Myotonic Dystrophy Foundation (MDF)
2025 MDF International Conference
Poster Abstracts

Poster # 5

Launch of Myotonic Dystrophy In Motion Month

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The first annual Myotonic Dystrophy In Motion Month took place in July 2024. With the intention of engaging the DM community in accessible, community-based exercise, and encouraging those living with DM to build a personal practice, this program was highly successful in its first year. In Motion Month had over 130 participants from 14 countries, and 32 states. Participants engaged in movement work through weekly exercise and informational sessions as well as an In Motion Buddy Network that paired community members based on their interests and movement abilities. Participants were motivated to find exercises that worked for them; 85% of respondents found the In Motion Month Community Sessions accessible, and 83% found them valuable. MDF published an infographic focused on the four pillars of movement based on our existing Exercise Guide for People Living with Myotonic Dystrophy. In addition to a month-long program, the informational and exercised based programming are available as an ever-green resource to the community through MDF's Digital Academy and received over 300 views in the first month alone.

Poster # 7

Development of an AAV-based microRNA gene therapy for Myotonic Dystrophy type 1

Authors: Martin Goulet, Basel T. Assaf, Bill McCarty, Giulio Tomassy, Wei Fan, Shuwen Cao, Alessandro Magli, Tracy Zhang, Kristen Boyle, James Cao, Robert Jackson, Jennifer Sullivan, Amy Frederick, Catherine O' Riordan, Christian Mueller

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Introduction: Myotonic Dystrophy Type 1 (DM1) is caused by a CTG repeat expansion in the DMPK gene. This mutation results in insoluble mRNA aggregates, sequestration of important splicing factors, and widespread RNA processing defects. Reduction of the DMPK mRNA by antisense siRNA or ASO has seen promising results in clinical trials. However, these modalities require repeated administrations.

Objective: For single administration, we tested SAR446268 an investigational gene therapy candidate containing a novel recombinant adeno-associated vector, AAV.SAN011, expressing an artificial miRNA, amiRDMPK, targeting DMPK mRNA.

Methodology: We analyzed the ability of amiRDMPK to silence the human DMPK transcript and correct splicing defects in patient-derived cells and in DMSXL mice carrying a mutant human DMPK gene. We also performed an intravenous SAR446268 dose-response and evaluated safety in non-human primates (NHP).

Results: AmiRDMPK treatment resulted in DMPK silencing and correction of mis-splicing in DM1 differentiated myotubes. In DMSXL mice, a single intravenous dose of SAR446268 silenced human DMPK mRNA in muscle, reduced mRNA aggregates, improved splicing abnormalities, cardiac function and electrical myotonia. In NHP, SAR446268 treatment resulted in a persistent dose-dependent expression of amiRDMPK and consequent silencing of DMPK mRNA expression by >75% in cardiac and all skeletal muscles surveyed relative to a control group. Administration of SAR446268 resulted in transient minor clinical pathology findings with no abnormal observations in either microscopic evaluation of peripheral organs, neurological assessments, cardiac biomarkers and electrocardiology.
Conclusions: In cellular and animal models of DM1 we demonstrated that SAR446268 reduces DMPK RNA levels, and improves molecular, pathological, and clinically relevant disease hallmarks. SAR446268 treatment is well tolerated in NHP and results in a dose-dependent silencing of DMPK mRNA in all major muscle groups.
Practical Implications: Overall, our data supports the efficacy and safety of a single dose SAR446268 as a potential gene therapy for DM1.

