboc D. Right ventricular mr abnormalities in myotonic dystrophy and relationship with intracardiac electrophysiologic test findings: Initial results. *Radiology*. 2002;224:231-235
29. Casella M, Dello Russo A, Pace M, Pelargonio G, Ierardi C, Sanna T, Messano L, Bencardino G, Valsecchi S, Mangiola F, Lanza GA, Zecchi P, Crea F, Bellocci F. Heart rate turbulence as a noninvasive risk predictor of ventricular tachyarrhythmias in myotonic dystrophy type 1. *J Cardiovasc Electrophysiol*. 2006;17:871-876
30. Bushby K, Muntoni F, Bourke JP. 107th enmc international workshop: The management of cardiac involvement in muscular dystrophy and myotonic dystrophy. 7th-9th june 2002, naarden, the netherlands. *Neuromuscul Disord*. 2003;13:166-172

Chapter 4

# Structural and functional cardiac changes in myotonic dystrophy type 1: a cardiac magnetic resonance imaging study

*Mieke C.E. Hermans[a], Catharina G. Faber[a], Sebastiaan C.A.M. Bekkers[b], Christine E.M. de Die-Smulders[c], Monique M. Gerrits[c], Ingemar S.J. Merkies[a,d], Gabriel Snoep, MD[e], Yigal M. Pinto[f], Simon Schalla[b]*

[a] Department of Neurology, Maastricht University Medical Centre, Maastricht
[b] Department of Cardiology, Maastricht University Medical Centre, Maastricht
[c] Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht
[d] Department of Neurology, Spaarne Hospital, Hoofddorp
[e] Department of Radiology, Maastricht University Medical Centre, Maastricht
[f] Department of Cardiology, Academic Medical Center, Amsterdam

*J Cardiovasc Magn Reson. 2012 Jul 24;14(1):48*

## Summary

Myotonic dystrophy type 1 (DM1) is a neuromuscular disorder with potential involvement of the heart and increased risk of sudden death. Considering the importance of cardiomyopathy as a predictor of prognosis, we aimed to systematically evaluate and describe structural and functional cardiac alterations in patients with DM1. Eighty DM1 patients underwent physical examination, electrocardiography (ECG), echocardiography and cardiac magnetic resonance imaging (CMR). Blood samples were taken for determination of NT-proBNP plasma levels and CTG repeat length. Functional and structural abnormalities were detected in 35 patients (44%). Left ventricular systolic dysfunction was found in 20 cases, left ventricular dilatation in 7 patients, and left ventricular hypertrophy in 6 patients. Myocardial fibrosis was seen in 10 patients (12.5%). In general, patients had low left ventricular mass indexes. Right ventricular involvement was uncommon and only seen together with left ventricular abnormalities. Functional or structural cardiac involvement was associated with age ($P$=0.04), male gender ($P$<0.001) and abnormal ECG ($P$<0.001). Disease duration, CTG repeat length, severity of neuromuscular symptoms and NT-proBNP level did not predict the presence of myocardial abnormalities. CMR can be useful to detect early structural and functional myocardial abnormalities in patients with DM1. Myocardial involvement is strongly associated with conduction abnormalities, but a normal ECG does not exclude myocardial alterations. These findings lend support to the hypothesis that DM1 patients have a complex cardiac phenotype, including both myocardial and conduction system alteration.

## Introduction

Myotonic dystrophy type 1 (DM1), or Steinert's disease, is an autosomal dominant inherited disorder caused by an unstable expansion of a repetitive trinucleotide sequence (CTG) on chromosome 19. The prevalence varies from 2.1-14.3 per 100 000.[1] DM1 is characterized by slowly progressive weakness of skeletal muscles, myotonia and involvement of several organ systems [1]. An earlier age of onset and increased severity of clinical symptoms has been observed in subsequent generations and is related to degree of CTG expansion.[2] Patients with DM1 usually die from respiratory or cardiac complications.[3, 4] Sudden death is considered to be the result of atrioventricular block or ventricular arrhythmias.[5] Recent studies showed that severe electrocardiographic (ECG) abnormalities and atrial arrhythmias are independent risk factors, although with moderate sensitivity, for sudden death in DM1 patients.[6] Although death from progressive heart failure is uncommon in patients with DM1 compared to other muscular dystrophies,[7,8] left ventricular systolic dysfunction is associated with an increased risk of overall mortality and sudden death.[9] Therefore, the picture emerges that DM1 patients have a complex cardiac phenotype including both the myocardium and the conduction system.
Considering the importance of cardiomyopathy as a predictor of prognosis, we aimed to measure cardiac function and detect structural abnormalities in patients with DM1. We used CMR imaging in the current study since CMR is an accurate and highly reproducible technique for the assessment of cardiac volumes, function, mass and focal fibrosis and the interstudy reproducibility in normal, dilated, and hypertrophic hearts was superior to 2-dimensional echocardiography.[10]

## Methods

### Patient selection

The protocol was approved by the local Medical Ethics Committee and each participant gave written informed consent. Patients older than 18 years of age were invited for a prospective, on-going study on cardiac involvement and early stratification of arrhythmogenic risk. Participants were recruited from the genetic register of the Maastricht University Medical Centre and through the Dutch neuromuscular patients' association (Vereniging Spierziekten Nederland, VSN). Subjects with previously implanted pacemakers or implantable cardioverter-defibrillators or with severe comorbidity leading to reduced life expectancy such as malignant disease or respiratory failure were excluded. During a 2 year period, 80 consecutive patients underwent CMR imaging and were enrolled in this study.

**Clinical examination**
A standardized interview was conducted in all participants to evaluate their clinical history and current symptoms. A neurological and cardiac evaluation was conducted by the same examiner (MH) in a predefined standardized fashion. The DM1 phenotype was established according to the commonly accepted classification based on the age at onset of symptoms: mild (late onset), classical (adult onset) and childhood/congenital type.[2] Skeletal muscle strength was manually tested and graded according to the Medical Research Council (MRC) 6-point grading system (0-5).[11] A total of 22 muscle groups were tested: neck flexors and extensors separately plus 10 bilateral muscles: 6 proximal muscle groups (shoulder abductors, elbow flexors, elbow extensors, hip flexors, knee extensors, knee flexors) and 4 distal muscle groups (wrist extensors, digits flexors, ankle dorsiflexors, ankle plantar flexors). Summation of the scores yields an extended MRC-sum score, ranging from 0 (paralytic) to 110 (normal strength).[12, 13] Furthermore, blood samples were taken to determine N-Terminal pro Brain Natriuretic Peptide (NT-pro-BNP) plasma levels and the length of the CTG repeat.
*CTG repeat length analysis.* Analysis of the CTG repeat length was performed on peripheral blood lymphocytes. Polymerase chain reaction followed by fragment length analysis was used to determine small allele lengths of 5 to 100 repeats, and Southern blotting was used to estimate repeat lengths >100. For purposes of statistical analysis, the CTG expansions were divided in 4 categories (<100; 100-250; 250-500; >500).

**Electrocardiography**
ECG was considered abnormal if signs of conduction disease (PR interval ≥210 ms, QRS duration ≥120 ms, left anterior or posterior fascicular hemiblock), hypertrophy (Sokolow-Lyon index ≥35 mm), myocardial infarction or rhythm other than sinus, were present.

**Echocardiography**
Echocardiography was used to exclude significant valvular disease, elevated right ventricular systolic pressure. Transthoracic echocardiograms were performed using a SONOS 5500 system with S3 transducer (Philips Medical Systems, Best, The Netherlands). Echocardiographic investigations were performed according to the recommendations of the American Society of Echocardiography.

**CMR**
Patients were examined in supine position with a clinical 1.5 T Gyroscan Intera MR scanner (Philips Medical Systems, Best, The Netherlands) equipped with a 5 channel cardiac surface coil. ECG-gated cine images were acquired

for functional analysis during multiple breath holds (10-13 seconds) using a steady-state free precession sequence (slice thickness 6 mm, slice gap 4 mm, TR/TE 3.8/1.9 ms, flip angle 50°, FOV 350 mm, matrix 256 x 256, 22-25 phases per cardiac cycle) in two-chamber, three-chamber and four-chamber view and a short-axis stacks covering the entire LV. For the detection of myocardial edema multislice short axis images were acquired using a dual-inversion black-blood T2-weighted sequence with fat suppression (slice thickness 8 mm, slice gap 2 mm, TR/TE 1600/100 ms, flip angle 90°, FOV 350 mm, matrix 512 x 512). After intravenous contrast administration (Gd-DTPA 0.2 mmol/kg) a Look-Locker sequence (slice thickness 10 mm, TR/TE 3.6/1.7 ms, flip angle 8°, FOV 370 mm, resolution 256 x 256, 39 phases, phase interval 15 ms) was applied to determine the inversion time (TI) to optimally "null" LV myocardium (typical TI range 200-280 ms) for the subsequent scan. To evaluate the presence of myocardial late gadolinium enhancement (LGE) a breath-hold 3D inversion recovery gradient echo sequence covering the entire LV (acquired slice thickness 12mm, reconstructed slice thickness 6mm, average TR/TE 3.9/2.4ms, multishot (50 profile/shot) segmented partial echo readout every heartbeat, flip angle 15 , field of view 400mm, matrix 256 x 256, acquired and reconstructed pixel size 1.56 x 1.56mm, typically 16-18 slices) was used with images in short-axis, two-chamber and four-chamber view, acquired 10 minutes after the administration of intravenous contrast.



**CMR data analysis**

MR images were analyzed with commercially available software (CAAS MRV 3.0, Pie medical imaging, Maastricht, The Netherlands). Endocardial and epicardial contours were manually traced in end-diastolic and end-systolic phases on short axis cine images to determine end-diastolic and end-systolic volume, ejection fraction and LV end-diastolic mass. Systolic LV dysfunction was defined as an ejection fraction <55% or regional wall motion abnormalities. LV and right ventricular (RV) dilatation were defined as enddiastolic volumes >2SD and RV systolic dysfunction as ejection fraction <2SD of mean reference values normalized for gender, body surface area and age.[14] We considered LV hypertrophy as increase in the LV mass and LV wall hypertrophy as wall thickness >12 mm. The presence and localization of edema or focal fibrosis was visually identified by a consensus of two independent experienced observers using the T2-weighted and LGE images. CMR was considered to be abnormal if regional or global dysfunction, ventricular dilatation, hypertrophy, or areas of fibrosis or edema were observed.

## Statistics

Descriptive statistics of clinical characteristics, electrocardiographic findings and cardiac magnetic resonance results are presented. Categorical variables

were summarized by frequency counts (percentage) and differences between groups were evaluated using chi-square tests. For continuous variables, results are presented as median (range) and comparison between categories was made with Mann-Whitney U tests. Multiple group comparisons were made with Kruskal-Wallis tests. All analyses were performed using SPSS software version 15.0. A *P*-value of <0.05 at a two-sided level was considered statistically significant.

## Results

### Patient characteristics

Characteristics of 80 DM1 patients (all Caucasian; 45 males and 35 females) are shown in table 4.1. No significant differences in clinical characteristics were found between men and women. As expected, with increasing CTG repeat length, the median age at onset and skeletal muscle strength score decreased (*P*<0.001). All patients were ambulant for short distances (<100 meter), but 16 subjects used mobility aids and 4 patients were confined to a wheelchair for longer distances.

**Table 4.1.** Clinical and genetic characteristics according to clinical phenotype category

| | Mild type<br>n = 9 | Classical type<br>n = 63 | Congenital/ childhood type<br>n = 8 | Total<br>n = 80 |
|---|---|---|---|---|
| Male | 5 (56%) | 33 (52%) | 7 (88%) | 45 (56%) |
| Age in years (range) | 60 (46-70) | 47 (24-64) | 32 (24-51) | 48 (24-70) |
| Age at onset in years (range) | 52 (50-65)* | 27 (10-51) | 6.5 (0-10) | 27 (0-65) |
| Muscle strength sum score (range) | 110 (107-110) | 96 (73-109) | 100 (78-109) | 98 (73-110) |
| Abnormal ECG | 1 (11%) | 41 (65%) | 7 (88%) | 49 (61%) |
| Abnormal CMR | 3 (33%) | 28 (44%) | 4 (50%) | 35 (44%) |

*age at onset of neuromuscular signs and symptoms could not be determined in 3 patients, since they were still asymptomatic.

Fatigue and dyspnoea were frequently reported symptoms: 48 patients were either dyspnoeic or fatigued after exertion and 10 subjects complained of both. No patient reported a history of syncope, severe palpitations at rest, angina pectoris or myocardial infarction. Mild peripheral oedema was seen in 4 patients, all of whom had normal systolic and diastolic LV function and normal NT-proBNP levels.

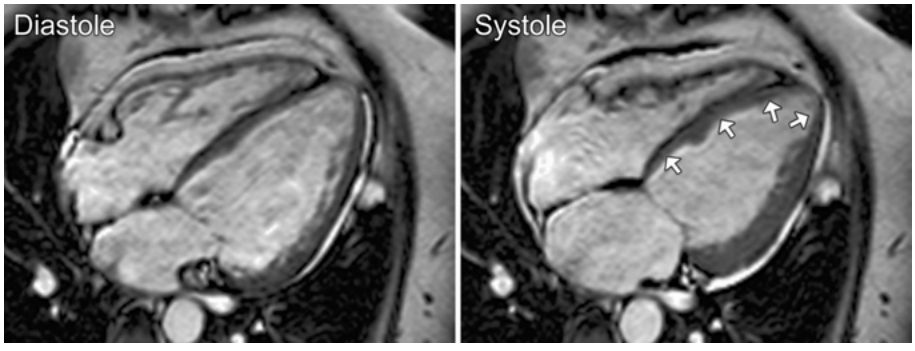
## CMR

Functional or structural abnormalities were detected with CMR imaging in 35 patients (44%). The results of CMR analysis are summarized in table 4.2. LV systolic dysfunction was found most frequently, being present in 20 patients. An example of LV dysfunction on CMR is shown in figure 4.1. Concomitant dilatation of the LV was found in 4 patients, while 3 patients had LV dilatation with preserved systolic function.

**Table 4.2.** Cardiac magnetic resonance imaging results

| | All n=80 | Male n=45 | Female n=35 |
|---|---|---|---|
| LV ejection fraction, % (range) | 58 (38-73) | 57 (45-73) | 61 (38-71) |
| LV systolic dysfunction, n (%) | 20 (25%) | 16 (36%) | 4 (11%) |
| LV enddiastolic volume, ml/m² (range) | 72 (38-117) | 77 (41-117) | 67 (38-104) |
| LV endsystolic volume, ml/m² (range) | 31 (11-63) | 35 (14-63) | 28 (11-56) |
| LV dilatation, n (%) | 7 (9%) | 6 (13%) | 1 (3%) |
| LV mass, g/m² (range) | 47(30-79) | 50 (36-79) | 41(30-67) |
| LV wall hypertrophy, n (%) | 6 (8%) | 4 (9%) | 2 (6%) |
| RV ejection fraction, % (range) | 64 (38-77) | 60 (38-77) | 67 (50-76) |
| RV systolic dysfunction, n (%) | 4 (5%) | 4 (9%) | 0 |
| RV enddiastolic volume, ml/m² (range) | 66 (40-117) | 71 (40-117) | 61 (40-102) |
| RV endsystolic volume, ml/m² (range) | 23 (10-66) | 28 (10-66) | 20 (10-46) |
| RV dilatation, n (%) | 1 (1%) | 1 (2%) | 0 |
| RV outflow tract, mm (range) | 26 (21-37) | 27 (22-37) | 25 (21-29) |
| Myocardial fibrosis, n (%) | 10 (13%) | 7 (16%) | 3 (9%) |

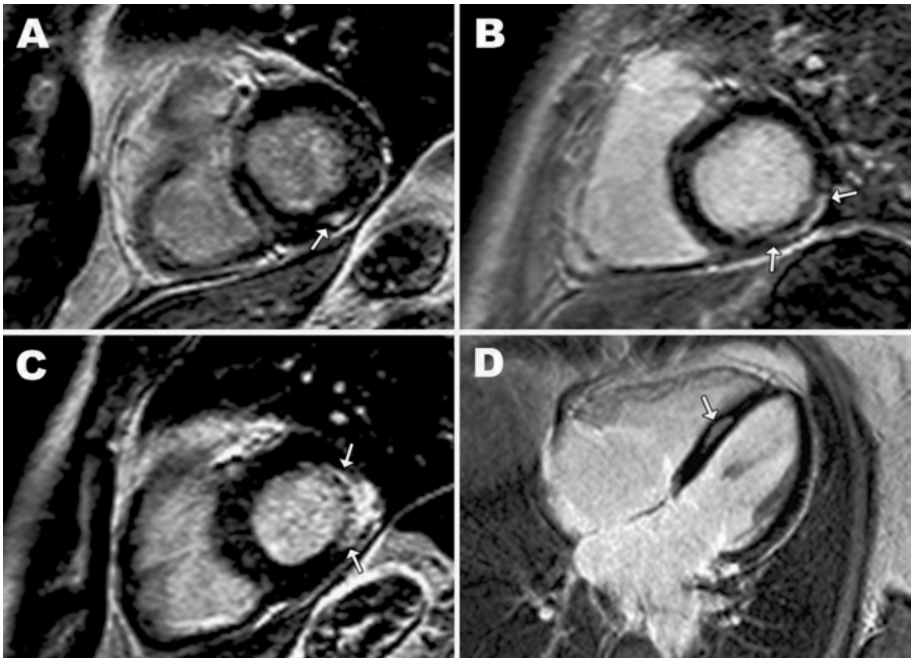
**Figure 4.1.** Ventricular dysfunction in myotonic dystrophy type 1




**Legend to figure 4.1**. Cine images in four-chamber long-axis view in diastole and systole of a patient with impaired systolic left ventricular function (ejection fraction 38%): septal and apical hypokinesia (arrows).

Regional LV hypokinesia was observed in 11 patients and co-localized with local thinning of the wall in 3 cases. LV hypertrophy was observed in 6 patients. None of the patients with LV hypertrophy had arterial hypertension. LV mass indexes of DM1 patients were remarkably low and the mean values differed significantly from values obtained from healthy volunteers (t-test, $P<0.001$).[14] Right ventricular dysfunction or dilatation was only present in patients with LV dysfunction. Abnormal myocardial function and structure was more frequent in men than in women ($P<0.001$) and associated with higher age ($P=0.04$), but not with duration of disease, muscle strength sum score or CTG repeat length. Focal myocardial fibrosis was detected on LGE images in 10 patients, most often as midmyocardial hyperenhancement of the septal segments and basal (inferio) lateral segments of the LV wall (n=8). Subendocardial and partly transmural hyperenhancement of the basal lateral wall was also found (n=2). Examples of selected CMR images are shown in figure 4.2. No patient had signs of myocardial oedema on T2-weighted images. No significant relationship between the presence of myocardial fibrosis and DM1 phenotype or CTG repeat length was observed.

**Figure 4.2.** Myocardial fibrosis in myotonic dystrophy type 1




**Legend to figure 4.2**. Late gadolinium enhancement (LGE) images in short axis (A, B and C) and 4-chamber long axis views (D) of 4 patients with myotonic dystrophy type 1. Between arrows are regions of increased signal intensity, indicating focal fibrosis, visible as midmyocardial enhancement to epicardial enhancement with endocardial sparing.

**ECG and echocardiography**

Electrocardiographic findings are summarized in table 4.3. An abnormal ECG was recorded in 49 patients (61%). All patients were in sinus rhythm except for 2 with atrial fibrillation. Conduction delay was present in 46 patients (58%) and 1 patient had abnormal Q-waves. There were no signs of hypertrophy on the ECGs. In general, patients with rhythm or conduction disturbances had more severe skeletal muscle weakness than those without (*P*=0.002). Of the 31 patients with normal ECGs, seven showed sinusbradycardia without conduction abnormalities (frequency 50-60 bpm, n=4; frequency <50 bpm, n=3). Echocardiography ruled out hemodynamically significant valvular disease or elevated right ventricular pressures in all patients.

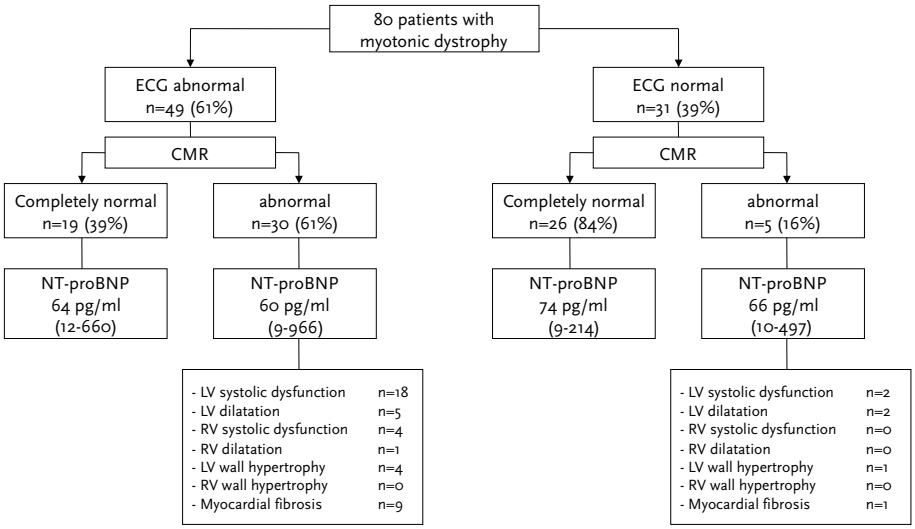


**Table 4.3.** Electrocardiography results

| | **n=80** |
|---|---|
| Frequency, bpm (range) | 70 (40-95) |
| Sinusbradycardia, n (%) | 14 (18%) |
| Atrial fibrillation, n (%) | 2 (3%) |
| PR interval, ms (range) | 200 (136-460) |
| Prolonged PR interval, n (%) | 30 (38%) |
| QRS duration, ms (range) | 100 (80-164) |
| Intraventricular conduction delay, n (%) | 26 (33%) |
| Left anterior fascicular block, n (%) | 6 (8%) |
| Left posterior fascicular block, n (%) | 1 (1%) |

A graphic reproduction of the cardiac evaluation is shown in figure 4.3. There was an association between ECG abnormalities and abnormal CMR findings (*P*<0.001). Patients with an abnormal ECG were more likely to have functional or structural cardiac abnormalities (OR 8.2; 95% CI 2.7-25.1). However, myocardial involvement was also seen in 5 out of 31 patients with a normal ECG. The sensitivity of the ECG to predict myocardial involvement in this selected population was 86%, with a specificity of 58%. Late gadolinium enhancement was found in 9 out of 49 patients with ECG abnormalities and only 1 of the 31 patients with a normal ECG. NT-proBNP levels did not significantly differ between patients with and without cardiac conduction disease or myocardial abnormalities. Complaints of fatigue and dyspnoea on exertion were not associated with abnormalities on ECG or imaging.

**Figure 4.3.** Graphic reproduction of cardiac evaluation




**Legend to figure 4.3**. The majority of patients with ECG abnormalities had functional or structural cardiac abnormalities. However, a substantial number of patients with normal ECG also showed myocardial alterations. NT-proBNP levels did not help to distinguish between patients with and without impaired myocardial functioning (presented as median (range)).

## Discussion

The principal finding of this study is that structural and functional myocardial abnormalities are frequent in DM1 patients. The presence of mild to moderate left ventricular systolic dysfunction, ventricular dilatation, myocardial hypertrophy or fibrosis was strongly associated with electrocardiographic conduction abnormalities. However, 16% of patients with a normal ECG still had myocardial alterations. These findings lend support to the concept that the myocardium is generally involved in the pathogenic process of DM1.

Myocardial involvement may be prognostic in predicting death in DM1.[9] CMR imaging is established in cardiomyopathies, because of its greater sensitivity and reproducibility than conventional diagnostic investigations (ECG and echocardiography) to demonstrate early abnormalities or subtle changes.[15] However, the cardiac magnetic resonance imaging phenotype of DM1 had not been well characterized. Initial descriptions of CMR findings in DM1 revealed structural abnormalities, but the number of investigated subjects was small and

functional analysis had not been carried out.[16] Left ventricular hypertrabeculation confirmed by CMR has been reported in 2 related patients with DM1.[17] Another study described a possible relationship between CMR abnormalities of the right ventricle and inducible arrhythmias at electrophysiological testing in DM1 patients.[18] Yet, no gadolinium contrast was used to visualize fibrosis and the induced ventricular arrhythmias were mostly non-sustained. We did not find any isolated remarkable abnormalities of the right ventricle or left ventricular hypertrabeculation in our large cohort.

Fibrosis is a frequent histopathological finding in individual DM1 cases.[19-23] Focal myocardial fibrosis as detected by LGE-CMR was present in 13% of our patients. As in other non-ischemic cardiomyopathies, late gadolinium enhancement was usually located in the interventricular septum and often limited to the mid-myocardium.[24, 25] An increased risk of sustained ventricular tachycardia and sudden death is associated with midwall fibrosis in patients with dilated cardiomyopathy.[26] Whether midwall fibrosis determined by CMR is a predictor of mortality in DM1 remains to be investigated.

The low prevalence of symptomatic heart failure in DM1 is usually partly attributed to the reduced cardiac demand due to diminished skeletal muscle activity.[27] This lower hemodynamic load in DM1 patients can also explain the low LV mass indexes found in this study.

Increasing age, male sex and ECG conduction abnormalities are all significantly associated with myocardial disease, whereas CTG repeat length and severity of muscular impairment are not. Male gender and age have been positively associated with arrhythmia and conduction abnormalities.[28] There is no consensus from the literature as to whether or not CTG repeat size has value as a prognostic indicator of conduction disturbances or cardiac event.[28-30] While age at onset of symptoms and severity of the phenotype correlate with the size of the CTG repeat, the association between the length of the CTG repeat measured in leukocytes and other symptoms of DM1 is more elusive. The heterogeneity of symptoms shown by patients with similar CTG repeat sizes can partly be explained by the presence of somatic mosaicism and somatic expansion over time.[31]

Structural and functional cardiac changes were found in patients with mild as well as severe neurological phenotypes. Duration of neuromuscular disease was not significantly related to cardiac disease, indicating that cardiac manifestations can precede, coincide with or succeed skeletal myopathy. It should however be stressed that recall of age at onset is sometimes poor and unreliable, as the diagnosis is often considerably delayed. Furthermore, duration of symptoms do not necessarily relate to the severity of neuromuscular symptoms as disease progression is highly variable. Symptoms of dyspnoea or fatigue were not associated with LV dysfunction and may therefore largely be ascribed to the

progressive physical disability of the muscular disease.
The current study is a descriptive study of a large cohort of patients with DM1 using state of the art diagnostic technology. A limitation of the descriptive survey is the absence of a comparison group or prognostic data allowing no inferences to be drawn about cause of disease and the predictive value of myocardial fibrosis or other CMR findings for identifying patients with DM1 who are at risk for cardiac death.

## Conclusion

Subclinical cardiomyopathy in patients with DM1 is frequently observed with CMR imaging. Screening for functional and structural cardiac disease should be considered in all patients since myocardial involvement can be overlooked by ECG alone. Whether the identification of structural or functional cardiac changes has prognostic implications for the prediction of disease progression or sudden death remains to be investigated in a long-term prospective study.

# References


1. Harper PS. Myotonic dystrophy. London: Saunders; 2001
2. Harley HG, Rundle SA, MacMillan JC, Myring J, Brook JD, Crow S, Reardon W, Fenton I, Shaw DJ, Harper PS. Size of the unstable ctg repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. *Am J Hum Genet*. 1993;52:1164-1174
3. de Die-Smulders CE, Howeler CJ, Thijs C, Mirandolle JF, Anten HB, Smeets HJ, Chandler KE, Geraedts JP. Age and causes of death in adult-onset myotonic dystrophy. *Brain*. 1998;121:1557-1563
4. Mathieu J, Allard P, Potvin L, Prevost C, Begin P. A 10-year study of mortality in a cohort of patients with myotonic dystrophy. *Neurology*. 1999;52:1658-1662
5. Lazarus A, Varin J, Ounnoughene Z, Radvanyi H, Junien C, Coste J, Laforet P, Eymard B, Becane HM, Weber S, Duboc D. Relationships among electrophysiological findings and clinical status, heart function, and extent of DNA mutation in myotonic dystrophy. *Circulation*. 1999;99:1041-1046
6. Groh WJ, Groh MR, Saha C, Kincaid JC, Simmons Z, Ciafaloni E, Pourmand R, Otten RF, Bhakta D, Nair GV, Marashdeh MM, Zipes DP, Pascuzzi RM. Electrocardiographic abnormalities and sudden death in myotonic dystrophy type 1. *N Engl J Med*. 2008;358:2688-2697
7. Cox GF, Kunkel LM. Dystrophies and heart disease. *Curr Opin Cardiol*. 1997;12:329-343
8. Bushby K, Muntoni F, Bourke JP. 107th enmc international workshop: The management of cardiac involvement in muscular dystrophy and myotonic dystrophy. 7th-9th june 2002, naarden, the netherlands. *Neuromuscul Disord*. 2003;13:166-172
9. Bhakta D, Groh MR, Shen C, Pascuzzi RM, Groh WJ. Increased mortality with left ventricular systolic dysfunction and heart failure in adults with myotonic dystrophy type 1. *Am Heart J*. 2010;160:1137-1141, 1141 e1131
10. Grothues F, Smith GC, Moon JC, Bellenger NG, Collins P, Klein HU, Pennell DJ. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. *Am J Cardiol*. 200211.
Medical research council. Aids to the examination of the peripheral nervous system. London: Her Majesty's Stationary Office; 1943:1-2
12. Kleyweg RP, van der Meche FG, Schmitz PI. Interobserver agreement in the assessment of muscle strength and functional abilities in guillain-barre syndrome. *Muscle Nerve*. 1991;14:1103-1109
13. Mathieu J, Boivin H, Meunier D, Gaudreault M, Begin P. Assessment of a disease-specific muscular impairment rating scale in myotonic dystrophy. *Neurology*. 2001;56:336-340
14. Hudsmith LE, Petersen SE, Francis JM, Robson MD, Neubauer S. Normal human left and right ventricular and left atrial dimensions using steady state free precession magnetic resonance imaging. *J Cardiovasc Magn Reson*. 2005;7:775-782
15. Verhaert D, Richards K, Rafael-Fortney JA, Raman SV. Cardiac involvement in patients with muscular dystrophies: Magnetic resonance imaging phenotype and genotypic considerations. *Circ Cardiovasc Imaging*. 2011;4:67-76
16. De Ambroggi L, Raisaro A, Marchiano V, Radice S, Meola G. Cardiac involvement in patients with myotonic dystrophy: Characteristic features of magnetic resonance imaging. *Eur Heart J*. 1995;16:1007-1010
17. Finsterer J, Stollberger C, Kopsa W. Familial left ventricular hypertrabeculation in myotonic dystrophy type 1. *Herz*. 2003;28:466-470
18. Vignaux O, Lazarus A, Varin J, Coste J, Carlier P, Argaud C, Laforet P, Weber S, Legmann P, Duboc D. Right ventricular mr abnormalities in myotonic dystrophy and relationship with intracardiac electrophysiologic test findings: Initial results. *Radiology*. 2002;224:231-235
19. Arnason G, Berge T, Dahlberg L. Myocardial changes in dystrophia myotonica. *Acta Med Scand*. 1964;176:536-538
20. Bulloch RT, Davis JL, Hara M. Dystrophia myotonica with heart block. A light and electron microscopic study. *Arch Pathol*. 1967;84:130-140
21. Motta J, Guilleminault C, Billingham M, Barry W, Mason J. Cardiac abnormalities in myotonic dystrophy. Electrophysiologic and histopathologic studies. *Am J Med*. 1979;67:467-473
22. Bharati S, Bump FT, Bauernfeind R, Lev M. Dystrophica myotonia. Correlative electrocardiographic, electrophysiologic, and conduction system study. *Chest*. 1984;86:444-450
23. Nguyen HH, Wolfe JT, 3rd, Holmes DR, Jr., Edwards WD. Pathology of the cardiac conduction system in myotonic dystrophy: A study of 12 cases. *J Am Coll Cardiol*. 1988;11:662-671

24. McCrohon JA, Moon JC, Prasad SK, McKenna WJ, Lorenz CH, Coats AJ, Pennell DJ. Differentiation of heart failure related to dilated cardiomyopathy and coronary artery disease using gadolinium-enhanced cardiovascular magnetic resonance. *Circulation*. 2003;108:54-59
25. Moon JC, McKenna WJ, McCrohon JA, Elliott PM, Smith GC, Pennell DJ. Toward clinical risk assessment in hypertrophic cardiomyopathy with gadolinium cardiovascular magnetic resonance. *J Am Coll Cardiol*. 2003;41:1561-1567
26. Assomull RG, Prasad SK, Lyne J, Smith G, Burman ED, Khan M, Sheppard MN, Poole-Wilson PA, Pennell DJ. Cardiovascular magnetic resonance, fibrosis, and prognosis in dilated cardiomyopathy. *J Am Coll Cardiol*. 2006;48:1977-1985
27. Phillips MF, Harper PS. Cardiac disease in myotonic dystrophy. *Cardiovasc Res*. 1997;33:13-22
28. Petri H, Vissing J, Witting N, Bundgaard H, Kober L. Cardiac manifestations of myotonic dystrophy type 1. *Int J Cardiol*. 2012;160:82-88
29. Sabovic M, Medica I, Logar N, Mandic E, Zidar J, Peterlin B. Relation of ctg expansion and clinical variables to electrocardiogram conduction abnormalities and sudden death in patients with myotonic dystrophy. *Neuromuscul Disord*. 2003;13:822-826
30. Clarke NR, Kelion AD, Nixon J, Hilton-Jones D, Forfar JC. Does cytosine-thymine-guanine (ctg) expansion size predict cardiac events and electrocardiographic progression in myotonic dystrophy? *Heart*. 2001;86:411-416
31. Martorell L, Monckton DG, Gamez J, Johnson KJ, Gich I, Lopez de Munain A, Baiget M. Progression of somatic ctg repeat length heterogeneity in the blood cells of myotonic dystrophy patients. *Hum Mol Genet*. 1998;7:307-312

Chapter 5

# Changes in global gene expression in hearts of myotonic dystrophy type 1 patients

*Mieke C.E. Hermans[a], Blanche Schroen[b], Catharina G. Faber[a], Georg Summer[b], Elizabeth A. McClellan[c], Andrew P. Stubbs[c], Jack Cleutjens[d], Ingemar S.J. Merkies[a,e], Stephane R.B. Heymans[b], Harry Crijns[f], Yigal M. Pinto[g]*

[a] Department of Neurology, Maastricht University Medical Centre, Maastricht, the Netherlands
[b] Centre for Heart Failure Research, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht
[c] Department of Bioinformatics, Erasmus Medical Centre, Rotterdam
[d] Department of Pathology, University Hospital Maastricht, Maastricht
[e] Department of Neurology, Spaarne Ziekenhuis Hoofddorp
[f] Department of Cardiology, Maastricht University Medical Centre, Maastricht
[g] Department of Cardiology, Academic Medical Centre, Amsterdam

*Submitted*

## Summary

Myotonic dystrophy type 1 (DM1) is an autosomal dominant inherited multisystemic disorder caused by a trinucleotide expansion in a noncoding region of the DMPK gene. A toxic RNA gain-of-function is assumed to lead to degeneration in many tissues. Cardiac involvement is a frequent complication, but the molecular mechanisms remain poorly defined. We assessed the gene expression patterns in human cardiac biopsies obtained from patients with DM1-related cardiomyopathy. Microarray analysis showed significant changes in the expression levels of 989 mRNAs and 16 microRNAs compared to controls. Bioinformatics analysis of gene expression data identified several pathological changes in DM1 hearts, including loss of DMPK, deregulation of mitochondrial oxidative phosphorylation, RNA processing, calcium signalling and cytoskeletal genes. These pathways largely overlap with those found in gene expression profiles of skeletal muscle. We show that gene expression levels in hearts of DM1 patients are compatible with proposed pathogenic mechanisms and that the observed changes may predispose DM1 hearts to conduction disturbances and impaired myocardial function. This suggests that similar mechanisms affect both skeletal and cardiac muscle in DM1.

## Introduction

Myotonic dystrophy type 1 (DM1) is an autosomal dominantly inherited multisystem disorder with a prevalence of 2.1-14.3 per 100 000 worldwide.[1] The diagnosis of DM1 is usually suspected in individuals with characteristic muscle weakness and myotonia and can be confirmed by detection of a CTG trinucleotide repeat in the 3' non-coding region of the dystrophia myotonica protein kinase (DMPK) gene.[2]

Apart from neuromuscular symptoms, patients have multiple organ manifestations including cardiac involvement. The severity of cardiac complications has become increasingly appreciated over the past several years. Myocardial fibrosis and degeneration of the cardiac conduction system result in progressive conduction abnormalities, atrial and ventricular arrhythmias, and ventricular dysfunction. Sudden death occurs in 20-30% of cases and is believed to result from fatal atrioventricular block or from ventricular tachyarrhythmia.[3, 4] Thus far, no intervention has effectively prevented sudden death in this population.

The major pathogenic mechanism involves a toxic gain-of-function of DMPK RNA transcripts containing an expanded CUG repeat (CUGexp). It has been shown that mutant DMPK transcripts accumulate in nuclear inclusions and interfere with activity, localization and/or steady-state levels of RNA-interacting proteins like CUG binding protein 1 (CUGBP1) and muscleblind-like 1 (MBNL1).[5] In turn, loss of function of MBNL1, and hyperphosphorylation and up-regulation of CUGBP1 disturb splicing of pre-mRNAs, suppress translation, and deregulate processing of microRNAs (miRNA).[5] In addition, it is assumed that reduction of DMPK protein levels itself and decreased expression levels of neighbouring genes may be involved.[6, 7]

However, it remains elusive which concepts mentioned above occur in the myocardium of DM1 patients with signs of cardiomyopathy. Exploring the changes in gene expression can identify molecular pathways associated with the clinical hallmarks of DM1 cardiomyopathy such as disturbed cardiac conduction and enhanced arrhythmogenesis. Therefore, we assessed the gene expression patterns in human cardiac biopsies obtained from patients with DM1-related cardiomyopathy. Transcript levels of both protein coding genes and miRNAs were determined in an unbiased manner to identify potential pathophysiological explanations for the observed cardiomyopathy.

## Methods

### Patient selection and cardiac muscle biopsies

A cohort of 170 patients with genetically proven DM1 was examined as part of a prospective, on-going study on cardiac involvement in DM1. The study protocol was approved by the institutional ethics committee of the Maastricht University Medical Centre and written informed consent was obtained from all participants.

ECG, echocardiogram, Holter examination and cardiac magnetic resonance imaging (CMR) was performed in each patient. Subjects were considered to have DM1-related cardiomyopathy when 1) QRS duration was >120 ms, or 2) ejection fraction (EF) was below 45% on echocardiography, or 3) there was myocardial late gadolinium enhancement seen at CMR, or 4) non-sustained ventricular tachycardia (VT) was found at Holter examination, defined as 3 or more ventricular beats with a rate of >120 beats/min and a duration of <30 s. Patients meeting one or more of these criteria were considered eligible for diagnostic electrophysiological studies. Between January 2007 and June 2009, 37 out of 50 eligible patients agreed to undergo invasive electrophysiological studies, and 27 of these patients gave informed consent to undergo invasive cardiac muscle biopsy during the procedure. Control biopsies were obtained from subjects who underwent diagnostic endomyocardial biopsies for idiopathic VT (n=4), for exclusion of arrhythmogenic right ventricular cardiomyopathy/ dysplasia (ARVC/D) (n=1) and for exclusion of cardiac sarcoidosis (n=1).

**Table 5.1.** Clinical data

| | **Patients n=27** | **Controls n=6** | **P-value** |
|---|---|---|---|
| Age in years, median (range) | 50 (26-63) | 36 (25-56) | 0.03 |
| Male, n (%) | 16 (59) | 4 (67) | ns |
| ECG | | | |
| PR in ms, median (range) | 210 (140-460) | 152 (114-202) | 0.004 |
| QRS in ms, median (range) | 120 (85-165) | 96 (82-114) | 0.001 |
| Echocardiography | | | |
| LVEF in %, median (range) | 58 (35-70) | 55 (48-66) | ns |
| LVEDD index in mm/m$^2$, median (range) | 24 (17-31) | 28 (22-31) | ns |
| CMR imaging | | | |
| fibrosis, n (%) | 4 (15) | 0 | na |

ns= not significant; na= not applicable

A total of 4-5 endomyocardial biopsies were taken from the septal wall of the right ventricle with a flexible bioptome by fluoroscopic guidance. Cardiac biopsies were immediately snap-frozen for RNA extraction, or formalin-fixed for histological analyses. Baseline characteristics of DM1 and control patients are provided in table 5.1.

**Histological analysis**

Paraffin-embedded tissue sections of 4 µm thickness were stained using Sirius red for collagen and CD3, CD4, CD8, CD20, CD45, and CD68 antibodies for inflammatory cells. The total tissue area of the myocardial biopsies on the histological slide was determined by morphometric analysis and the number of staining inflammatory cells were counted and expressed per $mm^2$, as described previously.[8] Collagen volume fraction was quantified as percentage Sirius red stained area per total myocardial tissue area, excluding perivascular and endocardial fibrosis.



**RNA isolation and microarray analysis**

Total RNA was isolated using the mirVana miRNA isolation kit (Ambion, Austin, TX) and hybridized to either Illumina Human miRNAv2 Expression Panel arrays or Illumina HumanHT-12 v3 Expression BeadChips by ServiceXS (Leiden, The Netherlands). The mRNA arrays contain 48 000 probes derived from the National Center for Biotechnology Information Reference Sequence (NCBI) RefSeq (Build 36.2, Release 22) and the UniGene (Build 199) databases. The miRNA arrays contained 858 human microRNA sequences described in Sanger Institute miRBase release 12.05, plus additional novel content derived using Illumina sequencing technologies, making up a total of 1146 assays per chip.

The lumi R package was used for annotation, quality control and normalization of both microarrays.[9] A quantile normalization was applied to the mRNA and miRNA arrays separately.[10] Differential expression was analysed using the limma R package.[11] mRNAs and miRNAs were considered differentially expressed with a corrected *P*-value <0.05.[12]

The resulting genes were analysed for enrichment in the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway terms,[13] using the Database for Annotation Visualization and Integrated Discovery (DAVID) tool.[14, 15] Differentially regulated genes were highlighted and their regulation direction indicated on the enriched KEGG pathways.

### Statistics

Descriptive statistics of clinical characteristics and histological findings are presented. Categorical variables were summarized by frequency counts (percentage) and differences between groups were evaluated using chi-squared tests. For continuous variables, results are presented as median (range) or mean (SD) and comparison between categories was made with unpaired t-tests or Mann-Whitney U tests. All analyses were performed using SPSS software version 20.0. A *P*-value of <0.05 at a two-sided level was considered statistically significant.

## Results

### Histological analysis

Histological analysis of biopsies of 24 patients with DM1 and 6 controls is summarized in table 5.2. The median collagen volume fraction (CVF) was 3.2% (range 0.4-8.8%) in patients with DM1 and 4.2% (range 2.2-7.9%) in controls (*P*=0.2). The CVF correlated neither with age nor with CMR measured LVEF or end-diastolic volume. Patients with inducible VT during the electrophysiological studies did not have more interstitial fibrosis in their biopsies than patients who did not. No patient had increased lymphocytic inflammation.

