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Most expression and splicing changes in myotonic dystrophy type 1 and type 2 skeletal muscle are shared with other muscular dystrophies

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Abstract

The prevailing pathomechanistic paradigm for myotonic dystrophy (DM) is that aberrant expression of embryonic/fetal mRNA/protein isoforms accounts for most aspects of the pleiotropic phenotype. To identify aberrant isoforms in skeletal muscle of DM1 and DM2 patients, we performed exon-array profiling and RT-PCR validation on the largest DM sample set to date, including Duchenne, Becker and tibial muscular dystrophy (NMD) patients as disease controls, and non-disease controls. Strikingly, most expression and splicing changes in DM patients were shared with NMD controls. Comparison between DM and NMD identified almost no significant differences. We conclude that DM1 and DM2 are essentially identical for dysregulation of gene expression, and DM expression changes represent a subset of broader spectrum dystrophic changes. We found no evidence for qualitative splicing differences between DM1 and DM2. While some DM-specific splicing differences exist, most of the DM splicing differences were also seen in NMD controls. *SSBP3* exon 6 missplicing was observed in all diseased muscle and led to reduced protein. We conclude there is no widespread DM-specific spliceopathy in skeletal muscle and suggest that missplicing in DM (and NMD) may not be the driving mechanism for the muscle pathology, since the same pathways show expression changes unrelated to splicing.

Keywords: Myotonic dystrophy; DM1; DM2; Aberrant isoform expression; Missplicing

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1. Introduction

Myotonic dystrophy (DM) is the most common adult-onset muscular dystrophy and characterized by a pleiotropic phenotype with multisystem involvement [1,2]. Although mutations have been identified, the pathomechanisms are incompletely understood, and presently there is no effective treatment. DM type 1 (DM1) and type 2 (DM2) are caused by different unstable, non-coding microsatellite expansions—$(CTG)_{DM1}$ in *DMPK* [3–5] and $(CCTG)_{DM2}$ in *CNBP* (*ZNF9*) [6,7]. Despite similar mutations, DM1 and DM2 are clinically distinct diseases and the basis for these differences is as yet unknown [2]. Since transcription of the mutant repeats into $(CUG)_{DM1}$/$(CCUG)_{DM2}$ appears to be necessary and sufficient to cause disease [8,9], the prevailing paradigm is that DM1 and DM2 are toxic RNA gain-of-function diseases [10–13]. Mutant RNAs form ribonuclear inclusions that sequester splice factors of the MBNL family, resulting in a number of embryonic/fetal isoforms being aberrantly expressed in adult tissues, with over 30 genes verified as missplicing to date [12,14–17]. Thus, efforts to understand the pathomechanisms in DM have so far focused primarily on aberrant splicing. However, while missplicing of the chloride channel *CLCN1* can account for the myotonia [18–22], the pathogenic roles of the other aberrantly spliced genes has not been experimentally demonstrated and remain circumstantial.

Evidence from several different animal models suggests that splicing, RNA foci, and muscle pathology are separable events [23–26] and strongly suggest the existence of additional pathomechanisms (recently reviewed in [2,27]). Moreover, studying human patients and mouse models of muscular dystrophies not associated with toxic RNAs, we and others recently reported that splicing changes may be a much more general phenomenon of muscle disease and can be secondary to muscle regeneration [28,29]. These findings raise questions concerning the extent to which missplicing plays a major role in the disease phenotype and whether expression or splicing changes in DM are specific to this disease or are common manifestations of dystrophic muscle in general. There is, as yet, no comprehensive study of expression and splicing for human DM skeletal muscle that addresses these issues, especially comparing DM1 to DM2, or DM (DM1 and/or DM2) to non-DM dystrophies. The purpose of our study was to elucidate the relative contributions of aberrant splicing and aberrant expression to the DM phenotype and to distinguish DM1- and DM2-specific events from those common to all dystrophic muscle. To this end, we performed global array-based expression and splicing profiling using the Affymetrix Exon 1.0 ST array. Along with skeletal muscle samples from DM1, DM2 and normal individuals, we also profiled samples from patients with non-DM inherited muscular dystrophies.

2. Materials and methods

2.1. Overall experimental design

After confirming molecular diagnosis using patient DNA [7,30], total RNA was extracted from skeletal muscle samples and used for both cDNA preparation and array hybridization [31]. Following analysis of the array data, pathway analysis was conducted on various gene lists resulting from these analyses and verification of aberrant splicing by RT-PCR was attempted for select genes identified as expressing alternative transcripts.

2.2. Patient samples

For the exon array profiling we used a panel of 28 retrospective skeletal muscle biopsies from DM1 ($n = 8$), DM2 ($n = 10$), Becker muscular dystrophy (BMD, $n = 3$), Duchenne muscular dystrophy (DMD, $n = 1$), tibial muscular dystrophy (TMD, $n = 2$) and normal skeletal muscle ($n = 4$). Patient samples were obtained through the active collaboration and sharing of patient samples within the European Neuromuscular Centre (ENMC) consortium on *DM2 and Other Myotonic Dystrophies* [32]. Following informed consent samples were collected with the appropriate oversight at the institutions of the various collaborating investigators. Normal control RNAs were purchased commercially (Ambion, Biochain, and Stratagene). A subset of these samples was used for alternative splicing verification by RT-PCR. Several additional patient samples were included in the splice validation panel, but not the array experiment. Samples and assays run are described in more detail in Table 1.

2.3. Verification of diagnosis for DM1 and DM2

Presence or absence of DM1 or DM2 expansion mutations were verified by PCR-based molecular genetic diagnostic procedures as previously described [7,30]. Two alleles observed at *DMPK* or *CNBP*/*ZNF9* was considered sufficient reason for exclusion of a diagnosis of DM1 or DM2, respectively. Repeat-primed PCR (RP-PCR) was performed on all samples and was used to distinguish between homozygous samples and those with amplification-resistant expansions.

2.4. RNA extraction

RNA was extracted using the TriZol Reagent according to the manufacturer's suggestions (Invitrogen, Carlsbad, CA) and further purified using the RNeasy kit (Qiagen Inc., Valencia, CA). The quality and integrity of the RNA was then analyzed on an Agilent Bio- Analyzer (RNA 6000 Nano LabChip). Total cellular RNA samples with a RIN (RNA integrity number) > 7 were used for further microarray studies [31].