Young Investigator!
Poster # 21
Myotonic Dystrophy type I (DM1) Impairs Cardiac Bioenergetics and Mitochondrial Fusion-Fission Dynamics
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Objectives: Impaired mitochondrial function has been demonstrated in the brain, skeletal muscle, and fibroblast of DM1 patients. These have, however, not been reported in the heart, nor have their potential contribution to the pathogenesis of DM1 cardiac dysfunctions been explored. We probed the bioenergetic profile of DM1-afflicted heart tissues and explored the mechanistic basis of DM1-induced cardiac bioenergetic defects.
Methods: We screened existing RNA-seq datasets to identify DM1-associated splicing defects of genes involved in mitochondrial functions and validated these events in hearts from DM1 patients and a doxycycline-inducible, cardiomyocyte-specific DM1 mouse model. Next, we performed extracellular flux analyses on cardiac mitochondrial fractions from control and DM1 mice. We quantified ATP and total NAD(H) concentrations, to determine efficiency of mitochondrial oxidative respiration (OXPHOS) in DM1-afflicted heart tissues. Finally, we performed TOM20 immunofluorescence staining with confocal microscopy to assess mitochondrial morphology in DM1 mice.
Results: We identified aberrant splicing of transcripts of mitochondria fission factor Mff and dynamin 1-like protein Dnm1l, the two most important components of mitochondrial fission pathway in DM1 RNA-seq datasets. We validated both events in hearts from human DM1 patients. Both events were recapitulated in hearts from MBNL1 KO mice, indicating a role of DM1-induced sequestration of MBNL1 in pathogenesis of these splicing defects. We identified the same splicing defects in hearts from DM1-afflicted mice, which correlates with increased mitochondrial fragmentation on TOM20 IF microscopy, impaired mitochondrial oxidative respiration on Seahorse extracellular flux analyses, and deficient ATP synthesis in DM1-afflicted mice hearts. Further, we found that forcing Mff and Dnm1l mis-splicing using splice site-targeting anti-sense oligonucleotide (ASO) recapitulated similar mitochondrial bioenergetic and fission defects, in the absence of DM1 in HL-1 cardiomyocytes.
Conclusion: DM1 impairs cardiac bioenergetic function potentially through the mis-splicing of Mff and Dnm1l, which are critical genes regulating mitochondrial fusion-fission dynamics.

Practical Implications: Reversal of DM1-induced mis-splicing of Mff and Dnm1l may be exploited as a therapeutic strategy to improve bioenergetic profile and alleviate cardiac dysfunction in DM1-afflicted heart tissues.

Young Investigator!

Poster # 22

A Novel Mouse Model for Studying the Neurological Manifestations of Myotonic Dystrophy Type 1

Authors: Larissa Nitschke and Thomas Cooper
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Objective: Myotonic Dystrophy Type 1 (DM1) is an autosomal dominant disorder caused by a CTG repeat expansion in the 3' untranslated region of the DMPK gene. While primarily known for skeletal muscle dysfunction, over 80% of DM1 patients exhibit neurological symptoms including sleep disturbances, mood disorders, anxiety, ADHD, autistic features and cognitive impairment. The severity of these symptoms varies with age of onset, with congenital and childhood-onset patients experiencing more severe neurological manifestations compared to those with adult-onset. Despite the high prevalence and impact of the neurological symptoms, the underlying mechanisms of DM1 brain disease remain poorly understood.

Methodology: To study DM1 brain disease, we developed a doxycycline-repressible DM1 mouse model, CUG960, that expresses 960 interrupted CUG repeats throughout the brain.

Results: The CUG960 mouse model exhibits widespread CUG repeat RNA expression in the brain and recapitulates key molecular DM1 features including nuclear RNA foci, MBNL sequestration, and alternative splicing defects. Phenotypically, the mice display reduced brain weight and behavioral abnormalities including hyperactivity and learning and memory deficits. Using the doxycycline-repressible system, we demonstrate that early suppression of CUG repeat RNA at conception or birth rescues brain weight changes. However, suppression at 4-weeks of age fails to improve brain weight, suggesting a critical time window for therapeutic intervention. Additionally, we show that CUG repeat RNA expression can be induced in adult mice, enabling investigations of both developmental and adult-onset disease mechanisms.

Conclusions: We generated a novel DM1 brain mouse model that enables temporal control of CUG repeat RNA expression and recapitulates key features of DM1 brain disease. Future studies will expand on our initial characterization, identify the molecular mechanisms underlying the behavioral phenotypes, define critical therapeutic time windows, and investigate age-dependent effects.

Practical Implications: This CUG960 mouse model provides a valuable tool to study the neurological manifestations of DM1 and facilitate the development of targeted therapies.

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Young Investigator!

