Myotonic Dystrophy Foundation (MDF)
2023 MDF Annual Conference
Poster Abstracts

1. **Characterization of sleep phenotypes and underlying mechanisms in mouse models of Myotonic Dystrophy Type 1** (Poster ID # 42)

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Format: Posters- Hybrid

ABSTRACT: Although Myotonic Dystrophy type 1 (DM1) patients present with a variety of symptoms spanning multiple body systems, excessive daytime sleepiness, and other debilitating CNS symptoms are largely understudied as compared to symptoms in peripheral tissues. Muscleblind-like (MBNL) 2 protein depletion has been shown to contribute to CNS symptoms in DM1 through the mis-splicing of different transcripts. Specifically, DM1 frontal cortex and MBNL2 knockout (KO) mice show mis-splicing of the Gamma2 subunit (GABRG2) of the GABAA receptor. The mis-splicing of this exon may contribute to changes in locomotor activity, GABA sensitivity, and sleep phenotypes. Here, we aim to study its contribution to sleep regulation using a high-throughput and highly sensitive system– the PiezoSleep system. This system allows for control of environmental factors, such as light, and records non-invasive sleep/wake parameters including sleep or wake bout length, percent asleep or awake, and total minutes slept. Preliminary data show that male MBNL2 KO mice exhibit significant sleep fragmentation during their inactive phase. Using this system, along with AAV-mediated perturbation of GABRG2 isoform levels, we aim to dissect the relationship between sleep and GABRG2 mis-splicing in a rigorous age- and sex-specific manner in models that recapitulate CNS defects, such as MBNL2 knockout and 480 CTG repeat knock-in mouse models.

2. **The role of Pvr in DM1** (Poster ID # 31)

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Format: Posters- Hybrid

ABSTRACT: Previous research using a mouse model of myotonic dystrophy type 1 (DM1) with severe muscle wasting showed deregulated platelet-derived growth factor receptor β (PDGFRβ) signaling. PDGFRβ is a receptor tyrosine kinase that regulates cell growth and survival. PDGFRb has been demonstrated to play a role in skeletal muscle hypertrophy and

impaired PDGFRβ signaling has been indicated in Duchenne Muscular Dystrophy, but its role in skeletal muscle wasting has not been extensively studied in DM1. Our lab uses a Drosophila (fruit fly) model of DM1 to assess how the homologous PDGF/VEGF Receptor (Pvr) is involved in skeletal muscle phenotypes associated with DM1. This model uses the Gal4/UAS system in fruit flies to express expanded CUG repeats to mimic DM1. The use of the fruit fly model allows us to study the signaling pathway in a more simplified view to understand its role in both normal development and DM1. The goal of this project is to modulate the expression of Pvr and its downstream components to determine whether Pvr signaling is required for proper skeletal muscle development and function in DM1 and unaffected flies. We show by flight testing and climbing assays that skeletal muscle performance is reduced in the fly model of DM1, consistent with previous findings. Though still in the process of modulating the Pvr levels in DM1, we expect that, if Pvr is involved in the progression of DM1, levels of activated Pvr will be reduced in the skeletal muscles of individuals with DM1, relative to controls; that inactivating Pvr in unaffected flies may lead to similar skeletal muscle structural and functional defects as observed in the DM1 flies; that RNAi knockdown of Pvr in DM1 flies will exacerbate the DM1 phenotype; and that inducing expression of constitutively active Pvr has the potential to rescue the DM1 phenotype.

3. **The Global Alliance for myotonic dystrophy awareness efforts to launch International Myotonic Dystrophy Awareness Day** (Poster ID # 75)

Kate Beck, Niv Joshi, Kleed Cumming, Mike Knaapen, Tanya Stevenson
Myotonic Dystrophy Foundation, Oakland, California, US
Format: Posters- Hybrid

ABSTRACT: The Global Alliance for Myotonic Dystrophy Awareness, which includes over 50 international nonprofit organizations, academic and research institutions, biotechnology and pharmaceutical companies, patient advocacy groups, and others focused on raising global awareness about myotonic dystrophy, united for the first time on Rare Disease Day in February of 2021 to declare International Myotonic Dystrophy Awareness Day be observed each September 15th. The purpose of raising DM awareness is to improve quality of life, the availability of essential resources, access to appropriate healthcare for individuals living with DM, and to help accelerate the research and drug development process by promoting understanding and support for the community across a broad range of people – the general public, policymakers, regulators, biopharmaceutical representatives, researchers, health care professionals, and anyone with an interest in changing the future of myotonic dystrophy. In 2021 the Global Alliance focused awareness efforts on establishing International Myotonic Dystrophy Awareness Day to be observed

each September 15th. The efforts included the adoption of the official International Myotonic Dystrophy Awareness Day logo, endorsements by federal and state governments and government officials, nearly 50 US and UK monuments, landmarks, and buildings lit in green, and grassroots awareness campaigns on social media and beyond. The Global Alliance continues its efforts in 2022 and 2023 by promoting general awareness while collectively focusing efforts on raising DM awareness amongst clinical care teams and preparing participants for clinical trials.

4. **Benefit of amlodipine or ranolazine on myotonic dystrophy bi-channelopathy** (Poster ID # 21)

Lily Cisco
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Format: Posters- Hybrid

ABSTRACT: Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy in adults. In DM1, there are hundreds of aberrantly spliced transcripts making it challenging to connect splice variants to disease manifestations. The mechanism behind the leading cause of mortality, muscle weakness, and atrophy, remains poorly understood. However, a correlation between altered splicing in transcripts central to excitation-contraction coupling and muscle weakness in DM1 patients has been made. In mice, the combination loss of the chloride channel ClC-1 (ClC-1-/-) and exon 29 deletion in the voltage-gated calcium channel CaV1.1 (CaV1.1Δ e29), results in significantly reduced survival. CaV1.1Δ e29/ClC-1-/- mice had a median life expectancy of 9 weeks, with none surviving beyond 14 weeks. Additionally, they exhibited significantly reduced body weight, respiratory function, muscle force generation, exacerbated myotonia, and prolonged time of righting reflex. Identifying CaV1.1Δ e29 as a potential DM1 therapeutic target, we administered FDA-approved calcium channel blocker amlodipine and the sodium channel blocker ranolazine to see if we could improve survival and other metrics of health. With daily treatment, CaV1.1Δ e29/ClC-1-/- mice had significantly improved survival, weight gain, and reduced time of righting reflex with amlodipine or ranolazine. Further, we found that amlodipine and ranolazine improved transient weakness and myotonia in in vitro muscle contraction experiments. Results demonstrate that targeting CaV1.1Δ e29 conductance and myotonia has therapeutic potential for DM1 patients. In future studies, we will test if amlodipine or ranolazine treatment could improve survival and health in DM1 mouse models that exhibit the full panoply of altered DM1 splicing and myopathic features.

5. **Senolytics targets defective muscle stem cells and restore myogenesis in Myotonic Dystrophy type 1**(Poster ID # 35)

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ABSTRACT: Progressive muscle weakness and atrophy are clinical hallmarks in the progression of myotonic dystrophy type 1 (DM1). Muscle stem cells, which contribute to skeletal muscle development, growth, and repair in adults, are also affected by this disease. However, the molecular mechanisms leading to this defective activity are still elusive. Here, we explored through an unbiased approach the molecular signature leading to impaired activity of muscle stem cells in DM1. Single-cell RNAseq data revealed the presence of a specific subset of DM1 myoblasts expressing a senescence signature, characterized by the high expression genes related to senescence-associated secretory phenotype (SASP). This senescent profile was confirmed at the protein level in vitro and in situ. Drug screening identified the BCL-XL inhibitor, A1155463, as a senolytic drug that can specifically target senescent DM1 myoblasts to induce their apoptosis and reduce their expression of SASP. Removal of senescent cells re-established the myogenic function of the remaining non-senescent DM1 myoblasts, which displayed improved proliferation and differentiation capacity in vitro; and enhanced engraftment in vivo. Altogether this study presents a well-defined senescent molecular signature in DM1 untangling part of the pathological

mechanisms observed in the progression of the disease, and we demonstrated the therapeutic potential of targeting these defective cells with senolytics to restore myogenesis.

6. **Selection of a patient-reported outcome measure to assess oropharyngeal dysphagia in myotonic dystrophy type 1** (Poster ID # 18)

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Format: Posters- Hybrid

ABSTRACT: Objective: Oropharyngeal dysphagia (OD) is common in patients with neuromuscular diseases (NMDs) such as oculopharyngeal muscular dystrophy (OPMD) and myotonic dystrophy type 1 (DM1) [1]. OD is important to assess because it may conduct to weight loss and aspiration pneumonia. Patient-reported outcome measures (PROMs) are useful to assess OD, but there is no specific PROM for DM1. This study aimed to identify PROMs that could be recommended to assess OD in patients with DM1. Methodology: A two-step literature review was conducted to identify dysphagia-related symptoms in DM1 and currently available PROMs for the assessment of OD. Content validity of PROMs was documented through content analysis. Results: Sixteen symptoms were identified, including impairments like food sticking in the throat, and coping strategies such as taking small bites and having liquids with meals to safer swallowing. Like in other NMDs, such as OPMD, coping strategies seem to help patients with DM1 to manage their symptoms and may be important to consider in the assessment of OD. The SWAL-QOL [2], the Dysphagia Handicap Index [3] and the Deglutition Handicap Index [4] were the 3 PROMs with the highest number of symptoms covered. However, they overlook coping strategies. The Dysphagiameter [5], a newly developed PROM for OPMD, had fewer symptoms covered but was the PROM that best included coping strategies. Conclusions: A consultation of experts and patients with DM1 is required to identify the key symptoms related to OD in DM1 and select the best PROM among the PROMs identified in this review.

1. Argov Z, de Visser M, Dysphagia in adult myopathies. Neuromuscular Disorders, 2021. 31(1): p. 5-20.
2. McHorney CA, Robbins J, Lomax K, Rosenbek JC, Chignell K, Kramer AE, et al., The SWAL-QOL and SWAL-CARE outcomes tool for oropharyngeal dysphagia in adults: III. Documentation of reliability and validity. Dysphagia, 2002. 17(2): p. 97-114.
3. Silbergleit AK, Schultz L, Jacobson BH, Beardsley T, Johnson AF, The Dysphagia Handicap Index: Development and validation. Dysphagia, 2012. 27(1): p. 46-52.
4. Woisard V, Andrieux MP, Puech M, [Validation of a self-assessment questionnaire for swallowing disorders (Deglutition Handicap Index)]. Revue de Laryngologie - Otologie - Rhinologie, 2006. 127(5): p. 315-325.

