Poster number 93

Characterization of the myotonic dystrophy type 2 miRNome

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ABSTRACT

Myotonic dystrophy type 2 (DM2) remains far less characterized than DM1, and the molecular mechanisms underlying its muscle pathology are still poorly understood. Although both diseases are caused by toxic repeat expansions that sequester MBNL proteins, the weaker correlation between MBNL loss and clinical severity in DM2 suggests the contribution of additional regulatory layers.

In DM1, microRNA (miRNA) dysregulation has been shown to exacerbate MBNL depletion and splicing defects, but its role in DM2 remains largely unexplored. Here, we performed integrated mRNA-seq and small RNA-seq on muscle biopsies from DM2 patients and controls to characterize transcriptomic alterations, aberrant splicing events, and miRNA dysregulation. By comparing DM2 splicing changes with the Splice Index developed for DM1, we identify both shared and distinct molecular features, providing new insight into DM2 pathogenesis and potential therapeutic targets.

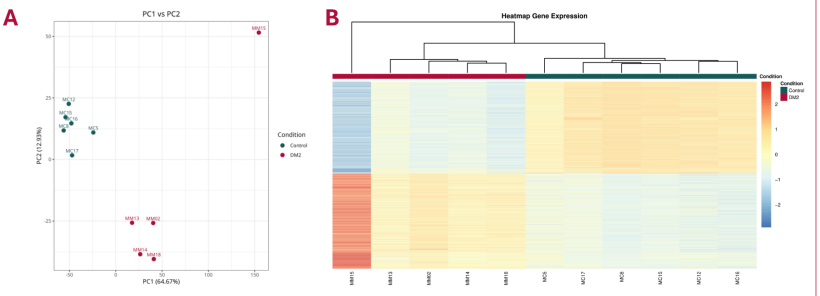
OBJECTIVES

1) To identify dysregulated miRNAs in DM2 with potential functional and therapeutic relevance.

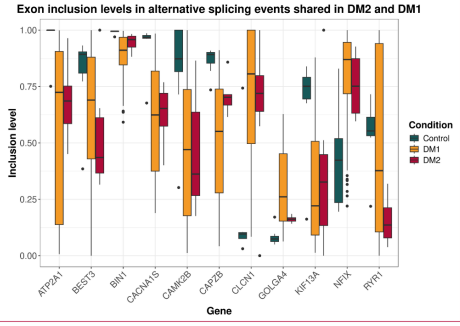
2) To characterize transcriptomic alterations and aberrant splicing events in DM2 muscle biopsies and assess their overlap with established DM1 splicing biomarkers.

PRELIMINARY RESULTS

1 mRNA-seq-based transcriptome profiling of DM2 in patient muscle biopsies and controls

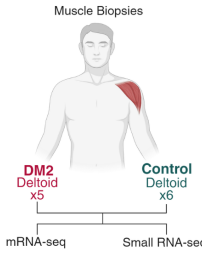


C Splicing events from the DM1 Splice Index evaluated in DM2 muscle biopsies



A. **Principal Component Analysis (PCA) of the transcriptomic profile.** Control samples (blue) and patient samples (red) cluster according to their group, except for MM15 that is an outlier.
B. **Heatmap of differentially expressed genes.** Control samples (blue) and patient samples (red) cluster according to their group, following opposite expression patterns. MM15 follows the same trend as other DM2 samples but with higher intensity.
C. **Boxplot of exon inclusion levels of Splice Index splicing events** across control samples (blue), DM2 patient samples (red), and all DM1 samples reported by Provenzano et al. (2025) (yellow). **Eleven of the 22 splicing events** described as biomarkers of disease progression **in DM1 are also altered in DM2 biopsies.**