**Table 5.2.** Histological analysis

| | Patients n=24 | Controls n=6 | P-value |
|---|---|---|---|
| CD3+, lymphocytes/mm², mean (SD) | 2.9 (2.1) | 5.2 (4.7) | ns |
| CD4+, lymphocytes/mm², mean (SD) | 1.4 (1.7) | 2.6 (3.1) | ns |
| CD8+, lymphocytes/mm², mean (SD) | 3.0 (3.9) | 3.0 (3.6) | ns |
| CD20+, lymphocytes/mm², mean (SD) | 0.04 (0.1) | 0 (0) | na |
| CD45+, lymphocytes/mm², mean (SD) | 7.7 (5.0) | 9.3 (4.9) | ns |
| CD68+, macrophages/mm², mean (SD) | 4.6 (3.4) | 5.0 (3.3) | ns |
| Interstitial collagen volume fraction | 3.2 (2.3) | 4.22 (2.2) | ns |

ns= not significant; na= not applicable

### Differential microarray analysis identifies cardiac deregulation of candidate genes

Microarray analysis showed significant changes in the expression levels of 989 mRNAs in hearts of DM1 patients as compared to controls. Sixteen miRNAs were deregulated (table 5.3).

First of all, levels of DMPK mRNA itself were significantly reduced in DM1 hearts. Concomitantly, the expression of the homeobox gene SIX5, which is located immediately downstream of DMPK, was also significantly reduced. On the other hand, gene expression level of the RNA-binding proteins MBNL1 and CUGBP1 were increased in DM1 hearts. In addition, expression levels of cardiac troponin T (TNNT2), which is aberrantly spliced in cardiac tissue of DM1 patients,[16] were also increased. Another up-regulated gene that has been implicated in DM1 is PDZ and LIM domain 3 (PDLIM3). Aberrant splicing of PDLIM3 transcripts was shown in DM1 skeletal muscle and may affect physiologic functions of heart muscle.[17] Interestingly, its family members PDLIM5 and PDLIM7 were down-regulated in our cardiac DM1 biopsies. In addition, we found that calsequestrin 1 (CASQ1) gene expression, which is up-regulated in DM skeletal muscle,[18] was also up-regulated in the hearts of DM1 patients.



**Table 5.3.** Deregulated miRNAs

| Name | Fold-change | P-value |
|---|---|---|
| hsa-let-7c* | -1.27 | 0.013 |
| hsa-miR-1236 | -1.13 | 0.046 |
| hsa-miR-1257 | -1.29 | 0.021 |
| hsa-miR-1283 | -1.18 | 0.011 |
| hsa-miR-1308 | -1.28 | 0.028 |
| hsa-miR-147b | -1.46 | 0.033 |
| hsa-miR-182* | -1.28 | 0.044 |
| hsa-miR-186* | -1.14 | 0.032 |
| hsa-miR-30b* | -1.27 | 0.011 |
| hsa-miR-32* | 1.36 | 0.049 |
| hsa-miR-516a-5p | -1.27 | 0.031 |
| hsa-miR-541 | -1.18 | 0.025 |
| hsa-miR-545 | -1.22 | 0.037 |
| hsa-miR-545:9.1 | -1.31 | 0.023 |
| hsa-miR-548b-3p | -1.28 | 0.001 |
| hsa-miR-767-5p | -1.34 | 0.029 |

Expression levels of non-muscle myosin heavy-chain gene MYH14, which are reduced in skeletal muscle of DM1 patients,[19] were also reduced in cardiac tissue. Finally, LIM domain binding 3 (LDB3) transcripts, which are aberrantly spliced in DM1 skeletal muscle,[20] were found up-regulated in hearts. Interestingly, mutations in this gene have been associated with myofibrillar myopathy and dilated cardiomyopathy.[21, 22]

**Pathway analyses reveal hidden cardiac pathology in DM1 patients**

Microarray data were subjected to pathway analysis using the KEGG pathway database.[23] Top enriched KEGG pathways included those for mitochondrial oxidative phosphorylation (OXPHOS), fatty acid metabolism, ribosome, spliceosome, proteasome and cardiac muscle contraction (figures 5.1-5.6). Most remarkably, many subunits of the OXPHOS chain were consistently down-regulated in DM1 hearts (figure 5.1). In addition, in-depth functional gene characterization of the list of differentially expressed genes revealed a number of interesting patterns in hearts of DM1 patients.

**Genes involved in calcium/cAMP signalling are up-regulated in DM1 hearts**

Calcium signalling, in particular the calcium/calmodulin pathway, is crucial both for cardiac contraction and relaxation and for the control of cardiac gene expression.

We found a general up-regulation of calcium/calmodulin-related genes in DM1 hearts, including the anchor protein AKAP1 involved in cAMP-signalling, the Aspartate beta-hydroxylase which regulates calcium homeostasis, the calcium/calmodulin-binding Caldesmon 1, the Calcium/calmodulin-dependent protein kinase type II subunits B and D and their inhibitor CAMK2N1, the calcium-binding protein P22 that resembles Calcineurin B and Calmodulin, the mitochondrial calcium-binding protein Calsequestrin 1, and finally the protein phosphatase PPM1K which has CAMK2G as a substrate (table 5.4).

**Table 5.4.** Deregulated calcium signalling and homeostasis genes

| Gene | Full name of protein | Fold-change | P-value |
|---|---|---|---|
| AKAP1 | A kinase (PRKA) anchor protein 1 | 1.38 | 0.040 |
| ASPH | aspartate beta-hydroxylase | 1.55 | 0.004 |
| CALD1 | caldesmon 1 | 2.07 | 0.002 |
| CASQ1 | calsequestrin | 2.25 | 0.008 |
| CAMK2B | calcium/calmodulin-dependent protein kinase II beta | 1.78 | 0.002 |
| CAMK2D | calcium/calmodulin-dependent protein kinase II delta | 1.44 | 0.044 |
| CAMK2N1 | calcium/calmodulin-dependent protein kinase II inhibitor 1 | 1.43 | 0.032 |
| CHP | calcium binding protein P22 | 1.26 | 0.030 |
| MAP6D1 | MAP6 domain containing 1 | -1.31 | 0.003 |
| PPM1F | protein phosphatase 1F (PP2C domain containing) | -1.57 | 0.005 |
| PPM1K | protein phosphatase 1K (PP2C domain containing) | 1.52 | 0.031 |

**mRNA processing and splicing are affected in DM1 hearts**

It is suggested that the CUGexp RNA associated with DM1 leads to a trans-dominant effect on normal RNA processing. In line with this, we found deregulation of genes involved in mRNA processing, splicing and RNA binding and stability (i.e. genes involved in mRNA surveillance and nonsense-mediated mRNA decay) in DM1 hearts (table 5.5).

Genes involved in mRNA processing are the cleavage and polyadenylation specificity factor CPSF1; the heterogeneous nuclear ribonucleoproteins HNRNPAB, HNRNPD, HNRNPU and HNRNPUL which are involved in pre-mRNA processing and other aspects of mRNA metabolism and transport; both the small nuclear ribonucleoprotein SNRPN and associated SNURF involved in pre-mRNA processing, possibly tissue-specific alternative splicing events; SSB involved in RNA metabolism; and finally the nuclear mRNA factory protein WBP11.

Genes involved in mRNA splicing included the aforementioned alternative splicing regulators MBNL1 and CUGBP1; the spliceosome RNA helicase BAT1; the DEAD box proteins DDX19B, -31, -46, and -51 and DHX37 and -40 which are RNA helicases implicated in a number of cellular processes involving alteration of RNA secondary structure such as translation initiation, nuclear and mitochondrial splicing, and ribosome and spliceosome assembly; the nuclear protein LUC7L3 possibly involved in spliceosome formation; the RNA-binding protein NONO with roles in transcriptional regulation and RNA splicing; the splicing factor SLU7; and finally the tRNA splicing endonuclease TSEN15.

Genes encoding for RNA binding and stabilizing proteins include the RNA binding HADHB which decreases the stability of some RNAs; Ligatin which contains PUA and SUI1 domains that may function in RNA binding and translation initiation; the RNA-binding motif proteins RBM42, RBMS1 and RBMX; the RNA binding and posttranscriptional regulator SAMD4A; the mRNA-binding protein SERBP1 involved in mRNA stability; the mRNA surveillance proteins UPF1, which is part of a post-splicing multiprotein complex; WDR61; and SMG7.

**DM1 heart gene expression analysis reveals protein synthesis, folding and degradation abnormalities**

KEGG pathway analysis identified genes encoding ribosomal proteins to be generally down-regulated (figure 5.3), which could signify a decrease in translational capacity. We found additional deregulated genes involved in ribosome assembly and function. These included the above-described DEAD and DEAH box proteins which are also involved in ribosome assembly, the snoRNP gene family members NOP10 and NOP56 which are required for ribosome biogenesis and Nucleolin which is involved in the synthesis and maturation of ribosomes.

**Table 5.5.** Deregulated RNA processing genes

| Gene | Full name of protein | Fold-change | P-value |
|---|---|---|---|
| ADPRHL1 | ADP-ribosylhydrolase like 1 | 1.87 | 0.0002 |
| ARL16 | ADP-ribosylation factor-like 16 | 1.65 | 0.013 |
| ARL2 | ADP-ribosylation factor-like 2 | -2.00 | 0.002 |
| ASAP3 | ArfGAP with SH3 domain, ankyrin repeat and PH domain 3 | -1.50 | 0.014 |
| BANF1 | similar to barrier-to-autointegration factor 1 | 1.57 | 0.006 |
| CUGBP1 | CUG triplet repeat, RNA binding protein 1 | 1.23 | 0.014 |
| CMPK1 | cytidine monophosphate (UMP-CMP) kinase 1, cytosolic | 1.47 | 0.021 |
| CPSF1 | cleavage and polyadenylation specific factor 1, 160kDa | -1.33 | 0.023 |
| DDX19B | DEAD (Asp-Glu-Ala-As) box polypeptide 19B | -1.23 | 0.049 |
| DDX31 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 31 | -1.22 | 0.006 |
| DDX46 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 46 | -1.35 | 0.004 |
| DDX51 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 51 | 1.50 | 0.049 |
| DHX37 | DEAH (Asp-Glu-Ala-His) box polypeptide 37 | -1.26 | 0.047 |
| DHX40 | DEAH (Asp-Glu-Ala-His) box polypeptide 40 | 1.15 | 0.032 |
| EXOSC1 | exosome component 1 | -1.33 | 0.016 |
| GEMIN6 | gem (nuclear organelle) associated protein 6 | -1.25 | 0.036 |
| HADHB | hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit | 1.32 | 0.014 |
| HNRNPAB | heterogeneous nuclear ribonucleoprotein A/B | -1.55 | 0.008 |
| HNRNPD | heterogeneous nuclear ribonucleoprotein D (AU-rich element RNA binding protein 1, 37kDa) | 1.37 | 0.006 |
| HNRNPU | heterogeneous nuclear ribonucleoprotein U (scaffold attachment factor A) | 2.06 | 0.008 |
| HNRNPUL1 | heterogeneous nuclear ribonucleoprotein U-like 1 | 1.57 | 0.0493 |
| HSPA1A | heat shock 70kDa protein 1A | 1.63 | 0.003 |
| IMP4 | IMP4, U3 small nucleolar ribonucleoprotein, homolog (yeast) | -1.52 | 0.011 |
| INTS1 | integrator complex subunit 1 | -1.31 | 0.029 |
| IPO11 | importin 11 | -1.23 | 0.016 |
| IPO7 | importin 7 | 1.38 | 0.013 |
| LGTN | ligatin | -1.20 | 0.045 |
| LUC7L3 | cisplatin resistance-associated overexpressed protein | 1.51 | 0.023 |
| MBNL1 | muscleblind-like 1 | 1.34 | 0.040 |
| MBP | myelin basic protein | -1.51 | 0.010 |
| MED10 | mediator complex subunit 10 | -1.47 | 0.003 |

| Gene | Full name of protein | Fold-change | P-value |
|---|---|---|---|
| NHP2L1 | NHP2 non-histone chromosome protein 2-like 1 (S. cerevisiae) | -1.50 | 0.029 |
| NONO | non-POU domain containing, octamer-binding | 1.40 | 0.011 |
| POLR3B | polymerase (RNA) III (DNA directed) polypeptide B | -1.35 | 0.011 |
| PRDM9 | PR domain containing 9 | -1.18 | 0.031 |
| PUS1 | pseudouridylate synthase 1 | -1.31 | 0.019 |
| RBM42 | RNA binding motif protein 42 | 1.39 | 0.030 |
| RBMS1 | RNA binding motif, single stranded interacting protein 1 | 1.40 | 0.004 |
| RBMX | RNA binding motif protein, X-linked | 1.24 | 0.032 |
| SAMD4A | sterile alpha motif domain containing 4A | -2.04 | 0.0006 |
| SERBP1 | SERPINE1 mRNA binding protein 1 | -1.25 | 0.005 |
| SLU7 | SLU7 splicing factor homolog (S. cerevisiae) | 1.40 | 0.002 |
| SMG7 | Smg-7 homolog, nonsense mediated mRNA decay factor (C. elegans) | -1.12 | 0.049 |
| SNRPN | small nuclear ribonucleoprotein polypeptide N | 1.61 | 0.004 |
| SNURF | SNRPN upstream reading frame protein | 1.65 | 0.017 |
| SSB | Sjogren syndrome antigen B (autoantigen La) | 1.20 | 0.047 |
| SSSCA1 | Sjogren syndrome/scleroderma autoantigen 1 | -1.24 | 0.029 |
| TADA3 | transcriptional adaptor 3 (NGG1 homolog, yeast)-like | 1.34 | 0.0496 |
| TAF6L | TAF6-like RNA polymerase II, p300/CBP-associated factor (PCAF)-associated factor, 65kDa | -1.23 | 0.035 |
| TCEB2 | transcription elongation factor B (SIII), polypeptide 2 (18kDa, elongin B) | -1.64 | 0.012 |
| TSEN15 | tRNA splicing endonuclease 15 homolog (S. cerevisiae) | -1.48 | 0.010 |
| UPF1 | UPF1 regulator of nonsense transcripts homolog (yeast) | -1.30 | 0.043 |
| WBP11 | WW domain binding protein 11 | 1.43 | 0.002 |
| WDR61 | WD repeat domain 61 | -1.19 | 0.044 |
| XAB2 | XPA binding protein 2 | 1.44 | 0.027 |

Following protein synthesis in ribosomes, correct protein folding is essential for normal protein function. Deregulated genes involved in protein folding included Calnexin which facilitates protein folding and assembly and is involved in quality control of folded proteins; the heat shock protein 70 family member HSPA1A which stabilizes existing proteins against aggregation and mediates the folding of newly translated proteins; and TCP1 which contributes to a complex that folds various proteins including actin and tubulin.

We identified the proteasome as another significantly deregulated pathway in DM1 hearts (figure 5.3). As for the ribosome, most proteasome genes were found to have reduced expression levels, including 6 of the 7 beta subunits of the 20S proteasome (PSMB2-7), and PSMB10, PSMC3 and PSMD4. Protein degradation is mediated by the proteasome on the one hand, and by ubiquitin ligases on the other hand. From the list of differentially expressed genes, we identified additional deregulated genes involved in proteasome function and ubiquitin ligation (table 5.6). These are the positive regulator of E3 ubiquitin ligases COPS8; the F-box protein family member FBXO6 which constitutes one of the four subunits of the ubiquitin protein ligase complex; the ring finger protein RNF7 which is an essential subunit of the F-box protein ubiquitin ligases; HSPA1A which is also involved in the ubiquitin-proteasome pathway; PSME3 which is part of the immunoproteasome; Ubiquilin 2 which functionally links the ubiquitination machinery to the proteasome to affect protein degradation, and valosin-containing protein which functions in the 26S proteasome in ubiquitin-dependent protein degradation. Two highly interesting down-regulated genes are the cathepsins B and L1, which are involved in intracellular protein catabolism, in particular of collagen and elastin, and have been implicated in myofibril necrosis in myopathies. Finally, we found up-regulation of Calpastatin, which is thought to affect the expression levels of genes encoding structural or regulatory proteins by proteolysis. YAF2 interacts with YY1, a zinc finger protein involved in negative regulation of muscle-restricted genes including sarcomeric actin, and facilitates proteolytic cleavage of YY1 by the calcium- activated protease. YAF2 down-regulation in DM1 hearts may result in excess YY1 levels, putatively decreasing actin levels.

**Cytoskeletal genes are deregulated in DM1 hearts**

The cytoskeleton provides structural support to the cell and the main types of fibres comprise microfilaments, microtubules, and intermediate filaments. Genes coding for structural constituents of cytoskeleton are crucial for cardiac myocyte functioning and were generally up-regulated in DM1 hearts. Besides the aforementioned LDB3, PDLIM3 and TNNT2 genes, these include the intermediate filament proteins vimentin and synemin; the microfilament protein actin; and the actin-binding proteins CAPZA1, cofilin 2 and profilin 2 involved in actin growth; the actin-binding nexilin, SORBS2, supervillin and tropomyosin; CCT7 and -8 involved in actin folding; CDC42 involved in actin polymerisation; the actin depolymerizing factor destrin involved in actin turnover; and intersectin 1, NEBL and WDR1 which are all involved in actin assembly.

**Table 5.6.** Deregulated protein folding and degradation genes

| Gene | Full name of protein | Fold-change | P-value |
|---|---|---|---|
| CANX | calnexin | -1.52 | 0.0002 |
| CAST | calpastatin | 1.52 | 0.012 |
| COPS8 | COP9 constitutive photomorphogenic homolog subunit 8 (Arabidopsis) | 1.24 | 0.047 |
| CTSB | cathepsin B | -1.43 | 0.035 |
| CTSL1 | cathepsin L1 | -1.43 | 0.030 |
| DDX19B | DEAD (Asp-Glu-Ala-As) box polypeptide 19B | -1.23 | 0.049 |
| DDX31 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 31 | -1.22 | 0.006 |
| DDX46 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 46 | -1.35 | 0.004 |
| DDX51 | DEAD (Asp-Glu-Ala-Asp) box polypeptide 51 | 1.50 | 0.049 |
| DHX37 | DEAH (Asp-Glu-Ala-His) box polypeptide 37 | -1.26 | 0.047 |
| DHX40 | DEAH (Asp-Glu-Ala-His) box polypeptide 40 | 1.15 | 0.0321 |
| FBXO6 | F-box protein 6 | -1.26 | 0.019 |
| HSPA1A | heat shock 70kDa protein 1A; heat shock 70kDa protein 1B | 1.63 | 0.003 |
| NCL | nucleolin | 1.51 | 0.002 |
| NOP10 | NOP10 ribonucleoprotein homolog (yeast) | -1.41 | 0.049 |
| NOP56 | NOP56 ribonucleoprotein homolog (yeast) | 1.54 | 0.008 |
| PSMB10 | proteasome (prosome, macropain) subunit, beta type, 10 | -1.55 | 0.049 |
| PSMB2 | proteasome (prosome, macropain) subunit, beta type, 2 | -1.43 | 0.003 |
| PSMB3 | proteasome (prosome, macropain) subunit, beta type, 3 | -1.44 | 0.012 |
| PSMB4 | proteasome (prosome, macropain) subunit, beta type, 4 | -1.28 | 0.009 |
| PSMB5 | proteasome (prosome, macropain) subunit, beta type, 5 | -1.28 | 0.028 |
| PSMB6 | proteasome (prosome, macropain) subunit, beta type, 6 | -1.39 | 0.005 |
| PSMB7 | proteasome (prosome, macropain) subunit, beta type, 7 | -1.26 | 0.039 |
| PSMC3 | proteasome (prosome, macropain) 26S subunit, ATPase, 3 | -1.36 | 0.037 |
| PSMD4 | proteasome (prosome, macropain) 26S subunit, non-ATPase, 4 | -1.32 | 0.045 |
| PSME3 | proteasome (prosome, macropain) activator subunit 3 (PA28 gamma; Ki) | 1.31 | 0.029 |
| RNF7 | ring finger protein 7 | -1.31 | 0.040 |
| TCP1 | hypothetical gene supported by BC000665; t-complex 1 | 1.33 | 0.010 |
| UBQLN2 | ubiquilin 2 | 1.47 | 0.002 |
| VCP | valosin-containing protein | -1.50 | 0.013 |
| YAF2 | YY1 associated factor 2 | -1.34 | 0.003 |

**Table 5.7.** Deregulated cytoskeletal genes

| Gene | Full name of protein | Fold-change | P-value |
|---|---|---|---|
| ACTC1 | actin, alpha, cardiac muscle 1 | 1.49 | 0.001 |
| CAPZA1 | capping protein (actin filament) muscle Z-line, alpha 1 | 1.17 | 0.0498 |
| CCT7 | chaperonin containing TCP1, subunit 7 (eta) | 1.29 | 0.048 |
| CCT8 | similar to chaperonin containing TCP1, subunit 8 (theta) | 1.27 | 0.017 |
| CDC42 | cell division cycle 42 (GTP binding protein, 25kDa) | 1.23 | 0.016 |
| CFL2 | cofilin 2 (muscle) | 1.14 | 0.031 |
| DSTN | destrin (actin depolymerizing factor) | 1.88 | 0.005 |
| ITSN1 | intersectin 1 (SH3 domain protein) | -1.51 | 0.007 |
| LDB3 | LIM domain binding 3 | 1.31 | 0.049 |
| MAP1LC3A | microtubule-associated protein 1 light chain 3 alpha | 1.57 | 0.021 |
| MAP1LC3C | microtubule-associated protein 1 light chain 3 gamma | -1.19 | 0.008 |
| MAST4 | microtubule associated serine/threonine kinase family member 4 | -1.69 | 0.008 |
| MB | myoglobin | -1.43 | 0.021 |
| MKLN1 | muskelin 1, intracellular mediator containing kelch motifs | -1.28 | 0.010 |
| MTMR14 | myotubularin related protein 14 | -1.57 | 0.007 |
| MTMR3 | myotubularin related protein 3 | 1.36 | 0.010 |
| MYBPC3 | myosin binding protein C, cardiac | -1.34 | 0.035 |
| MYF5 | myogenic factor 5 | -1.27 | 0.004 |
| MYH14 | myosin, heavy chain 14 | -1.30 | 0.003 |
| MYOZ2 | myozenin 2 | 1.59 | 0.016 |
| NEBL | nebulette | 1.91 | 0.00008 |
| NEXN | nexilin (F actin binding protein) | 1.54 | 0.029 |
| PDLIM3 | PDZ and LIM domain 3 | 2.28 | 0.004 |
| PFN2 | profilin 2 | 1.47 | 0.033 |
| SORBS2 | sorbin and SH3 domain containing 2 | 1.90 | 0.0047 |
| SPTAN1 | spectrin, alpha, non-erythrocytic 1 (alpha-fodrin) | 1.50 | 0.020 |
| SVIL | supervillin | 1.15 | 0.02 |
| SYNM | synemin, intermediate filament protein | 1.63 | 0.005 |
| TNNT2 | troponin T type 2 (cardiac) | 1.54 | 0.013 |
| TPM2 | tropomyosin 2 (beta) | 1.90 | 0.019 |
| TUBA3D | tubulin, alpha 3d; tubulin, alpha 3c | -1.89 | 0.007 |
| VIM | vimentin | 1.56 | 0.008 |
| WDR1 | WD repeat domain 1 | 1.24 | 0.033 |

Differentially expressed microtubule(-associated) genes include the microtubule-constituent tubulin TUBA3D; MAST4, with a possible broad range of neural functions; and MAP1LC3A and MAP1LC3C, which mediate the physical interactions between microtubules and components of the cytoskeleton and are involved in microtubule assembly, an essential step in neurogenesis. Other deregulated structural protein genes include Muskelin 1, which mediates cytoskeletal responses to the extracellular matrix component thrombospondin I; the myotubularin related proteins MTMR3 and -14 for which mutations have been associated with autosomal dominant centronuclear myopathy; the cardiac myosin binding protein C MYBPC3; the myogenic factor MYF5 involved in muscle differentiation; the myosin heavy chain MYH14; myozenin 2, which is important for calcineurin signalling as it tethers calcineurin to alpha-actinin at the z-line of the sarcomere of cardiac and skeletal muscle cells; and the cell stabilizing alpha spectrin SPTAN1 (table 5.7).



## Discussion

This is the first study that charts the myocardial mRNA and miRNA expression profiles of human DM1-related cardiomyopathy. Microarray analysis showed significant changes in the expression levels of 989 mRNAs and 16 miRNAs in hearts of DM1 patients as compared to controls. We show that mRNA expression levels in hearts of DM1 patients are compatible with proposed pathogenic mechanisms and that several pathological changes in DM1 hearts may have potential functional consequences.

First, we found reduced DMPK transcript levels in human DM1 hearts in accordance with studies in other tissues.[24] This suggests that the presence of a large number of repeats in the 3' untranslated region of the DMPK gene reduces both the synthesis and/or processing of DMPK mRNA. It has indeed been shown that CUGexp transcripts are processed into small RNAs that trigger down-regulation of mutant transcripts.[25] DMPK encodes several serine/threonine protein kinase isoforms that are necessary for ion homeostasis and remodelling of the cytoskeleton and could be a contributing factor in the cardiac involvement in of DM1.[26] Secondly, we found reduced cardiac transcript levels of SIX5, which is located immediately downstream of DMPK. These reduced levels may contribute to the molecular pathogenesis of cardiac disease, as experimental heterozygous loss of SIX5 in mice causes cataracts and cardiac conduction disease.[27-29]

Furthermore, the RNA binding proteins MBNL1 and CUGBP1 have an established role in DM1 pathogenesis. Their expression and stability was not altered in skeletal muscle biopsies from DM1 patients.[30] By contrast, both MBNL1 and

CUGBP1 mRNA levels were mildly up-regulated in heart samples from our DM1 patients. In addition, many mRNAs known to be aberrantly spliced in DM1 muscle were up-regulated, including TNNT2, PDLIM, LDB3 and CASQ1. This may possibly reflect a compensatory mechanism to overcome protein isoforms that do not provide the necessary functional properties. Alternative splicing has a major role in cardiac adaptive responses, and aberrant splicing has been associated with heart disease.[31]

miRNAs are small non-coding RNAs that regulate gene expression post-transcriptionally and may be involved in the regulation of the pathological pathways leading to cardiac dysfunction. Misregulation of miRNA-1 has been demonstrated in heart samples from patients with DM1 obtained at autopsy.[32] Our miRNA microarray data did not confirm this finding. In addition, the miRNAs that have been found to be deregulated in skeletal muscle of patients with DM1,[33] or other types of dystrophy,[34] were unaltered in the hearts of our patients with DM1-related cardiomyopathy. Most notably, miRNA-30b was down-regulated among a relatively small number of down-regulated miRNAs in our cardiac samples. This may be of interest since the miRNA-30 family inhibits CTGF and thereby may act as a natural inhibitor of fibrosis.[35] Loss of miRNA-30b may contribute to the fibrosis seen in DM1 hearts.

Furthermore, we identified deregulated pathways in cardiac muscle of DM1 patients through the analysis of gene expression profiles. Deregulated expression of genes involved in fatty acid metabolism and oxidative phosphorylation suggest altered energy metabolism. In addition, genes encoding proteasomes, ribosomes and spliceosomes were down-regulated suggesting concerted changes in RNA processing. Cytoskeletal genes were generally up-regulated, as were calcium signalling related genes. Interestingly, these pathways largely overlap with those found enriched in gene expression profiles of skeletal muscle from patients and mouse models of DM1, including calcium signalling and homeostasis, energy metabolism and ribosomal proteins.[18, 36]

It has been suggested that mitochondrial oxidative alterations can occur in DM1 and may be a possible mechanism underlying cell damage in skeletal muscle of DM1 patients.[37] However, direct evidence of a mitochondrial defect in DM1 has not yet been established and possible links between molecular abnormalities and mitochondrial dysfunction have still to be clarified in this disease. Here, we show global down-regulation of genes involved in the mitochondrial OXPHOS pathway in hearts of DM1 patients. In addition, we found reduced expression of the mitochondrial transcription factor TFAM mRNA, which is required for mtDNA transcription initiation and thus for the expression of several OXPHOS subunits.[38, 39] Mitochondrial dysfunction is common to skeletal muscle and neurological disorders. OXPHOS deficiency is also an established cause of cardiomyopathies and conduction abnormalities.[40] Our findings strongly

point towards DM1-dependent disturbance of cardiac mitochondrial function, putatively predisposing DM1 hearts to conduction disturbances and impaired myocardial function. In addition, abnormal cardiomyocyte calcium handling may further impact contractile dysfunction and render hearts susceptible to arrhythmias, although the precise mechanisms involved remain to be fully elucidated.

Recent evidence indicates that functional insufficiency of the ubiquitin-proteasome system plays a pathogenic role in a subset of heart diseases.[41, 42] We observed decreased levels of proteasomal and ubiquitin system-related genes in DM1 hearts, putatively compromising protein quality control in DM1 heart muscle cells and predisposing these patients to arrhythmias or progressive cardiomyopathy. Our data are not supported by findings in skeletal muscle of a transgenic DM1 mouse model, in which polyubiquitin/proteasome stress-signalling pathways were activated.[43]

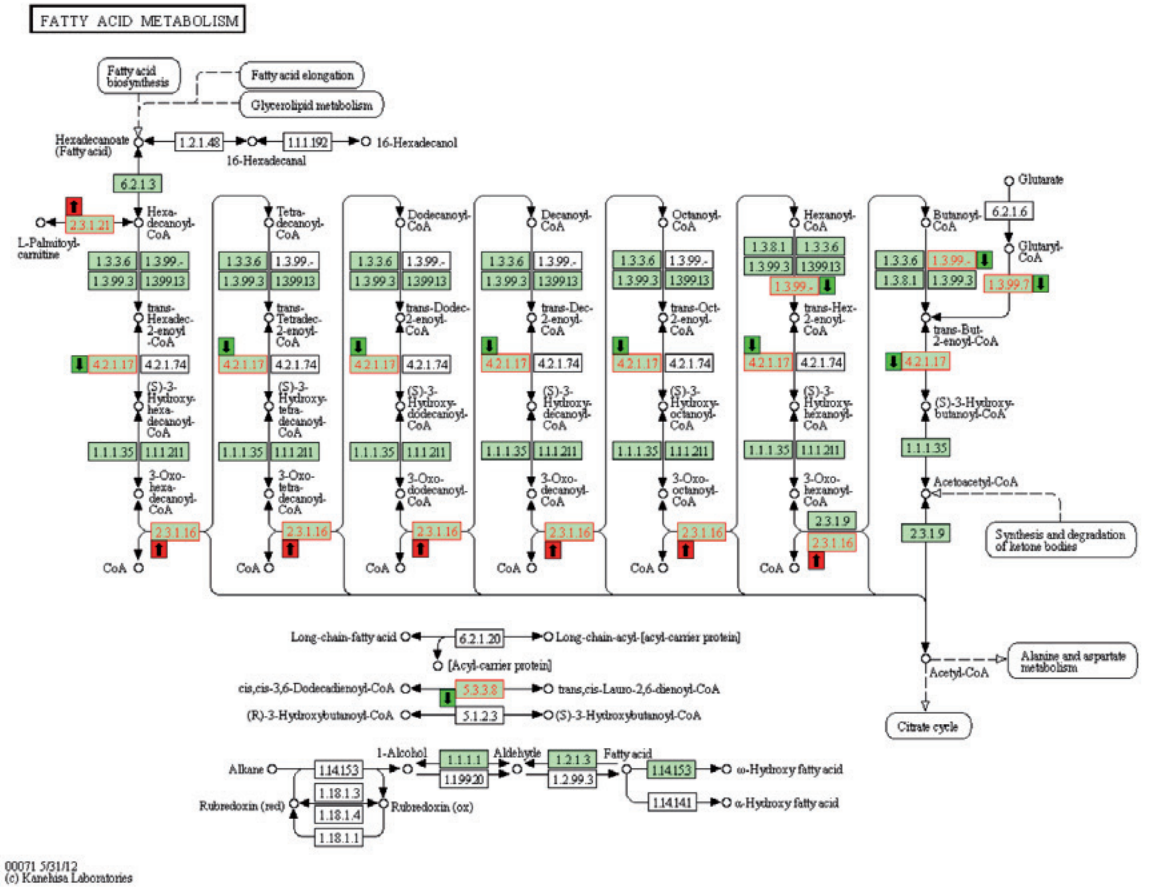
The endomyocardial biopsies used in this study have recognized limitations. First, sampling error may occur in case of local cardiac damage, such as focal fibrosis or focal inflammation. Secondly, cellular heterogeneity, i.e. muscular and endothelial as well as fibrotic components, can be obtained. Although endomyocardial biopsy is an invasive procedure with certain limitations, biopsies obtained from clinically stable DM1 patients allowed us to identify early changes in cardiac gene expression profiles in contrast to patients with end stage DM1. We were able to reproduce gene expression changes in DM1 cardiac tissue that have been directly linked to established concepts on DM1 pathophysiology, for instance the loss of DMPK and SIX5 expression.

As has been shown, the expansion mutation has complex effects on gene expression in DM1 hearts. Altered global gene expression has previously been shown in skeletal muscle biopsies of DM1 patients,[18, 20, 44] and in skeletal muscle of MBNL1 knock-out mice and of transgenic mice expressing non-coding CUG repeats.[36] These gene expression abnormalities may result from effects on transcription, RNA processing and RNA stability.[36] Transcription can be disrupted through several mechanisms, including transcription factor leaching from chromatin by expanded DMPK transcripts,[45] or MBNL1 loss of function.[36] While it is tentative to speculate that all mRNA expression changes are the direct consequence of the DMPK repeat expansion, secondary effects of cardiac dysfunction on these genes cannot be ruled out.

## Conclusion

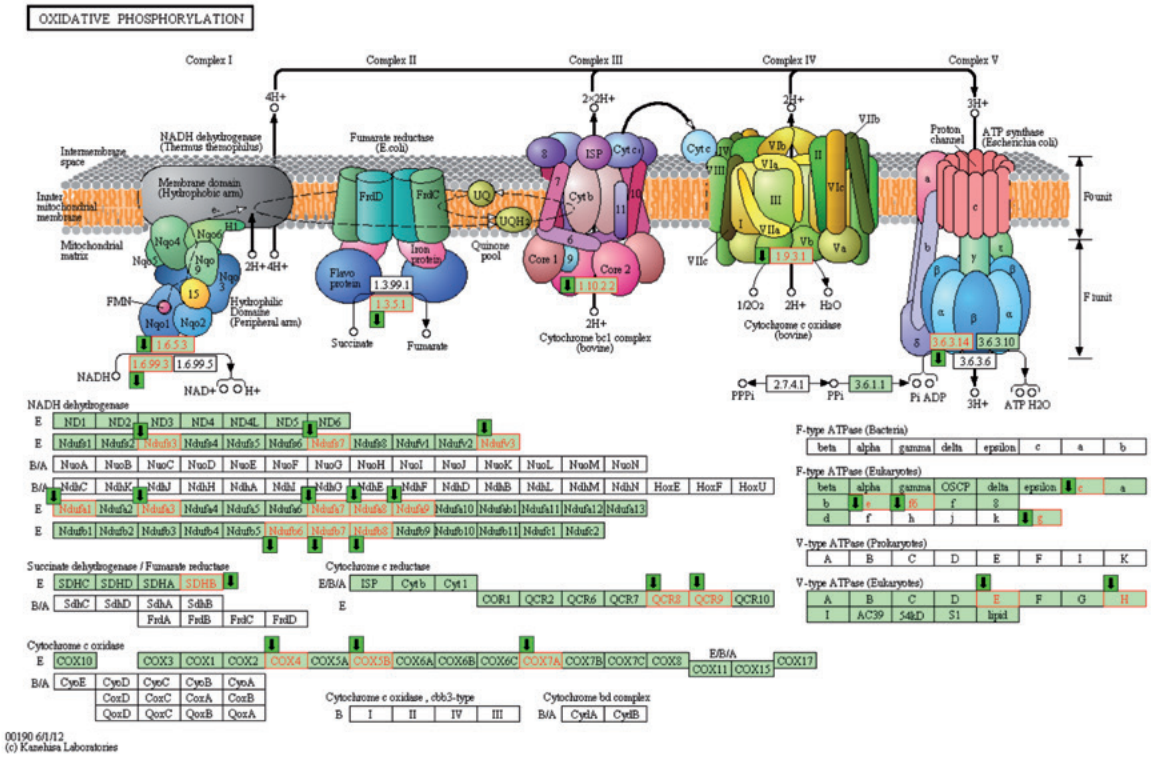
We demonstrate for the first time that cardiac involvement in DM1 is associated with distinct gene expression signatures reminiscent of those found in skeletal muscle and experimental models. Gene expression profiles identified several pathological changes in DM1 hearts with potential functional consequences, including loss of DMPK, altered RNA processing and deregulation of OXPHOS, calcium signalling and cytoskeletal genes. While DM1 patients exhibit heterogeneous cardiac manifestations, their cardiac gene expression profiles suggest a rather uniform DM1-associated cardiomyopathy.

**Figure 5.1.** Genes mapping to KEGG pathway for fatty acid metabolism



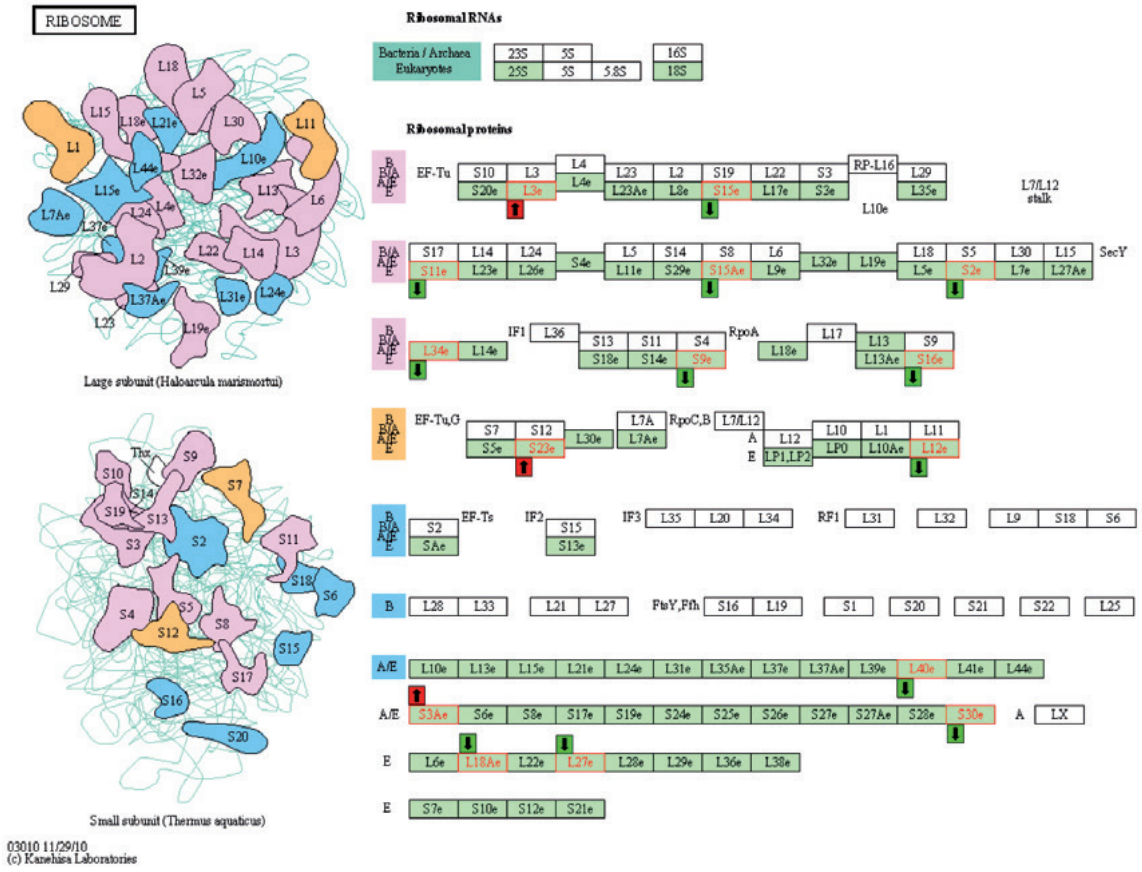

**Legend to figure 5.1.** Each green box is representative of a human protein for which a gene has been identified in the genome. The numbers in the boxes are the Enzyme Commission (EC) number for the proteins. Green and red arrows indicate down- and up-regulated genes respectively.

**Figure 5.2.** Genes mapping to KEGG pathway for oxidative phosphorylation system (OXPHOS)



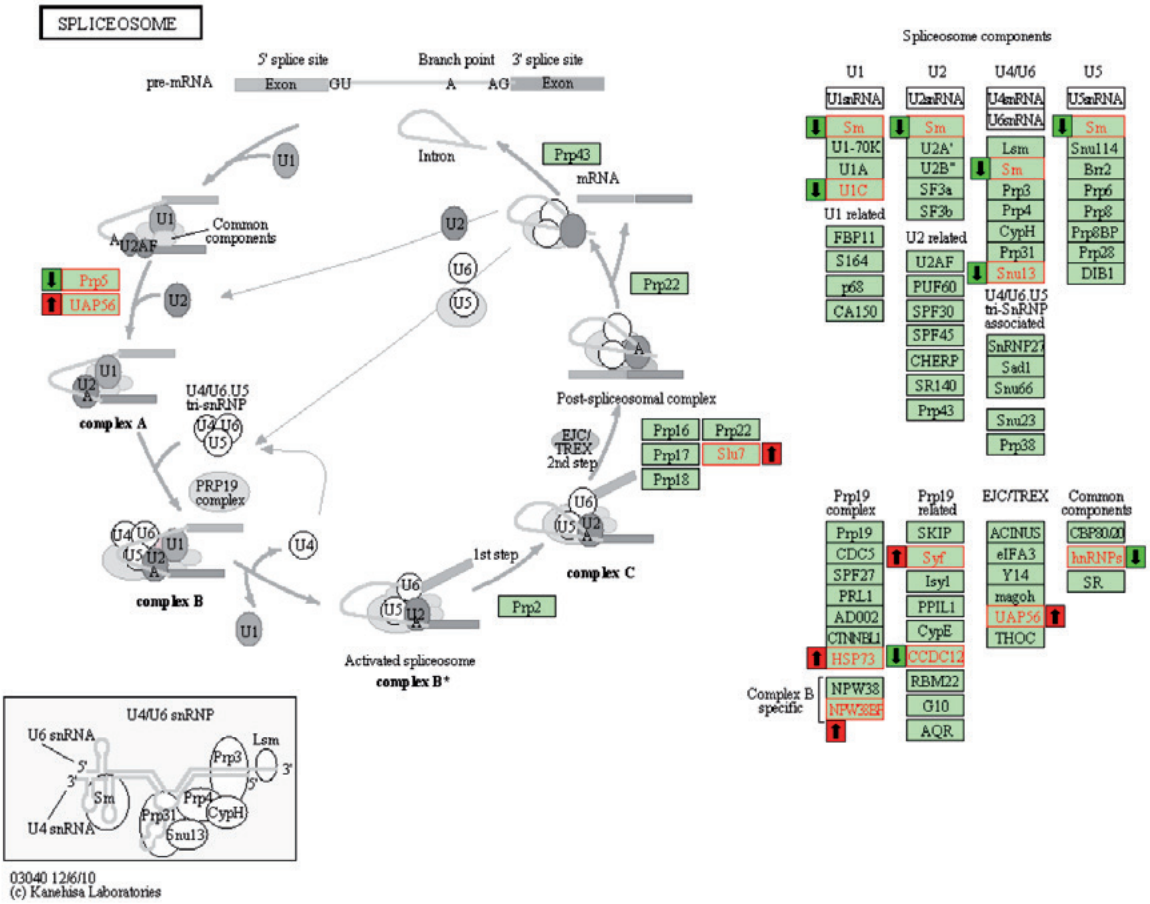

**Legend to figure 5.2.** Each green box is representative of a human protein for which a gene has been identified in the genome. The numbers in the boxes are the Enzyme Commission (EC) number for the proteins. Green and red arrows indicate down- and up-regulated genes respectively.