5. Côté C, Brais B, Batcho CS, Brisson JD, Youssof S, Sogbossi ES, et al., Measurement properties of the Dysphagiameter for the assessment of dysphagia in oculopharyngeal muscular dystrophy. Neuromuscular Disorders. (submitted).

7. **Regenerative failure in myotonic dystrophy: pathomechanisms and insights from a novel model of improved regeneration** (Poster ID # 14)

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Format: Posters- Hybrid

ABSTRACT: The hallmark feature of many muscular dystrophies including myotonic dystrophy (DM) is progressive muscle wasting and weakness. In contrast to other muscular dystrophies where repeated rounds of myofiber degeneration and regeneration occur, these repetitive cycles are rare in DM. Instead, there appears to be slow atrophy of the muscle due to impaired myofiber regeneration. It is hypothesized this is due to premature senescence of DM1 satellite cells or compromised satellite cell activation. Another issue concerns current DM1 therapies under development, which are designed to limit DMPK expression and prevent further muscle loss, but it is not clear to what degree pre-therapy muscle loss can be reversed to restore normal function. Therefore, it is critical to understand the mechanisms underlying the regenerative process and how muscle regeneration could be improved in DM. In recent years a new mammalian model, the African spiny mouse Acomys cahirinus, was discovered to display remarkable regenerative capabilities, including the ability to regenerate muscle from 4mm ear punch wounds, full thickness skin excisions, and repeated rounds of cardiotoxin injury. The objective of our research is to evaluate the contribution of regenerative deficits to muscle wasting in DM models and test the ability of pro-regenerative genes identified in Acomys to promote regeneration and prevent muscle wasting in DM models. Currently, muscle from DM1 models is being examined for regenerative deficits while potentially pro-regenerative genes from Acomys are identified by RNAseq. Subsequently, the ability of these genes to promote regeneration will be tested in muscle wasting models of DM.

8. **Investigating global and cell type-specific transcriptomic dysregulation in the DM1 brain** (Poster ID # 43)

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Format: Posters- Hybrid

ABSTRACT: Myotonic dystrophy type 1 (DM1) is a multisystemic disease caused by a CTG repeat expansion in the 3' UTR of the DMPK gene. The expanded repeats sequester the MBNL family of RNA binding proteins, resulting in dysregulation of splicing, among other perturbed pathways. While peripheral symptoms of DM1 are widely studied and linked to mis-splicing events, central nervous system (CNS) symptoms are reported by patients to be a highly significant burden to their everyday lives. However, the study of CNS involvement in DM1 is poorly understood and compounded by an extreme diversity of cell types. We performed bulk RNA-seq using postmortem tissue from up to 11 brain regions of 9 DM1 patients and quantitated splicing dysregulation to investigate transcriptomic dysregulation across the DM1 brain. We focused on five regions (caudate/ putamen/ accumbens, cerebellum, anterior hippocampus/ entorhinal cortex, amygdala, striate/ parastriate cortex) and compared to publicly available control RNA-seq data; we found 62 shared mis-splicing events across all 5 regions. Next, we sought to determine cell type-specific dysregulation in the CNS by performing single-nucleus RNA-seq using nuclei extracted from postmortem frontal cortex of DM1 patients and controls. We found an increase in microglial and endothelial cell composition in DM1 patients and dysregulation of gene expression in all cell types identified, with the greatest number of differentially expressed genes found in microglia and the greatest severity of gene expression dysregulation found in endothelial cells. Further investigation of cell type-specific transcriptomic dysregulation in the DM1 brain will characterize distinct cell types and how they contribute to disease mechanisms in the CNS.

9. **Methods of remote data collection and analysis** (Poster ID # 46)

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Format: Posters- Hybrid

ABSTRACT: Objective: Timed functional assessments can be performed in the home environment under direct supervision by videoconferencing. We developed standard operating procedures and then examined interrater reliability for analyzing data collected at remote study visits. Methodology: Participants in the REACH DM1 study (Remote assessments and genetic analysis in patients with Myotonic Dystrophy Type 1) completed remote study visits under video supervision. Video-hand-opening-time (VHOT), Timed-Up-And-Go (TUG), 9- hole-peg-test (9HPT), and 10-meter-walk/run-test (10MWRT) were video recorded. The recordings were viewed offline by 2 separate raters to determine timed function, using pre-specified criteria for quality and selection of start and end points. Results: Within the first cohort of the REACH DM1 study, a total of 400 videos were captured and analyzed. Preliminary analysis shows that the interrater reliability was excellent for 9HPT (ICC 0.97, n=21), TUG (ICC 0.92, n=22), 10MWRT (0.97, n=15) and VHOT (ICC 0.94, n=20). Conclusion: Using stringent rating criteria, the timing of video-recorded functional assessments in subjects with DM1 is stable between different raters.

10. **Comparing intellectual disability and non-intellectual disability in children with congenital myotonic dystrophy** (Poster ID # 48)

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Format: Posters- Hybrid

ABSTRACT: Introduction: Children with congenital myotonic dystrophy (CDM) display varying cognitive and adaptive impairments. Some face significant challenges, while others have milder impairments and better overall function. Identifying distinctive characteristics that determine cognitive abilities is crucial for clinical care, daily activities, and quality of life. Objective: Differentiate cognitive ability levels in pediatric CDM, identify group differences, and explore associations between participant characteristics and neuropsychological outcomes. Methods: Thirty-eight children with CDM and 29 controls completed neuropsychological assessment of intelligence (IQ), adaptive (AB) and executive (EF) behaviors, autism, and sleep. DSM 5 diagnostic criteria for Intellectual Disability (ID) was used to group CDM participants as ID or Non-Intellectual Disability (NID). Group differences were determined using parametric and nonparametric tests. Correlations were used to identify relationships between participant characteristics, outcome measures, and cognitive levels. Results: CDM participants exhibited significantly low IQ, impaired AB and EF, and elevated autism and sleep symptoms. CTG repeat length correlated moderately with IQ and visuospatial skills; IQ and AB were strongly correlated. Over half met DSM 5 criteria for ID, displaying significant deficits in IQ, AB, EF, sleep, and speech delay. ID strongly correlated with IQ and AB, and moderately with CTG repeat length and initiating

behavior. Positive autism screens were more prevalent in ID group. Conclusion: The study provides evidence of different cognitive ability levels, including ID, in pediatric CDM. Executive functioning, speech delay, daytime sleepiness, and neurodevelopmental symptoms contribute significantly to cognitive and adaptive deficits in CDM cases with ID. Comorbid autism is more likely in CDM cases with ID.

11. **Scalable kinematic analysis using smartphone videos: Towards movement biomarkers for neuromuscular diseases** (Poster ID # 66)

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Format: Posters- Hybrid

ABSTRACT: Myotonic Dystrophy (DM1) and Facioscapulohumeral Dystrophy (FSHD) are progressive genetic neuromuscular disorders that lead to muscle atrophy, imbalances, and weakness, significantly impacting activities of daily living and independence. In DM1, muscle weakness typically emerges from the distal to proximal regions, resulting in challenges related to balance, foot drop, gait inefficiency, as well as upper limb weakness, often manifesting first as hand/grip weakness. Conversely, FSHD primarily displays muscle weakness that originates proximally, affecting the upper arms, shoulder girdle, and ankle dorsiflexors. This weakness progresses to difficulties in tasks like raising hands to the mouth or overhead and gait abnormalities. The assessment of movement plays a pivotal role in evaluating function and strength. Commonly used timed functional tests, such as sit-to-stand, the 10-meter walk, and timed up-and-go (TUG), provide insights into functional mobility. However, the evaluation of movement has typically relied on clinician-rated scales or patient-reported outcomes, lacking the ability to objectively capture compensatory mechanisms and changes in movement quality. Quantitative human movement assessments are vital to assessing quality of movement such as asynchronous movements and muscle compensations associated with progressive weakness. OpenCap, a new tool enabling 3D movement analysis from smartphone videos, combines the accuracy of motion capture with the scalability required for clinical practice and clinical trials. OpenCap is a cloud-based software platform for estimating human motion, musculoskeletal forces, and neuromuscular control using computer vision algorithms and deep learning models to estimate 3D joint positions from video, a biomechanical model and inverse kinematics to estimate physics-based musculoskeletal simulations. This video-based motion capture has potential to provide metrics previously only attainable from large motion capture laboratories, but the clinical value of video-based metrics remains unproven. Current methods to assess movement are clinician-rated scales that have limitations requiring specific NMD expertise and clinical judgment that have the risk of greater variability and

less objectivity than objective movement metrics. Greater variability with measurements may impact the ability to detect change in clinic and power clinical trials. 3D motion capture can detect subtle biomechanical and kinetic changes arising from progression of movement-related conditions like neuromuscular diseases. To tackle this interdisciplinary challenge, we established a collaborative partnership between engineers and clinical researchers specializing in neuromuscular diseases. Initial needs finding revealed that clinicians can observe changes in movement quality that are undetected by existing trial endpoints such as timed functional tests (TFTs) and upper limb clinical outcome assessments. Our objective is to determine the feasibility of OpenCap to differentiate between NMD conditions and determine specific movement metrics most correlated to functional ability and patient-reported outcomes using the ACTIVLM. Methods: We used OpenCap to create rapid, in-depth movement assessments for two neuromuscular diseases: myotonic dystrophy (DM) and facioscapulohumeral muscular dystrophy (FSHD). Specifically, we investigated whether statistical models using OpenCap kinematic features can (1) differentiate between conditions and (2) predict patient-reported outcomes more accurately than models using timed functional tests. We conducted a large-scale, parallel data collection (seven OpenCap stations) over three days (12 hours total). Results: Eighty-eight participants (27 DM, 23 FSHD, 38 control) completed 12 activities in an average of 20 minutes. Six OpenCap features differentiated patient cohorts 20% (95% confidence interval: 3.7%, 36.3%) more accurately than did six timed functional tests using linear discriminant analysis. OpenCap features predicted the ACTIVLIM aggregated patient-reported outcome metric with 22% (95% confidence interval: -10.3%, 50.6%) lower RMSE than did timed functional tests using linear regression. Conclusions: These results are consistent with the hypothesized disease specificity and salience of video-derived kinematic features. More broadly, OpenCap's convenience may accelerate large-scale studies necessary for novel biomarker creation as a complementary measure to current validated clinical outcomes and remote measures in DM and FSHD.

