Generation and Characterization of a DM2 BAC Mouse Model

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1 Abstract

Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are multisystemic diseases caused by CTG•CAG (DM1) or CCTG•CAGG (DM2) repeat expansion mutations located in the dystrophia myotonica protein kinase (DMPK) or cellular nucleic acid binding (CNBP) genes, respectively. While RNA gain of function effects play a role in disease, bidirectional transcription and repeat associated non-AUG (RAN) translation may also contribute to DM. RAN translation of sense (CCUG) and antisense (CAGG) expansion transcripts produce sense (LPAC) and antisense (QAGR) RAN proteins that accumulate in DM2 human autopsy brains. LPAC is found in neurons, astrocytes, and glia in gray matter while QAGR is found in oligodendrocytes located in white matter. Both LPAC and QAGR RAN proteins, which are toxic to cells in culture, are found in brain regions that show pathologic changes suggesting they contribute to CNS features of the disease (Zu et al. 2017, Neuron 95:1292-1305). Understanding the role of RAN proteins in DM2 and developing therapeutic approaches requires animal models that mirror DM2 patient disease features. Using a DM2 patient-derived bacterial artificial chromosome (BAC) library approach our lab has successfully generated two separate lines of transgenic DM2 mice. Both DM2 BAC mouse lines contain the entire CNBP gene with large repeat expansions (~750 CCTG). Substantial flanking sequences on the BAC are likely to contain endogenous promoter sequences to more accurately recapitulate endogenous human expression patterns. RT-PCR shows that both transgenic lines express the *CNBP* transgene in brain and muscle. We also show these mice develop CCUG RNA foci. We are currently characterizing these mice for other DM2-relevant molecular, histopathological and behavioral features. Our goal is that these mice will be a useful tool to better understand the molecular mechanisms of disease and for the development of therapeutic strategies.

2 Background and Introduction

Disease Mechanisms in Myotonic Dystrophy Type 2

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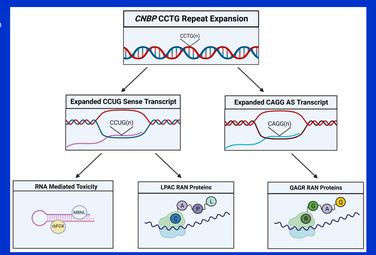

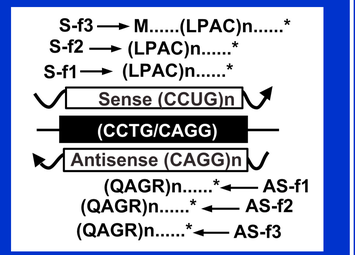

Zu et al. *Neuron* 2017

Fig. 1 DM2 disease mechanisms. *CNBP* expansion RNA aggregates into RNA foci, sequestering RNA binding proteins, leading to dysregulation of splicing and post-transcriptional processing. Expansion RNAs also undergo RAN translation, generating toxic RAN proteins. The DM2 CCTG·CAGG repeat expansion is bidirectionally transcribed and sense and antisense transcripts undergo RAN translation and the production of Leu-Pro-Ala-Cys (LPAC) and Gln-Ala-Gly-Arg (QAGR) proteins.

LPAC and QAGR RAN Proteins Accumulate in Human DM2 Brain Regions with Pathological Changes

Zu et al. *Neuron* 2017

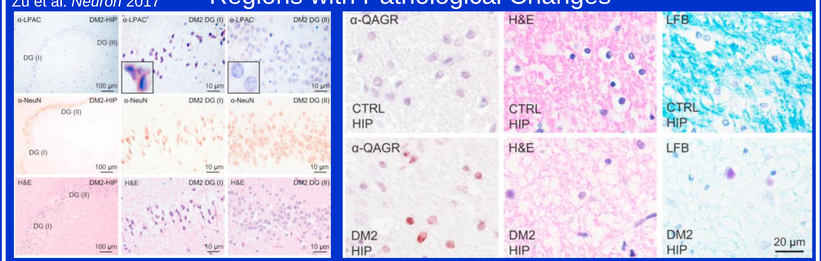

Fig. 2 LPAC and QAGR RAN proteins in DM2 patient brains. LPAC and QAGR aggregates accumulate in DM2 patient brains. LPAC is found in grey matter regions undergoing necrosis. In contrast, QAGR is most prominently found in white matter regions associated with demyelination.