**Figure 5.3.** Genes mapping to KEGG pathway for ribosome



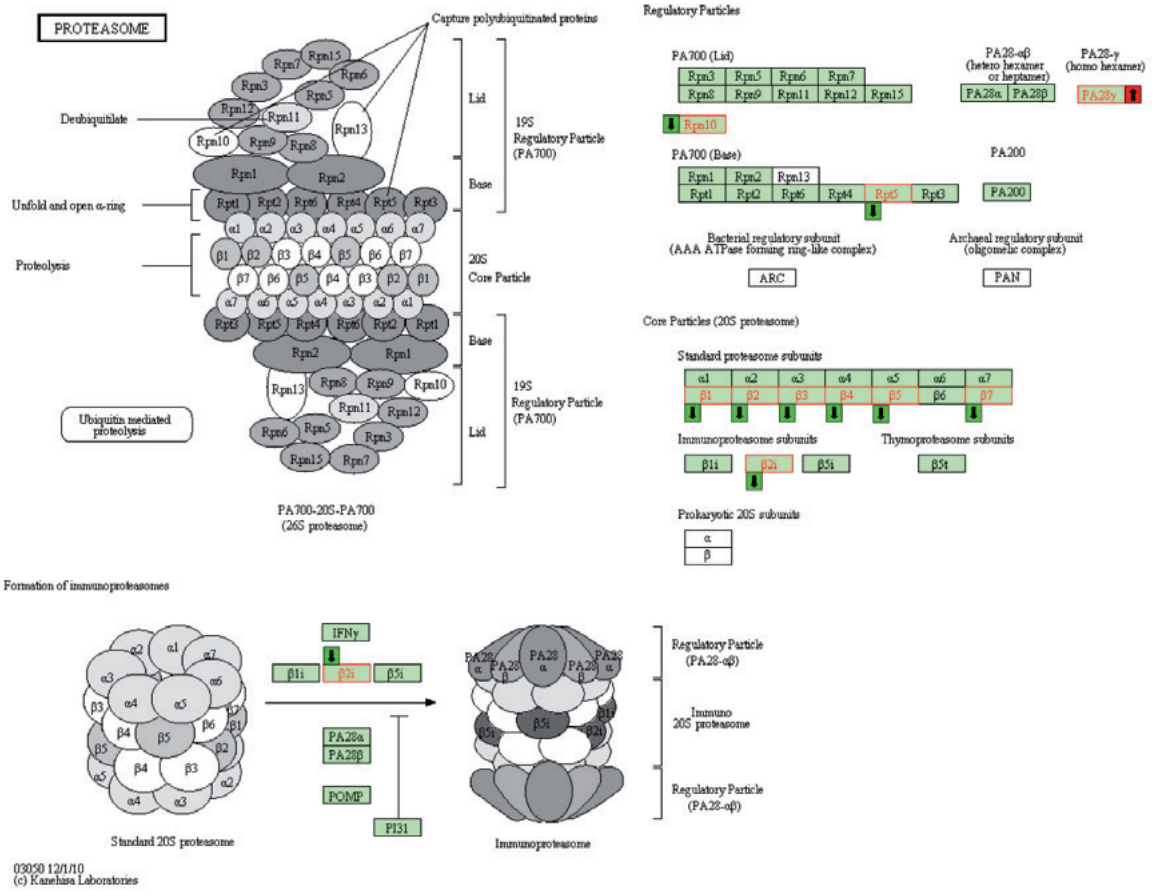

**Legend to figure 5.3.** Each green box is representative of a human protein for which a gene has been identified in the genome. The numbers in the boxes are the Enzyme Commission (EC) number for the proteins. Green and red arrows indicate down- and up-regulated genes respectively.

**Figure 5.4.** Genes mapping to KEGG pathway for spliceosome



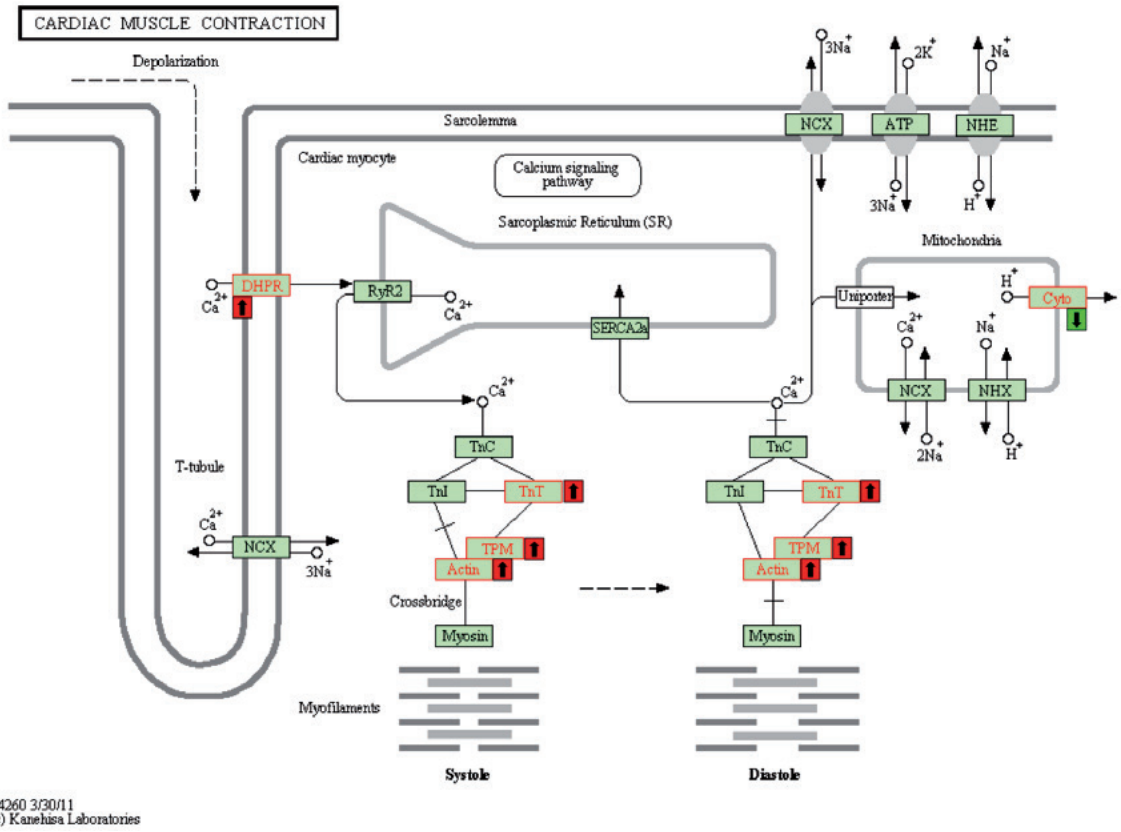

**Legend to figure 5.4.** Each green box is representative of a human protein for which a gene has been identified in the genome. The numbers in the boxes are the Enzyme Commission (EC) number for the proteins. Green and red arrows indicate down- and up-regulated genes respectively.

**Figure 5.5.** Genes mapping to KEGG pathway for proteasome




**Legend to figure 5.5.** Each green box is representative of a human protein for which a gene has been identified in the genome. The numbers in the boxes are the Enzyme Commission (EC) number for the proteins. Green and red arrows indicate down- and up-regulated genes respectively.

**Figure 5.6.** Genes mapping to KEGG pathway for cardiac muscle contraction




**Legend to figure 5.6.** Each green box is representative of a human protein for which a gene has been identified in the genome. The numbers in the boxes are the Enzyme Commission (EC) number for the proteins. Green and red arrows indicate down- and up-regulated genes respectively.

# References


1. Harper PS. Myotonic dystrophy. London: Saunders; 2001
2. Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, Aburatani H, Hunter K, Stanton VP, Thirion JP, Hudson T, et al. 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*. 1992;69:385
3. de Die-Smulders CE, Howeler CJ, Thijs C, Mirandolle JF, Anten HB, Smeets HJ, Chandler KE, Geraedts JP. Age and causes of death in adult-onset myotonic dystrophy. *Brain*. 1998;121:1557-1563
4. Mathieu J, Allard P, Potvin L, Prevost C, Begin P. A 10-year study of mortality in a cohort of patients with myotonic dystrophy. *Neurology*. 1999;52:1658-1662
5. Sicot G, Gourdon G, Gomes-Pereira M. Myotonic dystrophy, when simple repeats reveal complex pathogenic entities: New findings and future challenges. *Hum Mol Genet*. 2011;20:R116-123
6. Berul CI, Maguire CT, Gehrmann J, Reddy S. Progressive atrioventricular conduction block in a mouse myotonic dystrophy model. *J Interv Card Electrophysiol*. 2000;4:351-358
7. Klesert TR, Otten AD, Bird TD, Tapscott SJ. Trinucleotide repeat expansion at the myotonic dystrophy locus reduces expression of dmahp. *Nat Genet*. 1997;16:402-406
8. Dennert R, Velthuis S, Schalla S, Eurlings L, van Suylen RJ, van Paassen P, Tervaert JW, Wolffs P, Goossens VJ, Bruggeman C, Waltenberger J, Crijns HJ, Heymans S. Intravenous immunoglobulin therapy for patients with idiopathic cardiomyopathy and endomyocardial biopsy-proven high pvb19 viral load. *Antivir Ther*. 2010;15:193-201
9. Du P, Kibbe WA, Lin SM. Lumi: A pipeline for processing illumina microarray. *Bioinformatics*. 2008;24:1547-1548
10. Bolstad BM, Irizarry RA, Astrand M, Speed TP. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics*. 2003;19:185-193
11. Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. *Stat Appl Genet Mol Biol*. 2004;3:Article3
12. Benjamini Y, Hochberg Y. Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J.R. Statist. Soc. B*. 1995;57:289-300
13. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M. Kegg for integration and interpretation of large-scale molecular data sets. *Nucleic Acids Res*. 2012;40:D109-114
14. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using david bioinformatics resources. *Nat Protoc*. 2009;4:44-57
15. Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: Paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res*. 2009;37:1-13
16. Philips AV, Timchenko LT, Cooper TA. Disruption of splicing regulated by a cug-binding protein in myotonic dystrophy. *Science*. 1998;280:737-741
17. Ohsawa N, Koebis M, Suo S, Nishino I, Ishiura S. Alternative splicing of pdlim3/alp, for alpha-actinin-associated lim protein 3, is aberrant in persons with myotonic dystrophy. *Biochem Biophys Res Commun*. 2011;409:64-69
18. Vihola A, Sirito M, Bachinski LL, Raheem O, Screen M, Suominen T, Krahe R, Udd B. Altered expression and splicing of ca(2+) metabolism genes in myotonic dystrophies dm1 and dm2. *Neuropathol Appl Neurobiol*. 2012;[Epub ahead of print]
19. Rinaldi F, Terracciano C, Pisani V, Massa R, Loro E, Vergani L, Di Girolamo S, Angelini C, Gourdon G, Novelli G, Botta A. Aberrant splicing and expression of the non muscle myosin heavy-chain gene myh14 in dm1 muscle tissues. *Neurobiol Dis*. 2012;45:264-271
20. Vihola A, Bachinski LL, Sirito M, Olufemi SE, Hajibashi S, Baggerly KA, Raheem O, Haapasalo H, Suominen T, Holmlund-Hampf J, Paetau A, Cardani R, Meola G, Kalimo H, Edstrom L, Krahe R, Udd B. Differences in aberrant expression and splicing of sarcomeric proteins in the myotonic dystrophies dm1 and dm2. *Acta Neuropathol*. 2010;119:465-479
21. Vatta M, Mohapatra B, Jimenez S, Sanchez X, Faulkner G, Perles Z, Sinagra G, Lin JH, Vu TM, Zhou Q, Bowles KR, Di Lenarda A, Schimmenti L, Fox M, Chrisco MA, Murphy RT, McKenna W, Elliott P, Bowles NE, Chen J, Valle G, Towbin JA. Mutations in cypher/zasp in patients with dilated cardiomyopathy and left ventricular non-compaction. *J Am Coll Cardiol*. 2003;42:2014-2027
22. Selcen D, Engel AG. Mutations in zasp define a novel form of muscular dystrophy in humans. *Ann Neurol*. 2005;57:269-276

23. www.genome.jp/kegg
24. Carango P, Noble JE, Marks HG, Funanage VL. Absence of myotonic dystrophy protein kinase (dmpk) mrna as a result of a triplet repeat expansion in myotonic dystrophy. *Genomics*. 1993;18:340-348
25. Krol J, Fiszer A, Mykowska A, Sobczak K, de Mezer M, Krzyzosiak WJ. Ribonuclease dicer cleaves triplet repeat hairpins into shorter repeats that silence specific targets. *Mol Cell*. 2007;25:575-586
26. Oude Ophuis RJ, Wijers M, Bennink MB, van de Loo FA, Fransen JA, Wieringa B, Wansink DG. A tail-anchored myotonic dystrophy protein kinase isoform induces perinuclear clustering of mitochondria, autophagy, and apoptosis. *PloS one*. 2009;4:e8024
27. Wakimoto H, Maguire CT, Sherwood MC, Vargas MM, Sarkar PS, Han J, Reddy S, Berul CI. Characterization of cardiac conduction system abnormalities in mice with targeted disruption of six5 gene. *J Interv Card Electrophysiol*. 2002;7:127-135
28. Klesert TR, Cho DH, Clark JI, Maylie J, Adelman J, Snider L, Yuen EC, Soriano P, Tapscott SJ. Mice deficient in six5 develop cataracts: Implications for myotonic dystrophy. *Nat Genet*. 2000;25:105-109
29. Sarkar PS, Appukuttan B, Han J, Ito Y, Ai C, Tsai W, Chai Y, Stout JT, Reddy S. Heterozygous loss of six5 in mice is sufficient to cause ocular cataracts. *Nat Genet*. 2000;25:110-114
30. Nezu Y, Kino Y, Sasagawa N, Nishino I, Ishiura S. Expression of mbnl and celf mrna transcripts in muscles with myotonic dystrophy. *Neuromuscul Disord*. 2007;17:306-312
31. Guo W, Schafer S, Greaser ML, Radke MH, Liss M, Govindarajan T, Maatz H, Schulz H, Li S, Parrish AM, Dauksaite V, Vakeel P, Klaassen S, Gerull B, Thierfelder L, Regitz-Zagrosek V, Hacker TA, Saupe KW, Dec GW, Ellinor PT, MacRae CA, Spallek B, Fischer R, Perrot A, Ozcelik C, Saar K, Hubner N, Gotthardt M. Rbm20, a gene for hereditary cardiomyopathy, regulates titin splicing. *Nat Med*. 2012;18:766-773
32. Rau F, Freyermuth F, Fugier C, Villemin JP, Fischer MC, Jost B, Dembele D, Gourdon G, Nicole A, Duboc D, Wahbi K, Day JW, Fujimura H, Takahashi MP, Auboeuf D, Dreumont N, Furling D, Charlet-Berguerand N. Misregulation of mir-1 processing is associated with heart defects in myotonic dystrophy. *Nat Struct Mol Biol*. 2011;18:840-845
33. Perbellini R, Greco S, Sarra-Ferraris G, Cardani R, Capogrossi MC, Meola G, Martelli F. Dysregulation and cellular mislocalization of specific mirnas in myotonic dystrophy type 1. *Neuromuscul Disord*. 2011;21:81-88
34. Eisenberg I, Eran A, Nishino I, Moggio M, Lamperti C, Amato AA, Lidov HG, Kang PB, North KN, Mitrani-Rosenbaum S, Flanigan KM, Neely LA, Whitney D, Beggs AH, Kohane IS, Kunkel LM. Distinctive patterns of microrna expression in primary muscular disorders. *Proc Natl Acad Sci U S A*. 2007;104:17016-17021
35. Duisters RF, Tijsen AJ, Schroen B, Leenders JJ, Lentink V, van der Made I, Herias V, van Leeuwen RE, Schellings MW, Barenbrug P, Maessen JG, Heymans S, Pinto YM, Creemers EE. Mir-133 and mir-30 regulate connective tissue growth factor: Implications for a role of micrornas in myocardial matrix remodeling. *Circ Res*. 2009;104:170-178
36. Osborne RJ, Lin X, Welle S, Sobczak K, O'Rourke JR, Swanson MS, Thornton CA. Transcriptional and post-transcriptional impact of toxic rna in myotonic dystrophy. *Hum Mol Genet*. 2009;18:1471-1481
37. Siciliano G, Mancuso M, Tedeschi D, Manca ML, Renna MR, Lombardi V, Rocchi A, Martelli F, Murri L. Coenzyme q10, exercise lactate and ctg trinucleotide expansion in myotonic dystro38. Larsson NG, Wang J, Wilhelmsson H, Oldfors A, Rustin P, Lewandoski M, Barsh GS, Clayton DA. Mitochondrial transcription factor a is necessary for mtdna maintenance and embryogenesis in mice. *Nat Genet*. 1998;18:231-236
39. Shi Y, Dierckx A, Wanrooij PH, Wanrooij S, Larsson NG, Wilhelmsson LM, Falkenberg M, Gustafsson CM. Mammalian transcription factor a is a core component of the mitochondrial transcription machinery. *Proc Natl Acad Sci U S A*. 2012;109:16510-16515
40. Berardo A, Musumeci O, Toscano A. Cardiological manifestations of mitochondrial respiratory chain disorders. *Acta Myol*. 2011;30:9-15
41. Schlossarek S, Carrier L. The ubiquitin-proteasome system in cardiomyopathies. *Curr Opin Cardiol*. 2011;26:190-195
42. Li YF, Wang X. The role of the proteasome in heart disease. *Biochim Biophys Acta*. 2011;1809:141-149
43. Vignaud A, Ferry A, Huguet A, Baraibar M, Trollet C, Hyzewicz J, Butler-Browne G, Puymirat J, Gourdon G, Furling D. Progressive skeletal muscle weakness in transgenic mice expressing ctg expansions is associated with the activation of the ubiquitin-proteasome pathway. *Neuromuscul Disord*. 2010;20:319-325
44. Botta A, Vallo L, Rinaldi F, Bonifazi E, Amati F, Biancolella M, Gambardella S, Mancinelli E, Angelini C, Meola G, Novelli G. Gene expression analysis in myotonic dystrophy: Indications for a common molecular pathogenic pathway in dm1 and dm2. *Gene Expr*. 2007;13:339-351
45. Ebralidze A, Wang Y, Petkova V, Ebralidse K, Junghans RP. Rna leaching of transcription factors disrupts transcription in myotonic dystrophy. *Science*. 2004;303:383-387

PART III

# Outcome measures in myotonic dystrophy type 1

Chapter 6

# Outcome measures in adults with myotonic dystrophy type 1

*Mieke C. E. Hermans[a], Floriaan G.C.M. De Kleermaeker[b], Ingemar S.J. Merkies[a,c], Catharina G. Faber[a]*

[a]Department of Neurology, Maastricht University Medical Centre, Maastricht
[b]Department of Neurology, Catharina Hospital, Eindhoven
[c]Department of Neurology, Spaarne Hospital, Hoofddorp

*Manuscript in preparation*

## Summary

Myotonic dystrophy type 1 (DM1) is a dominantly inherited multisystemic disorder. New and emerging therapeutic interventions require high quality outcome measures to determine the effect in future clinical trials. Various measurement instruments are available, but no systematic evaluation has been conducted to determine the scientific soundness of these scales in the DM1 population.

In this study, a comprehensive systematic literature search was conducted with predefined selection strategy to identify measurement instruments applied in clinical trials with DM1 patients. An overwhelming amount of health measures have been used. A total of 141 measures were evaluated in terms of applicability, validity, reliability and responsiveness. Very few outcome measures fulfilled the complete spectrum of clinimetric requirements. Recommendations are made to adopt modern rather than classical clinimetric approaches when constructing, evaluating and selecting outcome measures and to assemble a core set of outcome measures at various levels which can be applied in future therapeutic trials to improve comparability of the obtained results between trials.

## Introduction

Myotonic dystrophy type 1 (DM1) is a progressive autosomal dominant multisystem disorder with a prevalence of 2.1-14.3 per 100 000.[1] DM1 is caused by an unstable CTG-repeat expansion in the myotonic dystrophy protein kinase (DMPK) gene on chromosome 19.[2] The disease is characterized primarily by myotonia and muscle weakness of facial, axial and distal limb muscles. Other clinical features include cataracts, gastro-intestinal complaints, cardiac involvement, endocrine dysfunction, respiratory problems, excessive daytime sleepiness, and impairments in cognitive executive functions.[1] An earlier age of onset and increased severity of clinical symptoms has been observed in subsequent generations and is related to degree of CTG expansion. DM1 leads to physical impairment, activity limitations and participation restrictions, and decreased health-related quality of life.[3-6]

Future clinical trials evaluating the efficacy of (non-)pharmacological interventions aim at improving health outcome in patients with DM1. The selection of the most appropriate instrument for measuring a specific outcome is not easy with the wide range of measurement instruments available. However, high quality outcome measures are important since the validity of inferences from these trials is directly dependent on the quality of the applied instrument.[7] Selected outcome measures therefore need to be evaluated for their clinical applicability and scientific soundness, meeting the demands of being simple, communicable, valid, reliable, and responsive.

This review provides an overview of measurement instruments applied in clinical studies of patients with DM1 from January 1948 until March 2012 and summarizes the clinimetric properties of the selected instruments. Recommendations are made to adopt modern rather than classical clinimetric approaches when constructing outcome measures for DM1 and to use a certain degree of standardization to facilitate comparison of future clinical trial results and improve communication in clinical practice.



## Methods

Since the number of outcome measures in DM1 is probably infinite when covering all multisystemic clinical features, we focused on non-invasive, bedside clinical outcome measures. To identify outcome measures used in trials with DM1 patients between January 1948 and March 2012, a systematic literature search was performed in PubMed. Selection was based on the keywords and MESH terms myotonic dystrophy type 1, DM1, and human. Furthermore the bibliographies of all articles were checked. A sample population of at least

10 patients was required for inclusion in this review. Outcome measures for patients younger than 18 years of age were excluded. Studies included in this review were selected independently by two authors (FDK, IM). All selected outcome measures, primary and secondary, were categorized according to the International Classification of Functioning, Disability and Health (ICF) framework,[8] and quality of life concept,[9] and subsequently evaluated in terms of clinical applicability and scientific soundness.[10]

## Results

### General aspects

A total of 781 articles were identified and 141 outcome measures fulfilled the predefined criteria. All measurement instruments are systematically presented in table 6.1. A total of 137 (97%) outcome measures purely represented one level of outcome, while 4 (3%) were constructed by using a combination of items representing various levels of outcome (composite measures).

### Impairment measures

**Muscle strength.** Muscle strength measurements have had most attention in studies evaluating scientific clinimetric soundness in DM1. The widely used Muscular Impairment Rating Scale (MIRS) is a simple, valid and reliable clinical outcome for the measurement of muscular impairment to monitor major stages of progression within this population. However, the MIRS and other manual muscle testing scores may lack sensitivity to detect subtle changes in muscle strength. The quantitative muscle testing of ankle eversion and dorsiflexion with a hand held dynamometer seems to be a promising method to assess distal muscle weakness as a preliminary study showed good reliability and the ability to discriminate between healthy and DM1 patients with different levels of impairments.[11] However, future research should be done to better characterize the intertesters reproducibility and responsiveness. Quantitative muscle testing has also been applied in other muscle groups.[12]

**Myotonia.** Well-developed myotonia measurements are scarce. Computerized handgrip myometry is quite sophisticated to apply and strong evidence for validity and reliability is lacking.[13, 14] Furthermore, relaxation time after ulnar nerve stimulation cannot be simply applied in clinical practice and lacks evidence for responsiveness.[15, 16]

**Fatigue.** With exception of a good reliability of the Krupp's Fatigue Severity Scale in DM1,[17] there is no evidence for proper clinimetric quality of fatigue scales for this patient population.

**Sleepiness.** The Daytime Sleepiness Scale is specifically devised to assess the level of daytime sleepiness in patients with DM1 and has demonstrated good validity, reliability and simplicity.[17, 18] To date, no responsiveness studies have been conducted. The widely applied Epworth Sleepiness Scale demonstrated to be inferior to the Daytime Sleepiness Scale in DM1[17] and less sensitive to changes than the more robust Maintenance of Wakefulness Test (MWT).[19]

**Pain.** Clinimetric qualities of pain scales in DM1 patients have not been reported. Validity and reliability of a Numerical Rating Scale for pain have been demonstrated in a general chronic pain population, but the generalizability of these findings to other populations, like DM1 patients, is unknown.[20] The McGill Pain Questionnaire (MPQ) as well as the Brief Pain Inventory (BPI) have been proved to be valid and reliable in limb loss, cerebral palsy, spinal cord injury, general pain populations,[21-28] but have not been evaluated in DM1.

**Psychological distress.** Many psychological functioning scales have been applied in studies with DM1 patients. Many of these measures, like the Hospital Anxiety and Depression Scale (HADS), Hamilton Anxiety Rating Scale, Montgomery and Asberg Depression Rating Scale (MADRS), Depressive Mood Scale (EHD), Tyrer Anxiety Scale and General Health Questionnaire-12 (GHQ-12), have been extensively evaluated and shown to be valid, reliable, simple or responsive to some extent, but data obtained in the DM1 population are missing.[29-37]



### Activity and participation measures

Only the 6-minute walking test (6-MWT) and the Purdue Pegboard have been properly evaluated for its clinimetric properties in DM1 patients showing moderate to good reliability and responsiveness.[38, 39] The self-report Activities of Daily Living (ADL) has been shown to have a good construct validity and more sensitivity for changes over time than the Mental adjustment to cancer (MAC) scale, the Psychosocial well-being questionnaire and the ADL staircase in muscular dystrophy patients, including patients with DM1.[40, 41]

### Quality of life measures

Compared to measures of functioning and disability, quality of life seems to be neglected in clinical trials. The SF-36 and Sickness Impact Profile (SIP) are most widely used quality of life measures in DM1, probably because there is a lot evidence of clinimetric quality in various other populations.[9, 42-44] However, no quality of life measures have been appropriately evaluated for their clinimetric soundness in DM1.

## Discussion

An overwhelming amount of outcome measures have been used in studies involving DM1 patients over the last 60 years as demonstrated in table 6.1. This large pool of measurement instruments hampers comparison of the results of clinical trials. More important is the fact that clinimetric evidence is poor or incomplete for most measurement instruments. In particular, responsiveness studies are lacking.[45] At the impairment level of outcome, the MIRS has shown to be simple, communicable, valid and reliable. However, it is a single item scale, having limited ability to detect difference between individuals and detect subtle changes over time.[46] The MIRS may therefore not be suitable for efficacy measurements in short-term therapeutic trials.[47] Quantitative muscle testing using a hand-held myometer may seem to offer an alternative to evaluate as it can detect small changes in distal muscle strength. However, minimal changes do not necessarily correlate with noticeable changes for the patient.
At the activity level of outcome, the 6-minute walking test and the Purdue Pegboard bimanual were reliable, feasible and sensitive enough to detect clinical changes in subjects with DM1.[38, 39]
The SF-36 is one of the most widely used generic health status measure. Normative data are available from general population surveys that can be used to determine whether a group or individual in question scores above or below the average. However, the SF-36 is not disease-specific making it less efficient for more subtle changes experienced by DM1 patients.
Since clinimetric properties are dependent on the study population, evaluation of clinimetric properties of measurement should be performed in the population in which the instrument is intended to be used. However, many of the outcome measures that have been used in studies with DM1 patients have only been evaluated in populations other than DM1.

**New therapies require identification of high quality outcome measures**
Although no effective treatment is available in clinical practice, research advances for potential molecular therapeutic targets for DM1 are promising.[48] Preclinical studies mainly focus on selective elimination or neutralization of the toxic expanded CUG repeat transcripts,[49, 50] alteration of RNA binding proteins levels,[51, 52] or alteration of downstream splicing targets.[53] In addition, (non-) pharmacological symptom management may improve health status. However, before we are able to evaluate these therapies in clinical trials, appropriate measurement instruments should be selected. Furthermore, longitudinal studies are needed to study the natural progression of DM1. High quality measures of disease modification are mandatory in order to accurately detect clinically meaningful changes of therapeutic intervention.
In this perspective, researchers have started to developed new outcome

measures. A new disability scale was developed specifically for DM1 patients by Contardi et al.[54] Good results for interobserver agreement (ICC=0.72-0.97), intra-observer reliability, internal consistency (Cronbach's α>0.73) and external validity are shown. The scale is easy and simple to use, requiring approximately 15 minutes, and proved to be sensitive to clinical changes over a maximum period of 12 months. However, results are based just on a small cohort (n=33), and therefore the scale needs to be further evaluated in a larger population. Other impairment and activity measures were developed, but all have limitations for implementing in clinical practice for DM1 population since they are not specifically developed for DM1 patients,[55, 56] or time consuming and difficult to apply.[57]

**Ordinal outcome measures have limitations**

Most health-related outcomes, such as daily functioning or quality of life cannot be measured directly. Therefore, these variables are measured indirectly by how they manifest using rating scales. The majority of health-related outcome measures in DM1 are multi-item rating scales. Besides poor evaluation of clinimetric quality aspects, a major limitation of these scales is that they are all based on the classical test theory (CTT) and all provide ordinal measurement.[58] The value of numbers used in an ordinal scale and the numerical distance between each point in a scale is unknown. Furthermore, a sum of item scores is often calculated, but the relevance of each item to the variable of interest is not necessarily equal.[43] Ordinal scales are currently used in an inappropriate way by addressing obtained scores as interval measures and exposing them to parametric analyses.

**Shifting from classic to modern clinimetric approach**

Considering these shortcomings, it is clear that a modern scientific approach is needed for health evaluation in clinical trials. The most widely used approach is the Rasch unidimensional measurement model.[59, 60] This statistical technique models the probability of an individual's response to an item. Once data fit Rasch model expectations, an interval-scale measure of a latent variable can be estimated from an observed raw score. These interval level variables can be used in parametric statistics. Furthermore, they enable individual patient assessment in addition to comparisons at the group level and enable scales measuring the same health construct to be equated on a common metric.

We started in 2008 with an evaluation and development program of outcome measures in DM1, aiming to present new outcome measures for clinical trials based on modern test theory. Recently, two scales specifically designed for patients with DM1 have been developed using the Rasch Model. These are the Myotonic Dystrophy type 1 Activity and Participation Scale (DM1-Activ)[61] and the

Fatigue and Daytime Sleepiness Scale (FDSS).[62] The DM1-Activ compromises 20 items at the activity and participation level. High internal consistency (person separation index=0.95) and good test-retest reliability values of item difficulty hierarchy and patient location were demonstrated. Patient measures had acceptable correlations with MRC sum scores and MIRS grades (intraclass correlation coefficient=0.69 and 0.71, respectively), indicating good external construct validity. The FDSS is composed of 12 items selected from the Epworth Sleepiness Scale, Daytime Sleepiness Scale and Fatigue Severity Scale. High internal consistency (person separation index=0.80) and validity were demonstrated. Further research is needed to determine the responsiveness of these scales. However, its use is recommended in future clinical trials and follow-up studies since the scales are developed and validated for DM1 and overcomes the limitations of ordinal based measures.

## Conclusion

It is strongly recommended to develop a core set of outcome measures covering the most relevant ICF concepts which can be applied in future therapeutic trials with DM1 patients to improve comparability of the obtained results. Since ordinal-based outcome measures have serious weaknesses, we propose to move from classic to modern clinimetric approach when constructing outcome measures.

**Table 6.1.** Overview of outcome measures in DM1

| | Outcome Measure | Validity | Reliability | Sensitivity to change and responsiveness* |
|---|---|---|---|---|
| **Impairment measures** | | | | |
| | **Muscle strength** | | | |
| 1 | Muscular Impairment Rating Scale (MIRS)[38, 63-84‡] | Construct validity:<br>- correlation with MMT (Spearman's r=–0.81 to –0.88, *P*< 0.001)[47]<br>- correlation with FSI (Spearman's r=0.50, *P*<0.001)[47]<br>- correlation with eight timed functional tasks (Spearman's r=0.34 to 0.67, *P*< 0.001-0.012)[47] | Intraobserver reliability: Cohen's κ=0.74[47]<br>Interobserver reliability: Cohen's κ=0.77-0.79[47] | Lacks sensitivity to detect subtle changes[47] |
| 2 | Medical Research Council (MRC) grade[85-92] | - | Intra-/interobserver reliability: MRC grade 4 is covered by a wide range of muscle strength. Subdivision to 4+ and 4- expands the scale but will decrease intraobserver and interobserver reliability[92] | Lacks sensitivity to detect subtle changes in muscle strength[92] |
| 3 | Modified-modified Medical Research Council Scale (MM-MRCS)[11] | - | - | Unsuitable for detecting the small changes in strength compared to quantitative muscle testing[11] |
| 4 | Medical Research Council Sum Score (MSS)[93-96] | - | Interobserver reliability: ICC> 0.84[96] | - |
| 5 | MRC-Megascore[97, 98] | - | - | - |
| 6 | Manual Muscle Testing (12 muscles) according to Daniels and Worthingham[57] | - | - | - |
| 7 | Manual Muscle Testing (11 muscles)[99, 100] | Construct validity: correlation with MIRS (Spearman's r=-0.81 to -0.88, *P*<0.001)[47] | Intraobserver reliability: ICC=0.93[47]<br>Interobserver reliability: ICC=0.87[47] | - |
| 8 | Modified Manual of Muscle Testing (30 muscles) according to Ahlskong[101] | - | - | - |

| | Outcome Measure | Validity | Reliability | Sensitivity to change and responsiveness* |
|---|---|---|---|---|
| 9 | Manual Muscle Testing (26 muscles) according to Personius[13, 102] | | - | - |
| 10 | Quantitative scale from the Center for Myopathies Investigation and Treatment (CIM)[103] | - | - | - |
| 11 | Five point muscular involvement scale[104] | - | - | - |
| 12 | Walton Score[105] | - | - | - |
| 13 | Functional Impairment Scale according to Harper[106] | - | - | - |
| 14 | Muscular involvement score[107] | - | - | - |
| 15 | Muscle weakness score[108] | - | - | - |
| 16 | Cybex II isokinetic dynamometer[109-111] | - | - | - |
| 17 | Grippit instrument [82, 112] | - | Test-retest reliability:<br>- Grip force left hand (ICC ≥0.61),right hand (ICC ≥0.81)[39]<br>- Pinch grip force left hand (ICC ≥0.41), right hand (ICC≥0.61)[39]<br>Interrater reliability:<br>- Grip force bilateral (ICC≥0.81)[39]<br>- Pinch grip force bilateral (ICC≥0.61)[39] | RC left hand=23.15 N, RC right hand=29.65 N[39]§ |
| 18 | Jamar dynamometer[99, 100, 113] | - | - | - |
| 19 | Hand-grip Dynamometer (Kratos Equipamentos Industriais Ltd)[114] | - | - | - |
| 20 | Handgrip strain gauge (Rank Stanley Cox)[15] | - | - | - |
| 21 | Hand held dynamometer (Takei and Company)[92] | - | - | - |
| 22 | Dynamometer (Penny and Giles transducers Ltd.)[15] | - | - | - |

| | Outcome Measure | Validity | Reliability | Sensitivity to change and responsiveness* |
|---|---|---|---|---|
| 23 | Chatilon push-pull hand-held dynamometer (ankle dorsiflexion & eversion)[11, 12] | Face validity: patients had lower mean peak torque values compared to controls ($P$<0.001)[11] | Test-retest reliability:<br>- Ankle dorsiflexion ($R^2$=0.90-0.96)[11]<br>- Ankle eversion ($R^2$=0.89-0.94)[11]<br>Interrater reliability:<br>- Ankle dorsiflexion (Pearson's r=0.7-0.93)[11]<br>- Ankle eversion (Pearson's r=0.72-0.94)[11] | Able to detect small changes between groups (SEM=1-1.3 Nm)[11]¶ |
| 24 | Multi Muscle Tester M3 Diagnos System[96] | - | - | - |
| 25 | Hand held dynamometer (not specified)[115] | - | - | - |
| 26 | Strain gauge dynamometer (not specified)[111] | - | - | - |
| 27 | Nicholas electronic handheld dynamometer[100] | - | - | - |
| 28 | Fixed dynamometer (Biomech Designs Ltd)[13, 102] | - | - | - |
| 29 | Baseline Pinch Gauge (Fabrication Enterprises Inc.)[99, 100] | - | - | - |
| 30 | Dynamometer model IDDK (bite force)[114] | - | - | - |
| 31 | Test of involuntary isometric muscle force[15] | - | - | - |
| | **Myotonia** | | | |
| 32 | Clinical relaxation time after making fist[116-118] | - | - | - |
| 33 | Percussion myotonia thenar eminence (time in seconds)[117] | - | - | - |
| 34 | Relaxation time with surface electrodes after MVC[119] | - | - | - |
| 35 | Mean score of 3 clinical relaxation times and 3 EMG relaxation times[120] | - | - | - |
| 36 | Hand opening time (10 times)[121] | - | - | - |

| | Outcome Measure | Validity | Reliability | Sensitivity to change and responsiveness* |
|---|---|---|---|---|
| 37 | Computerized handgrip myometry[14, 102, 122] | Construct validity: correlation between handgrip myotonia and grip PF (r=-0.42, *P*=0.03), MMT (r=-0.37, *P*<0.05), and QST (r=-0.42, *P*=0.03)[13] | Test-retest reliability:<br>- Peakforce (r=0.96, *P*<0.0001)<br>- Relaxation time (r=0.76-0.77, *P*<0.001)[13] | Lacks sensitivity to detect mild degrees of myotonia[13] |
| 38 | Relaxation time after ulnar nerve stimulation[15, 16] | Face validity: RT longer in patients than controls (*P*=0.0006-0.07)[16]# | Test-retest reliability: pearson's r=0.972, *P*<0.0005[16] | - |
| | **Fatigue** | | | |
| 39 | Krupp's Fatigue Severity Scale (FSS)[6, 17, 73, 78, 80] | - | Test-retest reliability: ICC=0.88 and Goodman-Kruskal's γ coefficient=0.55-0.88[17]<br>Internal consistency: (Cronbach's α=0.86)[17] | - |
| 40 | Checklist individual strength subscale fatigue (CIS-fatigue)[88, 89, 91, 123-126] | - | - | - |
| 41 | 4 item abbreviated fatigue questionnaire (AFQ)[91] | - | - | - |
| 42 | 13 item fatigue questionnaire[127] | - | - | - |
| 43 | VAS-fatigue[128] | - | - | - |
| 44 | Physiological fatigue and activation failure measurement (central/peripheral)[91] | - | - | - |
| | **Sleepiness** | | | |
| 45 | Daytime Sleepiness Scale (DSS)[18, 78, 128, 129] | Construct validity: normalized factor loadings 0.6-0.8[18] | Test-retest reliability: ICC=0.82 and Goodman-Kruskal's γ coefficient=0.60–0.86[17]<br>Internal consistency: Cronbach's α=0.72[18] | - |
| 46 | Epworth Sleepiness Scale (ESS)[19, 78, 83, 90, 91, 128-136] | - | Test-retest reliability: ICC=0.68 and Goodman-Kruskal's γ coefficient=0.54-0.90[17]†<br>Internal consistency: Cronbach's α=0.24[17] | ESS score showed non-significant reduction after modafinil treatment[19] |

| | Outcome Measure | Validity | Reliability | Sensitivity to change and responsiveness* |
|---|---|---|---|---|
| 47 | Pittsburgh Sleep Quality Index (PSQI)[78, 129] | - | - | - |
| 48 | Stanford Sleepiness Scale (SSS)[131] | - | - | - |
| 49 | Maintenance of Wakefulness Test (MWT)[19, 135] | - | - | MWT significantly prolonged after modafinil treatment[19] |
| 50 | VAS-effect scale[135] | - | - | - |
| 51 | Daily Sleep Diary (DSD)[90, 137] | - | - | - |
| 52 | Self-Observation List[89] | - | - | - |
| 53 | Maudsley Hospital sleep questionnaire[127] | - | - | - |
| 54 | Sleep Questionnaire and Assessment of Wakefulness (SQAW)[128, 136] | - | - | - |
| 55 | Ullanlinna-Narcolepsy Scale[78] | - | - | - |
| | **Pain** | | | |
| 56 | Numeric Rating Scale[138-141] | - | - | - |
| 57 | VAS-pain[89] | - | - | - |
| 58 | McGill Pain Questionnaire (MPQ)[126] | - | - | - |
| 59 | Brief Pain Inventory (BPI)+ *modified version*[138-141] | - | - | - |
| | **Psychological distress** | | | |
| 60 | Symptom Checklist-90 (SCL-90)+ *revised version* (SCL-90-R)[88, 89, 128] | - | - | - |
| 61 | Beck Depression Inventory (BDI)[78, 82, 83, 88, 90, 140, 142, 143] | - | - | - |
| 62 | Beck Depression Inventory for Primary Care (BDI-PC)[89] | - | - | - |
| 63 | Hamilton Depression Rating Scale (HDRS)[5, 135, 137, 144] | - | - | - |
| 64 | Profile of Mood States (POMS)[131, 145] | - | - | - |

| | Outcome Measure | Validity | Reliability | Sensitivity to change and responsiveness* |
|---|---|---|---|---|
| 65 | Montgomery and Asberg Depression Rating Scale (MADRS)[144] | - | - | - |
| 66 | Self-administered depression rating scale[143] | - | - | - |
| 67 | Depressive Mood Scale[144] | - | - | - |
| 68 | Physical Anhedonia Scale (PAS)[144] | - | - | - |
| 69 | Social Anhedonia Scale (SAS)[144] | - | - | - |
| 70 | Hospital Anxiety and Depression Scale (HADS)[90, 115, 142, 146] | - | - | - |
| 71 | Irritability-Depression-Anxiety scale (IDA-scale)[147] | - | - | - |
| 72 | Symptom Rating Test (SRT)[148] | - | - | - |
| 73 | Hamilton Rating Scale for Anxiety[5, 149] | - | - | - |
| 74 | Covi Brief Anxiety Scale[144] | - | - | - |
| 75 | Tyrer Anxiety Scale[144] | - | - | - |
| 76 | State-Trait Anxiety Inventory Form Y (STAI-Y)[5, 149] | - | - | - |
| 77 | Abrams and Taylor scale for emotional blunting (AT)[144] | - | - | - |
| 78 | General Health Questionnaire-12 (GHQ-12)[88, 146] | - | - | - |
| 79 | Psychological Distress Index of Santé-Québec (IDPESQ-14)[150] | - | Internal consistency: Cronbach's $\alpha$=0.94[150] | - |
| 80 | Rosenberg Self-Esteem Scale[150] | - | Internal consistency: Cronbach's $\alpha$=0.86[150] | - |
| 81 | International Personality Disorder Examination[151] | - | - | - |
| 82 | Schedule for Affective Disorders and Schizophrenia (SADS)[148] | - | - | - |
| 83 | Apathy Evaluation Scale (AES)[127, 137] | - | - | - |

| | Outcome Measure | Validity | Reliability | Sensitivity to change and responsiveness* |
|---|---|---|---|---|
| 84 | Temperament and Character Inventory (TCI)[152, 153] | - | - | - |
| 85 | Beck Hopelessness Scale (BHS)[146] | - | - | - |
| **Activity and Participation measures** | | | | |
| | **Activity limitations** | | | |
| 86 | A four point muscular disability rating scale[154, 155] | - | - | - |
| 87 | Disability score according to Beijersbergen[156] | - | - | - |
| 88 | Clinical disability scale[157] | - | - | - |
| 89 | Self-report ADL scale[3, 40, 41, 158] | - | - | - |
| 90 | Activity of Daily Living Staircase[3, 40, 41] | - | - | - |
| 91 | Activities of daily living (ADL) score[101] | - | - | - |
| 92 | 50-item questionnaire of instrumental activities of daily living (IADL)[90] | - | - | - |
| 93 | Katz ADL Index[3, 73] | - | - | - |
| 94 | Frenchay Activities Index (FAI)[73] | - | - | - |
| 95 | Functional Disease Severity Scale by Scott[96] | - | - | - |
| 96 | Neuromuscular Symptom and Disability Functional Score (NSS)[96] | - | - | - |
| 97 | VAS (activities of daily living)[159, 160] | - | - | - |
| 98 | Function in daily life activities[111] | - | - | - |
| 99 | Timed functional testing (TFT)[99, 102] | - | Test-retest reliability: ICC=0.67-0.96[47]** | - |
| | **Activity limitations-mobility** | | | |
| 100 | Endurance and fatigability test[15] | - | - | - |
| 101 | Gait and running test[15] | | | |
| 102 | Brooke's grading scale for mobility[40, 82] | - | - | - |

| | Outcome Measure | Validity | Reliability | Sensitivity to change and responsiveness* |
|---|---|---|---|---|
| 103 | Level of functional deficit according to Zellwenger and Hanson's classification[148] | - | - | - |
| 104 | Rivermead Mobility Index[73, 90, 115] | - | - | - |
| 105 | Walking time over 20 m with one turn[90] | - | - | - |
| 106 | Walking time over 30 m[111] | - | - | - |
| 107 | 6-minute walking test[73, 102, 132] | - | Test-retest reliability: $ICC_{2.1}$=0.99[38] | - Acceptable sensitivity to detect changes between groups (SEM=12 meter)[38]<br>- RC=33 meter [38]§ |
| 108 | Performance Oriented Mobility Assessment (POMA)[115] | - | - | - |
| 109 | Barthel index[161] | - | - | - |
| 110 | Timed-stands test (TST)[73, 132] | - | - | - |
| 111 | Timed up-and-go test (TUG)[132] | - | - | - |
| 112 | Battery of functional tests[15] | - | - | - |
| 113 | Gait and activity indices measured by using the Step WatchTM step activity monitor (SAM) (Cymatech, Seattle, Washington, USA)[115] | - | - | - |
| 114 | Actometer (Actilog V3.0)[89] | - | - | - |
| | **Activity limitations – motor skills** | | | |
| 115 | Purdue Pegboard bimanual[78, 102] | - | Test-retest reliability: left hand (ICC=0.63), right hand (ICC=0.67)[39]<br>Interrater reliability left hand: (ICC=0.47), right hand (ICC=0.73)[39] | RC left hand=1.75, RC right hand=2.14[39]§ |
| 116 | Nine-Hole Peg Test (NHPT)[73] | - | - | - |
| 117 | Motor Function Measurement Scale (MFM scale)[81] | - | - | - |
| 118 | The Appel ALS rating scale[100, 110] | - | - | - |

| | Outcome Measure | Validity | Reliability | Sensitivity to change and responsiveness* |
|---|---|---|---|---|
| | **Participation restrictions** | | | |
| 119 | Assessment of Life Habits (LIFE-H)[6, 162] | - | Test retest reliability: total and subscores (ICC=0.80-0.91)[163]<br>Interrater reliability: total and subscores (ICC=0.86-0.92)[163] | - |
| 120 | Novel structured interview to measure an increase in spontaneous activity[134] | - | - | - |
| 121 | Sonderen Social Support Inventory (SSL)[89] | - | - | - |
| **Quality of Life measures** | | | | |
| | **General** | | | |
| 122 | SF-36 Mental Health scale[5, 6, 19, 100, 123, 125, 126, 128, 132, 135, 139, 141, 164] | - | - | - |
| 123 | Sickness Impact Profile (SIP)[40, 89, 102, 158, 165-167] | - | - | - |
| 124 | Western Ontario and McMaster University Ostheoarthritis Index (WOMAC)[109] | - | - | - |
| 125 | Problem Elicitation Technique (PET)[109] | - | - | - |
| 126 | The Quality of Life Profile[41] | - | - | - |
| 127 | RAND-36 questionnaire[131, 134, 140] | - | - | - |
| 128 | Modified activities-specific balance confidence scale (ABC-UK)[115] | - | - | - |
| | **Psychosocial wellbeing** | | | |
| 129 | Measure of the quality of the Environment (MQE)[6] | - | - | - |
| 130 | Multidimensional Scale of Perceived Social Support (MSPSS)[139] | - | - | - |
| 131 | (Catastrophizing subscale of the) Coping Strategies Questionnaire (CSQ) [139, 141] | - | - | - |

| | Outcome Measure | Validity | Reliability | Sensitivity to change and responsiveness* |
|---|---|---|---|---|
| 132 | Mental Adjustment to Cancer (MAC) scale[3, 167] | - | - | - |
| 133 | Psychosocial Well-Being Questionnaire[3] | - | - | - |
| 134 | Chronic Pain Coping Inventory (CPCI)[139, 141] | - | - | - |
| 135 | Survey of Pain Attitudes (SOPA)[139, 141] | - | - | - |
| 136 | Pain Catastrophizing Scale[141] | - | - | - |
| 137 | Kaasa test[40, 167] | - | - | - |
| **Composite level measures** | | | | |
| 138 | A 3-point-scale for exploration of patients' global assessment of treatment[96] | - | - | - |
| 139 | ICF-Checklist (modified version)[74] | - | - | - |
| 140 | Treatment effect questionnaire[15] | - | - | - |
| 141 | Functional status questionnaire[111] | - | - | - |