12. **Generation and characterization of a DM2 mouse model** (Poster ID # 41)

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Format: Posters- Hybrid

ABSTRACT: Objective: Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are multisystemic diseases caused by CTG or CCTG repeat expansions located in the DMPK or CNBP genes, respectively. RNA gain of function effects, bidirectional transcription, and

repeat associated non-ATG (RAN) translation are found in DM1 and DM2. In DM2, RAN translation of sense (CCUG) and antisense (CAGG) expansion transcripts produce (LPAC) and (QAGR) RAN proteins. LPAC and QAGR proteins are toxic to cells and found in brain regions with neuropathological changes and white matter loss. Understanding the role of RAN proteins in DM2 and developing therapeutic approaches requires animal models that mirror DM2 patient disease features. Methodology: Using a bacterial artificial chromosome (BAC) approach we generated two independent lines of DM2 BAC transgenic mice. We are characterizing these mice for RNA foci using HCR FISH, repeat instability by long-range PCR, and histopathological and behavioral features. Results: Both DM2 mouse lines contain the entire CNBP gene with substantial flanking sequence and unstable expanded repeats ranging from ~470 to over 2000 CCTGs. HCR-FISH detects signal in transgenic mice for CCUG repeats in skeletal muscle and brain. Both sense LPAC and antisense QAGR RAN proteins accumulate in brain tissue. These mice demonstrate substantial repeat instability in both germline and somatic tissues. Conclusions: We have generated a novel DM2 BAC transgenic mouse model that shows a number of disease relevant molecular phenotypes. We are continuing to characterize these mice and hope that this model will provide a useful tool for better understanding the molecular mechanisms of DM2 and therapy development.

13. **Development of patient-reported outcome measures to assess fatigue, sleepiness, and apathy in DM1** (Poster ID # 19)

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Format: Posters- Hybrid

ABSTRACT: Fatigue, sleepiness, and apathy (lack of motivation, interest) are common symptoms in myotonic dystrophy type 1 (DM1). These symptoms adversely affect many spheres of life in patients with DM1, such as employment. Due to their serious impacts on person lives, they are thought to be one of the next targets for a cure. However, these symptoms can have different meaning from a disease to another. In DM1, it is still unknown how they are experienced by patients. In addition, no DM1-specific tool exists to assess these symptoms. This is an essential aspect for assessment of treatment effectiveness. The current project will aim to 1) define in detail the fatigue, sleepiness, and apathy concepts based on the experience of persons living with DM1 and 2) develop questionnaire that will assess these three concepts. The first objective will be met by searching the literature, and consulting patients and experts to provide definitions for each of the concepts that are meaningful in DM1. To meet the second objective, we will identify existing questionnaires and create a pool of possible items. Experts will be consulted to assess the relevance of each preselected items. The questionnaire will be tested among 100 participants and will

be refine throughout the process. It is expected to obtain a final version of the questionnaire in French and English. Ultimately, we expect it to become a reference tool to assess fatigue, sleepiness, and apathy in the DM1 population in clinical practice and to be used in therapeutic trials to measure drug effectiveness.

14. **Assessing therapeutic potential and mechanism of action of novel small molecules in myotonic dystrophy type 1** (Poster ID # 36)

Jesus Frias
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Format: Posters- Hybrid

ABSTRACT: Objective: Myotonic dystrophy type 1 (DM1) is a multisystemic autosomal dominant disease that results in myotonia, cardiac conduction defects, muscle wasting, and weakness. DM1 is caused by CTG repeat expansions in the 3' UTR of the DMPK gene which produce toxic expansion RNAs that sequester the MBNL family of alternative splicing regulators, leading to the dysregulation of alternative splicing, which has been correlated to many DM1 disease symptoms (Reviewed by Reddy at al., 2019). Our group has focused on small molecules, such as diamidines, as possible DM1 therapeutics. Unfortunately, these compounds are often toxic or demonstrate only modest splicing rescue (Siboni et al., 2015; Jenquin et al., 2018). To overcome these barriers, we have developed a series of novel small molecules, called modified polycyclic compounds (MPCs), which improve splicing rescue and reduce toxicity. Methodology: We treated DM1 patient-derived fibroblasts and myotubes with MPCs, extracted RNA, and analyzed changes in alternative splicing events and expression of DMPK and MBNL transcripts via RT-qPCR. One MPC was tested in HSA-LR mice with daily 10mg/kg IP injections for 5 days and muscles from the mice were analyzed as previously described for cells. Results: Multiple MPCs (HM19B, 33, 43) rescued mis-splicing in the nanomolar range with maximum rescue in the 8-16nM (fibroblasts) or 62.5-125nM (myotubes) range. Excitingly, HM19B partially rescued mis-splicing in the TA of HSA-LR mice. Lead MPCs decrease CUG transcript levels in human cells and mouse tissue, while upregulating MBNL1/2 only in human cells, suggesting multiple mechanisms of action. Conclusion: Our preliminary data suggest that our novel MPCs may represent therapeutic candidates for the treatment of DM1.

15. **Genetic modifiers in myotonic dystrophy type 1: Insights from a region with a strong founder effect** (Poster ID # 51)

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Format: Posters- Hybrid

ABSTRACT: Myotonic dystrophy type 1 (DM1) is an autosomal dominant inherited disorder for which symptoms, severity, and progression are highly variable among patients. Despite large interindividual variations, it is thought to be caused by the expansion of a CTG repeat in the DMPK gene. However, this expansion only partly explains variable symptoms onset, and progression of the disease. In consequence, other genetic mechanisms such as modifier genes, might be involved and could help to better explain the CTG instability and the disease severity. The Saguenay-Lac-St-Jean (SLSJ) region of Quebec (Canada) has the highest incidence of DM1 worldwide, with a frequency of ~ 1/630, due to its strong founder effect. We hypothesize that the high prevalence and the availability of data from different sources will allow us to identify modifier genes more easily in this population. Whole-genome genotyping has been performed for 200 DM1 patients from the SLSJ population and will be combined with the deep genealogical data provided by BALSAC. BALSAC is a population database built at UQAC based on vital records allowing the reconstruction of the entire genealogy of the Quebec population of European descent from the beginning of the colonization in the 17th century until the present day. Using genotypes, we inferred identical-by-descent (IBD) segments for patients and controls from the Cartagene database with refinedIBD and found that 25% of the patients' pairs shared an IBD segment on chromosome 19 surrounding the DMPK gene. We looked at the specific haplotypes in this genomic region and found 3 distinct networks of haplotypes, corresponding to the already described one major and two minor entries of the variant in the SLSJ region. The genealogical data will be combined with the genotypes and patients' clusters sharing the same haplotype group and related through common ancestors will be identified. With this combined data we expect to find shared genomic regions IBD around the DMPK gene, but also around modifier genes that could have been inherited along with the expanded CTG repeat on DMPK gene. The identification of genetic modifiers shared by subgroups of genealogically linked patients could be the first step in initiating therapies or drug repurposing. Additionally, the identification of genetic modifiers associated with earlier onset of the disease could lead to early preventive therapies being offered to patients in the SLSJ region, but also elsewhere in the world.

16. **Supervised strength training in women with myotonic dystrophy type 1** (Poster ID # 40)

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ABSTRACT: Introduction: Myotonic dystrophy type 1 (DM1) is a hereditary disease characterized by muscular impairments. Fundamental and clinical positive effects of strength training have been reported in men with DM1, but its impact on women remains unknown. Objective: To evaluate the short and middle-term effects of 12 weeks supervised strength training on physical and neuropsychological health. Method: A group of women with DM1 performed a twice-weekly supervised resistance training program (3 series of 6-8 repetitions of squats, leg press, plantar flexion, knee extension, and hip abduction). Quantified muscle testing (QMT) of the lower limbs, one-repetition maximum (1-RM), 30-second sit-to-stand (30sSTS), staircase test, 10-meter walk test (10mWT), Marin apathy scale, DM1-activ, Lower extremity functional scale (LEFS), Fatigue and daytime sleepiness scale (FDSS) and Hospital anxiety and depression scale (HADS) were assessed before and after the intervention, as well as three and six months after completion of the training program. Muscle biopsies of the vastus lateralis were also taken before and after the training program to assess muscle fiber growth and mitochondrial respiration. Results: Eleven participants completed the program (attendance: 98.5%). QMT of hip extensors and knee extensors ($p<0.006$), apathy ($p=0.0005$), all 1-RM measures ($p<0.001$), and scores at LEFS ($p=0.003$), HADS ($P=0.009$), pain interference ($p=0.01$) and mitochondrial respiration ($p<0.05$) were significantly improved by training. Gains at QMT of hip extension,

1-RM of squat, HADS score, LEFS score, and pain interference were maintained up to six months after the training program. Conclusion: Strength training is an excellent therapeutic strategy for women with DM1.

17. **The role of dysregulated calcium homeostasis and membrane excitability in Myotonic Dystrophy myopathy** (Poster ID # 30)

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Format: Posters- Hybrid

ABSTRACT: Myotonic dystrophy type 1 (DM1) is a multisystem disorder that is characterized by myotonia and muscle weakness. Despite its prevalence in patient morbidity and mortality, the molecular underpinnings of muscle weakness and wasting are currently unknown. Additionally, the complex mechanism and heterogeneity of the disease presentation makes it difficult to link individual splice events to a disease manifestation. However, there has been a significant correlation between muscle weakness in DM1 patients and mis-splicing of proteins central to excitation-contraction (EC) coupling – specifically, CaV1.1, which leads to our question of whether CaV1.1 mis-splicing plays a central role in DM1 myopathy? The fetal isoform of CaV1.1 (CaV1.1Δe29) is a gain-of-function splice isoform, exhibiting a leftward shift in voltage-dependence of activation as well as an increase in calcium conductance. Together, we hypothesize that this gain-of-function property exacerbates myopathy through providing augmented calcium entry that results in sustained myotonic runs and calcium toxicity. To investigate this, we will utilize a mouse model that mimics the fetal splice pattern of CaV1.1 to breed with several established DM1 mouse models. Preliminary data suggest that forced expression of CaV1.1Δe29 contributes to a marked reduction in survival and weight. Future experiments will continue to characterize the role of CaV1.1Δe29 on muscle function and histopathology. The completion of this study will advance the field by elucidating the role of CaV1.1 mis-splicing in driving myopathy seen in individuals with DM1 and provide a potential new therapeutic target.