Poster # 28

AI-Derived Muscle Fat Fraction: Optimizing Biomarkers for Effective Trial Designs in Myotonic Dystrophies

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Objective: Myotonic dystrophy type 1(DM1) and type 2(DM2), the most common adult-onset muscular dystrophies, are characterized by progressive muscle weakness, myotonia, and multisystemic features. Muscle fat fraction(MFF), a proxy of intramuscular fat infiltration derived from magnetic resonance imaging(MRI), is a potential biomarker of disease severity. Artificial intelligence(AI)-based analysis enables streamlined MFF quantification, which could enhance biomarker utility in trials. However, its relationships with clinical endpoints have not been investigated in DM. The objective of this study is to examine the associations between AI-derived MFF and motor endpoints in DM1 and DM2.

Methodology: Lower extremity MRIs were obtained using a 3T scanner with a two-point, 3D-Dixon volumetric interpolated breath-hold examination (VIBE) protocol. Muscle compartments of participants were segmented from the thighs using TotalSegmentator1 and from the calves using Dafne2. Based on our previous work3, the most affected muscles (posterior calf compartment in DM1 and posterior thigh compartment in DM2) were selected to correlate with the six-minute walk distance (6MWT; meters), 10-meter walk test (10MWT; m/sec), 15-second step test, and grip strength (kg) using Spearman correlation coefficient(p).

Results: Of the seven DM1 (43% female) and nine DM2 (89% female) participants, mean age was 50±13years for DM1 and 62±12 for DM2, and disease duration was 18±14 and 20±12years, respectively. Average MFF of the posterior calf compartment in DM1 was 50±38%, and 46±18% for the posterior thigh compartment in DM2. MFF significantly correlates with 6MWT (p=-0.63, P-value=0.009), 10MWT (p=-0.66, P-value=0.005), the step test (p=-0.60, P-value=0.013), and grip strength (p=-0.70, P-value=0.003) in both DM1 and DM2.

Conclusions: MFF of the most affected muscle groups strongly associates with motor endpoints in DM.

Practical Implications: AI-based segmentation could provide an efficient method for trial designs. Larger and longitudinal studies are needed to validate this method as a sensitive and reliable biomarker of disease severity in DM.

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Young Investigator!

Poster # 31

Forced mis-splicing of Cav1.1 channels exacerbates diaphragmatic weakness in DM1 mouse models.

Authors: Sakura Hamazaki, Matthew Sipple, Lily Cisco, Jennasea Licata, Katherine Lupia, Zhenzhi Tang, Charles Thornton, John Lueck

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Myotonic dystrophy is a multisystem disorder that affects every organ in the body, with muscle weakness and wasting attributing to approximately 60% of patient mortality due to underlying

respiratory weakness. Despite this, the molecular mechanism involving muscle weakness and wasting remain poorly understood. Efforts by the Lueck and Thornton Labs have identified a combinatorial effect of L-type calcium channel (Cav1.1 with exclusion of exon 29; de29) aberrant splicing and loss of the skeletal muscle chloride channel (ClC-1) function results in severe skeletal muscle weakness and synthetic lethality in a reductionist mouse model. To better understand the role of Cav1.1 in DM1 myopathy, HSALR and Mbnl1-/- mouse models of DM1 were generated with forced expression of Cav1.1 de29. Mice expressing mis-spliced Cav1.1 in HSALR and Mbnl1-/- mice (Cav1.1 de29/Mbnl1-/- and Cav1.1 de29/HSALR) were characterized through survival, weight, time of righting reflex, and plethysmography, as well as ex vivo muscle contraction, in vivo muscle contraction with simultaneous EMG recordings, and histology. In efforts to uncover the cellular pathways involved in the significant myopathy observed in Cav1.1/Mbnl1-/- mice, mitochondrial morphology and proteomics was performed. Cav1.1 de29/Mbnl1-/- demonstrate a significant reduction in survival and respiratory muscle function, and exacerbated DM1 muscle histopathological features, that include significant increase in fibers containing central nucleation and fiber-type shifting. In contrast, Cav1.1 de29/HSALR exhibit only moderate disease aggravation, likely due to the lack of transgene expression in the diaphragm muscle. Completion of this study will advance the field by elucidating the role of Cav1.1 mis-splicing in driving DM1 myopathy and support Cav1.1 as a relevant therapeutic target.

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Young Investigator!