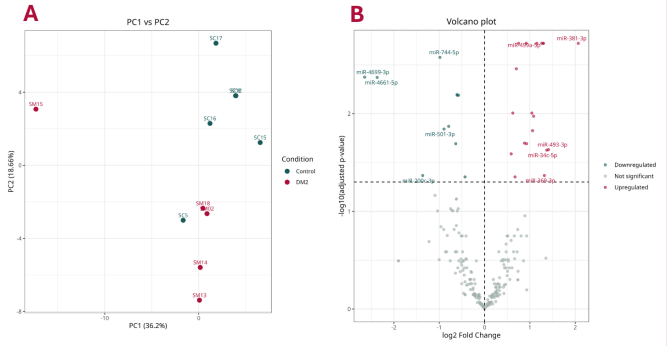
WORK FLOW



Sample name*	Sample Type	Gender	Age
M02	DM2	Female	52
M13	DM2	Female	69
M14	DM2	Female	53
M15	DM2	Female	75
M18	DM2	Female	37
C5	Control	Male	63
C8	Control	Male	24
C12	Control	Male	55
C15	Control	Female	34
C16	Control	Female	46
C17	Control	Female	37

*In the Results section, sample names are preceded by the prefix M (mRNA-seq) or S (small RNA-seq)

2 smallRNA-seq-based microRNAs analysis of DM2 in patient muscle biopsies and controls



A. **Principal component analysis (PCA) of microRNA profiles.** Control samples (blue) and DM2 samples (red) cluster separately, with SC5 lying close to the DM2 group and MM15 behaving as an outlier.

B. **Volcano plot of differentially expressed microRNAs between control and DM2 samples.** Downregulated microRNAs are shown in blue (n=13) and upregulated in red (n=18). A total of **31 dysregulated microRNAs were identified**. The names of the top 5 most significantly dysregulated microRNAs in DM2 biopsies are shown.

DISCUSSION

PCAs revealed clear clustering of DM2 patients and controls in both mRNA-seq and small RNA-seq datasets (**Figure 1A, 2A**). However, one sample (M15) consistently behaved as an outlier, showing different trends in both PCAs. Although its heatmap profile (**Figure 1B**) followed the same direction as other DM2 samples, it did so with markedly higher intensity. Additional biopsies and clinical/histological data are being analyzed to determine whether this pattern is unique or recurrent.

Regarding the 22 events described as biomarkers of disease progression in DM1 that constitute the Splice Index (Provenzano et al., 2025), half of the DM1-altered events were also detected in our DM2 samples (**Figure 1C**). Notably, Provenzano et al. used tibialis anterior muscle biopsies (a distal muscle), as distal muscles are more prominently affected in DM1, whereas our study is based on deltoid muscle biopsies (a proximal muscle), as proximal muscles are more prominently involved in DM2. This difference in muscle group may partly explain the partial overlap observed between the two datasets.

Lastly, 31 dysregulated microRNAs were identified (**Figure 2B**). The small sample size (n=5) likely limit detection and, therefore, larger cohorts are needed for reliable conclusions.

CONCLUSIONS

Integrated transcriptomic and miRNA profiling distinguishes DM2 muscle biopsies from controls and reveals partial overlap with DM1 splicing biomarkers. Our data indicate that DM2 shares selected splicing alterations with DM1 while exhibiting miRNA dysregulation profile.

These findings support the contribution of additional regulatory mechanisms beyond MBNL sequestration in DM2 and provide a molecular framework for future functional and therapeutic studies.

MAIN REFERENCE

Provenzano, M., Ikegami, K., Bates, K., Gaynor, A., Hartman, J. M., Jones, A., Butler, A., Berggren, K. N., Dekdebrun, J., Hung, M., Lapato, D. M., Kiefer, M., Thornton, C. A., Johnson, N. E., Hale, M. A., & Myotonic Dystrophy Clinical Research Network (DMCRN) (2025). The Splice Index as a prognostic biomarker of strength and function in myotonic dystrophy type 1. The Journal of clinical investigation, 135(4), e185426. https://doi.org/10.1172/JCI185426

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