18. **REACH DM1- Remote assessments and genetic analysis in patients with myotonic dystrophy type 1** (Poster ID # 45)

Johanna Hamel, Jeanne Dekdebrun, Erin Richardson, Katy Eichinger, Chandani Warnasoori, Christina Heil, Eleanor Stanton, Stella Deng, Charles Thornton
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Format: Posters- Hybrid

ABSTRACT: Objective: To assess feasibility of remote assessment of strength, function, multi-systemic disease manifestations, and CTG repeat size in people affected by myotonic dystrophy type 1 (DM1). Methodology: Subjects were identified through the National Registry and enrolled remotely. Participants received a toolkit including a tripod, a tablet equipped with software (Zoom for videoconferencing and REDCap for questionnaires), and devices for assessments of strength and function. A remote study visit (RSV) was conducted, including a structured interview to determine age of onset. Grip, pinch, and tongue strength, forced vital capacity (sitting and supine), 9-hole-peg-test, video-hand-opening-time, EKG, and timed-up-and-go (TUG) were determined under video supervision. The 10-meter-walk/run-test (10MWRT) was performed, safety and space permitting. Functional assessments were video-recorded for offline analysis. Participants completed questionnaires on disease burden, exercise, and RSV satisfaction. Participants wore activity monitors around wrist and waist for 7 days. Participants sent in blood samples for analysis of CTG repeat size. Results: Over nine months, 31 subjects (18 – 81 years) enrolled and completed the RSV. Symptom onset ranged from birth to 7th decade. Ten participants needed help from another person to complete the assessments. 10MWRT was completed in 23 participants. TUG and spirometry was completed in 30 subjects. No falls or injuries occurred. Genetic analysis is in progress, results will be presented. Questionnaires show high satisfaction with the research experience. Conclusions: Remote study of function and disease severity in DM1 is feasible. This novel platform provides opportunity to study large cohorts enabling broad participation while reducing the burden on individuals and families.

19. **Tackling challenges of large repeat amplification in DM1 knockin mouse model** (Poster ID # 24)

Christina Heil[1], Chandani Warnasooriya[1,2], Zhenzhi Tang[1,2], John L. Lueck[1,3], Charles A. Thornton[1,2]

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Format: Posters- Hybrid

ABSTRACT: Expanded CTG trinucleotide repeats in the 3'-untranslated region (3'UTR) of the myotonic dystrophy protein kinase (DMPK) gene are the genetic cause of myotonic dystrophy type I (DM1). Pathogenic repeat expansions can reach over 1,000 CTGs. Genetic analysis in DM1 requires amplification of the expanded repeats, but amplification of long stretches of repetitive, GC-rich genomic regions is challenging. Many people who show distinctive clinical signs being diagnosed with DM1 have never had genetic confirmation

due to lack of a reliable and accurate method for genetic analysis. Transgenic mice containing expanded CTG repeats of various sizes in the 3'UTR of Dmpk are being used to optimize amplification protocols. A genetic marker in those DM1 knockin mice allows us to determine relative frequency and assess amplification efficiency of expanded (mutant allele) vs. non-expanded (wild type allele) amplicons. We have also generated compound heterozygous mice carrying two different expanded repeat sizes on opposite alleles with distinct short sequence tags flanking the repeat tract on each allele. These model systems allow us to examine amplification efficiencies dependent on repeat size and to directly observe recombination events. These amplicons are subjected to long-read DNA sequencing to test its feasibility for DM1 genetic diagnostics. Long-read DNA sequencing provides high-quality sequence information of GC-rich repetitive regions up to several kilobase pairs, which is currently not accessible with any standard diagnostic method. This promises to be a reliable, high-throughput method for comprehensive analysis of repeat size, allelic heterogeneity, and repeat interruptions, all potential predictors of DM1 onset and severity.

20. **Alternative splicing dysregulation across small molecule treatments in the HSALR mouse model of myotonic dystrophy type 1** (Poster ID # 28)

Sawyer Hicks
The RNA Institute, State University of New York at Albany, New York, US
Format: Posters- Hybrid

ABSTRACT: Myotonic dystrophy type 1 (DM1) is a complex neuromuscular disorder caused by a CTG trinucleotide expansion in the 3'-untranslated region of the DMPK gene. The expression of CUG expansion RNAs leads to downstream aberrant alternative splicing and protein dysfunction. The HSALR mouse model, which expresses a human skeletal actin gene (ACTA1) with CTG expansions in the last exon, closely mimics the muscle phenotypes of DM1 and has been used extensively to investigate alternative splicing dysregulation and therapeutic strategies for DM1. Several small molecules have demonstrated success in rescuing alternative splicing dysregulation, including by binding toxic RNA structures preventing the formation of harmful RNA-protein complexes, or modulating RNA-binding proteins to restore their normal cellular processes. Our central hypothesis is that analysis of alternative splicing and gene expression across different therapeutic treatments in HSALR mice will provide a better understanding of the mechanisms of the therapeutic strategies. Our goal is to determine which alternative splicing events are rescued more readily and those events that are challenging to rescue. We will also determine which genes

and pathways are differentially expressed in response to treatment and if small molecules with different targets activate the same genes and pathways. To accomplish these goals, we analyzed publicly available RNA-seq datasets from HSALR mice treated with a range of small molecules, including both FDA-approved drugs and investigational small molecules. Analysis of alternative splicing events across treated HSALR mice identified splicing events that are consistently rescued across treatments and events that appear to be difficult to rescue. Our study seeks to provide a better understanding of the on-target and off-target effects of different therapeutic small molecules on alternative splicing events and differential gene expression in the DM1 HSALR mouse model. We believe that our study will guide future research in the development of therapeutic approaches for DM1.

21. PGN-EDODM1 nonclinical data demonstrate mechanistic and meaningful activity for potential treatment of myotonic dystrophy type 1 (Poster ID # 22)

Ashling Holland[1], Arnaud Klein[2], Pallavi Lonkar[1], Niels Svenstrup[1], Brijesh Garg[1], Denis Furling[2], Jaya Goyal[1]
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Format: Posters- Hybrid

ABSTRACT: PepGen's enhanced delivery oligonucleotide (EDO) cell-penetrating peptide technology is engineered to optimize tissue delivery and cellular uptake of therapeutic oligonucleotides. PGN-EDODM1 is being evaluated for the treatment of DM1. In nonclinical pharmacology studies, immortalized differentiated human DM1 myoblasts treated with PGN-EDODM1 resulted in dose-dependent reduction in toxic CUG repeat expansion in DMPK mRNA (pathogenic myonuclear foci) and liberation of sequestered MBNL1 protein from the foci, while DMPK levels remained unchanged. Liberation of MBNL1 is hypothesized to restore splicing profiles of multiple downstream transcripts; a central cause of DM1 pathology. Single intravenous (IV) dose of PGN-EDODM1 administered to HSALR transgenic mouse model of DM1 resulted in high muscle concentrations of PGN-EDODM1, resolution of myotonia, dose-dependent correction of mis-splicing, and no significant impact on HSA expression. Splicing correction in HSALR mice persisted for up to 24 weeks. Additional data from a repeat-dose study in HSALR mice with low PGN EDODM1 doses will be presented. Notably, repeat IV doses of PGN-EDODM1 administered to monkeys every 2 or 4 weeks did not result in decreases in DMPK transcript levels. Currently, there are no approved therapies for DM1. PGN-EDODM1 nonclinical pharmacology studies show considerable therapeutic potential.

22. **Targeting defective muscle stem cells as a new therapeutic approach for DM1** (Poster ID # 27)

Tatiana Koike[1], Talita Conte[1,2], Taeyeon Kim[1,2], Ines Mokhtari[1,2], Ornella Pellerito[1,2], Élise Duchesne[3], Nicolas Dumont[1,2]
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Format: Posters- Virtual

ABSTRACT: Background: Muscle stem cells (MuSC), the engine of muscle growth and repair, are dysfunctional in DM1. Our group and others showed that MuSC become senescent (irreversible cell cycle arrest) in DM1. These findings open new therapeutic avenue to target these defective cells to restore muscle function (1,2). Objective: To assess the therapeutic potential of senotherapeutic drugs, which can specifically target senescent cells, to restore MuSC myogenic capacity and enhance muscle function in DM1. Methodology: We used 2 different models: 1) isolated MuSC from DM1 patients and healthy controls were collected for analysis and drug screening; 2) DMSXL mice carrying the mutated DMPK gene with >1,000 CTG repeats as a preclinical model of DM1 to study the impact of senotherapeutics in vivo. Results: Our drug screen on different senotherapeutic drugs identified a few molecules that could eliminate senescent cells in DM1, especially A1155463 (BCL-XL inhibitor) which selectively eliminated senescent DM1 myoblasts, and reduce in their expression of a cocktail of harmful pro-inflammatory cytokines and enzymes, named the Senescence-Associated Secretory Phenotype (SASP). Moreover, the senostatics molecule Resolvin-D2 also showed potential to reduce SASP expression (e.g., TNFa). Both molecules restored myoblast proliferation and differentiation. Analysis of skeletal muscle from DMSXL mice show that they express higher levels of senescence markers (e.g., p21, 53), especially in males. Treatment of these mice with A1155463 or Resolvin-D2 enhanced muscle function. Conclusions: Senotherapeutics are a promising therapeutic approach to target defective MuSC, reduce SASP expression, and restore myogenesis.

1. Bigot, A. et al. Large CTG repeats trigger p16-dependent premature senescence in myotonic dystrophy type 1 muscle precursor cells. Am. J. Pathol. 174, 1435–1442 (2009).
2. Talita C. Conte, et al. Clearance of defective muscle stem cells by senolytics reduces the expression of senescence-associated secretory phenotype and restores myogenesis in myotonic dystrophy type 1. Nat. Commun. 2023.

23. **Extracellular RNA splice events in cerebrospinal fluid as candidate biomarkers of myotonic dystrophy type 1** (Poster ID # 39)

Preeti Kumari, Ningyan Hu, Alex Sizemore, Lauren Sullivan, Brigham Mckee, Parker Conquest, Thurman Wheeler
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Format: Posters- Hybrid

ABSTRACT: Objective: Alternative splicing is mis-regulated in post-mortem DM1 central nervous system (CNS) tissue. Extracellular RNA (exRNA) in cerebrospinal fluid (CSF) provides a source of CNS-derived splice events in living individuals that may serve as biomarker of DM1 disease activity. Methodology: We examined fresh CSF samples from unaffected controls (UA; N=14) from the MGH CSF biobank and DM1 individuals with mild CNS involvement (N=3). To separate extracellular vesicles (EVs) from cells contained in CSF, we used low speed centrifugation followed by filtration of the supernatant. EV size and concentration were measured using microscopy and particle tracking analysis software (NanoSight). We extracted exRNA from the EV pellet, produced cDNA, and quantified gene expression and splice events (% exon inclusion) by droplet digital PCR. Results: In CSF cells, total RNA content is 10 - 100-fold higher than exRNA. Normalized expression of both DMPK and CNBP was 50 - 60% higher in CSF exRNA than in CSF cells. Splicing is significantly different in CSF exRNA vs CSF cells. Alternative exons of GOLGA4, NUMA1, NCOR2 and MBNL2 transcripts were mis-spliced in CSF exRNA of DM1 vs UA. Inclusion of GOLGA4 alternative exon 23 shows no overlap between DM1 vs UA ($P<0.01$). Conclusions: Quantification of CNS-derived alternative splice products in CSF exRNA is feasible. Removal of leukocytes and erythrocytes normally found in CSF will enhance accurate quantification of CNS-derived splice events. GOLGA4 is a candidate biomarker of early CNS involvement in DM1, while NUMA1, NCOR2, and MBNL2 splice events are candidate biomarkers of disease severity.