Poster # 43

Quantification of the Warm-up Phenomenon of Handgrip Myotonia in Myotonic Dystrophy Type 1 Using VHOT

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Objective: The improvement of delayed muscle relaxation (myotonia) with repetitive movement, the "warm-up" phenomenon, is well known in people with myotonic dystrophy type 1 (DM1). Previous studies have quantified handgrip myotonia warm-up using an isometric handgrip myometer apparatus. Here, we aim to quantify myotonia and warm-up "at the bedside" using the video hand opening time (VHOT).

Methodology: During remote study visits of REACH DM1 study participants, two consecutive VHOT tests were recorded under video supervision, in rapid succession. The middle finger and thumb opening times in seconds were scored offline using standardized procedures we previously developed. Warm-up time was defined as the difference between the first and second VHOT trials. Subjects taking anti-myotonia drugs and subjects with absent or minimal myotonia (<3 seconds) were excluded.

Results: Thirty subjects (63% female) were included in the analysis. The mean age of participants was 46 years (SD=15). The average age of onset was 25 years (SD=14), and the average age of grip myotonia onset was 27 years (SD=14). Middle finger myotonia had a median duration of 6.9 seconds (Q1-Q3: 4.9-11.8). The middle finger warm-up had a mean of 4 seconds (SD=3.7). 60% of subjects experienced middle finger warm-up greater than 2 seconds. Thumb myotonia had a mean duration of 10.3 seconds (SD=5.0), with a warm-up median of 3 seconds (Q1-Q3: 1.6-6.1). Middle finger warm-up was strongly correlated to middle finger myotonia on the first VHOT trial (r= 0.77; 95%CI: 0.56-0.88, p <0.001). A moderate correlation was seen between thumb myotonia and thumb warm-up (r=0.59).

Conclusions: These data support VHOT as a measure for quantification of the myotonia warm-up phenomenon in DM1. These findings suggest that warm-up is proportional to the severity of myotonia, which is in line with prior studies.

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Poster # 55

The Global Alliance for International Myotonic Dystrophy Awareness

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International Myotonic Dystrophy Awareness Day and the Global Alliance for Myotonic Dystrophy Awareness aim to garner the attention of the wider general public, policy makers, regulators, biopharmaceutical representatives, researchers, health care professionals, and anyone with an interest in changing the future of myotonic dystrophy. Raising awareness of myotonic dystrophy will help improve service provision, basic research, drug development, and policymaking related to the disease. Increased funding for myotonic dystrophy research will improve health outcomes, reduce disability, and increase life expectancy for individuals living with the disease, and holds great promise for helping individuals with diseases with similar genetic bases, such as Fragile X syndrome and Huntington's disease. Since its inception in 2021, the Global Alliance for DM Awareness has grown to over 60 organizations and institutions around the world.

Poster # 56

Reliability of Remote Assessments in Myotonic Dystrophy type 1

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Objective: We previously reported the feasibility of remote study of function and disease severity in people with myotonic dystrophy type 1 (DM1). Here, we assess the test-retest reliability of remote assessment of strength and function.

Methodology: Subjects were identified through the National Registry and enrolled remotely. Participants received a toolkit including a tripod, a tablet equipped with software for videoconferencing, and devices for assessments of strength and function. Two consecutive remote study visits (RSV) were conducted within 3 months. At both RSVs, grip, pinch, and tongue strength, forced vital capacity (FVC, sitting and supine), sniff negative inspiratory pressure (SNIP), 9-hole-peg-test (9HPT), video-hand-opening-time, 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. Intraclass correlation coefficients (ICC) were calculated using a 2-way random effects model to evaluate the agreement between repeated measurements. ICCs were defined as excellent (>0.9), good (0.75-0.9), or moderate (0.5-0.75). Bland Altman Plots were generated to evaluate systemic bias.

Results: Forty individuals with DM1 (ages 19-81) participated in consecutive RSV, on average, 27 days apart. No falls or injuries occurred. Quantitative assessments were completed in >90% of the visits, except for 10MWRT (80.6%) and supine spirometry (87.5%). The ICC showed excellent reliability for sitting and supine FVC, 10MWRT at maximal speed, handgrip, and pinch testing. TUG and 9HPT showed good to excellent reliability. 10MWRT at comfortable speed, SNIP, tongue, and buccal strength testing showed moderate to excellent reliability.