* Sensitive to change indicates the ability to measure any change in health status, while responsiveness indicates the ability to detect meaningful change

† Goodman-Kruskal's γ was significant for ESS items 1, 2, 4, 5, and 7, but not for ESS item 8 (in a car, while stopped for a few minutes in traffic). In addition, Goodman-Kruskal's γ coefficient could not be computed for ESS items 3 and 6

‡ Muscular Impairment Rating Scale (MIRS) was called Muscular Disability Rating Scale (MDRS) in literature published before 2001

§ Repeatability coefficient is the difference required to detect a true difference between two measurements for the same subject

¶ The smallest mean group difference observed between DM1 patients was 2.3 Nm, a difference about twice than the Standard Error of Measurement (SEM)

# Different phases of relaxation were distinguished at 90, 50, 25, 10 and 5% of the peak force (PF). RT was determined after twitch and tetanic contraction of the first dorsal interosseous muscle. Separate P-values for each phase of relaxation were determined

** ICCs were calculated for each item separately: 0.96 for turning blocks and for climbing and descending four standard steps, 0.91 for assembling peg units, 0.81 for propelling a wheelchair 20 feet, 0.79 for walking 20 feet, 0.77 for cutting Theraplast, 0.71 for standing from lying supine, and 0.67 for standing from chair

**Legend to table 6.1.** Abbreviations: - = not available, VAS= Visual Analogue Scale; MWT= Maintenance of Wakefulness Test; MMT= Manual Muscle Testing; FSI= Functional Status Index; RT= Relaxation Time; N=Newton; SEM= Standard Error of Measurement; RC= repeatability coefficient; MVC: Maximal Voluntary Contraction; ICF= International Classification of Functioning, Disability and Health ICC= Intra class correlation coefficient

# References


1. Harper PS. Myotonic dystrophy. London: Saunders; 2001
2. Harley HG, Brook JD, Rundle SA, Crow S, Reardon W, Buckler AJ, Harper PS, Housman DE, Shaw DJ. Expansion of an unstable DNA region and phenotypic variation in myotonic dystrophy. *Nature*. 1992;355:545-546
3. Natterlund B, Gunnarsson LG, Ahlstrom G. Disability, coping and quality of life in individuals with muscular dystrophy: A prospective study over five years. *Disabil Rehabil*. 2000;22:776-785
4. Bostrom K, Ahlstrom G. Quality of life in patients with muscular dystrophy and their next of kin. *Int J Rehabil Res*. 2005;28:103-109
5. Antonini G, Soscia F, Giubilei F, De Carolis A, Gragnani F, Morino S, Ruberto A, Tatarelli R. Health-related quality of life in myotonic dystrophy type 1 and its relationship with cognitive and emotional functioning. *J Rehabil Med*. 2006;38:181-185
6. Gagnon C, Mathieu J, Jean S, Laberge L, Perron M, Veillette S, Richer L, Noreau L. Predictors of disrupted social participation in myotonic dystrophy type 1. *Arch Phys Med Rehabil*. 2008;89:1246-1255
7. Hobart JC, Cano SJ, Zajicek JP, Thompson AJ. Rating scales as outcome measures for clinical trials in neurology: Problems, solutions, and recommendations. *Lancet Neurol*. 2007;6:1094-1105
8. World Health Organization. International classification of functioning, disability and health. Geneva; 2001
9. Aaronson NK, Muller M, Cohen PD, Essink-Bot ML, Fekkes M, Sanderman R, Sprangers MA, te Velde A, Verrips E. Translation, validation, and norming of the dutch language version of the sf-36 health survey in community and chronic disease populations. *J Clin Epidemiol*. 1998;51:1055-1068
10. Streiner DL, Norman GR. Health measurement scales. A practical guide to their development and use. New York: Oxford University Press; 1995
11. Hebert LJ, Remec JF, Saulnier J, Vial C, Puymirat J. The use of muscle strength assessed with handheld dynamometers as a non-invasive biological marker in myotonic dystrophy type 1 patients: A multicenter study. *BMC Musculoskelet Disord*. 2010;11:72
12. Hiba B, Richard N, Hebert LJ, Cote C, Nejjari M, Vial C, Bouhour F, Puymirat J, Janier M. Quantitative assessment of skeletal muscle degeneration in patients with myotonic dystrophy type 1 using mri. *J Magn Reson Imaging*. 2012;35:678-685
13. Moxley RT, 3rd, Logigian EL, Martens WB, Annis CL, Pandya S, Moxley RTt, Barbieri CA, Dilek N, Wiegner AW, Thornton CA. Computerized hand grip myometry reliably measures myotonia and muscle strength in myotonic dystrophy (dm1). *Muscle Nerve*. 2007;36:320-328
14. Logigian EL, Martens WB, Moxley RTt, McDermott MP, Dilek N, Wiegner AW, Pearson AT, Barbieri CA, Annis CL, Thornton CA, Moxley RT, 3rd. Mexiletine is an effective antimyotonia treatment in myotonic dystrophy type 1. *Neurology*. 2010;74:1441-1448
15. Backman E, Henriksson KG. Effect of sodium selenite and vitamin e treatment in myotonic dystrophy. *J Intern Med*. 1990;228:577-581
16. Logigian EL, Moxley RTt, Blood CL, Barbieri CA, Martens WB, Wiegner AW, Thornton CA, Moxley RT, 3rd. Leukocyte ctg repeat length correlates with severity of myotonia in myotonic dystrophy type 1. *Neurology*. 2004;62:1081-1089
17. Laberge L, Gagnon C, Jean S, Mathieu J. Fatigue and daytime sleepiness rating scales in myotonic dystrophy: A study of reliability. *J Neurol Neurosurg Psychiatry*. 2005;76:1403-1405
18. Laberge L, Begin P, Montplaisir J, Mathieu J. Sleep complaints in patients with myotonic dystrophy. *J Sleep Res*. 2004;13:95-100
19. Talbot K, Stradling J, Crosby J, Hilton-Jones D. Reduction in excess daytime sleepiness by modafinil in patients with myotonic dystrophy. *Neuromuscul Disord*. 2003;13:357-364
20. Jensen MP, Turner JA, Romano JM, Fisher LD. Comparative reliability and validity of chronic pain intensity measures. *Pain*. 1999;83:157-162
21. Daut RL, Cleeland CS, Flanery RC. Development of the wisconsin brief pain questionnaire to assess pain in cancer and other diseases. *Pain*. 1983;17:197-210
22. Vanderiet K, Adriaensen H, Carton H, Vertommen H. The mcgill pain questionnaire constructed for the dutch language (mpq-dv). Preliminary data concerning reliability and validity. *Pain*. 1987;30:395-408
23. van der Kloot WA, Oostendorp RA, van der Meij J, van den Heuvel J. [the dutch version of the mcgill pain questionnaire: A reliable pain questionnaire]. *Ned Tijdschr Geneesk*. 1995;139:669-673

24. Tyler EJ, Jensen MP, Engel JM, Schwartz L. The reliability and validity of pain interference measures in persons with cerebral palsy. *Arch Phys Med Rehabil*. 2002;83:236-239
25. Coplan PM, Schmader K, Nikas A, Chan IS, Choo P, Levin MJ, Johnson G, Bauer M, Williams HM, Kaplan KM, Guess HA, Oxman MN. Development of a measure of the burden of pain due to herpes zoster and postherpetic neuralgia for prevention trials: Adaptation of the brief pain inventory. *J Pain*. 2004;5:344-356
26. Hanley MA, Jensen MP, Ehde DM, Hoffman AJ, Patterson DR, Robinson LR. Psychosocial predictors of long-term adjustment to lower-limb amputation and phantom limb pain. *Disabil Rehabil*. 2004;26:882-893
27. Zelman DC, Gore M, Dukes E, Tai KS, Brandenburg N. Validation of a modified version of the brief pain inventory for painful diabetic peripheral neuropathy. *J Pain Symptom Manage*. 2005;29:401-410
28. Raichle KA, Osborne TL, Jensen MP, Cardenas D. The reliability and validity of pain interference measures in persons with spinal cord injury. *J Pain*. 2006;7:179-186
29. Tyrer P, Owen RT, Cicchetti DV. The brief scale for anxiety: A subdivision of the comprehensive psychopathological rating scale. *J Neurol Neurosurg Psychiatry*. 1984;47:970-975
30. Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. *Br J Psychiatry*. 1979;134:382-389
31. Maier W, Buller R, Philipp M, Heuser I. The hamilton anxiety scale: Reliability, validity and sensitivity to change in anxiety and depressive disorders. *J Affect Disord*. 1988;14:61-68
32. Goldberg DP, Gater R, Sartorius N, Ustun TB, Piccinelli M, Gureje O, Rutter C. The validity of two versions of the ghq in the who study of mental illness in general health care. *Psychol Med*. 1997;27:191-197
33. Pevalin DJ. Multiple applications of the ghq-12 in a general population sample: An investigation of long-term retest effects. *Soc Psychiatry Psychiatr Epidemiol*. 2000;35:508-512
34. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the hospital anxiety and depression scale. An updated literature review. *J Psychosom Res*. 2002;52:69-77
35. Pais-Ribeiro J, Silva I, Ferreira T, Martins A, Meneses R, Baltar M. Validation study of a portuguese version of the hospital anxiety and depression scale. *Psychol Health Med*. 2007;12:225-235; quiz 235-227
36. Radat F, Lafittau M, Ouallet JC, Brochet B, Jouvent R. [validation of ehd self questionnaire in multiple sclerosis]. *Encephale*. 2007;33:49-57
37. Bernstein IH, Rush AJ, Stegman D, Macleod L, Witte B, Trivedi MH. A comparison of the qids-c16, qids-sr16, and the madrs in an adult outpatient clinical sample. *CNS Spectr*. 2010;15:458-468
38. Kierkegaard M, Tollback A. Reliability and feasibility of the six minute walk test in subjects with myotonic dystrophy. *Neuromuscul Disord*. 2007;17:943-949
39. Aldehag AS JH, Littorin S, Ansved T. Reliability of hand function testing instruments in patients with muscular dystrophies. *Int J Ther Rehabil*. 2008;15:211-219
40. Ahlstrom G, Gunnarsson LG. Disability and quality of life in individuals with muscular dystrophy. *Scand J Rehabil Med*. 1996;28:147-157
41. Natterlund B, Ahlstrom G. Activities of daily living and quality of life in persons with muscular dystrophy. *J Rehabil Med*. 2001;33:206-211
42. Bergner M, Bobbitt RA, Carter WB, Gilson BS. The sickness impact profile: Development and final revision of a health status measure. *Med Care*. 1981;19:787-805
43. Stucki G, Liang MH, Fossel AH, Katz JN. Relative responsiveness of condition-specific and generic health status measures in degenerative lumbar spinal stenosis. *J Clin Epidemiol*. 1995;48:1369-1378
44. Jensen MP, Turner JA, Romano JM. Changes in beliefs, catastrophizing, and coping are associated with improvement in multidisciplinary pain treatment. *J Consult Clin Psychol*. 2001;69:655-662
45. Liang MH. Evaluating measurement responsiveness. *J Rheumatol*. 1995;22:1191-1192
46. Hobart J. Rating scales for neurologists. *J Neurol Neurosurg Psychiatry*. 2003;74 Suppl 4:iv22-iv26
47. Mathieu J, Boivin H, Meunier D, Gaudreault M, Begin P. Assessment of a disease-specific muscular impairment rating scale in myotonic dystrophy. *Neurology*. 2001;56:336-340
48. Wheeler TM, Leger AJ, Pandey SK, MacLeod AR, Nakamori M, Cheng SH, Wentworth BM, Bennett CF, Thornton CA. Targeting nuclear rna for in vivo correction of myotonic dystrophy. *Nature*. 2012;488:111-115
49. Wheeler TM, Sobczak K, Lueck JD, Osborne RJ, Lin X, Dirksen RT, Thornton CA. Reversal of rna dominance by displacement of protein sequestered on triplet repeat rna. *Science*. 2009;325:336-339
50. Mulders SA, van den Broek WJ, Wheeler TM, Croes HJ, van Kuik-Romeijn P, de Kimpe SJ, Furling D, Platenburg GJ, Gourdon G, Thornton CA, Wieringa B, Wansink DG. Triplet-repeat oligonucleotide-mediated reversal of rna toxicity in myotonic dystrophy. *Proc Natl Acad Sci U S A*. 2009;106:13915-13920

51. Kanadia RN, Shin J, Yuan Y, Beattie SG, Wheeler TM, Thornton CA, Swanson MS. Reversal of rna missplicing and myotonia after muscleblind overexpression in a mouse poly(cug) model for myotonic dystrophy. *Proc Natl Acad Sci U S A*. 2006;103:11748-11753
52. Wang GS, Kuyumcu-Martinez MN, Sarma S, Mathur N, Wehrens XH, Cooper TA. Pkc inhibition ameliorates the cardiac phenotype in a mouse model of myotonic dystrophy type 1. *J Clin Invest*. 2009;119:3797-3806
53. Wheeler TM, Lueck JD, Swanson MS, Dirksen RT, Thornton CA. Correction of clc-1 splicing eliminates chloride channelopathy and myotonia in mouse models of myotonic dystrophy. *J Clin Invest*. 2007;117:3952-3957
54. Contardi S, Pizza F, Falzone F, D'Alessandro R, Avoni P, Di Stasi V, Montagna P, Liguori R. Development of a disability scale for myotonic dystrophy type 1. *Acta Neurol Scand*. 2012;125:431-438
55. Berard C, Payan C, Hodgkinson I, Fermanian J. A motor function measure for neuromuscular diseases. Construction and validation study. *Neuromuscul Disord*. 2005;15:463-470
56. Vandervelde L, van den Bergh PYK, Goemans N, Thonnard JL. Activlim: A rasch-built measure of activity limitations in children and adults with neuromuscular disorders. *Neuromuscul Disord*. 2007;17:459-469
57. Nitz JC, Burns YR, Jackson RV. A longitudinal physical profile assessment of skeletal muscle manifestations in myotonic dystrophy. *Clin Rehabil*. 1999;13:64-73
58. DeVellis RF. Classical test theory. *Med Care*. 2006;44:S50-59
59. Rasch G. Probalilistic models for some intelligence and attainment tests. Chicago: University of Chicago Press; 1980
60. Bond TG, C.M. Fox,. Applying the rasch model: Fundamental measurement for the human sciences. New York: Lawrence Erlbaum Associates; 2001
61. Hermans MC, Faber CG, De Baets MH, de Die-Smulders CE, Merkies IS. Rasch-built myotonic dystrophy type 1 activity and participation scale (dm1-activ). *Neuromuscul Disord*. 2010;20:310-318
62. Hermans MC, Merkies IS, Laberge L, Blom EW, Tennant A, Faber CG. Fatigue and daytime sleepiness scale in myotonic dystrophy type 1. *Muscle Nerve*. 2013;47:89-95
63. Begin P, Mathieu J, Almirall J, Grassino A. Relationship between chronic hypercapnia and inspiratory-muscle weakness in myotonic dystrophy. *Am J Respir Crit Care Med*. 1997;156:133-139
64. Bhakta D, Shen C, Kron J, Epstein AE, Pascuzzi RM, Groh WJ. Pacemaker and implantable cardioverter-defibrillator use in a us myotonic dystrophy type 1 population. *J Cardiovasc Electrophysiol*. 2011
65. Cudia P, Bernasconi P, Chiodelli R, Mangiola F, Bellocci F, Dello Russo A, Angelini C, Romeo V, Melacini P, Politano L, Palladino A, Nigro G, Siciliano G, Falorni M, Bongiorni MG, Falcone C, Mantegazza R, Morandi L. Risk of arrhythmia in type i myotonic dystrophy: The role of clinical and genetic variables. *J Neurol Neurosurg Psychiatry*. 2009;80:790-793
66. Dello Russo A, Pelargonio G, Parisi Q, Santamaria M, Messano L, Sanna T, Casella M, De Martino G, De Ponti R, Pace M, Giglio V, Ierardi C, Zecchi P, Crea F, Bellocci F. Widespread electroanatomic alterations of right cardiac chambers in patients with myotonic dystrophy type 1. *J Cardiovasc Electrophysiol*. 2006;17:34-40
67. Di Cori A, Bongiorni MG, Zucchelli G, Soldati E, Falorni M, Segreti L, Gemignani C, Siciliano A, Bovenzi FM, Di Bello V. Early left ventricular structural myocardial alterations and their relationship with functional and electrical properties of the heart in myotonic dystrophy type 1. *J Am Soc Echocardiogr*. 2009;22:1173-1179
68. Di Costanzo A, Di Salle F, Santoro L, Bonavita V, Tedeschi G. Dilated virchow-robin spaces in myotonic dystrophy: Frequency, extent and significance. *Eur Neurol*. 2001;46:131-139
69. Di Costanzo A, Santoro L, de Cristofaro M, Manganelli F, Di Salle F, Tedeschi G. Familial aggregation of white matter lesions in myotonic dystrophy type 1. *Neuromuscul Disord*. 2008;18:299-305
70. Groh WJ, Lowe MR, Simmons Z, Bhakta D, Pascuzzi RM. Familial clustering of muscular and cardiac involvement in myotonic dystrophy type 1. *Muscle Nerve*. 2005;31:719-724
71. Groh WJ, Lowe MR, Zipes DP. Severity of cardiac conduction involvement and arrhythmias in myotonic dystrophy type 1 correlates with age and ctg repeat length. *J Cardiovasc Electrophysiol*. 2002;13:444-448
72. Jaspert A, Fahsold R, Grehl H, Claus D. Myotonic dystrophy: Correlation of clinical symptoms with the size of the ctg trinucleotide repeat. *J Neurol*. 1995;242:99-104
73. Kierkegaard M, Harms-Ringdahl K, Holmqvist LW, Tollback A. Functioning and disability in adults with myotonic dystrophy type 1. *Disabil Rehabil*. 2011;33:1826-1836

74. Kierkegaard M, Harms-Ringdahl K, Widen Holmqvist L, Tollback A. Perceived functioning and disability in adults with myotonic dystrophy type 1: A survey according to the international classification of functioning, disability and health. *J Rehabil Med*. 2009;41:512-520
75. Kuo HC, Hsieh YC, Wang HM, Chuang WL, Huang CC. Correlation among subcortical white matter lesions, intelligence and ctg repeat expansion in classic myotonic dystrophy type 1. *Acta Neurol Scand*. 2008;117:101-107
76. Mathieu J, Allard P, Gobeil G, Girard M, De Braekeleer M, Begin P. Anesthetic and surgical complications in 219 cases of myotonic dystrophy. *Neurology*. 1997;49:1646-1650
77. Mathieu J, De Braekeleer M, Prevost C, Boily C. Myotonic dystrophy: Clinical assessment of muscular disability in an isolated population with presumed homogeneous mutation. *Neurology*. 1992;42:203-208
78. Minnerop M, Weber B, Schoene-Bake JC, Roeske S, Mirbach S, Anspach C, Schneider-Gold C, Betz RC, Helmstaedter C, Tittgemeyer M, Klockgether T, Kornblum C. The brain in myotonic dystrophy 1 and 2: Evidence for a predominant white matter disease. *Brain*. 2011;134:3530-3546
79. Pfeilsticker BH, Bertuzzo CS, Nucci A. Electrophysiological evaluation in myotonic dystrophy: Correlation with ctg length expansion. *Arq Neuropsiquiatr*. 2001;59:186-191
80. Pincherle A, Patruno V, Raimondi P, Moretti S, Dominese A, Martinelli-Boneschi F, Pasanisi MB, Canioni E, Salerno F, Deleo F, Spreafico R, Mantegazza R, Villani F, Morandi L. Sleep breathing disorders in 40 italian patients with myotonic dystrophy type 1. *Neuromuscul Disord*. 2012;22:219-224
81. Pruna L, Chatelin J, Pascal-Vigneron V, Kaminsky P. Regional body composition and functional impairment in patients with myotonic dystrophy. *Muscle Nerve*. 2011;44:503-508
82. Winblad S, Jensen C, Mansson JE, Samuelsson L, Lindberg C. Depression in myotonic dystrophy type 1: Clinical and neuronal correlates. *Behav Brain Funct*. 2010;6:25
83. Zalonis I, Bonakis A, Christidi F, Vagiakis E, Papageorgiou SG, Kalfakis N, Manta P, Vassilopoulos D. Toward understanding cognitive impairment in patients with myotonic dystrophy type 1. *Arch Clin Neuropsychol*. 2010;25:303-313
84. Magri D, Piccirillo G, Bucci E, Pignatelli G, Cauti FM, Morino S, Latino P, Santini D, Marrara F, Volpe M, Antonini G, Testa M. Increased temporal dispersion of myocardial repolarization in myotonic dystrophy type 1: Beyond the cardiac conduction system. *Int J Cardiol*. 2012;156:259-264
85. Avaria M, Patterson V. Myotonic dystrophy: Relative sensitivity of symptoms signs and abnormal investigations. *Ulster Med J*. 1994;63:151-154
86. Bachmann G, Damian MS, Koch M, Schilling G, Fach B, Stoppler S. The clinical and genetic correlates of mri findings in myotonic dystrophy. *Neuroradiology*. 1996;38:629-635
87. Huber SJ, Kissel JT, Shuttleworth EC, Chakeres DW, Clapp LE, Brogan MA. Magnetic resonance imaging and clinical correlates of intellectual impairment in myotonic dystrophy. *Arch Neurol*. 1989;46:536-540
88. Kalkman JS, Schillings ML, Zwarts MJ, van Engelen BG, Bleijenberg G. Psychiatric disorders appear equally in patients with myotonic dystrophy, facioscapulohumeral dystrophy, and hereditary motor and sensory neuropathy type i. *Acta Neurol Scand*. 2007;115:265-270
89. Kalkman JS, Schillings ML, Zwarts MJ, van Engelen BG, Bleijenberg G. The development of a model of fatigue in neuromuscular disorders: A longitudinal study. *J Psychosom Res*. 2007;62:571-579
90. Phillips MF, Steer HM, Soldan JR, Wiles CM, Harper PS. Daytime somnolence in myotonic dystrophy. *J Neurol*. 1999;246:275-282
91. Schillings ML, Kalkman JS, Janssen HM, van Engelen BG, Bleijenberg G, Zwarts MJ. Experienced and physiological fatigue in neuromuscular disorders. *Clin Neurophysiol*. 2007;118:292-300
92. Whittaker RG, Ferenczi E, Hilton-Jones D. Myotonic dystrophy: Practical issues relating to assessment of strength. *J Neurol Neurosurg Psychiatry*. 2006;77:1282-1283
93. Bae JS, Kim OK, Kim SJ, Kim BJ. Abnormalities of nerve conduction studies in myotonic dystrophy type 1: Primary involvement of nerves or incidental coexistence? *J Clin Neurosci*. 2008;15:1120-1124
94. Hermans MC, Faber CG, Vanhoutte EK, Bakkers M, De Baets MH, de Die-Smulders CE, Merkies IS. Peripheral neuropathy in myotonic dystrophy type 1. *J Peripher Nerv Syst*. 2011;16:24-29
95. Verhagen WI, ter Bruggen JP, Huygen PL. Oculomotor, auditory, and vestibular responses in myotonic dystrophy. *Arch Neurol*. 1992;49:954-960
96. Walter MC, Reilich P, Lochmuller H, Kohnen R, Schlotter B, Hautmann H, Dunkl E, Pongratz D, Muller-Felber W. Creatine monohydrate in myotonic dystrophy: A double-blind, placebo-controlled clinical study. *J Neurol*. 2002;249:1717-1722

97. Meola G, Sansone V, Perani D, Scarone S, Cappa S, Dragoni C, Cattaneo E, Cotelli M, Gobbo C, Fazio F, Siciliano G, Mancuso M, Vitelli E, Zhang S, Krahe R, Moxley RT. Executive dysfunction and avoidant personality trait in myotonic dystrophy type 1 (dm-1) and in proximal myotonic myopathy (promm/dm-2). *Neuromuscul Disord*. 2003;13:813-821
98. Sansone V, Gandossini S, Cotelli M, Calabria M, Zanetti O, Meola G. Cognitive impairment in adult myotonic dystrophies: A longitudinal study. *Neurol Sci*. 2007;28:9-15
99. Mathieu J, Boivin H, Richards CL. Quantitative motor assessment in myotonic dystrophy. *Can J Neurol Sci*. 2003;30:129-136
100. Penisson-Besnier I, Devillers M, Porcher R, Orlikowski D, Doppler V, Desnuelle C, Ferrer X, Bes MC, Bouhour F, Tranchant C, Lagrange E, Vershueren A, Uzenot D, Cintas P, Sole G, Hogrel JY, Laforet P, Vial C, Vila AL, Sacconi S, Pouget J, Eymard B, Chevret S, Annane D. Dehydroepiandrosterone for myotonic dystrophy type 1. *Neurology*. 2008;71:407-412
101. Sugino M, Ohsawa N, Ito T, Ishida S, Yamasaki H, Kimura F, Shinoda 102. Heatwole CR, Eichinger KJ, Friedman DI, Hilbert JE, Jackson CE, Logigian EL, Martens WB, McDermott MP, Pandya SK, Quinn C, Smirnow AM, Thornton CA, Moxley RT, 3rd. Open-label trial of recombinant human insulin-like growth factor 1/recombinant human insulin-like growth factor binding protein 3 in myotonic dystrophy type 1. *Arch Neurol*. 2011;68:37-44
103. Wigg CM, Duro LA. The kohs' blocks test as an important instrument to investigate the visuo-spatial impairments in myotonic dystrophy. Part i. Quantitative and qualitative analysis. *Arq Neuropsiquiatr*. 1999;57:547-555
104. Clarke NR, Kelion AD, Nixon J, Hilton-Jones D, Forfar JC. Does cytosine-thymine-guanine (ctg) expansion size predict cardiac events and electrocardiographic progression in myotonic dystrophy? *Heart*. 2001;86:411-416
105. Lazarus A, Varin J, Ounnoughene Z, Radvanyi H, Junien C, Coste J, Laforet P, Eymard B, Becane HM, Weber S, Duboc D. Relationships among electrophysiological findings and clinical status, heart function, and extent of DNA mutation in myotonic dystrophy. *Circulation*. 1999;99:1041-1046
106. Logullo F, Censori B, Danni M, Del Pesce M, Di Bella P, Provinciali L. Peripheral neuropathy in myotonic dystrophy: Electrophysiological and clinical features. *Electromyogr Clin Neurophysiol*. 1992;32:515-520
107. Barreca T, Muratorio A, Sannia A, Murri L, Rossi B, Rolandi E. Evaluation of twenty-four-hour secretory patterns of growth hormone and insulin in patients with myotonic dystrophy. *J Clin Endocrinol Metab*. 1980;51:1089-1092
108. Bundey S. Clinical evidence for heterogeneity in myotonic dystrophy. *J Med Genet*. 1982;19:341-348
109. Lindeman E, Leffers P, Spaans F, Drukker J, Reulen J, Kerckhoffs M, Koke A. Strength training in patients with myotonic dystrophy and hereditary motor and sensory neuropathy: A randomized clinical trial. *Arch Phys Med Rehabil*. 1995;76:612-620
110. Lindeman E, Leffers P, Spaans F, Drukker J, Reulen J. Deterioration of motor function in myotonic dystrophy and hereditary motor and sensory neuropathy. *Scand J Rehabil Med*. 1995;27:59-64
111. Orndahl G, Grimby G, Grimby A, Johansson G, Wilhelmsen L. Functional deterioration and selenium-vitamin e treatment in myotonic dystrophy. A placebo-controlled study. *J Intern Med*. 1994;235:205-210
112. Winblad S, Lindberg C, Hansen S. Cognitive deficits and ctg repeat expansion size in classical myotonic dystrophy type 1 (dm1). *Behav Brain Funct*. 2006;2:16
113. Motlagh B, MacDonald JR, Tarnopolsky MA. Nutritional inadequacy in adults with muscular dystrophy. *Muscle Nerve*. 2005;31:713-718
114. Guimaraes AS, Carlsson GE, Marie SK. Bite force and handgrip force in patients with molecular diagnosis of myotonic dystrophy. *J Oral Rehabil*. 2007;34:195-200
115. Wiles CM, Busse ME, Sampson CM, Rogers MT, Fenton-May J, van Deursen R. Falls and stumbles in myotonic dystrophy. *J Neurol Neurosurg Psychiatry*. 2006;77:393-396
116. Grant R, Sutton DL, Behan PO, Ballantyne JP. Nifedipine in the treatment of myotonia in myotonic dystrophy. *J Neurol Neurosurg Psychiatry*. 1987;50:199-206
117. Gascon GG, Staton RD, Patterson BD, Konewko PJ, Wilson H, Carlson KM, Brumback RA. A pilot controlled study of the use of imipramine to reduce myotonia. *Am J Phys Med Rehabil*. 1989;68:215-220
118. Antonini G, Vichi R, Leardi MG, Pennisi E, Monza GC, Millefiorini M. Effect of clomipramine on myotonia: A placebo-controlled, double-blind, crossover trial. *Neurology*. 1990;40:1473-1474
119. Lewis I. Trial of diazepam in myotonia - a controlled clinical trial. *Neurology*. 1966;16:831-836
120. Leyburn P, Walton JN. The treatment of myotonia: A controlled clinical trial. *Brain*. 1959;82:81-91

121. Mielke U, Haass A, Sen S, Schmidt W. Antimyotonic therapy with tocainide under ecg control in the myotonic dystrophy of curschmann-steinert. *J Neurol*. 1985;232:271-274
122. Logigian EL, Blood CL, Dilek N, Martens WB, Moxley RTt, Wiegner AW, Thornton CA, Moxley RT, 3rd. Quantitative analysis of the "warm-up" phenomenon in myotonic dystrophy type 1. *Muscle Nerve*. 2005;32:35-42
123. Kalkman JS, Schillings ML, van der Werf SP, Padberg GW, Zwarts MJ, van Engelen BG, Bleijenberg G. Experienced fatigue in facioscapulohumeral dystrophy, myotonic dystrophy, and hmsn-i. *J Neurol Neurosurg Psychiatry*. 2005;76:1406-1409
124. Kalkman JS, Schillings ML, Zwarts MJ, van Engelen BG, Bleijenberg G. Influence of relatives on fatigue experienced by patients with facioscapulohumeral dystrophy, myotonic dystrophy and hmsn-i. *Eur Neurol*. 2006;56:24-30
125. Minis MA, Kalkman JS, Akkermans RP, Engels JA, Huijbregts PA, Bleijenberg G, Oostendorp RA, van Engelen BG. Employment status of patients with neuromuscular diseases in relation to personal factors, fatigue and health status: A secondary analysis. *J Rehabil Med*. 2010;42:60-65
126. Tieleman AA, Jenks KM, Kalkman JS, Borm G, van Engelen BG. High disease impact of myotonic dystrophy type 2 on physical and mental functioning. *J Neurol*. 2011;258:1820-1826
127. Rubinsztein JS, Rubinsztein DC, Goodburn S, Holland AJ. Apathy and hypersomnia are common features of myotonic dystrophy. *J Neurol Neurosurg Psychiatry*. 1998;64:510-515
128. Laberge L, Dauvilliers Y, Begin P, Richer L, Jean S, Mathieu J. Fatigue and daytime sleepiness in patients with myotonic dystrophy type 1: To lump or split? *Neuromuscul Disord*. 2009;19:397-402
129. Romigi A, Izzi F, Pisani V, Placidi F, Pisani LR, Marciani MG, Corte F, Panico MB, Torelli F, Uasone E, Vitrani G, Albanese M, Massa R. Sleep disorders in adult-onset myotonic dystrophy type 1: A controlled polysomnographic study. *Eur J Neurol*. 2011;18:1139-1145
130. Damian MS, Gerlach A, Schmidt F, Lehmann E, Reichmann H. Modafinil for excessive daytime sleepiness in myotonic dystrophy. *Neurology*. 2001;56:794-796
131. MacDonald JR, Hill JD, Tarnopolsky MA. Modafinil reduces excessive somnolence and enhances mood in patients with myotonic dystrophy. *Neurology*. 2002;59:1876-1880
132. Miyamoto T, Miyamoto M, Suga T, Aizawa C, Takekawa H, Hirata K. Methylphenidate hydrochloride for excessive daytime sleepiness in a patient with myotonic dystrophy. *Psychiatry Clin Neurosci*. 2002;56:271-272
133. Kumar SP, Sword D, Petty RK, Banham SW, Patel KR. Assessment of sleep studies in myotonic dystrophy. *Chron Respir Dis*. 2007;4:15-18
134. Wintzen AR, Lammers GJ, van Dijk JG. Does modafinil enhance activity of patients with myotonic dystrophy?: A double-blind placebo-controlled crossover study. *J Neurol*. 2007;254:26-28
135. Orlikowski D, Chevret S, Quera-Salva MA, Laforet P, Lofaso F, Verschueren A, Pouget J, Eymard B, Annane D. Modafinil for the treatment of hypersomnia associated with myotonic muscular dystrophy in adults: A multicenter, prospective, randomized, double-blind, placebo-controlled, 4-week trial. *Clin Ther*. 2009;31:1765-1773
136. Laberge L, Begin P, Dauvilliers Y, Beaudry M, Laforte M, Jean S, Mathieu J. A polysomnographic study of daytime sleepiness in myotonic dystrophy type 1. *J Neurol Neurosurg Psychiatry*. 2009;80:642-646
137. Di Costanzo A, Mottola A, Toriello A, Di Iorio G, Tedeschi G, Bonavita V. Does abnormal neuronal excitability exist in myotonic dystrophy? Ii. Effects of the antiarrhythmic drug hydroquinidine on apathy and hypersomnia. *Neurol Sci*. 2000;21:81-86
138. Jensen MP, Hoffman AJ, Stoelb BL, Abresch RT, Carter GT, McDonald CM. Chronic pain in persons with myotonic dystrophy and facioscapulohumeral dystrophy. *Arch Phys Med Rehabil*. 2008;89:320-328
139. Miro J, Raichle KA, Carter GT, O'Brien SA, Abresch RT, McDonald CM, Jensen MP. Impact of biopsychosocial factors on chronic pain in persons with myotonic and facioscapulohumeral muscular dystrophy. *Am J Hosp Palliat Care*. 2009;26:308-319
140. Suokas KI, Haanpaa M, Kautiainen H, Udd B, Hietaharju AJ. Pain in patients with myotonic dystrophy type 2: A postal survey in finland. *Muscle Nerve*. 2012;45:70-74
141. Nieto R, Raichle KA, Jensen MP, Miro J. Changes in pain-related beliefs, coping, and catastrophizing predict changes in pain intensity, pain interference, and psychological functioning in individuals with myotonic muscular dystrophy and facioscapulohumeral dystrophy. *Clin J Pain*. 2012;28:47-54
142. Nugent AM, Smith IE, Shneerson JM. Domiciliary-assisted ventilation in patients with myotonic dystrophy. *Chest*. 2002;121:459-464

143. Weber YG, Roebling R, Kassubek J, Hoffmann S, Rosenbohm A, Wolf M, Steinbach P, Jurkat-Rott K, Walter H, Reske SN, Lehmann-Horn F, Mottaghy FM, Lerche H. Comparative analysis of brain structure, metabolism, and cognition in myotonic dystrophy 1 and 2. *Neurology*. 2010;74:1108-1117
144. Bungener C, Jouvent R, Delaporte C. Psychopathological and emotional deficits in myotonic dystrophy. *J Neurol Neurosurg Psychiatry*. 1998;65:353-356
145. Gaul C, Schmidt T, Windisch G, Wieser T, Muller T, Vielhaber S, Zierz S, Leplow B. Subtle cognitive dysfunction in adult onset myotonic dystrophy type 1 (dm1) and type 2 (dm2). *Neurology*. 2006;67:350-352
146. Timman R, Tibben A, Wintzen AR. Myotonic dystrophy: The burden for patients and their partners. *J Rehabil Med*. 2010;42:823-830
147. Franzese A, Antonini G, Iannelli M, Leardi MG, Spada S, Vichi R, Millefiorini M, Lazzari R. Intellectual functions and personality in subjects with noncongenital myotonic muscular dystrophy. *Psychol Rep*. 1991;68:723-732
148. Colombo G, Perini GI, Miotti MV, Armani M, Angelini C. Cognitive and psychiatric evaluation of 40 patients with myotonic dystrophy. *Ital J Neurol Sci*. 1992;13:53-58
149. Antonini G, Mainero C, Romano A, Giubilei F, Ceschin V, Gragnani F, Morino S, Fiorelli M, Soscia F, Di Pasquale A, Caramia F. Cerebral atrophy in myotonic dystrophy: A voxel based morphometric study. *J Neurol Neurosurg Psychiatry*. 2004;75:1611-1613
150. Prevost C, Veillette S, Perron M, Laberge C, Tremblay C, Auclair J, Villeneuve J, Tremblay M, Mathieu J. Psychosocial impact of predictive testing for myotonic dystrophy type 1. *Am J Med Genet A*. 2004;126A:68-77
151. Delaporte C. Personality patterns in patients with myotonic dystrophy. *Arch Neurol*. 1998;55:635-640
152. Winblad S, Hellstrom P, Lindberg C, Hansen S. Facial emotion recognition in myotonic dystrophy type 1 correlates with ctg repeat expansion. *J Neurol Neurosurg Psychiatry*. 2006;77:219-223
153. Winblad S, Lindberg C, Hansen S. Temperament and character in patients with classical myotonic dystrophy type 1 (dm-1). *Neuromuscul Disord*. 2005;15:287-292
154. Finsterer J, Stollberger C, Blazek G, Spahits E. Cardiac involvement in myotonic dystrophy, becker muscular dystrophy and mitochondrial myopathy: A five-year follow-up. *Can J Cardiol*. 2001;17:1061-1069
155. Stollberger C, Finsterer J, Keller H, Mamoli B, Slany J. Progression of cardiac involvement in patients with myotonic dystrophy, becker's muscular dystrophy and mitochondrial myopathy during a 2-year follow-up. *Cardiology*. 1998;90:173-179
156. Takase S, Okita N, Sakuma H, Mochizuki H, Ohara Y, Mizuno Y, Sato T, Hanew K. Endocrinological abnormalities in myotonic dystrophy: Consecutive studies of eight tolerance tests in 26 patients. *Tohoku J Exp Med*. 1987;153:355-374
157. Sinforiani E, Sandrini G, Martelli A, Mauri M, Uggetti C, Bono G, Nappi G. Cognitive and neuroradiological findings in myotonic dystrophy. *Funct Neurol*. 1991;6:377-384
158. Bostrom K, Natterlund BS, Ahlstrom G. Sickness impact in people with muscular dystrophy: A longitudinal study over 10 years. *Clin Rehabil*. 2005;19:686-694
159. Tarnopolsky M, Mahoney D, Thompson T, Naylor H, Doherty TJ. Creatine monohydrate supplementation does not increase muscle strength, lean body mass, or muscle phosphocreatine in patients with myotonic dystrophy type 1. *Muscle Nerve*. 2004;29:51-58
160. Tarnopolsky MA, Roy BD, MacDonald JR. A randomized, controlled trial of creatine monohydrate in patients with mitochondrial cytopathies. *Muscle Nerve*. 1997;20:1502-1509
161. Abe K, Fujimura H, Toyooka K, Yorifuji S, Nishikawa Y, Hazama T, Yanagihara T. Involvement of the central nervous system in myotonic dystrophy. *J Neurol Sci*. 1994;127:179-185
162. Gagnon C, Mathieu J, Noreau L. Life habits in myotonic dystrophy type 1. *J Rehabil Med*. 2007;39:560-566
163. Gagnon C, Mathieu J, Noreau L. Measurement of participation in myotonic dystrophy: Reliability of the life-h. *Neuromuscul Disord*. 2006;16:262-268
164. Orngreen MC, Olsen DB, Vissing J. Aerobic training in patients with myotonic dystrophy type 1. *Ann Neurol*. 2005;57:754-757
165. Ahlstrom G, Gunnarsson LG, Kihlgren A, Arvill A, Sjoden PO. Respiratory function, electrocardiography and quality of life in individuals with muscular dystrophy. *Chest*. 1994;106:173-179
166. Osborne RJ, Lin X, Welle S, Sobczak K, O'Rourke JR, Swanson MS, Thornton CA. Transcriptional and post-transcriptional impact of toxic rna in myotonic dystrophy. *Hum Mol Genet*. 2009;18:1471-1481
167. Ahlstrom G, Sjoden PO. Coping with illness-related problems and quality of life in adult individuals with muscular dystrophy. *J Psychosom Res*. 1996;41:365-376

Chapter 7

# An introduction to the Rasch Measurement Model

*Mieke C.E. Hermans[a*], Ingemar S.J. Merkies[a,b], Catharina G. Faber[a]*

[a]Department of Neurology, Maastricht University Medical Centre, Maastricht
[b]Department of Neurology, Spaarne Hospital, Hoofddorp

*Supplement in Muscle and Nerve 2013 Jan;47(1):89-95*

## Introduction

Medical doctors are generally well-informed about the basic clinimetric requirements of outcome measures that should be fulfilled prior to use in clinical practice. A proper outcome measure should be simple (non-time consuming), communicable (easily interpretable), valid (measures what it purports to measure), reliable (stable or consistent scores over time or across raters), and responsive (captures relevant changes over time).[1, 2] However, current outcome measures produce ordinal scores that are not suitable for conventional statistical testing, since an ordinal score is not a quantitative measurement, like temperature in degrees Celsius or weight in kilograms, but just a statement that one thing is more than another. Modern clinimetric techniques set additional quality standards for outcome measures and attempt to create measurement at the interval level. This chapter is intended to inform the neuromuscular doctor about the various steps in the evaluation and construction of outcome measures using the Rasch method.[3] The construction of the Fatigue and Daytime Sleepiness Scale for patients with Myotonic Dystrophy type 1 (FDSS, chapter 10) will be used by way of illustration.