24. Modulations of the proteome following a 12-week strength training program in male DM1 patients (Poster ID # 26)

Cécilia Légaré[1,2,3], Amira Aoussim[2,3], Marie-Pier Roussel[3,4], Anne-Marie Madore[4], Mathieu Morissette[5], Catherine Laprise[4], Elise Duchesne[2,3]
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Format: Posters- Hybrid

ABSTRACT: There are clinical benefits arising from strength training for individuals with myotonic dystrophy type 1(DM1), but the molecular mechanisms behind those are not clearly understood. Objective: To identify the proteins that are modulated by a 12-week strength training program in male DM1 patients and the muscular histomorphological variables associated with these changes. Methodology: Vastus lateralis biopsies from 11 participants before and after a supervised 12-week strength training program were collected. Liquid chromatography-tandem mass spectrometry was used to identify proteins that were modulated by the training program. Results: A total of 44 proteins were significantly modulated by training. A review of the literature indicated that they were implicated in various biological subclasses involved in training-induced response including immunity, energy metabolism, apoptosis, insulin signaling, myogenesis, and muscle contraction. Atrophy and hypertrophy factors derived from the muscle biopsies collected at baseline for each participant were identified as variables explaining proteome modulation using linear models. Heatmap analyses showed heterogeneity in proteomic response to training within this population. We found six proteins that were modulated by training and were of particular interest in DM1: calpain-3 (CAN3; Muscle development, positive regulation of satellite cell activation), 14-3-3 protein epsilon (1433E; Insulin/Insulin-like growth factor, PI3K/Akt signaling), myosin-binding protein H (MYBPH; Regulation of striated muscle contraction), four and a half LIM domains protein 3 (FHL3; Muscle organ development), filamin-C (FLNC; Muscle fiber development) and cysteine and glycine-rich protein 3 (CSRP3). Conclusions: These proteins could potentially serve as biomarkers in DM1; however, further studies are required to validate these findings.

25. Individual transcriptomic response to strength training for patients with myotonic dystrophy type 1 (Poster ID # 29)

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ABSTRACT: Objective: To study potential effects of a 12-week strength-training program on myotonic dystrophy type 1 (DM1) patients. Methodology: Male DM1 patients previously underwent a 12-week supervised strength-training program and clinical assessment. RNA-sequencing was performed on vastus lateralis biopsies from male DM1 patients (n=9) before and after strength-training and male control patients (n=6) who did not participate in the program. Differential gene expression and alternative splicing were analyzed pre- and post-exercise in the DM1 patients as well as compared to controls. These were then correlated to clinical measurements of four lower-limb exercises. Results: All DM1 participants were shown to experience clinical improvements after completing the training program. Here, we observed substantial changes in DMPK gene expression across patients, but only at the individual level. Strength training improved missplicing of skipped-exon events back towards control levels as well as splicing dysregulation scores for almost all DM1 participants. A correlation between clinical changes and the percentage of differentially expressed genes rescued following training was observed across all samples. Heterogeneity was observed in splicing and differential gene expression response across DM1 patients. Conclusions: DM1 participants of a 12-week strength-training program showed both clinical and transcriptomic responses. Clinical improvement was seen along with the rescue of differentially expressed genes and an improvement in alternative splicing dysregulation. The transcriptomic response varied amongst DM1 individuals with these significant changes masked by grouped analysis suggesting a need for future studies to consider investigating DM1 at the individual level.

26. **A genome-wide RNAi knock-down screen identifies spliceosome proteins as modifiers of RNA toxicity in myotonic dystrophy** (Poster ID # 32)

Jiss Louis[1], Jesus A. Frias[2], Jacob Schroader[2], Lindsey A. Jones[1], Emily E. Davey[1], Claudia D. Lennon[1], Ty Hamel[2], Andy Berglund[1], Kaalak Reddy[1]
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Format: Posters- Hybrid

ABSTRACT: Introduction: Myotonic dystrophy type 1 (DM1) is caused by a CTG repeat expansion in the DMPK gene. Transcription of these repeats produces toxic CUG RNA which leads to the sequestration of MBNL proteins that triggers a global spliceopathy that has been linked to many DM1 symptoms. Methods: Using a previously developed DM1 HeLa cell line that stably expresses r(CUG)480 and r(CUG)0 control1, we conducted a genome-wide siRNA screen of over 16,000 genetic targets to identify novel factors that modify toxic CUG RNA outcomes. The top hits from this initial screen were subsequently tested in DM1 patient-derived myoblasts to determine if siRNA treatment rescued DM1 splicing markers and/or resulted in the upregulation of MBNL1/2. Results: Our genome-wide siRNA screen identified multiple RNA processing factors including core spliceosome proteins (snRNPs) belonging to Sm (SNRPD2), PRPF19 (BCAS2), U1(SNRPA, SNRPC), U2 (SF3B2, SF3B5) and U4/U6 (PRPF3, PRPF4) protein complexes. These factors when knocked down in the HeLa DM1 cell models, reduced CUG RNA levels, upregulated MBNL1 and MBNL2 mRNA levels, and promoted rescue of MBNL-dependent mis-splicing. We knocked down our lead candidate SNRPD2 (Small nuclear ribonuclear protein D2) in DM1 patient-derived myoblasts and showed a reduction in DMPK levels, upregulation of MBNL1 at mRNA levels, and rescued MBNL1-mediated splicing events. Our results suggest that in DM1 the stoichiometry of spliceosome proteins is imbalanced and can be restored with their siRNA knockdowns leading to splicing rescue and reduction in toxic RNA. We are currently working to elucidate the possible molecular mechanism(s) through which this spliceosome protein stoichiometry can regulate RNA toxicity and spliceopathy. Conclusions: Our study demonstrates the importance of spliceosome proteins in regulating the toxic CUG RNA levels and MBNL-dependent splicing, providing new insights into DM disease biology and could lead to the identification of novel drug targets for myotonic dystrophy.

1Reddy K, Jenquin JR, McConnell OL, Cleary JD, Richardson JI, Pinto BS, Haerle MC, Delgado E, Planco L, Nakamori M, Wang ET, Berglund JA. Proc Natl Acad Sci U S A. 2019 Oct 15;116(42):20991-21000

27. **Changes in lower extremity muscle fat fraction and motor performance in myotonic dystrophy type 1** (Poster ID # 20)

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Format: Posters- Hybrid

ABSTRACT: Objective: To examine 1-year changes in motor outcomes and muscle fat fraction (MFF) of the legs of myotonic dystrophy type 1 (DM1) participants. Methodology: The lower extremities were scanned with 3T MRIs using a two-point 3D Dixon protocol. Muscle volumes of individual muscles (5 slices, 15mm thickness) were segmented from the proximal, middle, and distal levels of the thighs and calves and aggregated by muscle compartment. Six-minute walk distance test(6MWT; meters), 15-second step test, 10-meter walk time test(10MWT; seconds), and grip strength(kg) were measured. To assess changes in MFF and motor performance in the DM1 group, t-tests, and Wilcoxon signed-rank tests were used, respectively. Results: DM1 participants(n=7) at baseline were age(mean±SD) 50±13, 43% female, BMI of 26.0±3.6kg/m2, and a symptom duration of 19±14 years. Of the five participants with follow-up MRI, four had a 1-year follow-up and one a 2-year follow-up. There was no change in any motor outcomes (6MWT: 12.3 meters; 10MWT: 1.4 seconds; step test: -0.8; grip strength -0.6 kg; all P>0.05). Baseline MFF was 31±21% at the thigh, and 42±31% at the calf. The thigh MFF increased 0.9%/year (P=<0.001), and the calf MFF increased 1.7%/year(P=<0.001). MFF increased on all compartments of the thighs (anterior: 0.8%/year P=0.023, medial: 1.3%/year P=0.031, and posterior: 0.8%/year P=0.010) and calves (anterior: 2.1%/year P=0.003, lateral: 2.8%/year P=0.031, and posterior 1.0%/year P=0.005). The individual muscles with the highest mean changes in MFF were lateral gastrocnemius(1.6±1.7%/year), peroneus (1.5 ±1.7%/year), and extensor digitorum longus(1.3±1.2%/year) muscles. Conclusions: MFF could serve as a sensitive biomarker of disease progression in DM1.

28. **Evaluation of MSH3 as a genetic modifier of trinucleotide repeat instability in myotonic dystrophy** (Poster ID # 55)

Alexandra Marrero Quiñones
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Format: Posters- Hybrid

ABSTRACT: Introduction: Unstable CTG repeat expansions in Myotonic Dystrophy Type 1 (DM1) can lead to genetic anticipation. Most significant is the huge expansions that occur from mothers with low repeats to children with congenital myotonic dystrophy (CDM). Although disruptions in DNA repair pathways are suspected contributors of this rapid intergenerational expansion, the molecular pathogenesis of this phenomenon remains unknown. Objective: This project aims to evaluate predicted deleterious variants in populations of DM1 families with rapid intergenerational repeat expansion, defined as a 10-fold expansion between generations. This may identify potential therapeutic targets while providing insight into the role of trinucleotide repeat instability (TNRI) in the progression of disease between populations. Methodology: We previously performed whole exome sequencing on 10 mother-offspring dyads with DM1 and CDM rapid intergenerational expansion. Predicted deleterious variant effects will be functionally defined through in vitro biochemical assessments and various cell models of repeat instability. Results: Our sequencing data revealed two MSH3 rare and potentially deleterious variants of interest, defined as MAF<0.01 and SIFT>0.9. These DNA mismatch repair (MMR) protein variants segregate intergenerationally along the affected DMPK allele in a CDM population enriched for rapid genetic anticipation. DNA repair ability of MMR deficient LoVo cells was rescued by transient transfection with canonical MSH3 measured as mean %Tail DNA via Comet Assay. Conclusion: Evaluation of the clinically observed MSH3 deleterious variants is ongoing. We hypothesize that aberrant MSH3 function will result in global decrease in DNA repair and exacerbate the rate of the TNRI contributing to rapid expansion in DM1.