3 Generation of DM2 BAC Mice

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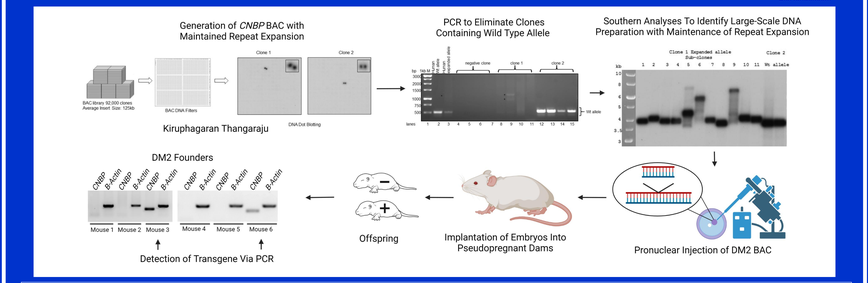

Fig. 3 Generation of DM2 BAC Mice. BAC library was generated from a DM2 patient lymphoblastoid cell line (LCL) and screened for *CNBP*. Positive clones were screened to identify those with an expansion. Expansion positive BAC DNA was isolated and used for pronuclear injection. BAC-injected embryos were transferred to pseudopregnant dam. 133 mice were screened for CNBP. Two founders positive for the *CNBP* gene were identified by PCR.

4 DM2 Founder Lines Contain *CNBP* and Flanking Regions

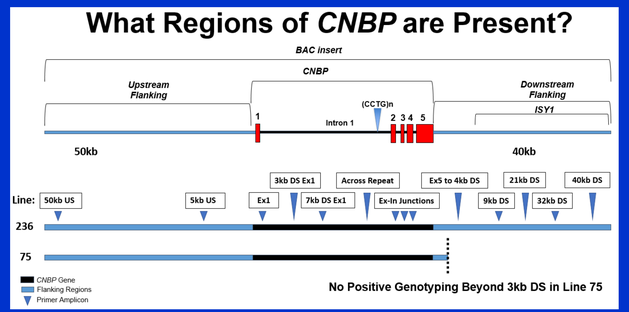

Fig. 4 PCR mapping of BAC transgenes in DM2 founder lines. Line 236 contains all regions of the BAC, which includes *CNBP*, 50kb and 40kb of upstream and downstream sequence, respectively. Line 75 contains all of *CNBP*, 50kb of upstream flanking region, and 3kb downstream flanking region. The differences in flanking regions between line 75 and 236 may differentially affect transgene expression. Line 75 does not contain as much 3' flanking sequence, and line 236 may express the downstream *ISY1* gene.

Human Transgenic *CNBP* RNA is Expressed in Muscle and Brain

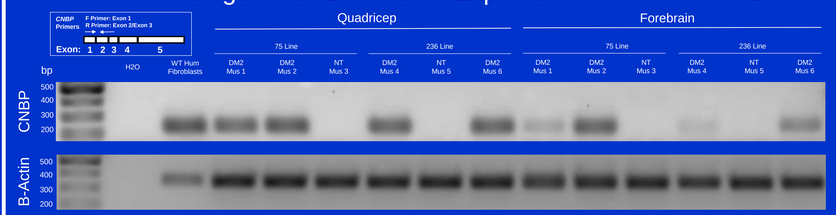

Fig. 5 Comparison of human *CNBP* expression between muscle (quadriceps) and brain (forebrain). RT-PCR was performed on quadriceps and forebrain RNA and shows *CNBP* expression in transgene positive but not non-transgenic littermates.

5 Repeat Expansion Shows Modest Instability Between Generations

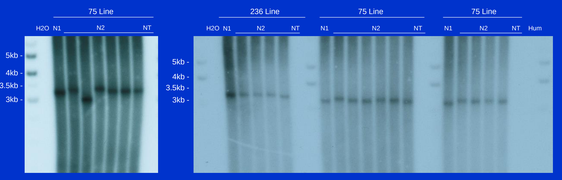

Fig. 6 Long-range PCR of N1 and N2 DM2 BAC mice. N2 litters show expansions and contractions between generations compared to their N1 parent. The repeat expansions in the N1 mice were ~750 repeats.

CCUG RNA Foci in DM2 BAC Mouse Muscle

Fig. 7 RNA FISH showing CCUG RNA foci. 3 month old DM2 BAC mouse quadricep muscle probed with $CAGG_{(4)}$ - Cy3.

6 Conclusions

- We have successfully generated two DM2 BAC transgenic mouse lines each containing the entire *CNBP* gene and ~750 CCTG repeats
  - Line 75 includes 50 kb upstream and 3kb downstream
  - Line 236 includes 50 kb upstream and 40 kb downstream (including *ISY1*)
- Transgenic *CNBP* is expressed in muscle (quadriceps) and brain (forebrain)
- Modest intergenerational repeat instability is seen with contractions and expansions in individual litters
- CCUG RNA foci are found in skeletal muscle (quadriceps)
- We are currently characterizing the molecular and behavioral phenotypes of these novel DM2 mice

Acknowledgments

Funding from the Myotonic Dystrophy Foundation for Dr. Kiruphagaran Thangaraju is gratefully acknowledged.

Support from the UF Graduate Program in Biomedical Sciences is greatly appreciated.