**Limitations of multi-item ordinal scales**

Some variables (e.g., height and weight) can be measured directly. Other variables (e.g., disability, fatigue or quality of life) are measured indirectly by how they manifest. Therefore, we need a method to transform the manifestations of these "latent" variables into numbers that can be taken as measurements.[4] Rating scales are a means of measuring a latent variable. The construction of these scales has been based on the classical test theory (CTT).[5] Outcome measures based on CTT are composed of multiple items with ordered (Likert-type) response options and create measurement at an ordinal level. Consequently, the value of numbers used in an ordinal scale and the numerical distance between each point in a scale is unknown. Therefore, a change in scale scores at one level in the scale (say from 1 to 2) is not necessarily equivalent to a change between two other points in the scale (say from 3 to 4) (figure 7.1). Moreover, sum of the item scores are often calculated, but the relevance of each item to the variable of interest is not necessarily equal.[6] Ordinal scales are currently used in an inappropriate way by addressing obtained scores as interval measures and exposing them to parametric analyses. Therefore, CTT-based scales may hamper the comparison between patients and various studies.

**Figure 7.1.** The Fatigue Severity Scale

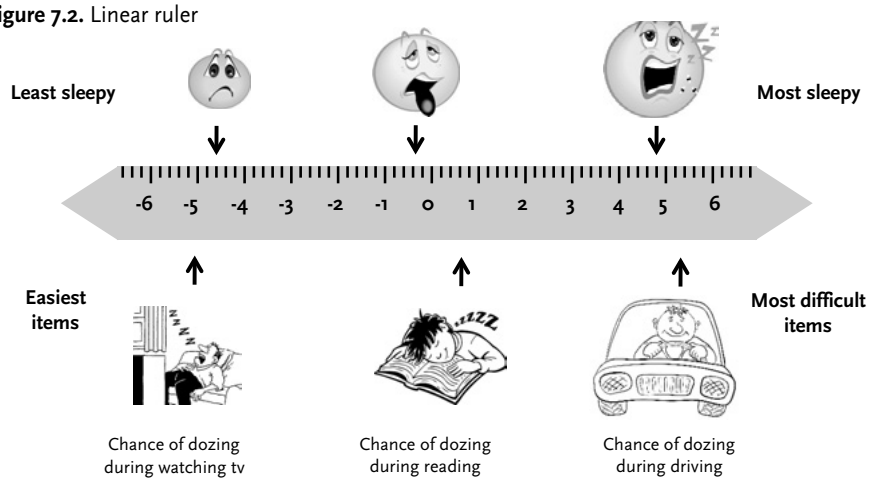
| | Disagree | | | | | | Agree |
|---|---|---|---|---|---|---|---|
| My motivation is lower when I am fatigued | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Exercise brings on my fatigue | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| I am easily fatigued | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Fatigue interferes with my physical functioning | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Fatigue causes frequent problems for me | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| My fatigue prevents sustained physical functioning | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Fatigue interferes with carrying out certain duties and responsibilities | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Fatigue is among my three most disabling symptoms | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Fatigue interferes with my work, family, or social life | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

**Legend to figure 7.1.** The fatigue severity scale is an outcome measure based on classical test theory. The true difference between response categories is not known. A one point difference in score is not necessarily equivalent along the range of scores.

## Rasch measurement model

Considering the shortcomings of the CTT based measures, it is clear that a modern scientific approach is needed for the evaluation and construction of outcome measures to improve the findings in observational and interventional trials. Using interval instead of ordinal measures would give a more accurate reflection of disease impact, differences between individuals and groups, and treatment effects. The Rasch method is a technique to estimate measures at the interval level from obtained ordinal data.[3, 6] Rasch analysis of the FDSS was performed using the Rasch unidimensional measurement model (RUMM2020).[7] The Rasch model is a tool to investigate outcome measures based on the logical assumption that persons with high levels of whatever construct that is being measured (for example, fatigue or sleepiness) should have an increased probability, relative to persons with low levels, of getting a higher score on any item (for example, fall asleep during reading). Similarly, one item being more difficult than another means that for any person the probability of affirming the second item is the greater one.[3] The Rasch model separates persons according their level of the measured construct and items according their difficulty and places both patient and item estimates on the same log-odds units (logit) scale, thereby creating measurement at the interval level (figure 7.2). This can only be obtained when the data fulfil all the Rasch model's

**Figure 7.2.** Linear ruler




**Legend to figure 7.2.** The Rasch model compares the item response patterns of individuals to the entire sample of patients being examined in order to estimate person location (level of sleepiness) and item difficulty (chance of dozing during various activities), and places both item and person estimates on the same logit scale.

expectations. Items and persons not fulfilling the model criteria should be evaluated and removed one by one if needed.

**Ordering item and person estimates on the same ruler.**

The statistical calculations for ordering the location of the persons and the difficulty of the items are based on the so-called Guttmann scaling.[8] As illustrated in figure 7.3, items are arranged in an order so that a person who agrees with a particular item also agrees with items of lower rank-order. Persons are also ordered according to their person location: patient E has a higher sum score than others, indicating a higher level of the measured trait than the others patients.

**Figure 7.3.** Guttman scaling

| | Increasing item difficulty → | | | | |
|---|---|---|---|---|---|
| | Item 1 | Item 2 | Item 3 | Item 4 | sum score |
| Person A | 0 | 0 | 0 | 0 | 0 |
| Person B | 1 | 0 | 0 | 0 | 1 |
| Person C | 1 | 1 | 0 | 0 | 2 |
| Person D | 1 | 1 | 1 | 0 | 3 |
| Person E | 1 | 1 | 1 | 1 | 4 |

Increasing patient location ↓

**Legend to figure 7.3.** Items can be ranked from most easy (coded 01111) to most difficult (coded 00001). Persons with a higher sum score have a higher level of the measured trait.

Figures 7.4A-C illustrate the statistical steps taken by the Rasch model to order the item and patient estimates on a same metric. Suppose we are examining 100 patients experiencing excessive daytime sleepiness and we want to look at the "chance of dozing" during everyday activities using a rating scale consisting of 4 items (activity A, B, C, and D) with 4 ordinal response options ranging from 0 (would never doze), to 1 (slight change of dozing), to 2 (moderate chance of dozing), and to 3 (high chance of dozing) (figure 7.4A).

**Figure 7.4A.** Rating scale measuring the chance of dozing during activities

| | Never<br>0 | Slight chance<br>1 | Moderate chance<br>2 | High chance<br>3 |
|---|---|---|---|---|
| Activity A | | | | |
| Activity B | | | | |
| Activity C | | | | |
| Activity D | | | | |

The obtained scores after completion of the scale by 100 persons are summarized in figure 7.4B. Item A turns out to be the "easiest item" based on the highest percentage (95%) of patients examined choosing response option 3 (high chance of dozing) for this item. Conversely, 98 of the 100 patients scored 0 (would never doze) on item B, making this the most difficult item. Since 50% of the patients had scored 0 (would never doze) on item D and only 10% on Item C, it is obvious that Item D is ranked as an more difficult item than item C. Figure 7.4C shows the ordering of the items by the Rasch model on the same ruler.

**Figure 7.4B.** Ordering of items according to difficulty

| | Never<br>0 | Slight chance<br>1 | Moderate chance<br>2 | High chance<br>3 |
|---|---|---|---|---|
| Activity A | | 1 | 44 | 95 |
| Activity B | 98 | 2 | | |
| Activity C | 10 | 40 | 36 | 14 |
| Activity D | 50 | 35 | 10 | 5 |

**Most difficult item** **Easiest item**

**Figure 7.4C.** Ordering of items on a logit scale

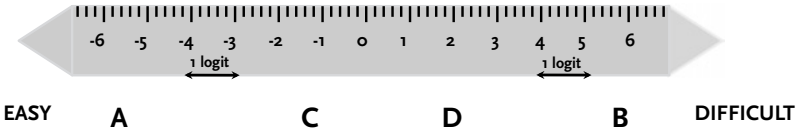



Figure 7.4D-E illustrate the ordering of the persons on the same ruler. As an example, we will compare the results of 3 patients completing the 4-item rating scale measuring chance of dozing during activities. Figure 7.4D shows the same rating scale, but now the items are listed in order of difficulty. The scores of patients I, II and III are shown. Patient I scored response option 3 (high chance of dozing) for all items. Based on the scoring results it can be concluded that patient I has the highest level of the measured construct, i.e. high level of sleepiness. Patient III had the lowest scores compared to the others. Figure 7.4E shows the ordering of patients by the Rasch model on the same ruler. The corresponding location of items and persons (expressed as logit) are also calculated by the model depending on the interaction between the items and patients.

The Rasch model estimates the person location and item difficulty, thereby creating measurement at the interval level, provided that the ordinal obtained raw data fulfil the Rasch model's expectations, like good item and person statistical fit, threshold ordering, no item bias or local dependency and unidimensionality.

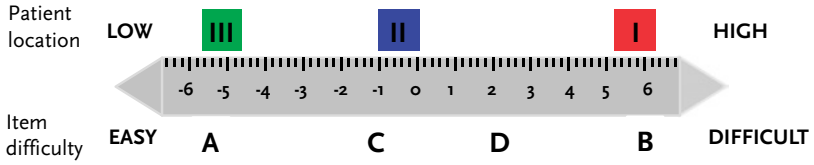
**Figure 7.4D.** Ordering of patients according to level of sleepiness

| | Never<br>0 | Slight chance<br>1 | Moderate chance<br>2 | High chance<br>3 |
|---|---|---|---|---|
| Activity A | | X | | X X |
| Activity B | X | | X | X |
| Activity C | X | X | | X |
| Activity D | X | X | | X |

Increasing item difficulty

**Patient I** **Patient II** **Patient III**

**Figure 7.4E.** Ordering of patients on a logit scale



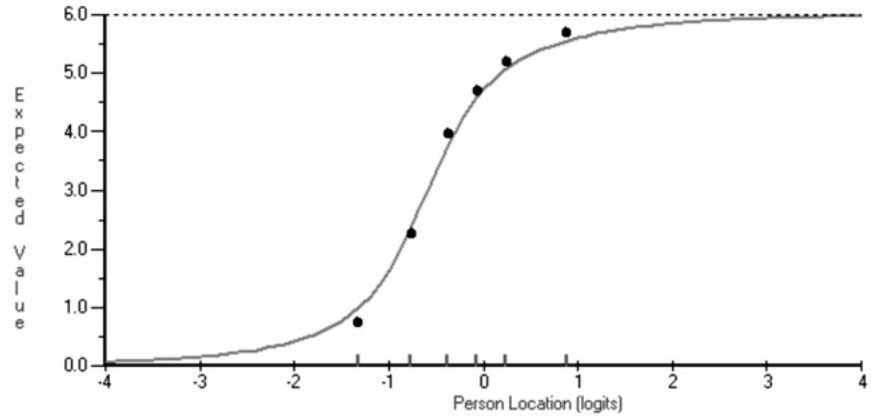

## Fit statistics

A variety of fit statistics determine whether the observed data do not significantly deviate from the expected response pattern calculated by the mathematical (Rasch) model. Three overall fit statics are generally considered. Two are item-person interaction statistics, which are a summary of all the individual item and person fit residuals (i.e. item or person deviations from model expectations). The fit residuals are then standardized to approximate a z-score representing a standardised normal distribution. For model fit, a mean of 0 and a standard deviation (SD) of 1 would be expected, although SD<1.4 is usually accepted. A third overall fit statistic is an item-trait interaction statistic reported as a chi-square, and reflects whether the hierarchical ordering of the items is consistent for different groups of responders across the trait (called class intervals). This is calculated by summing all the chi-square values for each of the individual items and calculating the significance value using the summated degrees of freedom. A non-significant chi-square indicates the required property of invariance.[9]

Fit statistics at the individual item and person level are also available as residual values, acceptable within the range ±2.5, and chi-square statistics, reflecting the deviation from the model by class intervals for each item. Chi-square statistics should indicate non-significant deviation the from model, after multiple testing Bonferroni corrections. Individual item fit is also examined graphically by plotting the observed item responses for class intervals against the expected model item characteristic curve (figure 7.5).[10] Items with good fit will show each plot on the expected model curve.

In addition to these overall fit statistics a Person Separation Index (equivalent to Cronbach's alpha) is available. A value of 0.7 is the minimum required for group comparisons.[10]

**Figure 7.5.** Item Characteristic Curve (ICC) for an item



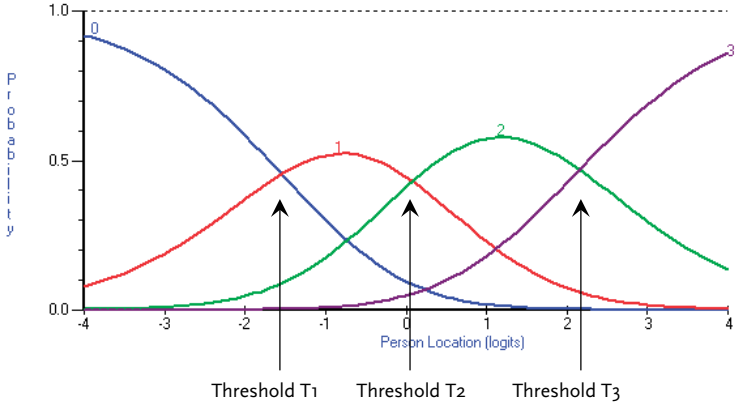

**Legend to figure 7.5.** Item Characteristic Curve showing the relationship between item score and person location estimate. The grey curved line represents where expected scores of an item lie if the item is functioning properly. The black dots represent the observed scores of persons within six class intervals.

## Threshold ordering

For polytomous scale (i.e. items having more than two score categories), response category structure should be examined for correct ordering. This can be verified by inspection of category probability curves. The category probability curve is a graph showing the relation between the probability of a given category as a function of person location. Thresholds are the boundaries between response categories and correspond with the location on the trait continuum at which it is equally likely that a person will be classified into adjacent categories (figure 7.6). Ordered thresholds represent an increase in level of the measured trait for each category. For example, a higher numbered category in the Daytime Sleepiness Scale (DSS) is thought to imply more excessive daytime sleepiness. Where thresholds are ordered, a person location between category boundaries will give that category the greatest probability of being observed.[11]

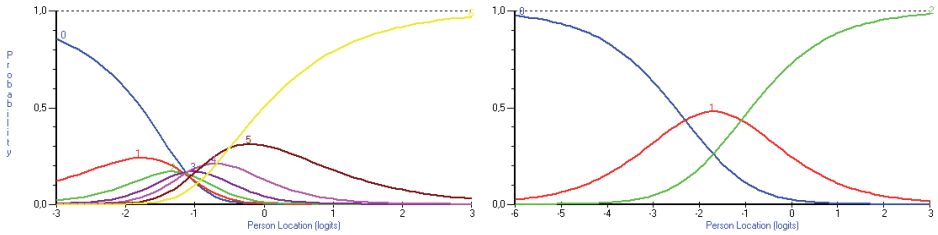
Disordered thresholds are a common source of item misfit and occur when respondents use the response options inconsistently. Persons can experience difficulties discriminating between response options when there are too many response options or when the labeling of response options is potentially confusing or open to misinterpretation. Disordered thresholds can usually be solved by collapsing categories. The number of patients in each class interval should always be checked before collapsing categories (figure 7.7).

**Figure 7.6.** Category probability curves with ordered thresholds




**Legend to figure 7.6.** Category probability curves for an item with 4 response categories. Thresholds are ordered (T1<T2<T3) and each category has a certain distance across the trait where it is the most probable response.

**Figure 7.7.** Category probability curves with disordered thresholds

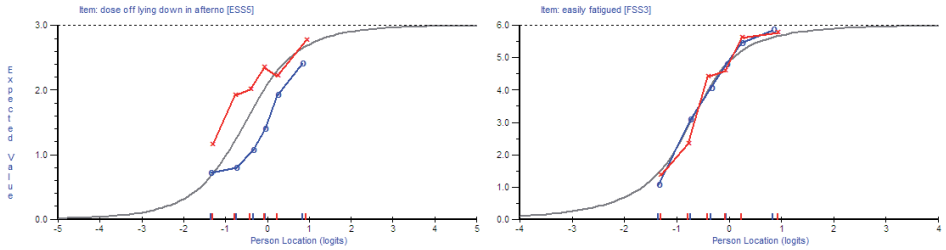



**Legend to figure 7.7.** Category probability curves for an item with 7 response categories. Left panel: thresholds were disordered and crowded together. Note that categories 1, 2 and 3 would never be the most probable response. Furthermore, categories 4 and 5 correspond to only a very narrow interval on the variable. Right panel: response options for all items were rescored in order to obtain ordered thresholds. The original 7 response options (coded 1-2-3-4-5-6-7) were collapsed into 3 categories (coded 0-0-1-1-1-2-2).

## Differential item functioning

Differential item functioning (DIF or item bias) occurs when different groups within the sample (e.g., men and women) with equal levels of the measured trait respond in a different manner to an individual item.[12] For example, Dutch and Canadian patients with equal levels of excessive daytime sleepiness may respond systematically different to an item in the Epworth Sleepiness Scale (figure 7.8). By contrast, the expected score on an item from the Fatigue Severity Scale was the same across countries for persons with the same level of fatigue (figure 7.8).

**Figure 7.8.** Differential item functioning




**Legend to figure 7.8.** Item Characteristic Curve displays the expected value for the item in relation to the person location. Left panel: respondents in Canada (red line) have a higher probability of affirming this item than Dutch respondents (blue line), indicating uniform differential item functioning. Right panel: no differential item functioning was observed across countries for this item.

Two types of DIF may be identified. Uniform DIF occurs where a particular subgroup shows a consistent systematic difference in their responses to an item, across the whole range of the attribute being measured. When there is non-uniformity in the differences between the group, this is referred to as non-uniform DIF. Uniform DIF can be resolved by separately calibrating the item for each group. With non-uniform DIF removal of the item is often the only solution.

## Local independence

Local independence of items is an assumption in Rasch model.[7] This assumption is violated when items in a test are related to each other. The classic example of this is where several walking items are included in the same scale (figure 7.9).[13]

**Figure 7.9.** Local dependence

| Does your health now limit you in these activities? If so, how much? | Yes, limited a lot | Yes, limited a little | No, not limited at all |
|---|---|---|---|
| Walking more than a amile? | 1 | 2 | 3 |
| Walking several hunderd yards? | 1 | 2 | 3 |
| Walking one hunderd yards? | 1 | 2 | 3 |

**Legend to figure 7.9.** An example of the SF-36 health status questionnaire was chosen to illustrate local dependence. If a patient scores 3 (no, not limited at all) on item "walking more than a mile", this automatically will determine the response on the other two questions "walking several hundred yards" and "walking one hundred yards". Since the results of these items are linked in some way, the obtained scores will inflate reliability and the final scale score in a particular direction.

Such sets of items inflate classic reliability and affect parameter estimation in Rasch analysis.[14] Local dependency can be identified through inspection of the residual correlation matrix. Item sets with correlations <0.28 are considered acceptable. In cases of local dependency, the researcher may consider to remove items or to create subsets of correlating items to improve model fit.

**Unidimensionality**

The Rasch model requires that all items in the questionnaire work together to measure a single underlying construct. For example, when we are interested in fatigue, no questions about quality of life should be addressed. A principal component analysis (PCA) of the residuals is conducted to detect any signs of multidimensionality. The absence of any meaningful pattern in the residuals will be deemed to support the assumption of unidimensionality of the scale.[15] This is formally tested by allowing the item loadings on the first factor of the PCA of the residuals to determine two subtests of items (defined by positive and negative loadings on the first residual component). A series of independent t-tests is then undertaken to see if the person estimates derived from these subsets significantly differ. The percentage of tests outside the range ±1.96 should not exceed 5% or the associated binomial proportions confidence interval should overlap the 5% expected value for the scale to be unidimensional.[14, 15]



## Conclusion

The Rasch model can be seen as the ideal response pattern, where persons with high level of the measured trait should have a higher probability of receiving a higher score on any item compared to persons with lower levels. Also any person should always have a greater probability of receiving a higher score on an easier item than on a more difficult one. In the model, person location and item difficulty are estimated separately. All data should fulfil the Rasch model's expectations like good item and person statistical fit, threshold ordering, absence of item bias, local independence and unidimensionality. Only then an interval-scaled measure of a latent variable can be estimated from an observed raw score, giving meaning to the scores and allowing comparison of (change in) scores. Since logits are difficult to interpret intuitively, it is more useful to convert the patient logit measures into a scale score with positive and integer values, like a centile metric score.

# References


1. Streiner DL, Norman GR. Health measurement scales. A practical guide to their development and use. New York: Oxford University Press; 1995
2. Feinstein A. Clinimetrics. New Haven, Connecticut: Yale University Press; 1987
3. Rasch G. Probabilistic models for some intelligence and attainment tests. Chicago: University of Chicago Press; 1980
4. Hobart JC, Cano SJ, Zajicek JP, Thompson AJ. Rating scales as outcome measures for clinical trials in neurology: Problems, solutions, and recommendations. *Lancet Neurol*. 2007;6:1094-1105
5. DeVellis RF. Classical test theory. *Med Care*. 2006;44:S50-59
6. Stucki G, Daltroy L, Katz JN, Johannesson M, Liang MH. Interpretation of change scores in ordinal clinical scales and health status measures: The whole may not equal the sum of the parts. *J Clin Epidemiol*. 1996;49:711-717
7. Andrich D, Lyne A, Sheridan B, Luo G. Rumm 2020. 2003
8. Altman DG. Practical statistics for medical research. London: Chapman and Hall; 1991
9. Pallant JF, Tennant A. An introduction to the rasch measurement model: An example using the hospital anxiety and depression scale (hads). *Br J Clin Psychol*. 2007;46:1-18
10. Fisher WP. Reliability statistics. *Rasch Measure Trans*. 1992;6:238
11. Andrich D, Luo G. Conditional pairwise estimation in the rasch model for ordered response categories using principal components. *J Appl Meas*. 2003;4:205-221
12. Dorans NJ, Holland PW. Dif detection and description: Mantel-haenszel and standardisation. In: Holland PWW, H., ed. Differential item functioning. Hillsdale: Lawrence Erlbaum Associates; 1993:36-66
13. Ware Jr JE, Kosinski M, Keller DS. Sf-36 physical and mental health summary scales: A user's manual. Boston: The Health Assessment Lab, New England Medical Center; 1994
14. Tennant A, Conaghan PG. The rasch measurement model in rheumatology: What is it and why use it? When should it be applied, and what should one look for in a rasch paper? *Arthritis Rheum*. 2007;57:1358-1362
15. Smith EV, Jr. Detecting and evaluating the impact of multidimensionality using item fit statistics and principal component analysis of residuals. *J Appl Meas*. 2002;3:205-231

Chapter 8

# Rasch-built Myotonic Dystrophy type 1 Activity and Participation Scale (DM1-Activ)

*Mieke C.E. Hermans[a], Catharina G. Faber[a], Marc H. De Baets[b,c], Christine E.M. de Die-Smulders[d], Ingemar S.J. Merkies[a,e]*

[a]Department of Neurology, Maastricht University Medical Centre, Maastricht
[b]School for Mental Health and Neuroscience, University of Maastricht, Maastricht
[c]Neuroimmunology Group, Biomedical Research Institute, Hasselt University, Hasselt
[d]Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht
[e]Department of Neurology, Spaarne Hospital, Hoofddorp

*Neuromuscul Disord. 2010 May;20(5):310-8*

## Summary

We describe the development of an outcome measure of activity and participation for patients with myotonic dystrophy type 1 using the Rasch measurement model. A 49-item questionnaire was completed by 163 DM1 patients. Data were subsequently analyzed with Rasch software to design the item set to fit model expectations. Through systematic investigation of response category ordering, model fit, item bias, and local response dependency, we succeeded in constructing a 20-item unidimensional scale of activity and participation (DM1-Activ). High internal consistency (PSI=0.95) and good test-retest reliability values of item difficulty hierarchy and patient location were demonstrated. Patient measures had acceptable correlations with MRC sum scores and MIRS grades (ICC=0.69 and 0.71 respectively), indicating good external construct validity. DM1-Activ is a practical, reliable and valid outcome measure that fulfils all clinimetric requirements. Further evaluation of this scale is needed to provide a nomogram for clinical use.

## Introduction

Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy in adults with a prevalence of 2.1-14.3 in 100 000.[1] The most noticeable symptoms are myotonia and progressive weakness of facial, axial and distal muscles. In addition, systemic manifestations such as cataracts, endocrine dysfunction and cardiac involvement may occur. Based on age at onset and severity of symptoms, four clinical types can be distinguished: congenital type, childhood-onset type, adult-onset (classical) type and late-onset (mild) type.[2] The clinical course is usually slowly progressive, but DM1 may be extremely disabling when more generalized limb weakness and respiratory muscle involvement develop. Systemic manifestations may also have a considerable effect on the patient's health status.

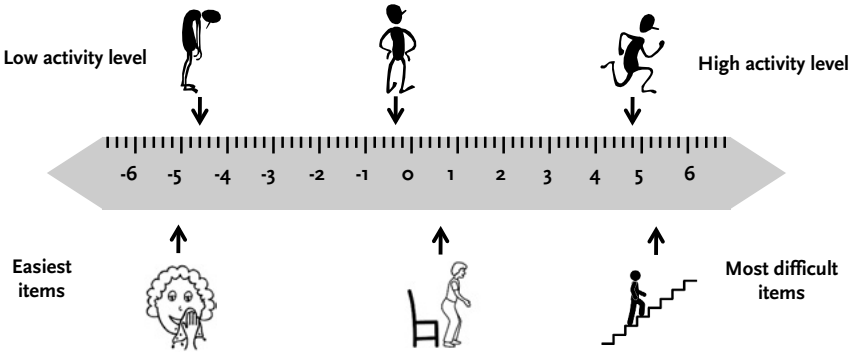
As a result, DM1 leads to physical impairment, activity limitations and participation restrictions with reduced quality of life expectations.[3-6] At present, no curative treatment is available. However, development of innovative strategies for targeted therapy has begun since studies on pathogenesis have elucidated a novel disease mechanism, in which mutant RNA has deleterious effects on gene expression.[7, 8] Preclinical studies have already demonstrated that myotonia can be reduced by antisense oligonucleotides correcting abnormal regulation of alternative splicing of muscle-specific chloride channel.[9] In order to evaluate future therapeutic interventions in randomized controlled trials, high quality outcome measures are needed to demonstrate relevant changes at various levels of outcome.[10, 11]

Functioning and disability of DM1 patients have been assessed in studies using a variety of (composite) outcome measures.[3, 4, 12-18] Only a few scales have been formally evaluated in terms of scientific soundness.[19] The muscular impairment rating scale (MIRS), an ordinal 5-point grading system based particularly on clinical muscle strength evaluation, has been most widely used in DM1.[12, 20] It can easily monitor major stages of disease, but does not refer to the functional impact of muscle weakness. Still, the MIRS is shown to be valid, reliable and to correlate with functional scores.[20] The motor function measure demonstrated good validity and reproducibility in patients with neuromuscular disorders.[17] However, this scale takes 36 min to complete and only 29 DM1 patients were included in its validation study. The Life Habits measure demonstrated good reliability scores, but its use is limited since the presented domains have up to 10 response options, assuming that patients are able to discriminate between the degrees of difficulty when performing a life habit.[18]

The construction of these ordinal scales has been based on the classical test theory (CTT). DeVellis has summarized various aspects of CTT including its advantages and disadvantages.[21] Outcome measures based on CTT may

constitute items that are arbitrarily collected and patients are requested to complete all items, even though some may be irrelevant or inappropriate for their level of ability. Furthermore, a sum of item scores is often calculated and the obtained data are often addressed as forming an interval range. Moreover, these scales may be prone to differential sensitivity, where for example a 3-point change at the centre of the sum score range may represent a different true score than a 3-point change at one of the extremes.[21, 22] Therefore, CTT-based scales may hamper the comparison between patients with different functional levels.
Considering these shortcomings, it is clear that a modern scientific approach is needed for health evaluation in clinical trials. The most widely used approach is that of Rasch unidimensional measurement model.[23, 24] The Rasch model shows what should be expected in responses to items if interval scale measurement is to be achieved. This statistical technique compares the item response patterns of individuals to the entire sample to estimate person ability and item difficulty and places both item and person estimates on the same logit scale (figure 8.1). Once data fit Rasch model expectations, logits of person estimates can be used as an interval level variable in parametric statistics.[24, 2]

**Figure 8.1.** Linear ruler




**Legend to figure 8.1.** The Rasch model places both item and person parameter estimates on the same interval scale. The units of this scale are log-odds-units or logits.

Recently, a Rasch-built measure of activity limitations for patients with neuromuscular disorders (ACTIVLIM) has been presented.[26] Despite having good scientific soundness, this scale should be considered as a generic activity measure for neuromuscular disorders, not particularly targeting a specific neuromuscular disease. Only 17% of the patient sample had DM1. In the current study, we aimed to develop a patient-based disease-specific outcome measure at the activity and participation level of outcome in DM1 patients (DM1-Activ)

using the Rasch model and evaluate its validity and reliability in these patients. We hypothesized that a disease-specific activity and participation scale for DM1 patients would lead to differences in items selection and difficulty of selected items.

## Methods

### Participants

Patients older than 18 years with genetically proven DM1 were invited for a prospective, on-going study on cardiac involvement. Participants were recruited from the genetic register of the Maastricht University Medical Centre and through the Dutch neuromuscular patients' association. According to the protocol, subjects with previously implanted pacemakers or cardioverter-defibrillators and patients with severe comorbidity who had life expectancies of less than 3 years were not eligible for participation in the study. The protocol was approved by the local Medical Ethics Committee. Informed consent was obtained prior to entrance in the study. A total of 163 patients with DM1 were included.

### Questionnaire development

For the construction of the DM1-Activ scale, accepted development procedures were applied.[19, 27] These procedures consist of four stages:

**Stage I**: the International classification of functioning, disability and health (ICF) frame was used to provide a definition of functioning and disability associated with health.[11] Disability should be described in terms of activity limitations and participation restrictions. The ICF item list for activities and participation was examined to design the patient-based scale.

**Stage II**: a systematic PubMed search was performed, reviewing scales for measuring of functional outcome used in studies with DM1 patients. Papers published over the last decades in English were identified using the following keywords: clinical studies, trial, myotonic dystrophy type 1, scales, disability, validity, and reliability. Items representing activity limitations or participation restrictions were, if not yet, identified and added to the questionnaire.

**Stage III**: additional items were collected during routine examination of DM1 patients at our institute. Efforts were made to describe these potential items in a short, simplistic and unambiguous way. Selected items were subjected to expert judgment and adaptations were made according to their suggestions, hereby obtaining face and content validity for the pre-phase scale.[19]

**Stage IV**: the constructed pre-phase DM1-Activ scale contained 49 items with 5 response options: unable to perform (0), able to perform, but with great

difficulty (1), able to perform, but with some difficulty (2), able to perform, but with little difficulty (3), easy to performed without difficulty (4). An item was scored (6) if it was not applicable to the patient.

**Procedures**

The pre-phase DM1-Activ questionnaire was completed by 163 DM1 patients. A random sample of 138 patients was re-examined one year later for test-retest reliability studies.[28] Sample description is provided in table 8.1. Standardized neurological examination was conducted by one examiner (MH). The degree of muscle weakness was manually tested and graded according to the Medical Research Council (MRC) 6-point grading system.[29] A total of 22 muscle groups were tested: neck flexors and extensors separately plus 10 bilateral muscles: 6 proximal muscle groups (shoulder abductors, elbow flexors, elbow extensors, hip flexors, knee extensors, knee flexors) and 4 distal muscle groups (wrist extensors, digits flexors, foot dorsiflexors and plantar flexors). Summation of the scores yielded an MRC sum score ranging from 0 (paralytic) to 110 (normal strength).[30] The muscular impairment rating scale (MIRS) grade was also assessed.[20] Its score ranges from 1 (no clinical muscular impairment) to a maximum of 5 (severe proximal weakness).[12]

**Table 8.1.** Patient sample description

| | First sample | Second sample (for test-retest reliability) |
|---|---|---|
| Number of patients | 163 | 138 |
| Age (years) | | |
| mean (SD), range | 44.2 (11.7), 18-69 | 46.0 (11.5), 19-70 |
| Gender (%) | | |
| Female | 79 (48.5) | 70 (50.7) |
| Male | 84 (51.5) | 68 (49.3) |
| Diagnosis type (%) | | |
| mild type | 13 (8.0) | 10 (7.3) |
| adult type | 134 (82.2) | 116 (84.0) |
| childhood/congenital type | 16 (9.8) | 12 (8.7) |
| Duration of symptoms (years) | | |
| mean (SD), range | 17.6 (10.3), 0-48 | 19.4 (10.1), 0-49 |

**Rasch analysis**

Obtained pre-phase DM1-Activ data were subjected to the Rasch unidimensional measurement model software (RUMM2020), which allows for a unified

approach to several measurement issues.[25, 31] A sample size of at least 150 patients is needed to estimate item difficulty, with 99% confidence that item calibrations are stable within ±0.5 logit, given appropriate targeting.[32] Items not fulfilling the following requirements were removed from the pre-phase DM1-Activ scale or adjusted to fit the model.

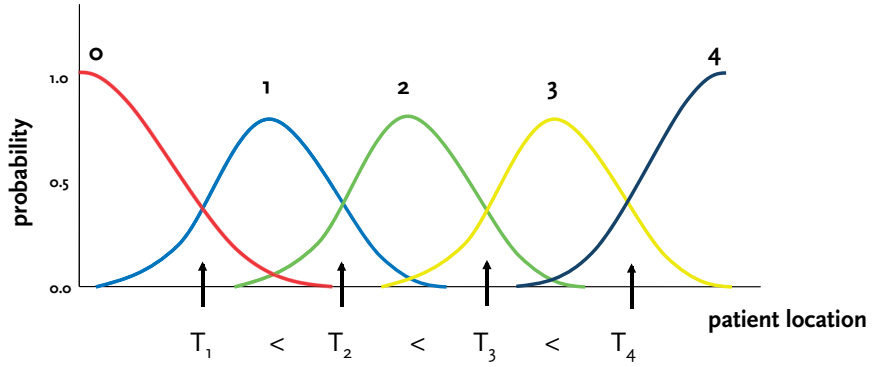
**Fit statistics**. Three overall fit statistics are considered to test whether the observed data meet the model expectations, which are based on a probabilistic form of Guttmann scaling.[33] Two are item-person interaction statistics transformed to approximate a z-score, representing a standardized normal distribution. Therefore, if the items and persons fit the model perfectly we would expect to see a mean of approximately zero and a standard deviation (SD) of 1. A third fit statistic score is an item-trait interaction statistic reported as a chi-square, reflecting the degree of invariance across the trait to be measured (i.e. activity and participation level). A non-significant chi-square indicates that the hierarchical ordering of the items remains the same at different levels of the underlying trait, thus fulfilling the required property of invariance.

**Fit residuals**. Individual person and item fit statistics are presented, both as residuals (a summation of individual patient and item deviations from expected model scores) and as a chi-square statistic. In the former case, residuals between ±2.5 are considered adequate fit to the model. In the latter case a chi-square statistic is available for each item. Summation of the overall chi-square for items gives the item-trait interaction statistic. Item misfit to the model can also be examined graphically when observed model fit for groups of responders representing different ability levels across the trait (called class intervals) are plotted against the expected model item characteristic curve. Items with good fit will show each plot on the expected model curve. Over-discrimination of an item is suggested by highly negative fit residuals and means that the observed responses form a steeper line than the expected item characteristic curve. It indicates that the item does not add to the information gained from the other items. Highly positive fit residual suggest low levels of discrimination and poor fit to the model.

**Internal reliability**. An estimate of the internal consistency reliability of the scale is available as a person separation index (PSI). The estimates on the logit scale for each person are used to calculate reliability. A PSI of ≥0.7 is consistent with the scale being able to differentiate at least 2 groups of patients and considered the minimum requirement for measurement.[34]

**Threshold examination**. Proper ordering of response categories should be verified. The term threshold refers to the point between two adjacent response categories where either response is equally probable (figure 8.2). One of the most common sources of item misfit concerns respondents' inconsistent use of response options, resulting in disordered thresholds.

**Figure 8.2.** Category probability curves with ordered thresholds




**Legend to figure 8.2.** Ideal category probability curves for an item with five response categories. The y-axis represents the probability of obtaining a response category and the x-axis represents the patient's level of ability. The term threshold refers to the point between two adjacent response options and corresponds to the level of ability at which a person is equally likely to obtain one of two successive scores. Where thresholds are ordered, a person location between category boundaries ensures that the probability of a response in that category is larger than of any other single category.

Disordered thresholds occur when respondents have difficulties discriminating consistently between response options. This can occur when there are too many response options, or when the labelling of options is potentially confusing or open to misinterpretation (e.g. great - some - little). Collapsing of categories where disordered thresholds occur, may improve overall fit to the model.

**Item bias**. A scale should always work in the same way irrespective of which group within the sample is being examined.[35] For example, if the probability of responding to an item is systematically different between men and women with equal levels of disability, then this item would be considered to display differential item functioning (DIF=item bias). DIF was checked with regard to five personal factors, categorized as follows: age (<30 years, 30-50 years, ≥50 years), gender, diagnosis type (mild type, adult type, childhood/congenital type), duration of complaints (<5 years, 5-10 years, 10-20 years, ≥20 years) and degree of education (elementary school, high school, university). DIF can be detected by analysis-of-variance and was conducted for each item comparing scores across each level of personal factor and across different class intervals.

**Local dependency**. Local response dependency arises when items are linked such that the response on one item may be dependent upon the response to another. This affects the estimation of test information and item discrimination parameters, hereby inflating reliability of the final scale score.[36]

Residual correlations between the individual items were examined to detect local dependency (defined as a correlation ≥0.3).

**Test for unidimensionality**. When issues of fit, DIF and threshold disordering have been resolved, it is necessary to perform a principal component analysis of the residuals. The absence of any meaningful pattern in the residuals will support the assumption of local independence and consequently the unidimensionality of the scale.[37] This is formally tested by allowing the factor loadings (correlation between items and the first residual factor) to determine subsets of items. A series of t-tests is then undertaken to see if the person estimate (the logit of 'person ability' or, in this case 'degree of activity and participation performance') derived from these two subsets of items significantly differs. The percentage of tests outside the range ±1.96 should not exceed 5% OR the binominal confidence interval should overlap the 5% expected value for the scale to be unidimensional.[37]

### External construct validity and reliability

The external construct validity of the final DM1-Activ scale was assessed by correlations with the MIRS and MRC sum score (intraclass correlation coefficient reported after applying regression analyses with restricted cubic spline functions on transformed DM1-Activ scores).[38] Moreover, test-retest reliability studies were performed to investigate whether hierarchy of item difficulty and patient ability location were consistent over time.[28] Reliability was quantified by calculation of the intraclass correlation coefficient using a one-way random effects analysis-of-variance model for group comparison.