Conclusions: This data further demonstrates the feasibility of remote study of DM1 disease severity. Test-retest reliability of remote assessments of strength and function is acceptable. This data supports this novel platform to study large cohorts, enabling broad participation while reducing the burden on individuals and families.

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Young Investigator!

Poster # 60

Impact of myotonia on transcript expression and splicing in skeletal muscle.

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Myotonia is one of the characteristic manifestations of DM1 in skeletal muscle. This muscle hyper-excitability caused by altered CLCN1 splicing has traditionally been viewed purely as a symptom of the disease; however, studies have indicated that myotonia alone alters transcript expression and shifts fiber-type distributions, indicating a potential larger role in exacerbating disease severity in DM1. Accordingly, we hypothesize that myotonia plays a key role in modulating other skeletal muscle manifestations of DM1, including weakness and wasting. To extract the role of myotonia in DM1 myopathy, we developed a myotonia resistant mouse model with the genomic deletion of the exon 7a sequence in the Clcn1 gene (Clcn1deltaE7a). By crossing the Clcn1deltaE7a mouse model with the Mbnl1KO model of DM1, we were able to eliminate myotonia and directly compare changes in muscle physiology, histopathology, and the transcriptome between myotonic (Mbnl1KO) and non-myotonic (Mbnl1KO;Clcn1deltaE7a) progeny. We observed significant differences in muscle function, histopathology and transcript expression/splicing between non-myotonic Mbnl1KO;Clcn1deltaE7a mice and the myotonic Mbnl1 KO mice. Overall, Mbnl1KO;Clcn1deltaE7a mice showed phenotypes closer to wild-type with improved rotarod performance and increased in vitro muscle force generation. We also observed dampened histopathological changes in central nucleation, fiber-type distribution, and myofiber morphology in non-myotonic versus myotonic Mbnl1KO mice. Furthermore, by completing deep RNA sequencing on muscle from these mice, we observed changes in transcript expression and splicing that were driven by the presence or absence of myotonia that likely underlie these physiologic and histologic changes seen in Mbnl1 KO muscle. Overall, the results of this study further substantiates the growing evidence that myotonia may play an important role in the development of the progressive myopathy in DM1 and highlights the need for further investigation of anti-myotonic medications as a long-term myo-protective strategy in DM1.

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Young Investigator!

Poster # 62

Central Nervous System Involvement of Myotonic Dystrophy in the National Registry

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Objective: The National Registry has enabled studies of disease progression in myotonic dystrophy type 1 (DM1) and type 2 (DM2).1-3 Here, we describe outcomes related to central nervous system (CNS) symptoms as captured by the National Registry questionnaire, with an aim to evaluate progression over time and potential impact on other health outcomes.
Methodology: Registry members provide updates on disease progression through annual surveys. The survey includes the Epworth Sleepiness Scale (ESS), medications, employment status, counseling, and non-invasive ventilation (NIV) use. Herein, we included members with symptomatic DM1 and symptomatic DM2. Individuals with congenital DM1 were not included. Baseline and last follow-up forms were analyzed. Presence of excessive daytime sleepiness (EDS) was defined as an ESS score =10. CNS symptoms and psychotropic medication use were also collected. Statistical modeling will assess associations and progression.
Results: Data were available for 1032 members with DM1 and 266 with DM2. The median time between enrollment and last follow-up was 9 years (DM1, Q1-Q3, 4-13) and 8 years (DM2, Q1-Q3, 4-13). Members were on average 45 (DM1) vs. 58 (DM2) years old, with 53% (DM1) vs. 61% (DM2) females. Median age at symptom onset was 25 years (DM1, Q1-Q3, 16-35) vs. 36 years (DM2, Q1-Q3, 25-48). EDS was present in 55% of DM1 members at baseline and 65% at the last follow-up. Of those, 21% used NIV at baseline and 35% at the last follow-up. In DM2, EDS was present in 34% at baseline and 42% at the last follow-up. Further analyses will assess CNS symptoms, psychotropic medications, counseling, NIV use, and impact on other health outcomes.
Conclusions: CNS symptoms are prevalent, variable, and impactful in both types of DM. This study will guide Registry survey modifications to monitor CNS outcomes more extensively in the future.
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Young Investigator!
Poster # 65
Sexual and pelvic floor function in women with muscular dystrophy
Authors: Jeanne Dekdebrun, Martina Anto-Ocrah, Samia Lopa, Erin Richardson, Loralei L. Thornburg, and Johanna I Hamel
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Objective: Myotonic dystrophy type 1 (DM1) and type 2 (DM2) cause skeletal and smooth muscle dysfunction, impacting pregnancy and delivery. Effects on women's health beyond reproduction are largely unknown. This study evaluates sexual and pelvic floor function in at-birth females with DM1, DM2, and facioscapulohumeral muscular dystrophy (FSHD), the latter having skeletal but no smooth muscle involvement.
Methodology: We conducted a cross-sectional survey, including the Female Sexual Function Index (FSFI), Urinary Distress Inventory (UDI), Pelvic Organ Prolapse Distress Inventory (POPDI), and Colorectal-Anal Distress Inventory (CRADI), each scored 1-100, as well as the combined Pelvic Floor Distress Inventory (PFDI), containing POPDI, CRADI, and UDI. Outcomes were compared between groups using the Kruskal-Wallis test, with higher scores suggesting more dysfunction in all indexes except FSFI.
Results: 106 people with DM1, 68 with DM2, and 109 with FSHD returned surveys. The average age was 46.9, 55.2, and 52.3 years (p=0.0005), with symptom onset at 27, 31, and 22 years for DM1, DM2, and FSHD. The total PFDI score was higher in DM1 (55.2) and DM2 (59.3) compared to FSHD (35.4) (p=0.002). People with DM scored higher on individual indexes of UDI (p=0.005) and POPDI (25, 22, and