29. **Quercetin selectively reduces expanded repeat RNA levels in models of myotonic dystrophy** (Poster ID # 34)

Subodh Mishra[1], Sawyer M. Hicks[1], Jesus A. Frias[1], Sweta Vangaveti[1], John D. Cleary[1], Masayuki Nakamori[2], Kaalak Reddy[1], Andrew Berglund[1]
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Format: Posters- Hybrid

ABSTRACT: Objective: Myotonic dystrophy (DM) is the leading cause of adult-onset muscular dystrophy. DM type 1 (DM1) and DM type 2 (DM2) are two genetically distinct forms of DM. DM1 is caused by CTG repeat expansion in 3' UTR of the DMPK gene, and DM2 is caused by CCTG repeat expansion in intron 1 of the CNBP gene. CUG and CCUG repeat RNA expansions sequester the MBNL family of alternative splicing regulatory proteins into ribonuclear foci, leading to pathogenic mis-splicing. Currently, there are no

FDA-approved disease-targeting treatments available for DM. The current study identified quercetin as a promising lead molecule as a disease-targeting therapeutic for DM. Methods: Our lab has developed a HeLa screening cell line that permits the ratio-metric evaluation of toxic r(CUG)480 levels compared to an r(CUG)0 control1. This cell line was used to screen the NCI natural product library. Patient-derived fibroblast (DM1 and DM2) and muscle cell lines (DM1) were used to measure mis-spicing rescue and DMPK/CNBP transcripts levels. DM1 mice were used to measure the CTG transcripts levels, mis-splicing rescue, and myotonia. Results and conclusion: Screening in the HeLa cells revealed the dietary flavonoid quercetin as a selective modulator of toxic CUG RNA levels. Quercetin treatment reduced the toxic RNA level and alleviated the MBNL-dependent mis-splicing in DM1 and DM2 patient-derived cells. EMIQ is a bioavailable form of quercetin, and its treatment in DM1 mice reduced the toxic CUG RNA levels, mitigated mis-splicing, and reduced myotonia. The excellent safety profile of quercetin with little to no adverse effects and tentative mechanism of action positions quercetin as a potentially safe compound for therapeutic consideration for DM.

30. **The compensatory mechanism of MBNL paralogs and its role in myotonic dystrophy type 1** (Poster ID # 15)

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Format: Posters- Hybrid

ABSTRACT: Objective: The effect of pathogenic genetic variations can be compensated by paralogs with redundant functions. An example of such compensation are the paralogs of the Muscleblind Like (MBNL) family of RNA-binding proteins. Loss of MBNL1 increases MBNL2 in tissues where Mbnl2 expression is low. As such, Mbnl1-/- mice develop mild phenotypes, while Mbnl1-/-; Mbnl2+/- mice display severe phenotypes recapitulating Myotonic Dystrophy Type 1 (DM1), a multisystemic disorder in which an expanded CUG RNA repeat sequesters the MBNL paralogs. The mechanism by which MBNL2 is upregulated and the impact on DM1 pathogenesis remain unknown. Methodology: In this study, we used molecular and cellular assays upon Mbnl1 knockdown and knockout in cell culture and in vivo to uncover the mechanism by which loss of MBNL1 upregulates MBNL2. Results: We found that MBNL1 represses the inclusion of Mbnl2 exon 9, which results in an MBNL2 protein isoform that contains a PEST degradation signal in its C-terminal region. Upon loss of MBNL1, inclusion of Mbnl2 exon 9 increases, shifting the reading frame to an alternative C-terminus lacking the PEST domain, thereby increasing MBNL2 levels. We further found that the inclusion of Mbnl2 exon 9 is increased in human DM1 tissues and in a DM1 mouse model in which MBNL2 protein is upregulated. Conclusions: This study

uncovered the mechanism by which loss of MBNL1 upregulates MBNL2 and suggests that the compensatory mechanism is active in DM1. Future work will investigate the importance of the compensatory mechanism in DM1 and explore its utilization for therapeutic purposes.

31. **Choroid plexus mis-splicing and altered cerebrospinal fluid composition in myotonic dystrophy type 1** (Poster ID # 23)

Curtis Nutter, Benjamin M. Kidd, Helmut A. Carter, Johanna I. Hamel, Philip M. Mackie, Nayha Kumbkarni, Mackenzie L. Davenport, Dana M. Yuyn, Adithya Gopinath, Peter D. Creigh, Łukasz J. Sznajder, Eric T. Wang, Laura P. W. Ranum, Habibeh Khoshbouei, John W. Day, Jacinda B. Sampson, Stefan Prokop, Maurice S. Swanson
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Format: Posters- Hybrid

ABSTRACT: Myotonic dystrophy type 1 (DM1) severely affects the brain with an unusual cohort of symptoms, including hypersomnia, executive dysfunction, as well as early onsets of tau/MAPT pathology and cerebral atrophy. To address the molecular and cellular events that lead to these pathological outcomes, we generated a mouse Dmpk CTG expansion knock-in model and identified choroid plexus epithelial cells as particularly affected by the expression of toxic CUG expansion RNAs. Alternative splicing analysis was performed on lateral and hindbrain choroid plexi from DM1 mouse models. To determine if transcriptome changes also occurred in the human disease, we obtained post-mortem choroid plexus for RNA-seq from neurologically unaffected and DM1 donors. To test that choroid plexus transcriptome alterations resulted in altered CSF composition, we obtained CSF via lumbar puncture from patients with DM1 and non-DM patients, and western blot and osmolarity analyses were used to test CSF alterations predicted by choroid plexus transcriptome analysis. We determined that CUG RNA induced toxicity was more robust in the lateral choroid plexus of Dmpk CTG knock-in mice. Impaired transitions to adult splicing patterns during choroid plexus development were identified in Mbnl2 knockout mice. Whole transcriptome analysis of DM1 choroid plexus revealed disease-associated RNA expression and mis-splicing events. Based on these RNA changes, predicted alterations in ion homeostasis, secretory output and CSF composition were confirmed by analysis of DM 1 CSF. Our results implicate choroid plexus spliceopathy and concomitant alterations in CSF homeostasis as an unappreciated contributor to DM 1 CNS pathogenesis. Funding: NIH (NS048843 and NS103172 to M.S.S.), an MDF postdoctoral fellowship (C.A.N.), MDF and UF McKnight Brain Institute predoctoral fellowships (B.M.K.), a MDA Development Grant

(MDA546770 to Ł.J.S.), and a Schmitt Program in Integrative Neuroscience (SPIN) pilot award (J.I.H.).

32. **Myotonic Dystrophy Family Registry: Current summary of patient demographics and disease characteristics** (Poster ID #76)

Sofia Olmos, Mindy Kim, Kleed Cummings, Tanya Stevenson
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Format: Posters- Hybrid

ABSTRACT: Introduction: The Myotonic Dystrophy Family Registry (MDFR), launched in March 2013, is a patient self-reported online database that collects information on the impact and scope of myotonic dystrophy (DM) from the perspective of patients and their families. This information is meant to help better understand myotonic dystrophy, aid researchers in developing new, effective treatments and help identify participants for research studies and clinical trials. Objectives: Describe the demographics of registrants as well as selected self-reported data about symptoms, device use and burden of disease. Methods: For this report, data elements gathered from 2013 to 2023 and reported to the Registry by 2260 DM affected individuals worldwide, were compiled and analyzed. Results: Diagnoses represented in the registry include 1,101 people with adult-onset DM1, 171 with Juvenile onset DM1, 437 with adult-onset DM2, and 323 with congenital myotonic dystrophy. Enrollees are primarily from USA (79.3%) and 88% of all participants self-identify as White. The aggregate data show that 67% of respondents have difficulty walking, 75% have myotonia, 72% have daytime sleepiness, 70% experience pain and 76% report some degree of fatigue. Further results within each DM diagnosis type are shown, as well as data on additional symptoms and burden of disease parameters (quality of life). Conclusion: Fatigue, myotonia, daytime sleepiness, pain and difficulty walking were the most frequently reported symptoms by registrants. Analysis of symptom prevalence, device use and quality of life measurements show a substantial burden of disease in a significant proportion of DM patients.

33. **Connecting cell-type specific gene-expression patterns in DM-model zebrafish to sleep and circadian disruptions and enhanced therapeutic development** (Poster ID # 54)

Alexa Orsino, Dylan Farnsworth
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Format: Posters- Hybrid

ABSTRACT: Deciphering the complex underlying mechanisms and gene dysregulation patterns in DM requires understanding which organs are primarily affected, which cell types in those organs are pathogenic, and what pathways within those affected cells contribute to clinical outcomes. This knowledge is especially critical for developing safe effective therapies that precisely target disease-specific factors and cell types. In DM, a critical gap in knowledge is the lack of comprehensive compendium of cell types organized by gene expression patterns on a genome-wide scale for either DM patients or animal models of DM. Our research utilizes single-cell RNA sequencing (scRNA-seq) and new DM zebrafish models to comprehensively identify transcriptionally unique cell types and disease-specific gene expression patterns across development. This knowledge is leveraged to specifically test the hypothesis that gut disorders present in both DM patients and DM-model zebrafish are linked to disruptions in circadian gene expression and sleep patterns. Zebrafish are ideally suited to behavioral assays for sleep, genetic assays for circadian oscillations, and the characterization of gut function and inflammation levels. This model system will also enable us to test the effectiveness of small molecules in relieving these symptoms using DM model zebrafish and existing libraries of drugs. Taken together, this work will comprehensively define disease-specific cell-type effects in a vertebrate model system and provide insight for the development of improved therapeutics to tackle this disorder.

34. The role of MBNL in smooth muscle function and DM1 gastrointestinal pathologies (Poster ID # 13)

Janel AM Peterson[1], Andrew N Miller[1], Brandon Nguyen[1], Krishnakant G Soni[2], Geoffrey A Preidis[2], Thomas A Cooper[1]

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Format: Posters- Hybrid

ABSTRACT: Despite gastrointestinal (GI) disturbances affecting more than 80% of individuals with myotonic dystrophy type 1 (DM1), the mechanism leading to GI dysfunction remains unknown1,2. Our goal is to understand how muscleblind-like (MBNL) loss of activity, a primary mechanism of DM1 pathology, affects GI smooth muscle function. We assessed GI motility in mice with tamoxifen inducible, smooth muscle specific loss of MBNL1 and MBNL2 (smoCRE;dKO mice). Upper GI transit was evaluated by quantifying movement of FITC dye after 25 minutes, and lower GI motility was measured by bead expulsion assay. In addition to gross anatomical measurements and histological evaluation of muscularis thickness, ex vivo force transduction assays were performed to measure jejunal contractility. DM1-associated splicing changes were evaluated by RT-PCR using GI

smooth muscle RNA. Progression of gavaged FITC dye is significantly delayed and bead latency is significantly increased in smoCRE;dKO mice compared to controls, indicating delays in upper and lower GI transit. Small intestine length is significantly reduced and duodenal muscularis thickness is increased in smoCRE;dKO animals, indicating functional disruption without cell loss that suggests intrinsic defects in smooth muscle function. Ex vivo force transduction experiments show significantly decreased jejunal contraction amplitude and activity at baseline and in response to a cholinergic stimulus. SmoCRE;dKO mice also share homologous misregulated splicing events previously identified in mouse MBNL KO and human DM1 striated muscle tissues. These results demonstrate that smooth muscle specific loss of MBNL alters GI motility through intrinsic myogenic defects, supporting a key role for MBNL in proper GI function.