### Statistics

Rasch analysis was performed with a partial credit model using RUMM2020.[31] Further analyses were undertaken using Stata Statistical Software, release 10.0 for Windows XP. Bonferroni corrections were applied to adjust the *P*-values for multiple testing.[39]

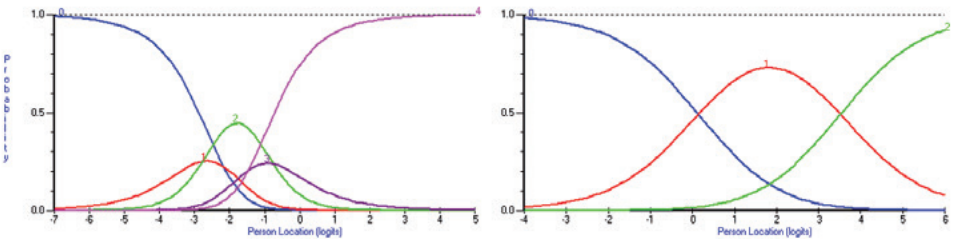
## Results

### Rasch analysis of the pre-phase DM1-Activ scale

The pre-phase 49-items DM1-Activ scale did not meet Rasch model expectations (table 8.2, analysis 1). The item fit residual SD value deviated from the expected value of 1. Patients showed reasonable model fit (mean residual -0.035, SD 1.123). The item-trait chi-square probability was significant, indicating lack of invariance of item difficulty across the scale. Category probability curves showed disordered thresholds for 33 items, particularly in the mid response category area

(figure 8.3). This indicated an inability of the patients to differentiate between the options: able to perform, but with great difficulty (1); able to perform, but with some difficulty (2); and able to perform, but with little difficulty (3). The ordered thresholds of the remaining 16 items were extremely close to each other in the mid response area. Furthermore, the test for unidimensionality on the initial item set indicated that a degree of multidimensionality was apparent.

**Figure 8.3.** Disordered thresholds




**Legend to figure 8.3.** Category probability curves for item "are you able to do sports" is presented as an example of disordered thresholds. Left panel: thresholds are disordered, e.g. response categories one and three have never the greatest probability of being observed. Right panel: threshold ordering is restored after rescoring response categories.

**Table 8.2.** Rasch analysis findings during the construction of the activity and participation scale for myotonic dystrophy type 1 patients

| Analysis | Item fit residuals | | Person fit residuals | | Item-trait Chi²-probability | | PSI | unidimensionality t-tests (CI) |
|---|---|---|---|---|---|---|---|---|
| nr | Mean | SD | Mean | SD | DF | *P*-value | | |
| 1 | 0.068 | 1.571 | -0.035 | 1.123 | 98 | <0.000001 | 0.98 | 0.21 (0.175-0.244) |
| 2 | -0.163 | 1.318 | -0.304 | 1.050 | 98 | <0.000001 | 0.98 | 0.22 (0.189-0.258) |
| 6 | -0.161 | 1.263 | -0.311 | 1.028 | 90 | 0.025 | 0.98 | 0.21 (0.182-0.252) |
| 16 | -0.115 | 1.229 | -0.273 | 0.915 | 68 | 0.028 | 0.97 | 0.15 (0.118-0.187) |
| 21 (final) | -0.117 | 1.225 | -0.284 | 0.916 | 40 | 0.089 | 0.95 | 0.08 (0.046-0.116) |

## Fitting data to the Rasch model

A stepwise description of the various Rasch analyses will be given. Items not fulfilling Rasch model criteria were analysed and removed one by one if needed. Changes and fit statistics of individual remaining items and overall model fit were monitored at each step.

**Step 1**: to maintain interpretability, response options for all items were re-scored by collapsing the 5-point scale into 3 response categories as follows: (0)=(0) unable to perform; (1-3)=(1) able to perform, but with difficulty; (4)=(2) easy to perform, without difficulty. As a result, all disordered thresholds were restored resulting in a slight improvement of the SD of the items and patients (table 8.2, analysis 2).

**Step 2**: uniform DIF was detected with regard to degree of education for the items "able to drive a car" and "able to visit friends/family". Item "able to dry yourself" showed non-uniform DIF by age. These 3 items were also removed leading to further improvement of model (table 8.2, analysis 6).

**Step 3**: after accomplishing these steps, individual fit statistics of 14 items still showed misfit to the model. Six items demonstrated under-discrimination and 8 items showed over-discrimination. These items were removed one by one, starting with the item with strongest misfit (item "ability to eat").

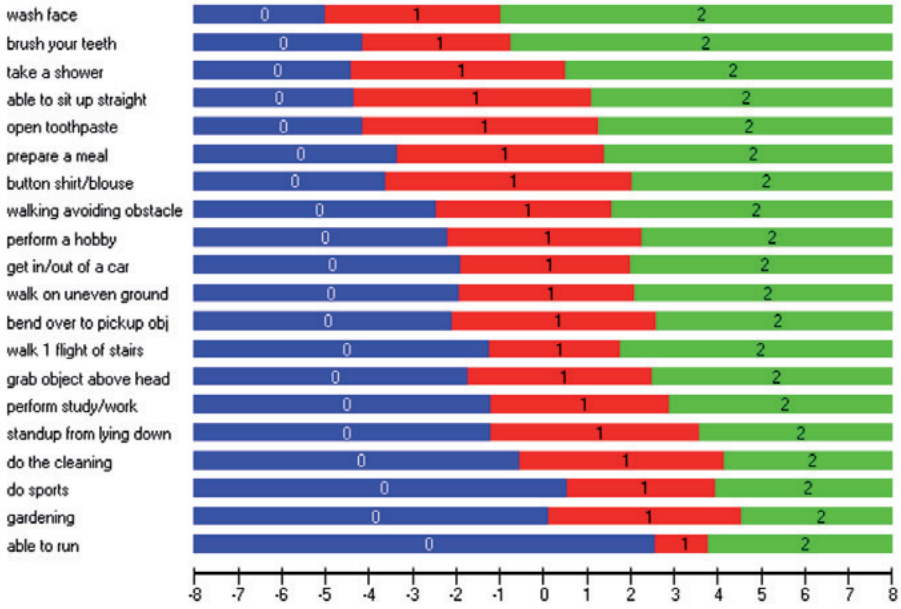
**Step 4:** a systematic evaluation of the correlation matrix (starting at correlations ≥0.7, then ≥0.6, through to ≥0.3) was performed. Item characteristic curves of these items were subsequently inspected to select the items that best fit the expected model curve. The highest correlation was seen between item "able to walk up ½ flight of stairs" and "able to walk up 1 flight of stairs" ($r$=0.756). Eventually, a total of 12 items were removed on the basis of local response dependency.

**Clinimetric properties of the final DM1-Activ**

After completing all these steps, a 20-item activity and participation scale was created and demonstrated good fit without any DIF or local dependency (table 8.2, final analysis). All items of the final scale had fit residuals within range ±2.5 with probability scores higher than Bonferroni cut-off $P$-value, indicating no significant deviation from the model (table 8.3). The person separation index was 0.95, demonstrating substantial internal consistency reliability. Principal component analysis of the residuals identified two subsets of items consisting of the 6 most positive and the 6 most negative loading items. t-Tests between person estimates from these two subsets of items demonstrated a proportion of 0.08 (95% CI 0.046-0.116) of the $t$-values falling outside the ±1.96 range, supporting acceptable unidimensionality of the scale.

The threshold distribution map of the final Rasch-built DM1-Activ scale shows the expected response to a given item as a function of the underlying activity and participation measure (figure 8.4). Item difficulty ranges from -2.98 to +3.17 logits and patient location from -4.684 to +6.364 logits. Only 0.63% of patients could not perform the tasks at all (floor effect) and 8.6% performed all activities without any problems (ceiling effect). Figure 8.5 provides the threshold distribution plot showing acceptable targeting between patient and item locations.

**Figure 8.4.** Threshold map of the 20-item DM1-Activ




**Legend to figure 8.4.** The expected response for every person on each item is given as a function of the underlying activity and participation measure (0=unable to perform, 1=able to perform, but with difficulty, 2=easy to perform, without difficulty). Items are ordered by increasing difficulty.

## External construct validity and reliability

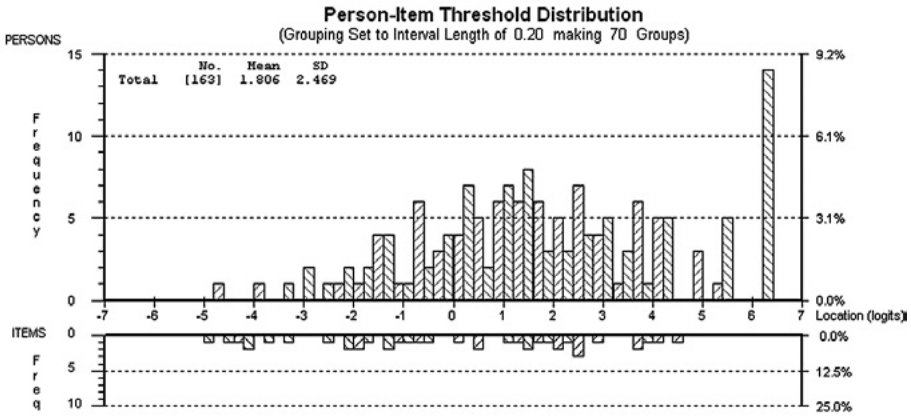
The DM1-Activ demonstrated acceptable correlations with the MRC sum score and the MIRS (intraclass correlation coefficient 0.69 for MRC sum score, 0.71 for MIRS; $P<0.0001$), indicating good external construct validity (figure 8.6). MRC sum score and MIRS findings explained 52.2% of the DM1-Activ findings. Test-retest reliability studies of item difficulty and patient location estimates are graphically represented in figure 8.7 demonstrating DIF over time for the items' and patients' location. Almost all 20-items were located within the 95% CI lines, indicating ideal invariance. Intraclass correlation coefficient was 0.97 for the item estimates and 0.93 for patient locations.

**Table 8.3.** Final fit statistics for the 20-item activity and participation scale for myotonic dystrophy type 1 patients

| Item | Location[a] | SE | Residual[b] | P-value[c] |
|---|---|---|---|---|
| Are you able to... | | | | |
| wash your face? | -2.98 | 0.249 | -1.117 | 0.102158 |
| brush your teeth? | -2.455 | 0.233 | -0.497 | 0.633135 |
| take a shower? | -1.951 | 0.209 | -0.71 | 0.056922 |
| sit up straight? | -1.621 | 0.204 | 1.315 | 0.915187 |
| open toothpaste? | -1.443 | 0.202 | 2.157 | 0.139324 |
| prepare a meal? | -0.969 | 0.197 | 0.479 | 0.668827 |
| button your shirt/blouse? | -0.788 | 0.2 | 0.571 | 0.368305 |
| walking avoiding obstacles? | -0.432 | 0.184 | -2.24 | 0.042261 |
| perform a hobby? | 0.032 | 0.193 | -0.023 | 0.728921 |
| get in/out of a car? | 0.038 | 0.178 | -0.628 | 0.958695 |
| walk on uneven ground? | 0.088 | 0.181 | -1.062 | 0.578111 |
| bend over to pick up object? | 0.241 | 0.185 | -0.978 | 0.372796 |
| walk one flight of stairs? | 0.268 | 0.169 | -1.808 | 0.060336 |
| grab an object above head? | 0.383 | 0.18 | 1.243 | 0.738543 |
| perform study/work? | 0.848 | 0.22 | 0.668 | 0.342525 |
| stand up from lying down? | 1.197 | 0.185 | 2.23 | 0.007782 |
| do the cleaning? | 1.81 | 0.191 | -1.246 | 0.738787 |
| do sports? | 2.24 | 0.181 | 0.298 | 0.46463 |
| do the gardening? | 2.324 | 0.195 | -0.469 | 0.167667 |
| run? | 3.17 | 0.17 | -0.513 | 0.788601 |

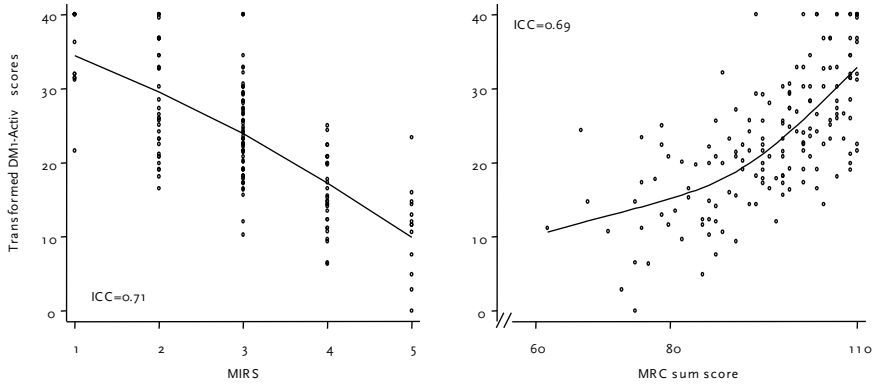
[a]expressed in linear log-odds units (logits) with mean item location set by convention at 0. [b]residuals summarize the deviation of observed from expected responses and lie within the recommended range of -2.5 and +2.5. [c]Bonferroni corrected probability scores were not significant, indicating no deviation from the model expectations.

**Figure 8.5.** Targeting graph of the DM1-Activ



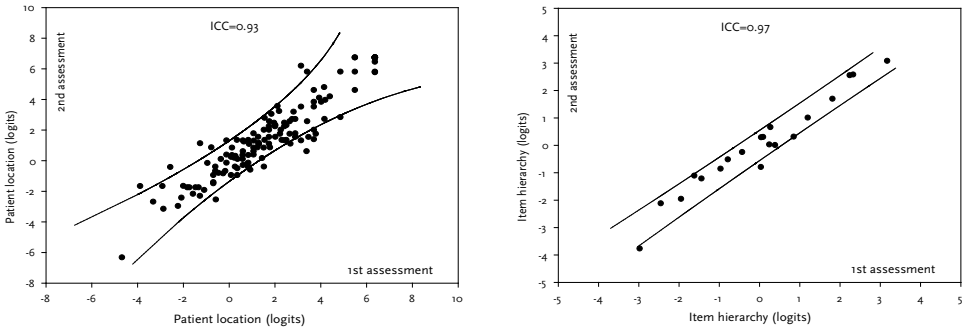

**Legend to figure 8.5.** Patient locations and item thresholds are displayed in the upper and the lower part of the graph, respectively. A well-targeted scale should include a set of items that span the full range of person estimates.

**Figure 8.6.** Relationship between DM1-activ measures and muscle strength scores




**Legend to figure 8.6.** DM1-Activ measures correlate significantly with MIRS grades (left panel) and the MRC sum score (right panel). A progressive decrease in activity and participation level was seen on the DM1-Activ Scale with increased muscular impairment. ICC=intraclass correlation coefficient.

**Figure 8.7.** Test-retest reliability




**Legend to figure 8.7.** Patient measures (left panel) and item difficulty hierarchy (right panel) in the first and second assessment are located within the 95% confidence intervals of the ideal invariance (solid lines). ICC=intraclass correlation coefficient.

## Discussion

We constructed an activity and participation measure for DM1 patients using the Rasch model (appendix A). The 20 items selected for the final DM1-Activ scale show no evidence of item bias or local dependency and demonstrate unidimensionality. Internal reliability of the DM1-Activ was high, indicating good differentiation between patients with different levels of disability. The observed invariance in item difficulty hierarchy after a delay of one year indicates that the questionnaire is reproducible over time. Furthermore, the estimated level of activity and participation remained relatively stable over a one-year period. In order to study the natural course of functional changes in DM1, measurements should be conducted over a much longer period of time.

As hypothesized in the introduction, most DM1-Activ items turned out to be different than those of the ACTIVLIM.[26] Five items were virtually the same (i.e. washing one's face, take a shower, walking outdoors, walking upstairs, and running), but demonstrated different difficulty levels. Moreover, some items of the DM1-Activ assess aspects of social participation. Based on these findings, a disease-specific Rasch-built scale would target the impact of DM1 more appropriately.

Acceptable correlation was also demonstrated between the DM1-Activ scale and traditional impairment measures (MRC sum score and MIRS), supporting its construct validity. It should be noted that the MRC sum score is an ordinal rating scale with unequal intervals, although it can be argued that summed items approximate interval measurement levels enough to warrant parametric

statistics. The DM1-Activ is able to indirectly capture physical impairment that leads to problems in daily and social functioning. Similar associations between impairment, disability and handicap have been reported in patients with chronic immune-mediated neuropathies.[40] However, only 50% of the DM1-Activ data was explained by the MRC sum score and MIRS data, suggesting that other variables contribute to activity limitations and participation restrictions in DM1. Factors like fatigue, pain, sleep disorders, behavioural problems, and gastrointestinal complaints, have been demonstrated to contribute to the deficits in daily and social activities.[5, 41-43] Focusing on these aspects is considered essential to capture the whole impact of a debilitating illness such as DM1, thereby improving the knowledge for guidance of patients, relatives and caregivers.

An international workshop on management of DM1 patients in 2004 focused on discussing published outcome measures in this disorder.[44] A list of available impairment and disability scales was presented. Unfortunately, construction of all presented measures were based on the classical test theory (CTT).[21] It should be realized that all ordinal scales are nonlinear and some caution should be taken when changes in sum scores are considered, since the whole score may not equal the sum of the parts.[22]

There are some methodological limitations in the present study that should be addressed. First, the use of an MRC sum score should be considered a shortcoming, although this outcome measure has been shown to fulfil all scientific requirements including responsiveness.[30, 45, 46] We aimed to illustrate the translation of general muscle weakness, measured with the MRC sum score, into limitations in daily activities and social participation, assessed by the DM1-Activ, hereby underlining the importance of the different aspects of an individual's functioning as defined by the ICF model.[11] Second, the analyses of DIF should be interpreted with some caution, since the sample sizes of the comparative groups were relatively restricted. Third, the DM1-Activ demonstrated a ceiling effect in 1 out of 12 patients which could lead to problems in future intervention studies. Fourth, the responsiveness of this scale needs to be investigated in a longitudinal study, taking into account the concept of minimum clinical important difference (MCID) to improve interpretability.[47-49] MCID has been defined as "the smallest difference in score in the domain of interest which patients perceive as beneficial and which would mandate, ..., a change in the patient's management".[49] MCID may provide an additional benchmark for future randomized controlled trials in all chronic illnesses, and may help clinicians and researchers better understand the clinical relevance of changes in scores. Practical guidelines for assessment of the MCID have been provided.[49]

## Conclusion

The constructed DM1-Activ scale is a practical, reliable and valid outcome measure and may help clinicians and caregivers to focus more on ameliorating the enormous disabling impact of this disorder on patients' functionality. Further scientific evaluation of the DM1-Activ to enhance its calibration is currently being conducted, in order to provide a reliable raw score-to-logit score conversion table (nomogram) for use in future DM1 clinical observational and interventional studies.

# References


1. Harper PS. Myotonic dystrophy. London: Saunders; 2001
2. Harley HG, Rundle SA, MacMillan JC, Myring J, Brook JD, Crow S, Reardon W, Fenton I, Shaw DJ, Harper PS. Size of the unstable ctg repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. *Am J Hum Genet*. 1993;52:1164-1174
3. Natterlund B, Gunnarsson LG, Ahlstrom G. Disability, coping and quality of life in individuals with muscular dystrophy: A prospective study over five years. *Disabil Rehabil*. 2000;22:776-785
4. Bostrom K, Natterlund BS, Ahlstrom G. Sickness impact in people with muscular dystrophy: A longitudinal study over 10 years. *Clin Rehabil*. 2005;19:686-694
5. Gagnon C, Mathieu J, Jean S, Laberge L, Perron M, Veillette S, Richer L, Noreau L. Predictors of disrupted social participation in myotonic dystrophy type 1. *Arch Phys Med Rehabil*. 2008;89:1246-1255
6. Antonini G, Soscia F, Giubilei F, De Carolis A, Gragnani F, Morino S, Ruberto A, Tatarelli R. Health-related quality of life in myotonic dystrophy type 1 and its relationship with cognitive and emotional functioning. *J Rehabil Med*. 2006;38:181-185
7. Wheeler TM, Thornton CA. Myotonic dystrophy: Rna-mediated muscle disease. *Curr Opin Neurol*. 2007;20:572-576
8. Wheeler TM. Myotonic dystrophy: Therapeutic strategies for the future. *Neurotherapeutics*. 2008;5:592-600
9. Wheeler TM, Sobczak K, Lueck JD, Osborne RJ, Lin X, Dirksen RT, Thornton CA. Reversal of rna dominance by displacement of protein sequestered on triplet repeat rna. *Science*. 2009;325:336-339
10. Aaronson NK. Quality of life: What is it? How should it be measured? *Oncology (Williston Park)*. 1988;2:69-76, 64
11. World Health Organization. International classification of functioning, disability and health. Geneva; 2001
12. Mathieu J, De Braekeleer M, Prévost C, Boily C. Myotonic dystrophy: Clinical assessment of muscular disability in an isolated population with presumed homogeneous mutation. *Neurology*. 1992;42:203-208
13. Orndahl G, Grimby G, Grimby A, Johansson G, Wilhelmsen L. Functional deterioration and selenium-vitamin e treatment in myotonic dystrophy. A placebo-controlled study. *J Intern Med*. 1994;235:205-210
14. Jaspert A, Fahsold R, Grehl H, Claus D. Myotonic dystrophy: Correlation of clinical symptoms with the size of the ctg trinucleotide repeat. *J Neurol*. 1995;242:99-104
15. Lindeman E, Leffers P, Spaans F, Drukker J, Reulen J, Kerckhoffs M, Koke A. Strength training in patients with myotonic dystrophy and hereditary motor and sensory neuropathy: A randomized clinical trial. *Arch Phys Med Rehabil*. 1995;76:612-620
16. Walter MC, Reilich P, Lochmuller H, Kohnen R, Schlotter B, Hautmann H, Dunkl E, Pongratz D, Muller-Felber W. Creatine monohydrate in myotonic dystrophy: A double-blind, placebo-controlled clinical study. *J Neurol*. 2002;249:1717-1722
17. Berard C, Payan C, Hodgkinson I, Fermanian J. A motor function measure for neuromuscular diseases. Construction and validation study. *Neuromuscul Disord*. 2005;15:463-470
18. Gagnon C, Mathieu J, Noreau L. Measurement of participation in myotonic dystrophy: Reliability of the life-h. *Neuromuscul Disord*. 2006;16:262-268
19. Streiner DL, Norman GR. Health measurement scales. A practical guide to their development and use. New York: Oxford University Press; 1995
20. Mathieu J, Boivin H, Meunier D, Gaudreault M, Bégin P. Assessment of a disease-specific muscular impairment rating scale in myotonic dystrophy. *Neurology*. 2001;56:336-340
21. DeVellis RF. Classical test theory. *Med Care*. 2006;44:S50-59
22. Stucki G, Daltroy L, Katz JN, Johannesson M, Liang MH. Interpretation of change scores in ordinal clinical scales and health status measures: The whole may not equal the sum of the parts. *J Clin Epidemiol*. 1996;49:711-717
23. Rasch G. Probalilistic models for some intelligence and attainment tests. Chicago: University of Chicago Press; 1980
24. Bond TG, Fox CM. Applying the rasch model: Fundamental measurement for the human sciences. New York: Lawrence Erlbaum Associates; 2001
25. Pallant JF, Tennant A. An introduction to the rasch measurement model: An example using the hospital anxiety and depression scale (hads). *Br J Clin Psychol*. 2007;46:1-18

26. Vandervelde L, van den Bergh PYK, Goemans N, Thonnard JL. Activlim: A rasch-built measure of activity limitations in children and adults with neuromuscular disorders. *Neuromuscul Disord*. 2007;17:459-469
27. Bombardier C, Tugwell P. A methodological framework to develop and select indices for clinical trials: Statistical and judgmental approaches. *J Rheumatol*. 1982;9:753-757
28. Wright BD, Stone MH. Best test design. Chicago: Mesa Press; 1979
29. Medical Research Council. Aids to the examination of the peripheral nervous system. London: Her Majesty's Stationary Office; 1943:1-2
30. Kleyweg RP, van der Meche FG, Schmitz PI. Interobserver agreement in the assessment of muscle strength and functional abilities in guillain-barre syndrome. *Muscle Nerve*. 1991;14:1103-1109
31. Andrich D, Lyne A, Sheridan B, Luo G. Rumm 2020. 2003
32. Linacre JM. Sample size and item calibration stability. *Rasch Measure Trans*. 1994;7:28
33. Altman DG. Practical statistics for medical research. London: Chapman and Hall; 1991
34. Fisher WP. Reliability statistics. *Rasch Measure Trans*. 1992;6:238
35. Dorans NJ, Holland PW. Dif detection and description: Mantel-haenszel and standardisation. In: Holland PWW, H., ed. Differential item functioning. Hillsdale: Lawrence Erlbaum Associates; 1993:36-66
36. Embretson SE, Reise SP. Item response theory for psychologists. New Jersey: Lawrence Erlbaum Associates; 2000
37. Tennant A, Conaghan PG. The rasch measurement model in rheumatology: What is it and why use it? When should it be applied, and what should one look for in a rasch paper? *Arthritis Rheum*. 2007;57:1358-1362
38. Herndon JE, Harrell FE. The restricted cubic spline hazard model. *Commun Stat Theory Methods*. 1990:639-663
39. Bland JM, Altman DG. Multiple significance tests: The bonferroni method. *BMJ*. 1995;310:170
40. Merkies IS, Schmitz PI, van der Meche FG, Samijn JP, van Doorn PA. Connecting impairment, disability, and handicap in immune mediated polyneuropathies. *J Neurol Neurosurg Psychiatry*. 2003;74:99-104
41. Laberge L, Bégin P, Montplaisir J, Mathieu J. Sleep complaints in patients with myotonic dystrophy. *J Sleep Res*. 2004;13:95-100
42. Ronnblom A, Forsberg H, Danielsson A. Gastrointestinal symptoms in myotonic dystrophy. *Scand J Gastroenterol*. 1996;31:654-657
43. Kalkman JS, Schillings ML, van der Werf SP, Padberg GW, Zwarts MJ, van Engelen BG, Bleijenberg G. Experienced fatigue in facioscapulohumeral dystrophy, myotonic dystrophy, and hmsn-i. *J Neurol Neurosurg Psychiatry*. 2005;76:1406-1409
44. van Engelen BG, Eymard B, Wilcox D. 123rd enmc international workshop: Management and therapy in myotonic dystrophy, 6-8 february 2004, naarden, the netherlands. *Neuromuscul Disord*. 2005;15:389-394
45. Hughes RA, Donofrio P, Bril V, Dalakas MC, Deng C, Hanna K, Hartung HP, Latov N, Merkies IS, van Doorn PA. Intravenous immune globulin (10% caprylate-chromatography purified) for the treatment of chronic inflammatory demyelinating polyradiculoneuropathy (ice study): A randomised placebo-controlled trial. *Lancet Neurol*. 2008;7:136-144
46. van der Meche FG, Schmitz PI. A randomized trial comparing intravenous immune globulin and plasma exchange in guillain-barre syndrome. Dutch guillain-barre study group. *N Engl J Med*. 1992;326:1123-1129
47. Liang MH. Evaluating measurement responsiveness. *J Rheumatol*. 1995;22:1191-1192
48. Jaeschke R, Singer J, Guyatt GH. Measurement of health status. Ascertaining the minimal clinically important difference. *Control Clin Trials*. 1989;10:407-415
49. Sloan JS, T. Vargas-Chanes, D. Fridley, B. Practical guidelines for assessing the clinical significance of health related quality of life within clinical trials. *Drug Information Journal*. 2003;37:23-31

Chapter 9

# Reconstructing the Rasch-built Myotonic Dystrophy type 1 Activity and Participation Scale (DM1-Activ$^{c}$)

*Mieke C.E. Hermans[a], Ingemar S.J. Merkies[a,b], Catharina G. Faber[a]*

[a]Department of Neurology, Maastricht University Medical Centre, Maastricht
[b]Department of Neurology, Spaarne Hospital, Hoofddorp

*Manuscript in preparation*

## Summary

We have recently published the first Rasch-built activity and participation scale specifically designed for patients with myotonic dystrophy type 1 (DM1-Activ). However, strengthening of the scale was needed in a larger cohort of patients to enable its clinical application. We report the results of the reconstructed DM1-Activ for clinical use (DM1-Activ$^{c}$) through Rasch analyses on an expanded questionnaire containing 146 activity and participation inquiries that was completed by 340 patients with DM1. Through stepwise investigation including data quality control, model fit, response category ordering, local dependency and item bias, we succeeded in reconstructing the DM1-Activ$^{c}$ consisting of 25 items that showed good Rasch model fit, including validity, reliability, and unidimensionality. Also, a nomogram is provided to calculate interval-level logit scores from the obtained raw scores. The DM1-Activ scale has been reconstructed (DM1-Activ$^{c}$) and its use is recommended in future studies in patients with DM1.

## Introduction

At current stage, treatment of patients with myotonic dystrophy type 1 (DM1) is almost exclusively limited to symptomatic interventions without therapeutic options to reverse of slow down the progression of the illness. However, since genetically based new therapeutic treatments are emerging, solid outcome measures are needed to capture possible relevant clinical changes in patients with DM1 being exposed to these new therapies.[1, 2]
Recently, we have described the first Rasch-built activity and participation measure specifically designed for patients with myotonic dystrophy type 1 (DM1-Activ).[3, 4] The DM1-Activ fulfilled all model requirements and is thus far the only metric that has been extensively evaluated in this condition. However, further evaluation of the scale is needed in a much larger pool of patients to obtain a more stable model and provide a reliable raw score-to-logit score conversion table for use in future DM1 clinical studies.[3]
We have reconstructed the DM1Activ scale for clinical use (DM1-Activ$^c$) using a larger sample of patients with DM1 and a much broader pool of items representing activity and participation activities.[5]

## Methods

### Participants

Patients older than 18 years with genetically proven DM1 were recruited through the Dutch neuromuscular patients' association. The protocol was approved by the Medical Ethics Committee of our university. Written informed consent was obtained from all participants. A total of 340 patients with DM1 were included, fulfilling optimal sample size requirements for scale construction.[6]

### Questionnaire development

The questionnaire was constructed as previously described.[3] In addition to the 49 previously selected items from the pre-phase DM1-Activ scale, we collected extra activities and participation items from the WHO-ICF items list.[1, 5] The preliminary DM1-Activ$^c$ scale contained 146 activity and participation activities. Response options were: unable to perform (0), able to perform, but with difficulty (1), and able to perform, without any difficulty (2) and are based on the previously determined discriminative ability of DM1 patients.[3] An item was scored (3) if it was "not applicable" to the patient.
The preliminary DM1-Activ$^c$ was completed by 340 patients. A random sample of 223 patients completed the questionnaire ~4 weeks later (test-retest reliability). The original 20-item DM1-Activ scale was also completed separately (external construct validity).

### Rasch analysis

Obtained preliminary DM1-Activ$^{c}$ data were subjected to Rasch analysis to determine whether model expectations were met. Several aspects were addressed including fit statistics, ordered thresholds, local independency, differential item functioning (item bias: DIF) and unidimensionality. Item bias was checked on personal factors: age (<30 years, 30-50 years, >50 years), gender, diagnosis phenotype (mild type, adult type, childhood/congenital type), and degree of education (elementary school, high school, university). Items or patients not fulfilling these requirements were removed or adjusted to obtain good model fit, hereby creating an interval scale.[3, 7, 8]

### Validity and reliability

Internal consistency reliability of the DM1-Activ$^{c}$ scale was determined by calculating the Person Separation Index (PSI). The external construct validity of the DM1-Activ$^{c}$ scale was tested by correlations with the original 20-item DM1-Activ scale, using logits scores from both scales. Moreover, test-retest reliability studies were performed to investigate consistency of item difficulty hierarchy and patient ability locations. Reliability was quantified by calculation of the intraclass correlation coefficient using ANOVA for group comparison.[9, 10]

## Statistics

All analyses were conducted using the Rasch model (partial credit model), as implemented in the RUMM2030 software.[11] Further analyses were undertaken using Stata Statistical Software version 11.0 with Bonferroni corrections if needed.[12]

# Results

### General aspects and data quality control

The basic characteristics of the 340 patients are presented in the table 9.1. As part of data quality control, items with >10% missing values (n=29) and items with inadequate face validity (n=12) according to experts' opinion (CF, IM) were removed. In the model construction, items scored as (3) "not applicable" were interpreted as missing data. Also, 28 patients were removed prior to Rasch analyses (16 without age record, 6 with unknown phenotype classification, 3 with unknown gender, 2 with >10% uncompleted items, and 1 with unknown educational level). A total of 105 items and 312 patient records were finally subjected to Rasch analyses, continuously checking the distribution of persons within the class intervals.

**Table 9.1.** Patient sample description

| | n=340 |
|---|---|
| Age (years) | |
| mean (SD), range | 47.5 (12.5), 18-82 |
| Gender (%) | |
| female | 49.7% |
| male | 50.3% |
| Diagnosis type (%) | |
| mild | 12.1% |
| adult | 77.3% |
| child/congenital | 10.6% |
| Educational level (%) | |
| elementary school | 15.9% |
| high school | 69.4% |
| university | 14.7% |

### Rasch analysis of the pre-phase DM1-Activ$^{c}$ scale

The preliminary 105-items DM1-Activ$^{c}$ showed overall misfit. The mean residual for items was -0.085 (SD 1.213) and for persons -0.278 (SD 1.361), indicating reasonable model fit. However, the item-trait chi-square probability was significant ($P$<0.00001; degrees of freedom (DF) 420), indicating lack of invariance. No disordered thresholds were seen. A proportion of 0.14 (95% CI 0.11-0.17) of the t-tests performed fell outside the ±1.96 range, indicating multidimensionality.

### Fitting data to the Rasch model

The individual item fit statistics of 32 items demonstrated misfit to the model (having significant chi-square probability and/or fit residuals exceeding ±2.5) and were removed one by one (73 items remaining).

Local dependency was seen between many items (r≥0.3). Starting with the highest correlations (r≥0.7, then ≥0.6, through to ≥0.3), the item of each correlating set of items showing the least face validity according to experts' opinion (CF, IM) or over- or under-discrimination on its category probability curve, was removed (45 items removed stepwise; 28 items remaining).

One item showed item bias (uniform DIF) on age and diagnosis phenotype and two items had non-uniform DIF on diagnosis phenotype. All 3 items were removed. Hence, at this stage 25 items were kept. Item "able to run" demonstrated uniform DIF on age. This item was one of the most difficult activities to perform. Since we aimed to obtain a wide range of measurement, we decided to keep this item in the model. However, before splitting this item and creating 2 subgroups (age <30 years versus ≥30 years), a test for

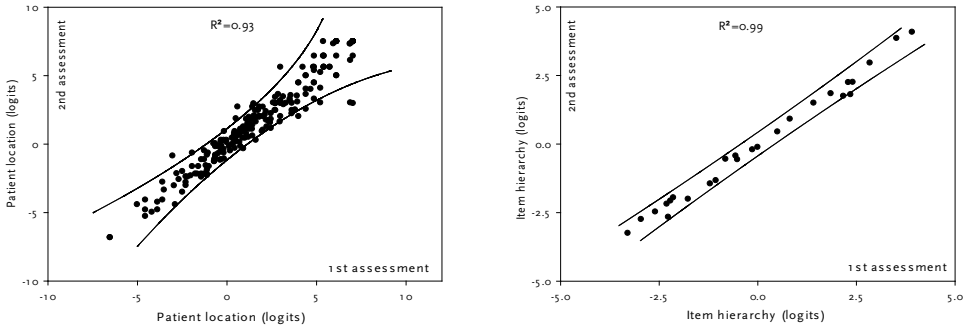
unidimensionality was performed, since RUMM2030 software does not provide the opportunity to do this after splitting an item.

**Clinimetric properties of the final DM-Activ$^c$**

The 25-item DM1-Activ$^c$ met all Rasch model expectations. The mean fit residual for items was -0.197 (SD 0.869) and for persons -0.273 (SD 0.774). The overall item-trait interaction chi-square probability was non-significant ($P$=0.24; DF 100), indicating the required property of invariance. The independent t-tests between person estimates from 7 most positive versus 7 most negative loaded items demonstrated a proportion of 0.067 (95% CI 0.043-0.091) falling outside the ±1.96 range, suggesting acceptable unidimensionality. Subsequently, the item "able to run" was split into 2 separate age-dependent items.

In the final DM1-Activ$^c$ scale (table 9.2), item "able to eat soup" (-3.305 logits) was the easiest item while "able to run" for patients ≥30 years (3.904 logits) was the most difficult item. Two patients (0.6%) demonstrated no activity limitations or participation restrictions (floor effect), while 23 patients (7.4%) had a maximum score (ceiling effect). Table 9.3 provides a nomogram allowing the translation of raw summed scores of the final DM1-Activ$^c$ (range 0 to 50) into logits, and subsequently into a more easily interpretable centile metric score ranging from 0 (most severe activity and social participation restrictions) to 100 (no activity and social participation limitations).

**Figure 9.1.** Test-retest reliability




**Legend figure 9.1.** Patient measures (left panel) and item difficulty hierarchy (right panel) in the first and second assessment are located within the 95% confidence intervals of the ideal invariance (solid lines). The fraction of variance is expressed by $R^2$.

**External construct validity and reliability**

The 25-item DM1-Activ[c] scale demonstrated high external construct validity ($R^2$=0.91 correlation with the 20-items DM1-Activ; *P*<0.0001).[3] Internal reliability was robust (PSI=0.95). The test-retest reliability for patient location and item difficulty was good ($R^2$=0.93 and $R^2$=0.99, respectively; *P*<0.0001; figure 9.1). All item and almost all patient locations were within the 95% CI lines, reflecting ideal invariance.

**Table 9.2**. Final fit statistics for the 25-item DM1-Activ[c] scale

| Item | Location[a] | SE | Residual[b] | P-value[c] |
|---|---|---|---|---|
| **Are you able to...** | | | | |
| eat soup? | -3.305 | 0.198 | -0.185 | 0.610198 |
| visit family or friends? | -2.967 | 0.176 | -0.113 | 0.653494 |
| care for your hair and body? | -2.608 | 0.18 | -0.136 | 0.793144 |
| dress your lower body? | -2.314 | 0.162 | -0.314 | 0.532921 |
| wash your upper body? | -2.278 | 0.164 | 0.076 | 0.933869 |
| take a shower? | -2.222 | 0.162 | -0.91 | 0.422836 |
| wash your lower body? | -2.15 | 0.16 | -0.549 | 0.80354 |
| get out of bed? | -1.777 | 0.151 | 1.836 | 0.003088 |
| move a chair? | -1.217 | 0.143 | -0.609 | 0.811204 |
| do the dusting/cleaning? | -1.071 | 0.145 | -0.743 | 0.514443 |
| do the shopping? | -0.825 | 0.137 | 0.818 | 0.452093 |
| tie the laces of your shoes? | -0.569 | 0.134 | 1.727 | 0.218672 |
| catch an object (e.g. a ball)? | -0.527 | 0.134 | 0.268 | 0.282685 |
| use dustpan and brush? | -0.145 | 0.132 | -0.821 | 0.605114 |
| empty dustbin? | -0.013 | 0.127 | -1.434 | 0.294051 |
| make up your bed? | 0.491 | 0.128 | -0.238 | 0.740776 |
| vacuum clean? | 0.807 | 0.124 | -1.127 | 0.195076 |
| serve coffee/tea on a tray? | 1.408 | 0.121 | -0.918 | 0.587366 |
| dance? | 1.844 | 0.125 | 0.149 | 0.08681 |
| stand up from squatting position? | 2.162 | 0.129 | 0.534 | 0.580312 |
| stand on one leg? | 2.287 | 0.126 | 1.141 | 0.100675 |
| run (*for those <30 years*)? | 2.343 | 0.486 | -2 | 0.823465 |
| walk uphill? | 2.399 | 0.133 | -1.085 | 0.628618 |
| walk 3 flights of stairs? | 2.832 | 0.134 | -1.114 | 0.140408 |
| carry and put down heavy object (10 kg)? | 3.509 | 0.139 | -0.674 | 0.244503 |
| run (*for those ≥ 30 years*)? | 3.904 | 0.148 | -0.599 | 0.709763 |

[a]expressed in linear log-odds units (logits) with mean item location set by convention at 0. [b]residuals summarize the deviation of observed from expected responses and lie within the recommended range of -2.5 and +2.5. [c]Bonferroni corrected probability scores were not significant, indicating no deviation from the model expectations.

**Table 9.3.** Nomogram

| Age <30 years | | | Age ≥30 years | | |
|---|---|---|---|---|---|
| Raw sumscore | Person location (logit) | Centile metric score | Raw sumscore | Person location (logit) | Centile metric score |
| 0 | -6,551 | 0 | 0 | -6,55 | 0 |
| 1 | -5,67 | 7 | 1 | -5,669 | 6 |
| 2 | -5,037 | 11 | 2 | -5,036 | 11 |
| 3 | -4,582 | 15 | 3 | -4,58 | 15 |
| 4 | KEY1 | KEY1 | 4 | KEY2 | KEY2 |
| 5 | -3,901 | 20 | 5 | -3,899 | 20 |
| 6 | -3,623 | 22 | 6 | -3,62 | 22 |
| 7 | -3,37 | 24 | 7 | -3,367 | 23 |
| 8 | -3,136 | 25 | 8 | -3,133 | 25 |
| 9 | -2,918 | 27 | 9 | -2,913 | 27 |
| 10 | -2,711 | 29 | 10 | -2,706 | 28 |
| 11 | -2,514 | 30 | 11 | -2,508 | 30 |
| 12 | -2,325 | 31 | 12 | -2,318 | 31 |
| 13 | -2,143 | 33 | 13 | -2,135 | 33 |
| 14 | -1,967 | 34 | 14 | -1,958 | 34 |
| 15 | -1,796 | 35 | 15 | -1,786 | 35 |
| 16 | -1,629 | 37 | 16 | -1,617 | 36 |
| 17 | -1,465 | 38 | 17 | -1,452 | 38 |
| 18 | -1,305 | 39 | 18 | -1,29 | 39 |
| 19 | -1,147 | 40 | 19 | -1,13 | 40 |
| 20 | -0,992 | 41 | 20 | -0,972 | 41 |
| 21 | -0,838 | 43 | 21 | -0,816 | 42 |
| 22 | -0,685 | 44 | 22 | -0,661 | 43 |
| 23 | -0,533 | 45 | 23 | -0,506 | 45 |
| 24 | -0,383 | 46 | 24 | -0,352 | 46 |
| 25 | -0,233 | 47 | 25 | -0,198 | 47 |
| 26 | -0,083 | 48 | 26 | -0,045 | 48 |
| 27 | 0,067 | 49 | 27 | 0,109 | 49 |
| 28 | 0,216 | 50 | 28 | 0,264 | 50 |
| 29 | 0,367 | 52 | 29 | 0,419 | 51 |
| 30 | 0,519 | 53 | 30 | 0,576 | 53 |
| 31 | 0,672 | 54 | 31 | 0,735 | 54 |
| 32 | 0,828 | 55 | 32 | 0,897 | 55 |
| 33 | 0,988 | 56 | 33 | 1,063 | 56 |
| 34 | 1,151 | 57 | 34 | 1,233 | 57 |
| 35 | 1,32 | 59 | 35 | 1,409 | 59 |
| 36 | 1,495 | 60 | 36 | 1,593 | 60 |
| 37 | 1,679 | 61 | 37 | 1,785 | 61 |

| Age <30 years | | | Age ≥30 years | | |
|---|---|---|---|---|---|
| Raw sumscore | Person location (logit) | Centile metric score | Raw sumscore | Person location (logit) | Centile metric score |
| 38 | 1,873 | 63 | 38 | 1,988 | 63 |
| 39 | 2,079 | 64 | 39 | 2,204 | 65 |
| 40 | 2,301 | 66 | 40 | 2,437 | 66 |
| 41 | 2,543 | 68 | 41 | 2,689 | 68 |
| 42 | 2,808 | 70 | 42 | 2,965 | 70 |
| 43 | 3,101 | 72 | 43 | 3,269 | 72 |
| 44 | 3,428 | 74 | 44 | 3,605 | 75 |
| 45 | 3,791 | 77 | 45 | 3,976 | 78 |
| 46 | 4,197 | 80 | 46 | 4,388 | 81 |
| 47 | 4,657 | 83 | 47 | 4,849 | 84 |
| 48 | KEY3 | KEY3 | 48 | KEY4 | KEY4 |
| 49 | 5,923 | 93 | 49 | 6,096 | 93 |
| 50 | 6,871 | 100 | 50 | 7,016 | 100 |

Translation of raw sum scores into logits or centile metric score is only possible if there are no missing data. KEYs are available by contacting the corresponding author.

## Discussion

In the current study, the reconstructed DM1-Activ[c] outcome measure for activity limitations and participation restrictions is presented (appendix B). This 25-item scale has been specifically designed for patients with DM1 and fulfilled all Rasch model expectations, with robust validity and reliability aspects. We have reconstructed the DM1-activ scale in a larger sample of patients in order to provide a better and more stable model with a nomogram for use in future DM1 clinical studies (table 9.3).

The internal reliability (PSI) of the DM1-Activ[c] scale was high, demonstrating discriminative ability of the scale to differentiate between patients with various levels of disability.[9] Its responsiveness is currently being examined as part of an ongoing natural course study. As previously stated, the concept of minimum clinically important difference (MCID) may also help clinicians and researchers to determine whether significant statistical differences between scores imply a clinically relevant change.[3] Practical guidelines for assessment of the MCID

have been provided and will be implemented in the natural course study.[13] Compared to the previously published 20-item DM1-Activ scale,[3] the current reconstructed DM1-Activ$^{c}$ contains five more items. The item difficulties of the DM1-Activ$^{c}$ cover a wider range than the DM1-Activ (7.209 logits versus 6.15 logits), thus providing a better targeting of patients. This was also reflected in a lower percentage of ceiling effect in DM1 patients (7.4% versus 8.6%).[3]

## Conclusion

The reconstructed activity and participation scale specifically designed for patients with DM1 (DM1-Activ$^{c}$) fulfills all modern clinimetric requirements. Its use is proposed in future clinical observational and interventional studies studies in DM1 patients.

## References


1. Streiner DL, Norman GR. Health measurement scales. A practical guide to their development and use. New York: Oxford University Press; 1995
2. Magana JJ, Cisneros B. Perspectives on gene therapy in myotonic dystrophy type 1. *J Neurosci Res*. 2011;89:275-285
3. Hermans MC, Faber CG, De Baets MH, de Die-Smulders CE, Merkies IS. Rasch-built myotonic dystrophy type 1 activity and participation scale (dm1-activ). *Neuromuscul Disord*. 2010;20:310-318
4. Rasch G. Probabilistic models for some intelligence and attainment tests. Chicago: University of Chicago Press; 1980
5. World Health Organization. International classification of functioning, disability and health. Geneva; 2001
6. Linacre JM. Sample size and item calibration stability. *Rasch Measure Trans*. 1994;7:28
7. Tennant A, Conaghan PG. The rasch measurement model in rheumatology: What is it and why use it? When should it be applied, and what should one look for in a rasch paper? *Arthritis Rheum*. 2007;57:1358-1362
8. Pallant JF, Tennant A. An introduction to the rasch measurement model: An example using the hospital anxiety and depression scale (hads). *Br J Clin Psychol*. 2007;46:1-18
9. Fisher WP. Reliability statistics. *Rasch Measure Trans*. 1992;6:238
10. Wright BD, Stone MH. Best test design. Chicago: Mesa Press; 1979
11. Andrich D, Lyne A, Sheridan B, Luo G. Rumm 2020. 2003
12. Bland JM, Altman DG. Multiple significance tests: The bonferroni method. *BMJ*. 1995;310:170
13. Sloan JA. Assessing the minimally clinically significant difference: Scientific considerations, challenges and solutions. *COPD*. 2005;2:57-62

Chapter 10

# Fatigue and Daytime Sleepiness Scale in Myotonic Dystrophy type 1

*Mieke C.E. Hermans[a], Ingemar S.J. Merkies[a,b], Luc Laberge[c,d], Eveline W. Blom[e], Allan Tennant[f], Catharina G. Faber[a]*

[a] Department of Neurology, Maastricht University Medical Centre, Maastricht
[b] Department of Neurology, Spaarne Hospital, Hoofddorp
[c] Département des sciences de la santé, Université du Québec à Chicoutimi, Canada
[d] ÉCOBES Recherche et transfert, Cégep de Jonquière, Jonquière, Canada
[e] Department of Clinical Genetics, Maastricht University Medical Centre, Maastricht
[f] Department of Rehabilitation Medicine, University of Leeds, Leeds, United Kingdom

*Muscle and Nerve 2013 Jan;47(1):89-95*

## Summary

Fatigue and excessive daytime sleepiness are frequent complaints in myotonic dystrophy type 1 (DM1) that often overlap. We aimed to construct a combined fatigue and daytime sleepiness rating scale for DM1 using the Rasch measurement model. Questionnaires, including the Epworth Sleepiness Scale, Fatigue Severity Scale, and Daytime Sleepiness Scale were completed by 354 patients. Data were subjected to Rasch analyses and tested for required measurement issues such as appropriate response categories, absence of item bias, local independence, and unidimensionality. The initial 22 items did not meet Rasch model expectations. After rescoring and removing misfitting items, the final 12-item scale showed good model fit and unidimensionality. High internal consistency (person separation index=0.80) and validity were demonstrated. The Rasch-built Fatigue and Daytime Sleepiness Scale, developed specifically for DM1 patients, provides interval measures on a single continuum. Its use is suggested in future clinical trials and therapeutic follow-up.

## Introduction

Myotonic dystrophy type 1 (DM1) is an autosomal dominant inherited multisystem disorder. It affects skeletal and smooth muscles, the heart, the eyes, the endocrine system and central nervous system. The diagnosis of DM1 is confirmed by molecular genetic testing of an expansion of a CTG trinucleotide repeat in the DMPK gene.[1] Based on age at onset and severity of symptoms, four clinical types can be distinguished: congenital type, childhood-onset type, adult-onset (classic) type and late-onset (mild) type.[2]

Excessive daytime sleepiness (EDS) is an important and common clinical feature of DM1 which occurs in about one third of patients.[3,4] Unlike narcolepsy, daytime sleepiness in DM1 patients is not episodic, and sleep tendency occurs when attention is not being held, rather than during activity. EDS can be a symptom of sleep-disordered breathing, chronic hypercapnia, or depression, but these conditions alone cannot entirely explain EDS in DM1.[5-7] Complaints of excessive fatigue appear even more frequently than those of EDS in patients with DM1.[8] DM1-related fatigue is characterized by a subjective lack of physical and/or mental energy. Although fatigue is an important symptom in any progressive physically disabling disease, it is more common in DM1 than in other neuromuscular disorders and may even be prominent when muscular impairment is relatively mild.[9] The presence of fatigue can have a major impact in daily life, general well-being, and social participation.[9]

EDS and fatigue have overlapping features, and both patients and physicians may have difficulty distinguishing between these 2 entities. Patients cannot always specify whether their complaint relates to sleepiness, fatigue, or both.[10] Several patient-based measures are available to evaluate daytime sleepiness or fatigue levels, but generally as separate entities. However, fatigue and EDS levels are associated in DM1 patients,[8,9] suggesting that available outcome measures do not necessarily represent separate constructs.

Current outcome measures are all ordinal scales based on classical test theory (CTT).[11] A major limitation of CTT is that scores create measurement at an ordinal level with unequal intervals that hamper accurate measurement of differences in scores and changes over time among individuals. Rasch analysis attempts to transform ordinal scores into interval measures that are scale independent and suitably accurate for individual patient assessment.[12,13] This methodology has previously been used to develop an outcome measure of activity limitations and participation restrictions for DM1 patients (DM1-Activ).[14] In view of the multisystem nature of this disease, health outcome measures at several levels of outcome are necessary for patient assessment. We aimed to construct a combined fatigue and daytime sleepiness rating scale (FDSS) for DM1 using the Rasch method, provided that fatigue and daytime sleepiness items address the same underlying health construct.

## Methods

### Participants and procedures

The study population comprised 354 adult DM1 patients (167 recruited at Maastricht University Medical Center in the Netherlands and 187 patients at Neuromuscular Clinic of the Centre de Santé et de Services Sociaux de Jonquière in Canada).[8] The study was approved by both local Medical Ethics Committees, and informed consent was obtained from all participants. A description of the patient sample is provided in table 10.1.

**Table 10.1.** Patient sample description

| | **Dutch population n=167** | **Canadian population n=187** |
|---|---|---|
| Age (years) | | |
| mean (SD), range | 44.1 (11.6), 18-69 | 46.0 (11.0), 20-80 |
| Gender (n, %) | | |
| female | 81 (48.5) | 115 (61.5) |
| male | 86 (51.5) | 72 (38.5) |
| Diagnosis type (n, %) | | |
| mild type | 14 (8.4) | 36 (19.3) |
| adult type | 137 (82.0) | 151 (80.7) |
| childhood/congenital type | 16 (9.6) | - |

The concept scale was composed of all 22 items from the Epworth Sleepiness Scale (ESS), Daytime Sleepiness Scale (DSS) and Fatigue Severity Scale (FSS). The ESS is a widely used questionnaire intended to measure daytime sleep propensity.[15, 16] Patients rate their chances of dozing on 8 everyday situations on a 4-point scale. Good reliability aspects have been demonstrated for the ESS in subjects with sleep-disordered breathing, primary sleep disorders and healthy subjects.[17-19] However, in DM1, weak internal consistency was reported, possibly because some items were irrelevant or inappropriate for these patients.[20] The 5-item DSS was built to assess daytime sleepiness in DM1 and showed good validity and reliability.[4, 20] The FSS is the questionnaire used most commonly to assess the impact of fatigue on daily activities. It contains 9 items, and each item is scored on a 7-point Likert scale. This scale has demonstrated high internal consistency and adequate validity.[21] An evaluation in DM1 patients showed good reliability of the FSS.[20]

All patients completed a questionnaire including socio-demographic and clinical information, and it containing ESS, FSS, and DSS items placed randomly. Patients were examined by a neurologist, and muscular impairment was categorized according to the muscular impairment rating scale (MIRS).[22]

### Rasch analysis

The Rasch unidimensional measurement model was used to construct the fatigue and sleepiness scale. Chapter 7 clearly explains the various steps of the Rasch method. In brief, the Rasch model can be seen as the ideal response pattern, where persons with a high level of the measured trait should have a higher probability of receiving a higher score on any item compared to persons with lower levels. Also, any person should always have a greater probability of receiving a higher score on an easier item than on a more difficult one.[12] In order to obtain an interval scale, the final scale should fulfill the model's expectations, like good item and person statistical fit, threshold ordering, lack of item bias or local dependency, and with demonstrated unidimensionality.[23-26] We assessed potential differential item functioning (DIF or item bias) for 6 person factors: gender, age groups (<30 years, 30-50 years, >50 years), diagnosis type (mild, adult, childhood/congenital), use of psychostimulants (yes, no), degree of education (elementary school, high school, university) and possible cultural differences (Dutch versus Canadian). Items and persons that did not fulfill Rasch model criteria were evaluated and removed one by one, if needed.

### Validity and reliability

The external construct validity of the scale was assessed by correlation with the MIRS. An estimate of the internal consistency reliability of the scale is available, based on the person separation index (PSI). PSI (equivalent to the Cronbach's alpha) should be greater than 0.7 for group comparison.[23]

## Statistics

All analyses were conducted using the Rasch model (partial credit model), as implemented in the RUMM2030 software.[25] Further analyses were undertaken using Stata Statistical Software version 11.0. One-way analysis of variance with corrections according to Bonferroni multiple testing was used to compare FDSS scores between subgroups.[27]



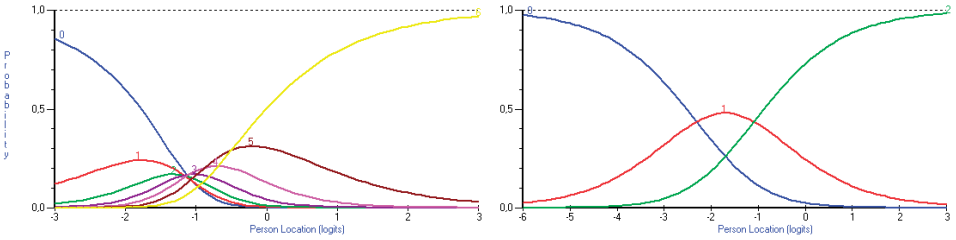
# Results

### Rasch analysis

The draft 22-item scale did not meet the Rasch model expectations. The item-trait chi-square probability was significant ($P$<0.00001), indicating lack of invariance of item difficulty across the scale. The mean residual for items was 0.24 (SD 2.44). The mean residual for persons was -0.26 (SD 1.14), indicating no serious misfit among respondents. The person separation index was 0.91. Thresholds were examined to see if disordering affected fit. Category probability

curves showed disordered thresholds for 4 ESS items, 1 DSS item and for all FSS items. In order to restore threshold ordering, response options for all items were rescored with the aim of creating a uniform set of response categories and strengthening the category frequencies.[28] For the ESS and DSS items, the original 4 response options (coded 0-1-2-3) were collapsed into 3 categories (coded 0-1-1-2). The original FSS items with 7-point response categories (coded 1-2-3-4-5-6-7) demonstrated a uniform disordered pattern and were subsequently collapsed into 3 categories (coded 0-0-1-1-1-2-2) (figure 10.1).

**Figure 10.1.** Disordered thresholds



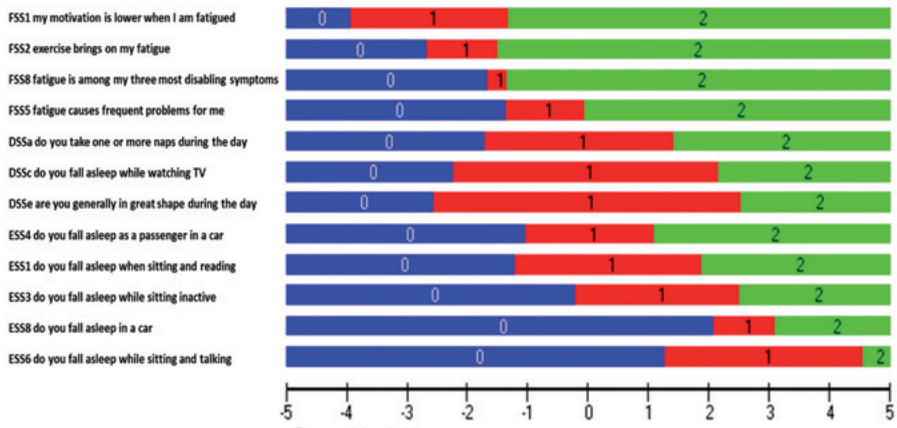

**Legend to figure 10.1.** Category probability curve for item FSS2 ("exercise brings on my fatigue") with 7 response categories (coloured curves correspond to score options 0 to 6) showing the relation between the probability of a given category as a function of person location. Left panel: thresholds were disordered and crowded together. Right panel: after rescoring into 3 response categories (coloured curves correspond to score options 0 to 2), showing ordered response categories and ordered thresholds. Note that each response category has a point along the fatigue and sleepiness continuum where it is the most likely response.

Next, we systematically checked for item misfit, local dependency and DIF. A total of 10 items were removed one by one based on the following: item ESS2 ("fall asleep while watching TV") was removed due to local dependency; items ESS5 ("fall asleep while lying down to rest in the afternoon when able"), EES7 ("fall asleep while sitting quietly after a lunch without alcohol"), DSSd ("difficulty being inactive for prolonged periods"), FSS4 ("fatigue interferes with my physical functioning"), FSS6 ("my fatigue prevents sustained physical functioning"), and FSS3 ("I am easily fatigued") were removed for showing significant misfit; item FSS7 ("fatigue interferes with carrying out certain duties and responsibilities") was removed due to item misfit and local dependency; items DSSb ("at times, sudden need to sleep during the day") and FSS9 ("fatigue interferes with my work, family or social life), showed differences in response between Dutch and Canadian patients with equal levels of excessive daytime sleepiness or fatigue (DIF related to cultural differences) and were also removed.

### Clinimetric properties of the final FDSS scale

After these steps, we succeeded in obtaining a 12-item scale that fulfilled all model expectations. Adequate item and patient fit statistics were obtained (mean fit residual -0.36 (SD 1.07) and mean fit residual -0.38 (SD 0.96), respectively). The overall item-trait interaction chi-square probability was non-significant (*P*= 0.61), thereby showing invariance. Finally, 2 item subsets were defined by the 4 most positive (ESS1, ESS3, ESS4, ESS6) and negative loading items (FSS1, FSS2, FSS5, FSS8) using a principal component analysis of residuals (see figure 10.2 for item identification). An independent t-test between person estimates from these 2 subsets of items demonstrated a proportion of 0.05 (95%CI: 0.03-0.08) of the tests falling outside the ±1.96 range, supporting unidimensionality of the scale.

**Figure 10.2.** Threshold map of the 12-item FDSS

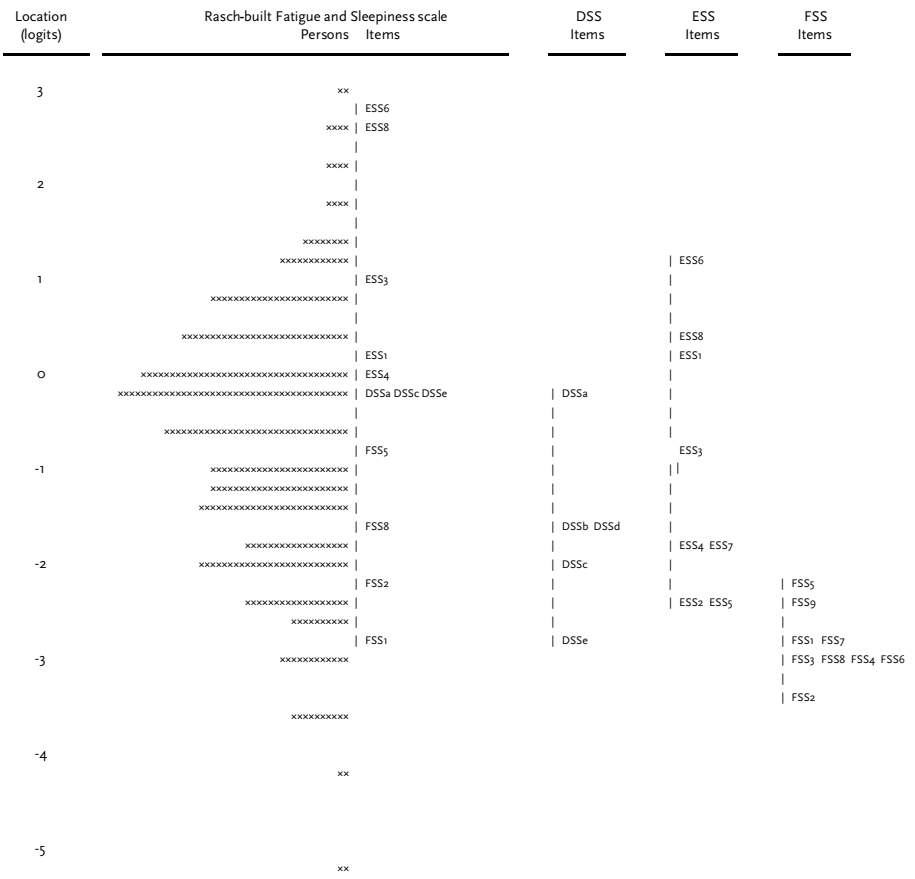



**Legend to figure 10.2.** Threshold map indicating patient's expected response for each item as a function of their level of fatigue and sleepiness (0 = seldom or never, 1 = sometimes, 2 = almost always). Items are ordered by increasing difficulty. Item "my motivation is lower when I am fatigued" was the easiest item and item "do you fall asleep while sitting and talking" the most difficult item.

The threshold distribution map of the FDSS shows the expected response to a given item as a function of the underlying fatigue and sleepiness measure (figure 10.2). Item difficulty ranged from -2.624 to +2.924 logits, and patient location ranged from -5.122 to +3.137 logits. Only 0.3% of patients were not fatigued or sleepy at all (floor effect), and 0.6% graded themselves as having the maximum score (ceiling effect). To compare targeting of the outcome measures, i.e. the difficulty range of outcome measures in relation to the levels of fatigue and sleepiness of the sample, various FDSS items were used for

anchoring each of the scales to the FDSS ruler separately. Figure 10.3 shows that the item locations of the ordinal DSS, ESS and FSS are poorly targeted to the sample population and cover a small range of measurement. By contrast, the targeting graph of the FDSS shows that the items are well spread across the continuum and sufficiently cover the patient sample (mean patient location of -0.38 logits).

**Figure 10.3.** Person and item distribution on a logit scale

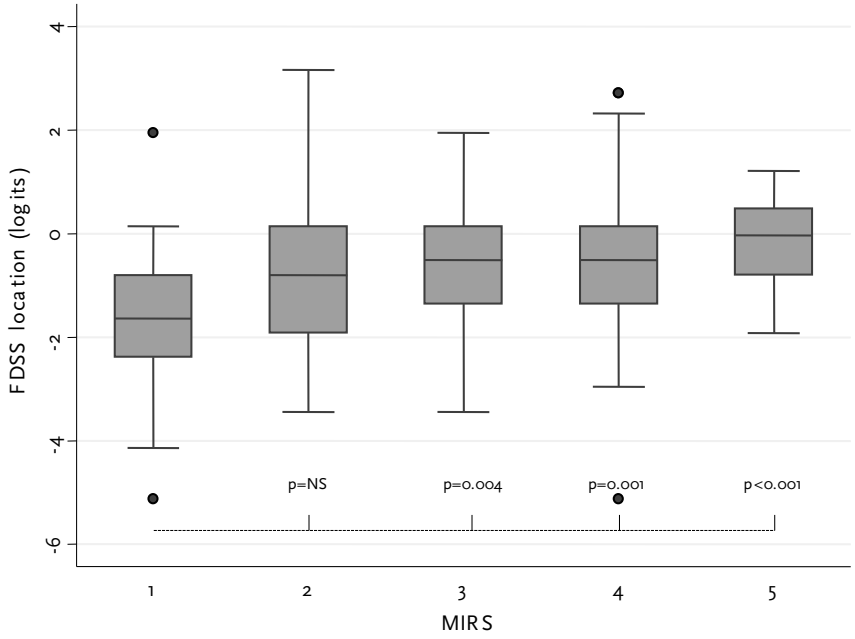



**Legend to figure 10.3.** The numbers -5 to 3 represent corresponding logits on a linear ruler (y-axis). The crosses under persons represent the number of patients having a person location at that point on the ruler. Item locations are presented on the right side. Positive values on the logit scale represent the more fatigued or sleepy patients and the more difficult items. Negative values on the logit scale represent the less fatigued or sleepy persons and the least difficult items. For a well-targeted scale (not too easy, not too hard), items should represent a wide range in difficulty.

**Validity and reliability**

The interval FDSS scores differed significantly between MIRS grades, a measure of the disease involvement (F= 5.77, *P*= 0.0002). A significant trend (*P*= 0.003) was seen toward higher fatigue and sleepiness levels in patients with more severe muscular impairment. However, at subgroup level, only the FDSS scores of patients with MIRS score 1 differed significantly from those with MIRS scores of 3, 4, and 5 (figure 10.4). There was no association between FDSS measures and CTG repeat length (F= 2.39, *P*= 0.07). The person separation index was 0.80, demonstrating acceptable internal consistency reliability.

**Figure 10.4.** Relationship between FDSS outcomes and MIRS grades




**Legend to figure 10.4.** FDSS values are presented in a box plot. A progressive increase in fatigue and sleepiness level was seen on the FDSS scale with increased muscular impairment demonstrated by higher MIRS grade. FDSS scores did not differentiate significantly between all subgroups.

## Discussion

We constructed a Rasch-built combined fatigue and daytime sleepiness scale (FDSS) specifically designed for patients with DM1 (appendix C). The FDSS is composed of items from the ESS, DSS, and FSS. The 12 items selected for the final scale fulfilled all Rasch model expectations, and the hierarchy of items was invariant across DM1 patients of different ages, gender, disease types, education levels, use of psychostimulants, and between patients from different countries (the Netherlands and Canada). Items retained for the FDSS are shown to measure a single construct combining aspects of sleep propensity as well as behavioural consequences of fatigue, which argues strongly in favour of a combined clinical outcome measure of these attributes. Fatigue items were the easiest items, while sleepiness items were the most difficult items. A significant association was found between FDSS measures and MIRS grade. This is in line with previous reports which showed that patients with fatigue and/or EDS had greater muscular impairment than patients without these symptoms.[4, 8]

Previous reports in inflammatory neuropathies and multiple sclerosis demonstrated the inability of patients to differentiate between the original FSS response categories.[29, 30] Similar disordered threshold patterns were seen in patients with DM1 and can only be visualized using a modern technique like the Rasch method. In addition, Rasch analysis allows one to investigate targeting. As shown in this study, conclusions based on FSS, ESS and DSS data may be questioned, since the sets of items might not be at the appropriate level of difficulty for the patients being examined (figure 10.3).

Interval measures can be deduced from FDSS raw scores and can be used for parametric statistical analyses. It is also possible to describe patients with a given sum score in functional terms using the logit measure or the modeled probability of a category score for each item (e.g., the percent difference in the probability of "fall asleep when sitting and reading"), thereby improving the interpretation of test scores and trial effects.[31]

The high person separation index is supportive of a good discriminatory capacity of the scale among patients with various degrees of fatigue and sleepiness, suggesting that the scale can measure change in fatigue induced by, for example, medical intervention. The FDSS scale would be a more suitable patient-based outcome measure for randomized trials of psychostimulants, as it is specifically devised and validated for DM1 and overcomes the limitations of ordinal based measures.

The ability of the FDSS to detect meaningful changes over time (responsiveness) needs further evaluation. Also, the use of this new scale should be complemented whenever possible by monitoring of multiple physiological parameters during

sleep (polysomnography), determination of physiologic sleepiness during the daytime (multiple sleep latency test), and assessment of potential immunologic and neuroendocrine disturbances, impaired psychophysiologic responses, and dysfunction in central and peripheral motor pathways.[32-34]

## Conclusion

This study addresses various measurement issues required to construct a clinically meaningful combined Fatigue and Daytime Sleepiness Scale (FDSS) specifically for patients with DM1 using Rasch analysis. The FDSS interval measure meets all Rasch model expectations and bypasses the difficulty of differentiating between fatigue and sleep problems by providing interval measures on a single continuum for both entities. Its use is therefore suggested in future clinical trials and follow-up studies in DM1 in order to determine its responsiveness.

# References


1. Harper PS. Myotonic dystrophy. London: Saunders; 2001
2. Harley HG, Rundle SA, MacMillan JC, Myring J, Brook JD, Crow S, Reardon W, Fenton I, Shaw DJ, Harper PS. Size of the unstable ctg repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. *Am J Hum Genet*. 1993;52:1164-1174
3. Rubinsztein JS, Rubinsztein DC, Goodburn S, Holland AJ. Apathy and hypersomnia are common features of myotonic dystrophy. *J Neurol Neurosurg Psychiatry*. 1998;64:510-515
4. Laberge L, Bégin P, Montplaisir J, Mathieu J. Sleep complaints in patients with myotonic dystrophy. *J Sleep Res*. 2004;13:95-100
5. van der Meche FG, Bogaard JM, van der Sluys JC, Schimsheimer RJ, Ververs CC, Busch HF. Daytime sleep in myotonic dystrophy is not caused by sleep apnoea. *J Neurol Neurosurg Psychiatry*. 1994;57:626-628
6. Bégin P, Mathieu J, Almirall J, Grassino A. Relationship between chronic hypercapnia and inspiratory-muscle weakness in myotonic dystrophy. *Am J Respir Crit Care Med*. 1997;156:133-139
7. Phillips MF, Steer HM, Soldan JR, Wiles CM, Harper PS. Daytime somnolence in myotonic dystrophy. *J Neurol*. 1999;246:275-282
8. Laberge L, Dauvilliers Y, Begin P, Richer L, Jean S, Mathieu J. Fatigue and daytime sleepiness in patients with myotonic dystrophy type 1: To lump or split? *Neuromuscul Disord*. 2009;19:397-402
9. Kalkman JS, Schillings ML, van der Werf SP, Padberg GW, Zwarts MJ, van Engelen BG, Bleijenberg G. Experienced fatigue in facioscapulohumeral dystrophy, myotonic dystrophy, and hmsn-i. *J Neurol Neurosurg Psychiatry*. 2005;76:1406-1409
10. Freal JE, Kraft GH, Coryell JK. Symptomatic fatigue in multiple sclerosis. *Arch Phys Med Rehabil*. 1984;65:135-138
11. DeVellis RF. Classical test theory. *Med Care*. 2006;44:S50-59
12. Rasch G. Probalilistic models for some intelligence and attainment tests. Chicago: University of Chicago Press; 1980
13. Bond TG, Fox CM. Applying the rasch model: Fundamental measurement for the human sciences. New York: Lawrence Erlbaum Associates; 2001
14. Hermans MC, Faber CG, De Baets MH, de Die-Smulders CE, Merkies IS. Rasch-built myotonic dystrophy type 1 activity and participation scale (dm1-activ). *Neuromuscul Disord*. 2010;20:310-318
15. Johns MW. A new method for measuring daytime sleepiness: The epworth sleepiness scale. *Sleep*. 1991;14:540-545
16. Miletin MS, Hanly PJ. Measurement properties of the epworth sleepiness scale. *Sleep Med*. 2003;4:195-199
17. Johns MW. Reliability and factor analysis of the epworth sleepiness scale. *Sleep*. 1992;15:376-381
18. Johns MW. Sleepiness in different situations measured by the epworth sleepiness scale. *Sleep*. 1994;17:703-710
19. Beiske KK, Kjelsberg FN, Ruud EA, Stavem K. Reliability and validity of a norwegian version of the epworth sleepiness scale. *Sleep Breath*. 2009;13:65-72
20. Laberge L, Gagnon C, Jean S, Mathieu J. Fatigue and daytime sleepiness rating scales in myotonic dystrophy: A study of reliability. *J Neurol Neurosurg Psychiatry*. 2005;76:1403-1405
21. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol*. 1989;46:1121-1123
22. Mathieu J, Boivin H, Meunier D, Gaudreault M, Begin P. Assessment of a disease-specific muscular impairment rating scale in myotonic dystrophy. *Neurology*. 2001;56:336-340
23. Fisher WP. Reliability statistics. *Rasch Measure Trans*. 1992;6:238
24. Dorans NJ, Holland PW. Dif detection and description: Mantel-haenszel and standardisation. In: Holland PWW, H., ed. Differential item functioning. Hillsdale: Lawrence Erlbaum Associates; 1993:36-66
25. Andrich D, Lyne A, Sheridan B, Luo G. Rumm 2020. 2003
26. Smith EV, Jr. Detecting and evaluating the impact of multidimensionality using item fit statistics and principal component analysis of residuals. *J Appl Meas*. 2002;3:205-231
27. Bland JM, Altman DG. Multiple significance tests: The bonferroni method. *BMJ*. 1995;310:170
28. Linacre JM. Optimizing rating scale category effectiveness. *J Appl Meas*. 2002;3:85-106

29. van Nes SI, Vanhoutte EK, Faber CG, Garssen M, van Doorn PA, Merkies IS. Improving fatigue assessment in immune-mediated neuropathies: The modified rasch-built fatigue severity scale. *J Peripher Nerv Syst*. 2009;14:268-278
30. Mills R, Young C, Nicholas R, Pallant J, Tennant A. Rasch analysis of the fatigue severity scale in multiple sclerosis. *Mult Scler*. 2009;15:81-87
31. van Hartingsveld F, Lucas C, Kwakkel G, Lindeboom R. Improved interpretation of stroke trial results using empirical barthel item weights. *Stroke*. 2006;37:162-166
32. Ono S, Takahashi K, Jinnai K, Kanda F, Fukuoka Y, Kurisaki H, Mitake S, Inagaki T, Yamano T, Nagao K. Loss of serotonin-containing neurons in the raphe of patients with myotonic dystrophy: A quantitative immunohistochemical study and relation to hypersomnia. *Neurology*. 1998;50:535-538
33. Romigi A, Izzi F, Pisani V, Placidi F, Pisani LR, Marciani MG, Corte F, Panico MB, Torelli F, Uasone E, Vitrani G, Albanese M, Massa R. Sleep disorders in adult-onset myotonic dystrophy type 1: A controlled polysomnographic study. *Eur J Neurol*. 2011
34. Yu H, Laberge L, Jaussent I, Bayard S, Scholtz S, Raoul M, Pages M, Dauvilliers Y. Daytime sleepiness and rem sleep characteristics in myotonic dystrophy: A case-control study. *Sleep*. 2011;34:165-170w

PART IV

# Summary

Chapter 11

# Summary, general discussion and future perspectives

This thesis describes a series of studies on myotonic dystrophy type 1 (DM1). In this chapter, an overview of this thesis is presented where the main findings and conclusions of each of the chapters are summarized and discussed.

**Chapter 1** is a general introduction illustrating the multisystemic characteristics of DM1. The underlying molecular mechanisms of DM1 are likely to be exceedingly complex as the mutation can affect gene expression in multiple ways. Misregulation of alternative splicing plays a central role in the development of DM1 symptoms, but major disease symptoms like muscle wasting and cardiac arrhythmias cannot be readily explained by any of the splicing changes identified to date. DM1 is a clinically heterogeneous disorder in which age at onset and severity of symptoms may vary widely. As a consequence, affected individuals need different approaches depending on the severity of their particular problems and their age. Despite the fact that a curative treatment is not available and affected individuals may not see the need for regular treatment or follow-up, structured multi-disciplinary management is of value as several measures can help diminish complaints, improve daily life, and prolong survival.[1]

## Cardiac disease

As prognosis of DM1 patients may be directly related to cardiac status, surveillance and timely management of cardiac complications are important. However, despite the high incidence of cardiac abnormalities in DM1, there is no consensus on the management of heart disease and the risk of sudden death may not be fully appreciated. The main objectives of the first part of this thesis were to determine the occurrence and predictors of sudden death, to establish the presence and nature of cardiomyopathy in DM1 and to describe changes in gene expression that may lead to cardiac dysfunction, in order to improve standard follow-up and treatment regimens.

**Chapter 2** gives a comprehensive overview of the extensive literature on hereditary muscular dystrophies associated with cardiac disease. The incidence and nature of cardiac involvement vary between different types of muscular dystrophies. Some types mainly lead to dilated cardiomyopathy, resulting in systolic dysfunction and heart failure, while others particularly affect the conduction system, leading to arrhythmias and sudden death. Molecular pathology, clinical aspects, cardiac findings and the mechanism and risk of cardiac death of different types of muscular dystrophies are summarized to provide insight into the present management of these diseases. However, recommendations for the investigation and treatment of cardiac involvement in muscular dystrophies are largely based on consensus statements, since published evidence of benefit

from any treatment is quite limited. Prospective well-coordinated multi-centre collaborative studies are mandatory to evaluate current strategies and to adjust guidelines for surveillance, heart failure treatment and device implantation.
Until recently, sudden death in DM1, defined as instantaneous unexpected death due to natural causes without symptoms of illness in the preceding 24 hours,[2] was thought to be primarily the result of conduction blocks. Current guidelines for device-based therapy of cardiac rhythm abnormalities recommend that: permanent pacemaker implantation may be considered for neuromuscular diseases such as myotonic dystrophy with any degree of AV block, with or without symptoms, because there may be unpredictable progression of AV conduction disease (level of evidence: B) or with bifascicular block or any fascicular block, with or without symptoms (level of evidence: C).[3]

**Chapter 3** shows that sudden death occurred in one third of all classified deaths in a retrospective study of 412 DM1 patients. Moreover, sudden death was certainly not always prevented by pacemakers. This high rate of sudden deaths demands for a new treatment strategy. ICDs have been increasingly used in patients with muscular dystrophies that predispose to sudden death.[4] The efficacy of an ICD in DM1 is suggested from nonrandomized observations of ventricular tachyarrhythmias and appropriate ICD therapy. The challenge remains to identify patients with a relatively good neuromuscular prognosis who would benefit from an ICD. Our case-control comparison of available electrocardiograms showed that QRS duration was significantly prolonged in patients with documented sudden death compared to controls and deaths from other causes. Others have identified a severe abnormality on ECG and a clinical diagnosis of atrial tachyarrhythmia as independent risk factors, although with moderate sensitivity, for sudden death in this population.[5] DM1 patients with this risk profile would be the ideal cohort for a long-term follow-up study to assess the survival benefit from ICD therapy versus pacemaker therapy, or alternatively, an insertable loop recorder. Other possible prognostic factors should be evaluated for their association with cardiac events. Because of the possibility of low event rates, the follow-up period should be adequate (e.g. 5-10 years).

**Chapter 4** describes the structural and functional myocardial abnormalities depicted by CMR imaging in patients with DM1. Although death from progressive heart failure is uncommon in patients with DM1 compared to other muscular dystrophies, myocardial involvement may also be prognostic in predicting overall mortality and sudden death in DM1.[6] CMR imaging has a greater sensitivity and reproducibility than conventional diagnostic investigations (ECG and echocardiography) to demonstrate early myocardial

abnormalities or subtle changes, but the CMR findings in DM1 had not been well characterized. We found that the presence of mild to moderate left ventricular systolic dysfunction, ventricular dilatation, myocardial hypertrophy or fibrosis on CMR imaging was strongly associated with electrocardiographic conduction abnormalities. However, a normal ECG did not exclude myocardial alterations. These findings lend support to the concept that DM1 patients have a complex cardiac phenotype, including both the myocardium and the conduction system. The predictive value of myocardial fibrosis or other CMR findings for identifying DM1 patients who are at risk for cardiac disease progression or sudden death remains to be investigated in a long-term prospective study.
CTG repeat length was not associated with myocardial involvement. There is no consensus from the literature as to whether or not CTG-repeat size has value as a prognostic indicator of cardiac disease.[7, 8] Indeed, CTG repeat analysis from peripheral blood leukocytes may be of limited value in individual patients because of the overlap between expansion sizes seen in different phenotypic groups (congenital type, childhood-onset type, adult-onset type, and late-onset type).[9] In addition, the size of the mutation observed in leukocytes does not necessarily reflect the CTG expansion size in the cardiac myocytes due to variability between tissues (somatic mosaicism),[10] which may explain why the CTG repeat length in peripheral blood leukocytes does not necessarily correlate with the severity and nature of symptoms.

**Chapter 5** reports the changes in gene expression in cardiac biopsies obtained from patients with DM1-related cardiomyopathy. Altered global gene expression has previously been shown in skeletal muscle biopsies of DM1 patients,[11-13] and in skeletal muscle of MBNL1 knock-out mice and of transgenic mice expressing non-coding CUG repeats.[14] Microarray analysis showed significant changes in the expression levels of 989 mRNAs and 16 miRNAs in hearts of DM1 patients as compared to control subjects. We were able to reproduce gene expression changes in DM1 cardiac tissue that have been directly linked to established concepts on DM1 pathophysiology, such as the loss of DMPK and SIX5 expression. Bioinformatics analysis of mRNA expression data identified several pathological changes, including disturbance of cardiac mitochondrial function, RNA processing, calcium signalling and expression changes in genes involved in cytoskeleton regulation. The observed changes may predispose DM1 hearts to conduction disturbances, arrhythmias and myocardial abnormalities. It would be interesting to identify gene expression profiles characteristic of disease subgroups to find prognostic or predictive molecular signatures as previously demonstrated in the context of cancer.[15]

## Outcome measures

Over time, DM1 leads to muscle and organ impairments causing progressive disability. Most of the treatment thus far is directed towards symptom management. In addition, advances in understanding of the complex pathogenesis have led to potential molecular therapeutic targets in preclinical studies.[16] Before being able to evaluate therapeutic interventions in clinical trials, consensus is needed on which core set of appropriate outcome measures should be used. In the second part of the thesis, we aimed to show the importance of modern clinimetric methods and construct new outcome measures for future DM1 studies.

**Chapter 6** provides an overview of outcome measures applied in clinical studies involving patients with DM1 over the last 60 years. A multitude of outcome measures have been used, which hampers comparison of different trials results. In addition, clinimetric properties, including validity, reliability and responsiveness, were incompletely or poorly evaluated for most of the measurement instruments. Another limitation of currently applied outcome measures was the fact that multi-item rating scales were all based on the classical test theory providing all ordinal measurement.[17] Ordinal scales are currently used in an inappropriate way by addressing obtained scores as interval measures and exposing them to parametric analyses. Recommendations are made to adopt modern rather than classical clinimetric approaches when constructing outcome measures for DM1 and to use a certain degree of standardization to facilitate comparison of future clinical trial results and improve communication in clinical practice.

**Chapter 7** is an introduction to the Rasch measurement model and informs the reader about the various steps in the evaluation and construction of outcome measures using Rasch analysis. This modern clinimetric technique sets additional quality standards for outcome measures and attempts to estimate an interval-scaled measure from an observed raw score, giving meaning to the scores and allowing comparison of (changes in) scores.[18]

**Chapter 8** describes the development of a new outcome measure of activity limitations and participation restrictions for patients with DM1 (DM1-Activ) using the Rasch measurement model. Although an interval measure at the activity level for patients with neuromuscular disorders (ACTIVLIM) is available,[19] a disease-specific scale is preferred over a generic scale for the evaluation of health outcomes. Patients with DM1 may experience different difficulties in daily and social functioning than patients with other neuromuscular disorders

(e.g. limb-girdle dystrophy), leading to differences in items selection and differences in weights (significance) of selected items. However, strengthening of the DM1-Activ scale was needed in a larger cohort of patients to enable its clinical application.

**Chapter 9** presents the reconstruction of the DM1-Activ scale for clinical use (DM1-Activ$^{c}$) through Rasch analyses on an expanded questionnaire in a larger population of patients with DM1. The new outcome measure to capture activity limitations and participation restrictions for patients with DM1 fulfils all requirements of the Rasch model and demonstrated stronger discriminatory capacity and reliability scores. A nomogram is provided to calculate interval estimates from the obtained raw scores which can be used in future clinical observational and interventional studies.