1. Heatwole, C. et al. Patient-reported impact of symptoms in myotonic dystrophy type 1 (PRISM-1). Neurology 79, 348–357 (2012).

2. Hilbert, J. E. et al. High frequency of gastrointestinal manifestations in myotonic dystrophy type 1 and type 2. Neurology 89, 1348–1354 (2017).

35. Timing is everything: Understanding sleep dysregulation in myotonic dystrophy (Poster ID # 44)

Belinda Pinto[1], Fangke Xu[2], Miguel Gutierrez[1], Andrew Morris[1], Andrew Liu[1], Karyn Esser[1], Ravi Allada[3], Eric Wang[1]

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Format: Posters- Hybrid

ABSTRACT: While a lot of attention has been given to understanding the basis of muscle pathology in Myotonic Dystrophy (DM), very little is known about the basis of the CNS symptoms in this disease, including excessive daytime sleepiness and sleep dysregulation. Sleep timing and structure are regulated by communication between the circadian network and the sleep-arousal pathways. Here, we describe our efforts to examine how circadian disruption contributes to sleep dysregulation in DM through studies in Drosophila and mouse models. Circadian activity analysis of a CTG250 expressing Drosophila model displays lengthening of the circadian period caused by perturbations to core clock proteins. To determine whether expanded CTG repeat expression perturbs circadian rhythms in mammals, we studied the DmpkCTG480 knock-in mouse model. Interestingly, we find that these mice display changes in circadian activity behavior with a shortening of the circadian period to ~23.5 hours. We are investigating the basis for this circadian disruption by

transcriptomic analysis of central and peripheral clock tissues and assessment of molecular rhythms of clock proteins via bioluminescence imaging of clock gene-luciferase fusion reporters. We are also extending these studies to examine circadian biomarkers in the DM patient population through urine-based ELISA assays. Taken together, our data show that circadian rhythms are disrupted in DM. Future studies will provide key insights into how circadian disruption contributes to excessive daytime sleepiness in DM.

36. **Single-administration of a cyclic peptide-conjugated CUG-repeat steric blocker rescues myotonia and molecular phenotypes in HSALR mice** (Poster ID # 47)

Emma Shea[1,2], Derek R. Muscato[2], Carmen Valero[2], Ryan P. Hildebrandt[2], Leanne M. Adams[2], Marina M. Scotti[2], Xiulong Shen[3], Mahboubeh Kheirabadi[3], Mark Wysk[3], Matthew Streeter[3], Natarajan Sethuraman[3], Ziqing Qian[3], Eric T. Wang[2]
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Format: Posters- Hybrid

ABSTRACT: In Myotonic Dystrophy type 1 (DM1), expanded CUG repeats in the DMPK transcript sequester MBNL splicing proteins. This causes molecular phenotypes including nuclear foci accumulation and aberrant splicing, leading to symptoms such as myotonia and muscle weakness. Multiple oligonucleotide therapeutics have been developed to either cleave DMPK mRNA or displace MBNL from the CUG repeats, but a major barrier to successful implementation of these approaches is efficient delivery to muscle tissue. Conjugation of oligonucleotides to antibodies, antibody fragments, and peptides to enhance delivery efficiency are currently in development by several groups. Here, we show that cyclic peptide oligonucleotide conjugates efficiently displace MBNL from expanded CUG repeats in human DM1 cells and mouse models of DM1. Nuclear CUG repeat foci were reduced in a CUG-repeat knock-in cell line (HeLa480) and patient-derived myoblasts, and splicing events were also assayed and rescued. A single intravenous administration into HSALR mice eliminated myotonia one week after injection, showed partial splicing rescue as early as one day after injection for some events, and revealed downregulation of the repeat-containing transcript. RNAseq analyses of nascent quadriceps transcripts reveal that some newly synthesized transcripts have corrected splicing patterns hours after injection, which suggests that transcript half-life influences overall speed to rescue. Live-cell RNA tracking experiments RNA imaging experiments are used to visualize cellular

activities after treatment. This study demonstrates the promise and potential of cyclic peptide oligonucleotide conjugates for the treatment of DM1.

37. **Myotonic dystrophy and mental health research** (Poster ID # 62)

Ruth Sheldon[1], Melissa Dixon[2], Benjamin Gallais[3], Benjamin Reynolds, Tanya Stevenson[1], Mindy Buchanan[1], Emily Romney[1]
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ABSTRACT: Myotonic dystrophy (DM) is a multisystemic neurological disease that can have far reaching mental health effects on an individual. Though largely absent from the literature, there are certain mental health conditions associated with a diagnosis of DM. Because individuals with DM have unique risk factors and susceptibilities an overview of potential mental health issues will allow individuals to anticipate, and prepare for, future mental health needs. The Myotonic Dystrophy Foundation (MDF) is consolidating a Myotonic Dystrophy and Mental Health Handbook (currently in development) designed for people living with DM and their caregivers focused on symptoms, diagnoses, and care recommendations. The authors of the Handbook will be facilitating the "DM and Mental Health: Your Questions Answered" session of this year's conference. Attendees are encouraged to submit their questions at the DM and Mental Health Research Poster (#62) in the Potomac Ballroom by 2:45 PM on Saturday.

38. **Isolating the role of myotonia in the pathogenesis of DM1 myopathy** (Poster ID # 38)

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Format: Posters- Hybrid

ABSTRACT: Myotonic dystrophy type 1 (DM1) is the most common adult-onset muscular dystrophy. DM1 patients possess >50 trinucleotide (CTG) repeats in the 3' UTR of the DMPK gene that when transcribed form toxic RNA hairpin structures that sequester RNA-binding proteins, such as the Muscleblind-like (MBNL) family of splicing factors. This leads to widespread changes in alternative splicing with the re-expression of fetal transcript isoforms resulting in dysfunction and disease. For example, altered splicing of transcripts for ClC-1, the main voltage-sensitive chloride channel on skeletal myofibers, has been directly linked to myotonia. Classically, myotonia in DM1 has been viewed exclusively as a

bothersome symptom afflicting DM1 patients. However, myotonia alone has been shown to cause changes in gene expression and recent work with splice-correcting ASOs has shown it to alter fiber-type distributions in DM1 mice. Therefore, we hypothesize that myotonia plays a central role in driving the other skeletal muscle pathology in DM1, namely weakness and wasting. To investigate this, we developed a novel mouse line resistant to myotonia caused by aberrant ClC-1 splicing to eliminate myotonia from established DM1 models. Thus far, we have observed significant physiologic, histologic, and splicing differences with the non-myotonic mice featuring phenotypes closer to wildtype. Overall, this research provides evidence that myotonia plays an important role in the development of progressive myopathy in DM1 and treatment of myotonia may provide myo-protective benefit to improve patient outcomes.

39. **Development of a novel fusion protein, JUV-161, that enhances muscle regeneration and function for treatment of myotonic dystrophy type 1** (Poster ID # 67)

Hee Ju Kim, Ashil Koranne , Zhihua Li, Thach Mai, Han Song, Trang Vuong, Rohit Jadhav, Dan Furlong, Mohammad Hassanipour, Vengadesh Karuppagounder, Mo Tabrizi, Jeremy O'Connell, Hanadie Yousef
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Format: Posters- Hybrid

ABSTRACT: Objective: Juvena Therapeutics developed JUV-161 as a recombinant fusion protein to agonistically target MAPK/ERK and PI3K/AKT regenerative cascades. These pathways are the major signaling mediators in the skeletal muscle to enhance myogenesis, and muscle survival/function. JUV-161 treatment could improve muscle strength, endurance, mass, and glucose regulation, leading to reduced atrophy and improve muscle function in adult-onset DM1 patients. Method: To advance the preclinical development of JUV-161, we developed a pan- inducible, TREDT960I transgenic mouse model containing a human genomic segment containing exons 11-15 of DMPK gene with 960 interrupted CTG repeats (CUG960) under direction of the tetO (tet-responsive element) promoter. This CUG960/+ murine model encompasses the key aspects of DM1 muscle deterioration, as shown using functional and histological testing. Results: Administration of JUV-161 in the DM1 mouse model resulted in significant improvements in the grip strength, coinciding with significantly increased tibialis anterior cross-sectional area and an increased density of fast-twitch type-2X/B muscle fibers in the soleus in both male and female mice. Binding assays and receptor sequence homology across species (BLASTP), reflected potential clinical translatability of the preclinical results obtained in a DM1 mouse model and the pharmacologically relevant species (Rat and Dog). Conclusions: The promising activity of

JUV-161 in preclinical and nonclinical studies, Juvena therapeutics is planning to evaluate the potential therapeutic benefit of JUV-161 in adult-onset DM1 patients in 2024. JUV-161 treatment could improve muscle strength, endurance, mass, and glucose regulation, leading to reduced atrophy together with faster walk speeds and reduced fall rates, in adult-onset DM1 patients. Funding: California Institute of Regenerative Medicine (CIRM TRAN1-12890); National Institute of Aging (NIA R43AG071181). Acknowledgments: The TREDT960I transgenic mouse model was licensed from the Baylor College of Medicine, and crossed with R26-M2rtTA(+/+) mice containing the reverse tetracycline trans-activator knock-in. The authors acknowledge scientific advice on DM1 model breeding and validation and support from Dr. Thomas Cooper, Baylor College of Medicine.

40. Topline data analysis of the phase 1/2 clinical trial evaluating AOC 1001 in adult patients with myotonic dystrophy type 1: MARINA (Poster ID # 25)

Nicholas Johnson[1], John W. Day[2], Johanna Hamel[3], Charles Thornton[3], S.H. Subramony[4], Payam Soltanzadeh[5], Jeffrey Statland[6], Matthew Wicklund[7], W. David Arnold[8], Miriam Freimer[8], Kelly DiTrapani[9], Carrie Heusner[9], Chao-Yin Chen[9], Brad McEvoy[9], Yiming Zhu[9], Li-Jung Tai[9], Elizabeth Ackermann[9]

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Format: Posters- Hybrid

ABSTRACT: Objective: The primary objective of this study is to evaluate the safety and tolerability of single and multiple ascending doses of AOC 1001 in adults with myotonic dystrophy type 1 (DM1). Background: DM1 is a rare, dominantly inherited, progressive neuromuscular disease caused by a toxic gain-of-function mutation in the DM1 protein kinase (DMPK) gene. AOC 1001, an antibody oligonucleotide conjugate (AOC) comprised of a DMPK siRNA conjugated to a humanized anti-transferrin receptor 1 antibody, was designed for functional delivery to muscle cells to reduce the toxic mutant DMPK mRNA and potentially address the pathology of DM1. Design: This phase 1/2 study (NCT05027269) is a randomized, placebo-controlled, double-blind trial in two parts. Part A was a single dose design. Part B is a multiple-ascending dose design with 3 cohorts (dose

levels), with quarterly doses and 1 booster after the first 6 weeks. The cohorts were initiated in a staggered fashion based on a safety data review of the preceding cohort(s). Secondary objectives include spliceopathy, pharmacokinetics, and pharmacodynamics (DMPK mRNA knockdown). Exploratory objectives include efficacy measures (myotonia, mobility, muscle strength, and muscle function), and patient-reported outcomes. The Study enrolled 38 adults aged 18 to 65 years with a genetic diagnosis of DM1. Results: The MARINA study is the first complete trial in the development of AOC 1001. This topline data analysis will include safety and tolerability, pharmacokinetics, pharmacodynamics, and the changes in RNA splicing from all cohorts. Conclusions: AOC 1001 represents a novel potential therapy addressing the underlying cause of DM1.

41. **Searching for Central Nervous System signatures in DM1 patients- Analysis of exosomal mRNAs from Cerebrospinal fluid** (Poster ID # 49)

Carmen Valero[1], Emily E. Davey[1], Belinda Pinto[1], Katharine A. Hagerman[2], Jacinda B. Sampson[2], John W. Day[2], Eric T. Wang[1]
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Format: Posters- Hybrid

ABSTRACT: Central Nervous System (CNS) symptoms are common in Myotonic Dystrophy (DM). In fact, many DM patients describe hypersomnia, memory deficits and difficulty focusing as highly debilitating. Cerebrospinal fluid (CSF) is a suitable liquid biopsy source to identify potential biomarkers of disease progression and response to therapeutic intervention. Exosomes are vesicles secreted from cells that contain microRNAs and mRNAs. However, transcriptome sequencing of mRNAs from CSF-derived exosomes has been technically challenging due to the low abundance of RNA. Thus, to date, most studies of CSF have focused on microRNA cargoes. We have recently developed a method to isolate exosomes, harvest their RNA cargoes, and deep sequence the polyA+ population, yielding full-length spliced and processed mRNAs. Signatures of CSF-derived exosomal mRNAs were compared between 5 healthy controls and 11 myotonic dystrophy type 1 patients. Among the most abundant detected mRNAs are included those typically expressed in the choroid plexus, and some show differential expression between DM1 patients and controls. Additionally, we observed distinct regulation patterns in genes encoding ribosomal protein subunits. As the CSF is produced by choroid plexus tissue, we will confirm whether transcriptomic changes detected in the CSF reflect changes in the choroid plexus of DM patient postmortem samples and DM CNS mouse models. Overall, we have established

methods to study mRNAs derived from CSF exosomes and will apply them to additional samples to gain further insights into potential biomarkers of CNS biology.

42. **Psychological health and quality of life in adult myotonic dystrophy** (Poster ID # 50)

Melissa Dixon, Kalista Vordos, Rebecca Felchar, Russell J. Butterfield
Department of Pediatrics, University of Utah, Salt Lake City, Utah, US
Format: Posters- Hybrid

ABSTRACT: Introduction: Myotonic Dystrophy (DM) symptom burden and disease progression play crucial roles in performance of activities of daily living (ADLs) and substantially impact psychological health and quality of life (QoL). Early and accurate identification of psychological and behavioral symptoms can greatly assist in targeted care. Objective: To better understand factors that influence and enhance psychological health and QoL in DM, validated surveys were used to collect information pertaining to psychological well-being and QoL concerns. Methods: PROMIS surveys were administered to 120 (DM1 N=87, DM2 N=33) adults with DM. Descriptive, parametric and non-parametric tests were conducted to describe the population and determine group differences by DM type (DM1, DM2). Results: Preliminary results show that overall, DM participants had significant impairments in physical function and sleep; significantly elevated levels of fatigue and a marked decrease in self-efficacy related to functioning in activities of daily living. Group differences were observed. Anxiety was significantly higher in DM1 compared to DM2. Pain was significantly greater and social participation was significantly less in DM2 compared to DM1. Conclusion: Preliminary findings indicate that individuals with DM face substantial impairments in physical function and sleep, heightened levels of fatigue, and lower self-efficacy for ADLs. Notable differences observed between DM1 and DM2 include significantly higher anxiety in DM1, and significantly greater pain and lower social participation in DM2. These results emphasize the unique challenges faced by individuals with DM1 and DM2 and highlight the importance of addressing physical and psychological factors to enhance well-being and QoL in DM populations.

43. **Physical activity and cognition as factors of function in myotonic dystrophy type 1** (Poster ID # 16)

Nicole White, Jeanne Dekdebrun, James Hilbert, Erin Richardson, Eleanor Stanton, Johanna Hamel, Katy Eichinger
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Format: Posters- Hybrid

ABSTRACT: Objective: Individuals with myotonic dystrophy type 1 (DM1) often present with cognitive involvement, commonly described as apathy. There is little information on the impact of cognition symptoms on physical activity (PA) within this population. Therefore, the objective of this study was to examine the relationships between cognition, physical activity, and function. Methods: This prospective observational study was conducted alongside an ongoing natural history study, during which assessments of strength (manual muscle testing) and function (10-meter walk/run, Timed Up and Go, and ascending 4 stairs) were performed. Over the following 6 weeks, patient reported measures of fatigue, apathy, and sleepiness were collected. PA in the natural environment was measured using wearable sensors (24-hour wear) and activity monitors (6-week wear). Descriptive statistics and Spearman's Rank Correlation were used to analyze the data. Results: 24 participants (63% female) were enrolled, with a step count completion rate of 67%. Correlations were found between different PA step count methods (patient-reported (PR, n=10)) and Fitbit (ρ=0.94, $p<0.001$), and Biostamp (ρ=0.78, p=0.008). PA (PR) was strongly correlated with apathy (ρ=0.74, p=0.002), and moderately correlated with strength (ρ=0.50, $p \leq 0.05$). In terms of function, PA was only associated with ascending 4 stairs (ρ=-0.5, p=0.04). Conclusions: Our findings suggest apathy impacts engagement in PA for individuals with DM1, with fatigue, sleepiness, strength, and function having a lesser effect. This may prove useful when developing targeted interventions to increase PA; however, additional studies are needed to explore this relationship further.

44. Analysis of the CUG repeat expansion DMPK transcript degradation in myotonic dystrophy type 1 (Poster ID # 59)

Xiaomeng Xing, J. David Brook
Institute of Genetics, School of Life Sciences, University of Nottingham, England, UK
Format: Posters- Virtual

ABSTRACT: Objective: Firstly, to explore the dynamics of RNA foci in DM1; secondly, to unravel the mechanisms involved in the transportation and degradation of mutant DMPK mRNAs; and finally, to investigate the role of MBNL proteins in these processes. Methodology: Stochastic optical reconstruction microscopy (STORM) has been applied to explore the RNA foci dynamics. Additionally, we seek to determine the key RNA decay factors responsible for the degradation of mutant DMPK mRNAs using lentiviral-shRNA delivery in DM1 cell lines and their derivatives. Results: 1) MBNL proteins hold multiple DMPK transcripts together to form large foci, and their absence not only prevents the

aggregation of multiple mutant DMPK mRNA molecules, but also promotes their degradation and their transportation to the cytoplasm; 2) Various RNA decay pathways play a critical role in reducing foci formation and promoting the degradation of mutant DMPK mRNAs. The sequestration of MBNLs to the mRNAs impedes its degradation process. Conclusions: My research uncovered valuable data on the RNA foci dynamics in DM1, revealing the intricate mechanisms that underlie their formation, stability, and turnover. My findings also contributed to delineate the complex pathways involved in the transportation and degradation of mutant DMPK mRNAs and provided insights into the critical role played by MBNL proteins in these processes. Studying the degradation mechanisms of mutant DMPK mRNAs in myotonic dystrophy may provide a foundation for comprehending the mechanisms of RNA degradation in other diseases caused by short tandem repeat (STR) mutations. Additionally, the use of cutting-edge STORM technology can provide a valuable tool for investigating RNA foci in other repeat expansion disorders.

45. **From myotube to patient: AOC 1001 demonstrates DMPK reduction and spliceopathy improvement in a phase 1/2 study in myotonic dystrophy type 1 (MARINA)** (Poster ID # 33)

Yiming Zhu, Tanya Kwan, Qingying Meng, Michelle Lee, Barbora Malecova, Rob Burke, Li-Jung Tai, Husam Younis, Art Levin, Mike Flanagan
Avidity Biosciences, San Diego, California, US
Format: Posters- Hybrid

ABSTRACT: Background: DM1 is a rare, autosomal dominant, progressive neuromuscular disease with no approved therapies. DM1 results from a mutation in myotonic dystrophy protein kinase (DMPK) mRNA leading to nuclear retention (nuclear foci) and splicing factors sequestration. AOC 1001, an antibody oligonucleotide conjugate (AOC), comprised of siRNA targeting DMPK mRNA (siDMPK) conjugated to humanized antibody targeting human transferrin receptor 1, is designed to reduce DMPK mRNA in muscle tissue. Method: AOC 1001 was evaluated in vitro in DM1 patient myoblasts and in a Phase 1/2 study in patients with DM1 (MARINA). Levels of DMPK mRNA, splicing events, and immunohistochemistry were evaluated in DM1 myoblasts treated with siDMPK. In the MARINA trial, muscle biopsy samples were evaluated for DMPK expression and splicing changes by qPCR and RNA sequencing, respectively. Results: In DM1 patient-derived myotubes, siDMPK achieved up to 75% DMPK mRNA knockdown, reduced nuclear foci by 50%, and mis-splicing improvements. In a first-in-human study (MARINA), AOC 1001 produced a ~40% mean reduction in DMPK mRNA after one or two doses of 1 and 2mg/kg treatment. Improvement in splicing dysregulation was evident based on correction of

multiple mis-spliced genes and increased normal isoforms of muscle-specific genes in DM1 participants who received AOC 1001. Conclusion: AOC 1001 treatment reduced DMPK mRNA and improved spliceopathy in DM1 patient skeletal muscle. This demonstrates the translation of potent in vitro activity to proof-of-mechanism in a first-in-human clinical study in DM1 participants. Altogether, this supports AOC 1001 as a potential therapy addressing the underlying cause of DM1.

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