**Chapter 10** describes the construction of a combined fatigue and daytime sleepiness scale for patients with DM1 (FDSS) using the Rasch Model. The FDSS interval measure bypasses the difficulty of differentiating between fatigue and sleep problems by providing interval measures on a single continuum for both entities. Currently used patient-based rating scales measure daytime sleepiness or fatigue levels as separate entities. However, excessive daytime sleepiness and fatigue have overlapping features and patients cannot always specify whether their complaint relates to sleepiness, fatigue, or both. Rasch analysis of the FDSS showed that the included fatigue and daytime sleepiness items address the same underlying health construct, which argues strongly in favour of a combined clinical outcome measure of these attributes. Compared to currently often used ordinal fatigue and sleepiness scales, the FDSS was better targeted to the sample population.
The capacity of these new Rasch-built outcome measures to detect changes over time (i.e. responsiveness) needs to be investigated in longitudinal studies. In order to be responsive, the outcome measure must detect change when it has occurred and it must remain stable when no change has occurred. However, it is also important to assess how relevant the changes are for patients. Therefore, responsiveness studies should also include determination of the minimal clinically important difference (MCID), which is defined as the smallest difference in score in the domain of interest which patients perceive as beneficial and which would mandate, in the absence of troublesome side effects and excessive costs, a change in the patient's management.[20] Therefore differences in scores smaller than the MCID are considered not important, independent of their statistical significance.

## Future perspectives

Although much has been learned about DM1 over the last 20 years, many important problems are still unsolved. The complexity and variability of disease manifestations in DM1 pose a challenge in anticipating all potential problems and implementing an integrated management approach of impairment, disability and quality of life. An optimal health management program should provide support to both the patient and family through an interdisciplinary team, which includes genetic and medical resources as well as community resources.[1]

DM1 is still relatively unknown among medical and paramedical resources. Many clinical manifestations of the disease, such as gastrointestinal and neuropsychiatric issues, are under-recognized and require further scientific and clinical research. Patients may consider gastrointestinal symptoms, particularly abdominal pain, diarrhoea and faecal incontinence, to be the most disabling consequence of the disease rather than their neuromuscular symptoms.[21] Limited information is available about the pathophysiology of gastro-intestinal dysfunction and the effect of treatments. In addition, central nervous system (CNS) involvement is another underestimated symptom of DM1 and poses great problems not only for patients but also for their relatives.[22] Increased apathy and personality characteristics may cause patients to lose interest in daily activities or relationships. Awareness of these neuropsychiatric problems is important in understanding related health and social consequences. In addition, intellectual disability, learning disabilities and/or behavioural problems are the principal concerns of children and adolescents with childhood-onset DM1. The wide range of CNS involvement in DM1 and the nature of its underlying neurological basis are only beginning to be understood.[23]

Future studies are needed to redefine guidelines concerning screening, follow-up and treatment of cardiac involvement in DM1 patients and to improve the identification of patients at risk of sudden death.[24] The diagnostic and therapeutic strategy used in our centre is presented in appendix D. Prospective observational clinical studies in DM1 patients who receive a pacemaker or ICD on the basis of a class I or IIa indication according to ACC/AHA guidelines[3] or a nonconventional indication, could help explore the prognostic value of several factors in predicting life-threatening arrhythmias.[25] Furthermore, studies with long-term recordings of implanted insertable loop recorders may also improve our insights in the electrophysiological abnormalities in these patients.[26] Additional studies of cardiac involvement should also address the efficacy and cost-effectiveness of ICD therapy in DM1 patients for primary prevention of sudden death. Finally, DM1 patients are also at high risk of respiratory failure and antiarrhythmia devices of any type in those with severe muscle involvement may not improve outcomes.[27]

A better understanding of the underlying molecular mechanisms of cardiac disease in DM1 is also essential for the development of rational approaches for the prevention and treatment of cardiac events. RNA sequencing may provide a more complete characterization of RNA transcripts, including improved detection of alternative splicing events and identification of previously unknown coding and non-coding RNA species.[2] In terms of treatment, several promising experimental strategies are obtained in cell-based and animal models that address the cause of DM1 rather than reduction of symptoms. Future challenges are to find efficient ways to systemically deliver active compounds *in vivo* that, for example, eliminate or neutralize the expanded DMPK transcript.[29] Before new treatments and therapies can be applied in clinical trial settings, the natural course of disease progression and treatment outcomes should be further explored. A core set of clinically meaningful and scientifically sound rating scales are required to evaluate the therapeutic efficacy of an intervention. Modern test theory models, such as the Rasch measurement model, are increasingly adopted in health outcomes research to strength clinimetric properties of newly developed or revised outcome measures. The increasing importance of high quality rating scales has also been recognized in other neuromuscular disorder, including Duchenne muscular dystrophy and immune-mediated peripheral neuropathies.[30, 31] Considering the multisystemic nature of DM1, health outcome measures of various aspects of DM1 disease manifestations, e.g. pain, muscle strength or psychological distress, are necessary for adequate patient assessment. In addition to self-reported measures, health status assessed by familial caregivers may reveal other relevant problems.

# References


1. Gagnon C, Noreau L, Moxley RT, Laberge L, Jean S, Richer L, Perron M, Veillette S, Mathieu J. Towards an integrative approach to the management of myotonic dystrophy type 1. *J Neurol Neurosurg Psychiatry*. 2007;78:800-806
2. World Health Organization. International statistical classification of diseases and related health problems 10th revision (icd-10). Geneva; 2010
3. Epstein AE, DiMarco JP, Ellenbogen KA, Estes NA, 3rd, Freedman RA, Gettes LS, Gillinov AM, Gregoratos G, Hammill SC, Hayes DL, Hlatky MA, Newby LK, Page RL, Schoenfeld MH, Silka MJ, Stevenson LW, Sweeney MO, Smith SC, Jr., Jacobs AK, Adams CD, Anderson JL, Buller CE, Creager MA, Ettinger SM, Faxon DP, Halperin JL, Hiratzka LF, Hunt SA, Krumholz HM, Kushner FG, Lytle BW, Nishimura RA, Ornato JP, Riegel B, Tarkington LG, Yancy CW. Acc/aha/hrs 2008 guidelines for device-based therapy of cardiac rhythm abnormalities: A report of the american college of cardiology/american heart association task force on practice guidelines (writing committee to revise the acc/aha/naspe 2002 guideline update for implantation of cardiac pacemakers and antiarrhythmia devices): Developed in collaboration with the american association for thoracic surgery and society of thoracic surgeons. *Circulation*. 2008;117:e350-408
4. Meune C, Van Berlo JH, Anselme F, Bonne G, Pinto YM, Duboc D. Primary prevention of sudden death in patients with lamin a/c gene mutations. *N Engl J Med*. 2006;354:209-210
5. Groh WJ, Groh MR, Saha C, Kincaid JC, Simmons Z, Ciafaloni E, Pourmand R, Otten RF, Bhakta D, Nair GV, Marashdeh MM, Zipes DP, Pascuzzi RM. Electrocardiographic abnormalities and sudden death in myotonic dystrophy type 1. *N Engl J Med*. 2008;358:2688-2697
6. Bhakta D, Groh MR, Shen C, Pascuzzi RM, Groh WJ. Increased mortality with left ventricular systolic dysfunction and heart failure in adults with myotonic dystrophy type 1. *Am Heart J*. 2010;160:1137-1141, 1141 e1131
7. Sabovic M, Medica I, Logar N, Mandic E, Zidar J, Peterlin B. Relation of ctg expansion and clinical variables to electrocardiogram conduction abnormalities and sudden death in patients with myotonic dystrophy. *Neuromuscul Disord*. 2003;13:822-826
8. Clarke NR, Kelion AD, Nixon J, Hilton-Jones D, Forfar JC. Does cytosine-thymine-guanine (ctg) expansion size predict cardiac events and electrocardiographic progression in myotonic dystrophy? *Heart*. 2001;86:411-416
9. Harley HG, Rundle SA, MacMillan JC, Myring J, Brook JD, Crow S, Reardon W, Fenton I, Shaw DJ, Harper PS. Size of the unstable ctg repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. *Am J Hum Genet*. 1993;52:1164-1174
10. Martorell L, Monckton DG, Gamez J, Johnson KJ, Gich I, Lopez de Munain A, Baiget M. Progression of somatic ctg repeat length heterogeneity in the blood cells of myotonic dystrophy patients. *Hum Mol Genet*. 1998;7:307-312
11. Botta A, Vallo L, Rinaldi F, Bonifazi E, Amati F, Biancolella M, Gambardella S, Mancinelli E, Angelini C, Meola G, Novelli G. Gene expression analysis in myotonic dystrophy: Indications for a common molecular pathogenic pathway in dm1 and dm2. *Gene Expr*. 2007;13:339-351
12. Vihola A, Bachinski LL, Sirito M, Olufemi SE, Hajibashi S, Baggerly KA, Raheem O, Haapasalo H, Suominen T, Holmlund-Hampf J, Paetau A, Cardani R, Meola G, Kalimo H, Edstrom L, Krahe R, Udd B. Differences in aberrant expression and splicing of sarcomeric proteins in the myotonic dystrophies dm1 and dm2. *Acta Neuropathol*. 2010;119:465-479
13. Vihola A, Sirito M, Bachinski LL, Raheem O, Screen M, Suominen T, Krahe R, Udd B. Altered expression and splicing of ca(2+) metabolism genes in myotonic dystrophies dm1 and dm2. *Neuropathol Appl Neurobiol*. 2012:[Epub ahead of print]
14. Osborne RJ, Lin X, Welle S, Sobczak K, O'Rourke JR, Swanson MS, Thornton CA. Transcriptional and post-transcriptional impact of toxic rna in myotonic dystrophy. *Hum Mol Genet*. 2009;18:1471-1481
15. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lonning PE, Brown PO, Borresen-Dale AL, Botstein D. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci U S A*. 2003;100:8418-8423
16. Foff EP, Mahadevan MS. Therapeutics development in myotonic dystrophy type 1. *Muscle Nerve*. 2011;44:160-169
17. DeVellis RF. Classical test theory. *Med Care*. 2006;44:S50-59

18. Rasch G. Probabilistic models for some intelligence and attainment tests. Chicago: University of Chicago Press; 1980
19. Vandervelde L, van den Bergh PYK, Goemans N, Thonnard JL. Activlim: A rasch-built measure of activity limitations in children and adults with neuromuscular disorders. *Neuromuscul Disord*. 2007;17:459-469
20. Jaeschke R, Singer J, Guyatt GH. Measurement of health status. Ascertaining the minimal clinically important difference. *Control Clin Trials*. 1989;10:407-415
21. Ronnblom A, Forsberg H, Danielsson A. Gastrointestinal symptoms in myotonic dystrophy. *Scand J Gastroenterol*. 1996;31:654-657
22. Hilton-Jones D. Myotonic dystrophy--forgotten aspects of an often neglected condition. *Curr Opin Neurol*. 1997;10:399-401
23. Harper PS. Myotonic dystrophy. London: Saunders; 2001
24. Petri H, Vissing J, Witting N, Bundgaard H, Kober L. Cardiac manifestations of myotonic dystrophy type 1. *Int J Cardiol*. 2012;160:82-88
25. Dello Russo A, Mangiola F, Della Bella P, Nigro G, Melacini P, Bongiorni MG, Tondo C, Calo L, Messano L, Pace M, Pelargonio G, Casella M, Sanna T, Silvestri G, Modoni A, Zachara E, Moltrasio M, Morandi L, Politano L, Palladino A, Bellocci F. Risk of arrhythmias in myotonic dystrophy: Trial design of the ramyd study. *J Cardiovasc Med (Hagerstown)*. 2009;10:51-58
26. Petri H, Vissing J, Witting N, Bundgaard H, Kober L. Response letter to "cardiac involvement in myotonic dystrophy type 1 - do not forget the loop recorder!". *Int J Cardiol*. 2012
27. Bhakta D, Shen C, Kron J, Epstein AE, Pascuzzi RM, Groh WJ. Pacemaker and implantable cardioverter-defibrillator use in a us myotonic dystrophy type 1 population. *J Cardiovasc Electrophysiol*. 2011
28. Ozsolak F, Milos PM. Rna sequencing: Advances, challenges and opportunities. *Nat Rev Genet*. 2011;12:87-98
29. Wheeler TM, Leger AJ, Pandey SK, MacLeod AR, Nakamori M, Cheng SH, Wentworth BM, Bennett CF, Thornton CA. Targeting nuclear rna for in vivo correction of myotonic dystrophy. *Nature*. 2012;488:111-115
30. van Nes SI, Faber CG, Merkies IS. Outcome measures in immune-mediated neuropathies: The need to standardize their use and to understand the clinimetric essentials. *J Peripher Nerv Syst*. 2008;13:136-147
31. Mayhew A, Cano S, Scott E, Eagle M, Bushby K, Muntoni F. Moving towards meaningful measurement: Rasch analysis of the north star ambulatory assessment in duchenne muscular dystrophy. *Dev Med Child Neurol*. 2011;53:535-542

Chapter 12

# Samenvatting

Dit proefschrift beschrijft een aantal studies over myotone dystrofie type 1 (DM1). In dit hoofdstuk wordt een overzicht gegeven van de belangrijkste bevindingen en conclusies van elk van de hoofdstukken.

**Hoofdstuk 1** is een algemene introductie waarin de complexe multisysteem pathologie van DM1 wordt geïllustreerd. DM1, ook wel de ziekte van Steinert genoemd, is de meest voorkomende erfelijke spierziekte bij volwassenen. Kenmerkende verschijnselen zijn een vertraagde ontspanning van skeletspieren (myotonie) en langzaam toenemende spierzwakte (dystrofie). Naast de myotonie en spierzwakte worden ook verschillende inwendige organen aangetast zoals het zenuwstelsel, het hart, de longen, de ogen en het maagdarmstelsel. DM1 wordt veroorzaakt door een verlenging van het aantal CTG trinucleotiden in het DMPK-gen. Het moleculaire mechanisme van DM1 is zeer complex doordat de mutatie genexpressies op verschillende manieren ontregeld. Een gestoorde regulatie van alternatieve splicing speelt een centrale rol bij het ontstaan van symptomen, maar belangrijke symptomen, zoals spierzwakte en hartritmestoornissen, kunnen tot nu toe niet worden gerelateerd aan een specifiek splicing defect. Het klinisch spectrum van DM1 is zeer heterogeen waarbij de beginleeftijd en ernst van symptomen sterk variëren. Men onderscheidt vier fenotypen: congenitale type, kindertype, klassieke of volwassen type en het lichte (Engels: 'mild') type. Ondanks het feit dat er geen curatieve behandeling voorhanden is en patiënten niet altijd overtuigd zijn van de noodzaak om regelmatig op controle te komen, is een gestructureerde multidisciplinaire aanpak van belang om klachten te verminderen, het dagelijks functioneren te verbeteren en de overleving te doen toenemen.

## Hartziekte

Aangezien de prognose van DM1 patiënten direct gerelateerd kan zijn aan de toestand van hun hart, zijn regelmatige controle en tijdige behandeling van cardiale complicaties belangrijk. Een actieve opsporing is noodzakelijk omdat cardiale betrokkenheid bij DM1 niet altijd gepaard gaat met symptomen en er geen duidelijk verband met de ernst van de neuromusculaire symptomen is. Ondanks de hoge incidentie van cardiale afwijkingen, is er geen consensus over de diagnostische en therapeutische aanpak van cardiale betrokkenheid en wordt het risico op plotse dood onvoldoende onderkend. De belangrijkste doelstellingen van het eerste deel van dit proefschrift waren het bepalen van het voorkomen en de voorspellers van plotse dood, het vaststellen van de aard van hartspierziekte (cardiomyopathie) bij DM1 patiënten en het beschrijven van veranderingen in genexpressie die kunnen leiden tot cardiale complicaties,

om zodoende een beter inzicht in de etiologie van deze ziekte te krijgen en de diagnostiek en behandeling van cardiale betrokkenheid te verbeteren.

**Hoofdstuk 2** geeft een uitgebreid overzicht van de omvangrijke literatuur over erfelijke spierdystrofieën die geassocieerd zijn met hartziekte. De incidentie en aard van cardiale problemen verschilt tussen verschillende typen spierdystrofieën. Sommige gaan gepaard met een dilaterende cardiomyopathie leidend tot systolische disfunctie en hartfalen, terwijl andere typen vooral gepaard gaan met geleidingsstoornissen, leidend tot ritmestoornissen en plotse dood. De moleculaire pathologie, klinische aspecten, cardiale onderzoeksbevindingen en het mechanisme en risico op hartdood van de verschillende typen spierdystrofieën wordt samengevat, om meer inzicht te krijgen in de huidige diagnostiek en behandeling van cardiale betrokkenheid. Desondanks zijn aanbevelingen voor de aanpak van hartziekte bij spierdystrofieën grotendeels gebaseerd op consensus omdat bewijs uit gepubliceerd wetenschappelijk onderzoek voor de effectiviteit van behandeling vrij beperkt is. Prospectieve en goed gecoördineerde multicenter studies zijn noodzakelijk om huidige behandelstrategieën te evalueren en richtlijnen en protocollen voor controle, hartfalen therapie en device implantatie aan te passen.
Tot voor kort werd aangenomen dat plotse dood bij DM1, gedefinieerd als een totaal onverwacht natuurlijk overlijden die zich voordoet binnen 24 uur na het begin van de symptomen, het gevolg is van geleidingsblokkades. Huidige richtlijnen stellen dan ook dat een pacemakerimplantatie overwogen dient te worden bij patiënten met een neuromusculaire ziekte zoals myotone dystrofie bij iedere graad van AV-blok in verband met de onvoorspelbare progressie geleidingsstoornissen (bewijsniveau B) of met een bifasciculair blok of ieder fasciculair blok met of zonder symptomen (bewijsniveau C).

**Hoofdstuk 3** laat zien dat in een retrospectieve studie van 412 DM1 patiënten plotse dood optreedt in een derde van alle geclassificeerde sterfgevallen. Bovendien werd plotse dood zeker niet altijd voorkomen door pacemakers. Deze hoge aantallen plotse doden vragen om een nieuwe behandelstrategie. ICD's worden in toenemende mate gebruikt bij patiënten met spierdystrofieën die geassocieerd zijn met plotse dood. De doeltreffendheid van een ICD bij DM1 wordt gesuggereerd uit niet-gerandomiseerde observaties waarin patiënten met ventriculaire aritmieën terechte ICD therapie hebben gekregen. De uitdaging blijft om patiënten te identificeren met een relatief goede neuromusculaire prognose die baat hebben bij een ICD. Uit onze case-controle analyse van beschikbare elektrocardiogrammen (ECGs) bleek dat de QRS-duur aanzienlijk verlengd was bij patiënten met gedocumenteerde plotse dood in vergelijking

met controles en sterfgevallen als gevolg van andere oorzaken. DM1 patiënten met dit risicoprofiel zouden moeten worden betrokken in lange termijn follow-up studies om het overlevingsvoordeel van ICD-therapie versus pacemaker therapie, of als alternatief, een implanteerbare loop recorder te beoordelen.

**Hoofdstuk 4** beschrijft de structurele en functionele afwijkingen van het myocard vastgesteld met cardiale MRI bij patiënten met DM1. Hoewel overlijden door progressief hartfalen zeldzaam is bij DM1 in vergelijking met andere spierdystrofieën, kan myocardiale betrokkenheid ook prognostische waarde hebben bij het voorspellen van de totale mortaliteit en plotse dood in DM1. Cardiale MRI heeft een grotere gevoeligheid en reproduceerbaarheid dan conventionele diagnostische onderzoeken (ECG en echocardiografie) voor het aantonen van vroege myocardiale afwijkingen of subtiele veranderingen, maar MRI bevindingen bij DM1 zijn nog niet goed gekarakteriseerd. We vonden dat de aanwezigheid van milde LV systolische disfunctie, ventriculaire dilatatie, myocardiale hypertrofie of fibrose op cardiale MRI sterk geassocieerd was met elektrocardiografische geleidingsstoornissen. Echter, een normaal ECG sluit myocardiale betrokkenheid niet uit. Deze bevindingen steunen de opvatting dat DM1 patiënten een complex cardiale fenotype hebben met zowel betrokkenheid van het myocard als het geleidingssysteem. De voorspellende waarde van myocardiale fibrose of andere cardiale MRI bevindingen voor het opsporen van DM1 patiënten met risico op cardiale ziekteprogressie of plotse dood dient nog te worden onderzocht in een langdurige prospectieve studie.

**Hoofdstuk 5** bestudeert de veranderingen in genexpressie in hartbiopten van patiënten met DM1-gerelateerde cardiomyopathie. Microarray analyse toonde significante veranderingen in de expressie niveaus van 989 mRNAs en 16 miRNAs in harten van DM1 patiënten in vergelijking met controle personen. We hebben in hartweefsel een aantal veranderingen in genexpressie gevonden, die in overeenstemming zijn met huidige pathofysiologische concepten, zoals het verlies van DMPK en SIX5 expressie. Bioinformatische analyse van mRNA expressie data identificeerde verscheidene pathologische veranderingen, zoals verstoring van cardiale mitochondriale functie, RNA processing, calcium signalering en veranderingen in expressie van genen betrokken zijn in de organisatie van het cytoskelet. Deze veranderingen kunnen in het hart van DM1 patiënten leiden tot geleidingsstoornissen, aritmie en myocardiale afwijkingen.

## Uitkomstmaten

Na verloop van tijd leidt DM1 tot ziekteverschijnselen van spieren en van tal van andere organen met aanzienlijke beperkingen in het dagelijks leven. De bestaande behandelingen zijn voornamelijk gericht op symptoombestrijding. Daarnaast heeft een beter begrip van de complexe pathogenese geleid tot de ontwikkeling van potentiële moleculaire targets voor therapeutische interventies in preklinische studies. Gezien deze recente ontwikkelingen in therapeutische mogelijkheden is het noodzakelijk dat er goede uitkomstmaten beschikbaar zijn om het effect van interventies op de functionele toestand van de patiënt in klinische trials te kunnen evalueren. De belangrijkste doelstellingen van het tweede deel van dit proefschrift waren het illustreren van het belang van nieuwe klinimetrisch methoden en het ontwikkelen van nieuwe klinische meetinstrumenten voor toekomstige DM1 studies.

**Hoofdstuk 6** geeft een overzicht van uitkomstmaten die in de afgelopen 60 jaar zijn toegepast in klinische studies met DM1 patiënten. Een zeer groot aantal verschillende meetinstrumenten is toegepast waardoor een vergelijking van studieresultaten niet goed mogelijk is. Bovendien zijn de klinimetrische eigenschappen van de meeste meetinstrumenten, zoals validiteit, betrouwbaarheid en responsiviteit, onvolledig of slecht geëvalueerd. Een andere beperking is het feit dat vragenlijsten op de klassieke test theorie gebaseerd zijn en enkel gegevens met een ordinaal meetniveau opleveren. Ordinale uitkomsten worden vaak onjuist gebruikt als interval meetniveau in parametrische analyses. Het gebruik van moderne klinimetrische technieken wordt daarom aanbevolen bij het evalueren en ontwikkelen van uitkomstmaten. Daarnaast wordt gepleit voor een gestandaardiseerde set van uitkomstmaten in toekomstige onderzoeken om vergelijking testresultaten en de communicatie in de klinische praktijk te vergemakkelijken.

**Hoofdstuk 7** is een introductie over het zogenaamde Rasch model en bespreekt in begrijpelijke taal de verschillende stappen van het beoordelen en ontwikkelen van een meetinstrument volgens de Rasch methode. Deze moderne klinimetrische techniek stelt aanvullende kwaliteitsvoorwaarden aan uitkomstmaten en kan, als de data voldoen aan de verwachtingen van het statistisch model, de moeilijkheidsgraad van items en het functionele niveau van patiënten hiërarchisch ordenen op een interval schaal, zodat metingen onderling vergelijkbaar zijn.

**Hoofdstuk 8** beschrijft de ontwikkeling en toetsing van een "activiteiten en participatie" schaal voor DM1 patiënten (DM1-Activ), gebaseerd op het Rasch model. Deze studie illustreert het belang van nieuwe klinimetrische methoden bij de ontwikkeling van meetinstrumenten voor trials met neurologische patiënten. Met de DM1-Activ vragenlijst kunnen functionele beperkingen in dagelijkse leven van DM1 patiënten worden beoordeeld. De DM1-Activ is een doelmatig, valide en betrouwbaar meetinstrument, dat voldoet aan moderne klinimetrische vereisten. Om klinische toepassing van de uitkomstmaat mogelijk te maken, moet de DM1-Activ schaal echter in een groter cohort van patiënten onderzocht te worden.

**Hoofdstuk 9** beschrijft de reconstructie van de DM1-Activ schaal voor klinisch gebruik (DM1-Activ$^{c}$) met een uitgebreidere vragenlijst en een grotere patiëntenpopulatie. De nieuwe uitkomstmaat voldoet eveneens aan de eisen van het Rasch model en heeft een sterker discriminerend vermogen en hogere betrouwbaarheid dan de DM1-Activ. Met het nomogram kan de ruwe score worden omgezet naar een interval niveau score die kan worden gebruikt in toekomstige klinische observatie en interventie studies.

**Hoofdstuk 10** beschrijft de ontwikkeling en toetsing van een gecombineerde "vermoeidheid en slaperigheid" schaal voor DM1 patiënten (FDSS), gebaseerd op het Rasch model. De FDSS omzeilt het probleem om vermoeidheid van slaperigheid te onderscheiden door een uitkomstmaat met een gemeenschappelijk onderliggend construct te veronderstellen. Bestaande vragenlijsten meten de ernst van slaperigheid en vermoeidheid als aparte entiteiten. Echter, overmatige slaperigheid overdag en vermoeidheid hebben veel overlappende kenmerken en patiënten kunnen niet altijd duidelijk maken of hun klachten berusten op slaperigheid, vermoeidheid of beide. Rasch analyse toonde aan dat vragen over slaperigheid en vermoeidheid inderdaad samen één construct meten. Dit pleit sterk voor een gecombineerde klinische uitkomstmaat voor deze symptomen. In vergelijking tot de veel gebruikte ordinale vragenlijsten om vermoeidheid en slaperigheid te meten, heeft de FDSS voor DM1 patiënten een groter bereik van makkelijke tot moeilijke vragen. Daardoor sluit de FDSS beter aan bij patiënten met verschillende niveaus van slaperigheid en/of vermoeidheid. Gebruik van deze uitkomstmaat wordt aanbevolen in toekomstige klinische trials bij DM1 patiënten.

**Hoofdstuk 11** geeft een samenvatting van de verschillende studieresultaten die in dit proefschrift zijn beschreven. De resultaten worden ter discussie gesteld en er worden aanbevelingen gedaan voor toekomstig onderzoek.

# Dankwoord

Het zal zeker al vaker gezegd zijn, maar een proefschrift maak je niet alleen. Velen hebben de afgelopen jaren bijgedragen aan de totstandkoming ervan. Een aantal personen wil ik in het bijzonder bedanken.

Allereerst bedank ik alle patiënten die aan dit onderzoek hebben deelgenomen. Ik heb veel bewondering voor jullie en de mantelzorgers. Het was voor velen een behoorlijke inspanning om jaarlijks helemaal naar Maastricht te komen en een lange dag onderzoeken te ondergaan. Dank voor jullie medewerking, geduld en interesse.

De promotor: Prof. dr. Y.M. Pinto, beste Yigal, ik wil je danken voor de gelegenheid om dit project aan te gaan. Je vertrek naar Amsterdam heeft een grote invloed gehad op het verdere verloop van dit onderzoek, maar het uiteindelijke resultaat mag er zijn. Bovendien kan een interuniversitaire samenwerking in de toekomst nog mooie dingen opleveren.

De copromotoren: Dr. C.G. Faber, beste Karin, je bent de motor van het neuromusculaire onderzoek in Maastricht. Met je enthousiasme weet je menig assistent te interesseren voor wetenschappelijk onderzoek in de neuromusculaire groep. Je bent het meest intensief bij mijn promotie betrokken geweest. Jouw deur staat altijd open, je bent ontzettend behulpzaam en je weet me altijd weer te motiveren.
Dr. I.S.J. Merkies, beste Ingemar, gaande weg mijn promotietraject ben je steeds meer betrokken geraakt bij mijn onderzoek. Toen ik stuurloos dreigde rond te dobberen, heb jij orde geschept en me richting gegeven. Je eigenzinnigheid en praktische instelling leiden tot een prettige samenwerking. Ik waardeer je vertrouwen in mijn kennis en kunde.

Mijn paranimfen: Mayienne, we zijn al jaren lotgenoten in het onderzoek en kamergenootjes op cursussen en congressen tijdens de opleiding neurologie. We hebben veel onderzoeksleed gedeeld en jij weet als geen ander hoe frustrerend het kan zijn. Maar het eind is in zicht, dus zet hem op! Angela, wat begon als twee onervaren dokters op een verpleegafdeling in het Cathrien, is ontaard in een bijzondere vriendschap. De vele koffiemomenten zorgden voor de nodige ontspanning en zelfreflectie. Ik hoop dat we nog lang op elkaars steun en vriendschap kunnen bouwen.

Prof. dr. M.H. De Baets, beste Marc, na een leuke wetenschapsstage neuro-immunologie heb je me met Karin in contact gebracht met een promotietraject en proefschrift als resultaat! Dank daarvoor.

Arts-assistenten en stafleden van de vakgroep neurologie wil ik bedanken voor de prettige werksfeer. Brigitte Brouwer, bedankt voor al je hulp en de prachtige informatieve website voor patiënten en hun (para)medische behandelaars.

Els Vanhoutte, Floris De Kleermaeker en Karen van Norden wil ik danken voor hun wetenschapsparticipatiestage. Els, je hebt als student een bijdrage geleverd aan dit onderzoek. Met jouw enthousiasme en energie heb je zelf in korte tijd een hoop weten te bereiken. Ik wens je een mooie toekomst als klinisch geneticus. Floris en Karen, leuk dat we jullie enthousiast hebben kunnen maken voor een neuromusculaire wetenschapsstage en bedankt voor werk dat jullie hebben verricht.

Een aantal cardiologen wil ik bedanken. Prof. dr. Harry Crijns en Dr. Laurent Pison voor het verrichten van elektrofysiologische onderzoeken en hartbiopten. Dr. Simon Schalla en Dr. Bas Bekkers voor hun tijd en geduld bij het (her) beoordelen van cardiale MRI's en zinvolle commentaar op het betreffende manuscript. Dr. Kevin Vernooy, voor de samenwerking op het maandelijkse Steinert spreekuur en het ontwikkelen van een lokaal cardiologie protocol voor diagnostiek en behandeling.

De afdeling genetica heeft eveneens een belangrijke bijdrage geleverd aan de totstandkoming van dit proefschrift. Prof. dr. C.E.M. de Die-Smulders, beste Christine, jouw database was het startpunt van dit onderzoek en daarom onmisbaar voor dit onderzoek. Prof. dr. Bert Smeets en Dr. Monique Gerrits wil ik bedanken voor hun inspanningen om de 'exacte' CTG expansies in leukocyten te bepalen ten behoeve van de reviewers.

Blanche Schroen, bedank ik voor het beheren en bewerken van ons kostbare hartweefsel en de prettige samenwerking bij het schrijven van het microarray manuscript. Jack Cleutjens voor de hulp bij de morfometrische analyses van de hartbiopten. Bioinformatics Georg Summer and Elizabeth McClellan, thank you for helping me out with the microarray data analysis.

Carla, we zijn blij met jou als nurse practitioner neuromusculaire ziekten. Charlotte en Miranda, het multidisciplinaire myotone dystrofie team levert maatwerk voor de patiënt en daarbij is jullie ondersteuning onmisbaar. Daniëlle, als research verpleegkundige heb je je hart al snel verloren aan deze patiënten

populatie. Het is jammer dat je niet meer tijd had om dit werk te kunnen blijven doen.

Medewerkers van het planbureau Hart en Vaat Centrum, bedankt voor jullie inzet en flexibiliteit bij het plannen van alle cardiologische onderzoeken. Polimedewerkers van het Hart en Vaat Centrum, klinische genetica en neurologie, dankzij jullie kon ik altijd wel ergens een ruimte vinden om patiënten te onderzoeken. Medewerkers van de vaatkamer, bedankt voor jullie hartelijke ontvangst bij de elektrofysiologische onderzoeken.

De Vereniging Spierziekten Nederland dank ik voor het verspreiden van proefpersonen informatie en vragenlijsten onder hun leden.

Marije, Suzanne, Marlies, Ellen, Bianca, Jacqueline en Santy, een clubje geneeskunde studenten dat dacht dat je als coassistent geen sociaal leven meer kon hebben. Hoewel iedereen inmiddels zijn eigen weg is gegaan en de frequentie van onze meetings afneemt, blijft het jaarlijks weekendje weg een vaste afspraak in onze drukke agenda's.

Lieve familie. Bedankt dat ik alle vrijheid heb gekregen en altijd mijn eigen keuzes kon maken. Mama en Paul, jullie vormen mijn thuis in Weert, een vertrouwde basis om op terug te vallen. Papa, ook al ben je er helaas niet meer, je blijft mijn voorbeeld van doorzettingsvermogen. Theo en Marjan, bedankt voor jullie interesse in mijn werk en onderzoek.

Lieve Matthijs, het beste van alles komt op het einde, in ieder geval in dit dankwoord. Je bent mijn steun bij teleurstellingen, mijn rustpunt bij stress en mijn helpdesk bij computerperikelen. Maar bovenal ben je de allerliefste zodat alles altijd weer goed komt. Ik houd van je!

# Curriculum Vitae

Mieke Hermans was born on the 26th of March 1981 in Weert, the Netherlands. She attended Gymnasium at the Bisschoppelijk College in Weert, where she graduated cum laude in 1998. The same year, she started her medical training at the Limburg's University Centre in Belgium (now Hasselt University). In 1999 she continued her training at the faculty of Medicine at the Maastricht University. She graduated cum laude in 2005 and started working as a clinical resident in neurology at the Catharina Hospital in Eindhoven. In 2006 she started her research on myotonic dystrophy at the department of neurology of the Maastricht University Medical Centre. She received a grant from the Prinses Beatrix Spierfonds to support her research. In 2009 she started her neurology training which she will finish in 2015.

Mieke Hermans werd op 26 maart 1981 geboren in Weert, Nederland. Ze ging naar het gymnasium aan het Bisschoppelijk College in Weert, waar ze cum laude haar diploma haalde in 1998. Dat jaar ging ze geneeskunde studeren aan het Limburg's Universitair Centrum in België (nu Universiteit van Hasselt). In 1999 vervolgde ze haar studie aan de faculteit der geneeskunde van de Universiteit Maastricht. Ze studeerde cum laude af in 2005 en ging als arts-assistent neurologie werken in het Catharina ziekenhuis in Eindhoven. In 2006 begon ze aan het onderzoek naar myotone dystrofie aan de afdeling neurologie van het Maastricht Universitair Medisch Centrum. Ze ontving een subsidie van het Prinses Beatrix Spierfonds. In 2009 begon ze aan de opleiding tot neuroloog welke ze in 2015 zal voltooien.

# List of publications

Fatigue and daytime sleepiness scale in myotonic dystrophy type 1.
**Hermans MC**, Merkies IS, Laberge L, Blom EW, Tennant A, Faber CG. *Muscle and Nerve 2013 Jan;47(1):89-95.*

Structural and functional cardiac changes in myotonic dystrophy type 1: a cardiovascular magnetic resonance study.
**Hermans MC**, Faber CG, Bekkers SC, de Die-Smulders CE, Gerrits MM, Merkies IS, Snoep G, Pinto YM, Schalla S. *J Cardiovasc Magn Reson.* 2012 Jul;14(1):48.

Modifying the Medical Research Council grading system through Rasch analyses.
Vanhoutte EK, Faber CG, van Nes SI, Jacobs BC, van Doorn PA, van Koningsveld R, Cornblath DR, van der Kooi AJ, Cats EA, van den Berg LH, Notermans NC, van der Pol WL, **Hermans MC**, van der Beek NA, Gorson KC, Eurelings M, Engelsman J, Boot H, Meijer RJ, Lauria G, Tennant A, Merkies IS; on behalf of the PeriNomS Study Group. *Brain* 2012 May;135(Pt 5):1639-49.

Peripheral neuropathy in myotonic dystrophy type 1.
**Hermans MC**, Faber CG, Vanhoutte EK, Bakkers M, De Baets MH, de Die-Smulders CE, Merkies IS. *J Peripher Nerv Syst* .2011 Mar;16(1):24-9.

Rasch-built Overall Disability Scale (R-ODS) for immune-mediated peripheral neuropathies.
van Nes SI, Vanhoutte EK, van Doorn PA, **Hermans MC**, Bakkers M, Kuitwaard K, C.G. Faber, I.S.J. Merkies. *Neurology* 2011 Jan;76(4):337-45.

Pain and autonomic dysfunction in patients with sarcoidosis and small fibre neuropathy.
Bakkers M, Faber CG, Drent M, **Hermans MC**, van Nes SI, Lauria G, De Baets M, Merkies IS. *J Neurol.* 2010 Dec;257(12):2086-90.

Hereditary muscular dystrophies and the heart.
**Hermans MC**, Pinto YM, Merkies IS, de Die-Smulders CE, Crijns HJ, Faber CG. *Neuromuscul Disord.* 2010 Aug;20(8):479-92.

Rasch-built myotonic dystrophy type 1 activity and participation scale (DM1-Activ).
**Hermans MC**, Faber CG, De Baets MH, de Die-Smulders CE, Merkies IS. *Neuromuscul Disord.* 2010 May;20(5):310-8.

Intraepidermal nerve fiber density and its application in sarcoidosis.
Bakkers M, Merkies IS, Lauria G, Devigili G, Penza P, Lombardi R, **Hermans MC**, van Nes SI, De Baets M, Faber CG. *Neurology* 2009 Oct;73(14):1142-8.

Constipation after botulinum toxin – an injection in lower and upper limb muscles, a transitory systemic autonomic adverse effect: report of three cases.
Vles GF, Vles JS, **Hermans MC**, Faber CG, de Louw A, Martina JD. *J Exp Clin Med*. 2009;26: 47–51.

Sudden death in myotonic dystrophy.
**Hermans MC**, Faber CG, Pinto YM. *N Engl J Med.* 2008 Oct;359(15):1626-8.

Revising two-point discrimination assessment in normal aging and in patients with polyneuropathies.
van Nes SI, Faber CG, Hamers RM, Harschnitz O, Bakkers M, **Hermans MC**, Meijer RJ, van Doorn PA, Merkies IS; PeriNomS Study Group. *J Neurol Neurosurg Psychiatry* 2008 Jul;79(7):832-4.

# Appendices

**Appendix A.** The Rasch-built activity and participation scale for patients with myotonic dystrophy type 1 for clinical use (DM1-Activ).

| | Item | Unable to perform (0) | Able to perform, but with difficulty (1) | Able to perform, without difficulty (2) |
|---|---|---|---|---|
| | **Are you able to...** | | | |
| 1 | wash your face? | ☐ | ☐ | ☐ |
| 2 | brush your teeth? | ☐ | ☐ | ☐ |
| 3 | take a shower? | ☐ | ☐ | ☐ |
| 4 | sit up straight? | ☐ | ☐ | ☐ |
| 5 | open toothpaste? | ☐ | ☐ | ☐ |
| 6 | prepare a meal? | ☐ | ☐ | ☐ |
| 7 | button your shirt/blouse? | ☐ | ☐ | ☐ |
| 8 | walking avoiding obstacles? | ☐ | ☐ | ☐ |
| 9 | perform a hobby? | ☐ | ☐ | ☐ |
| 10 | get in/out of a car? | ☐ | ☐ | ☐ |
| 11 | walk on uneven ground? | ☐ | ☐ | ☐ |
| 12 | bend over to pick up object? | ☐ | ☐ | ☐ |
| 13 | walk one flight of stairs? | ☐ | ☐ | ☐ |
| 14 | grab an object above head? | ☐ | ☐ | ☐ |
| 15 | perform study/work? | ☐ | ☐ | ☐ |
| 16 | stand up from lying down? | ☐ | ☐ | ☐ |
| 17 | do the cleaning? | ☐ | ☐ | ☐ |
| 18 | do sports? | ☐ | ☐ | ☐ |
| 19 | do the gardening? | ☐ | ☐ | ☐ |
| 20 | run? | ☐ | ☐ | ☐ |

**Appendix B**. The reconstructed Rasch-built activity and participation scale for patients with myotonic dystrophy type 1 for clinical use (DM1-Activ[c]).

| | Item | Unable to perform (0) | Able to perform, but with difficulty (1) | Able to perform, without difficulty (2) |
|---|---|---|---|---|
| | **Are you able to...** | | | |
| 1 | eat soup? | ☐ | ☐ | ☐ |
| 2 | visit family or friends? | ☐ | ☐ | ☐ |
| 3 | care for your hair and body? | ☐ | ☐ | ☐ |
| 4 | dress your lower body? | ☐ | ☐ | ☐ |
| 5 | wash your upper body? | ☐ | ☐ | ☐ |
| 6 | take a shower? | ☐ | ☐ | ☐ |
| 7 | wash your lower body? | ☐ | ☐ | ☐ |
| 8 | get out of bed? | ☐ | ☐ | ☐ |
| 9 | move a chair? | ☐ | ☐ | ☐ |
| 10 | do the dusting/cleaning? | ☐ | ☐ | ☐ |
| 11 | do the shopping? | ☐ | ☐ | ☐ |
| 12 | tie the laces of your shoes? | ☐ | ☐ | ☐ |
| 13 | catch an object (e.g. a ball)? | ☐ | ☐ | ☐ |
| 14 | use dustpan and brush? | ☐ | ☐ | ☐ |
| 15 | empty dustbin? | ☐ | ☐ | ☐ |
| 16 | make up your bed? | ☐ | ☐ | ☐ |
| 17 | vacuum clean? | ☐ | ☐ | ☐ |
| 18 | serve coffee/tea on a tray? | ☐ | ☐ | ☐ |
| 19 | dance? | ☐ | ☐ | ☐ |
| 20 | stand up from squatting position? | ☐ | ☐ | ☐ |
| 21 | stand on one leg? | ☐ | ☐ | ☐ |
| 22[a] | run ([a]**for those aged <30 years**)? | ☐ | ☐ | ☐ |
| 23 | walk uphill? | ☐ | ☐ | ☐ |
| 24 | walk 3 flights of stairs? | ☐ | ☐ | ☐ |
| 25 | carry and put down heavy object (10 kg)? | ☐ | ☐ | ☐ |
| 22[b] | run ([b]**for those aged ≥30 years**)? | ☐ | ☐ | ☐ |

**Appendix C.** The Rasch-built fatigue and daytime sleepiness scale for patients patients with myotonic dystrophy type 1 (FDSS).

| | Item | Seldom or never (0) | Sometimes (1) | Almost always (2) |
|---|---|---|---|---|
| 1 | My motivation is lower when I am fatigued | ☐ | ☐ | ☐ |
| 2 | Exercise brings on my fatigue | ☐ | ☐ | ☐ |
| 3 | Fatigue is among my three most disabling symptoms | ☐ | ☐ | ☐ |
| 4 | Fatigue causes frequent problems for me | ☐ | ☐ | ☐ |
| 5 | Do you take one or more naps during the day? | ☐ | ☐ | ☐ |
| 6 | Do you fall asleep while watching TV? | ☐ | ☐ | ☐ |
| 7 | Are you generally in great shape during the day? | ☐ | ☐ | ☐ |
| 8 | Do you doze off or fall asleep as a passenger in a car for an hour without a break? | ☐ | ☐ | ☐ |
| 9 | Do you doze off or fall asleep when sitting and reading? | ☐ | ☐ | ☐ |
| 10 | Do you doze off or fall asleep while sitting inactive in a public place? | ☐ | ☐ | ☐ |
| 11 | Do you doze off or fall asleep in a car, while stopped for a few minutes in traffic? | ☐ | ☐ | ☐ |
| 12 | Do you doze off or fall asleep while sitting and talking to someone? | ☐ | ☐ | ☐ |

Appendix D. Algorithm for cardiac assessment in patients with myotonic dystrophy type 1.