12.5 for DM1, DM2, and FSHD, p=0.001), but not CRADI. Those with DM1 scored higher on FSFI (24) compared with DM2 (13) and FSHD (18) (p=0.02).
Conclusions: Patients with DM1 and DM2 report greater pelvic floor dysfunction than FSHD, primarily related to prolapse and urinary distress. This may be attributed to the preferentially affected muscles in each disease. Sexual well-being was lower in DM2 and FSHD (vs. DM1). Multivariate analysis of factors such as age, childbirth, and hormonal treatments is planned.
Practical Implications: These data will contribute to understanding the impact of these diseases on pelvic wellness and sexuality.

Young Investigator!
Poster # 66
Smartphone video-based functional assessments for myotonic dystrophy
Author: Scott Uhlrich Selim Gilon
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Objective: The lack of sensitive functional outcome measures for myotonic dystrophy (DM) hinders drug development. Existing timed functional tests (TFTs) lack sensitivity to changes in movement quality, and they focus on a single activity, making them unrepresentative of overall daily function. Multi-activity rating scales are more comprehensive, but they are subjective and lack sensitivity to short-term changes. We aim to create more sensitive and comprehensive functional outcome measures by using smartphone video-based biomechanical analysis. Using our two-camera OpenCap algorithm [1], we have created movement biomarkers that can reproduce TFTs and are equally repeatable but can better distinguish individuals with DM from those with facioscapulohumeral dystrophy [2]. The objective of this study is to enable promising movement biomarkers like this to be measurable using one instead of two smartphones.
Methodology: We created OpenCap Monocular, software to compute human motion and musculoskeletal forces from a single smartphone video. We use a computer vision model that estimates a human mesh from video [3], followed by a two-stage optimization, where we refine the human pose and global position. Finally, we use musculoskeletal modeling and simulation [1,4,5] to estimate joint angles and musculoskeletal forces from the human mesh. Here we validate against lab-based motion capture for walking and squatting for one individual.
Results: For walking and squatting, OpenCap Monocular had mean absolute differences from marker-based motion capture and force plates of 6.6° for kinematics, and 7.7%bodyweight for ground reaction forces.
Conclusions: These errors are slightly greater than the two-camera OpenCap solution (5.2° error) that we have previously used to study DM [1]. Practical Implications Video-based functional assessments have the potential to be more sensitive to natural disease progression and intervention effects, accelerating drug discovery. Our method for quantifying motion and musculoskeletal forces from a single camera could enable regular in-home functional assessments and decentralized clinical trials.
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Poster # 74

Theta Oscillations as a Biomarker of Cognitive Dysfunction in Myotonic Dystrophy Type 1: Insights from Neuropsychological and Neuroimaging Correlates

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Objective: This study investigates the role of theta oscillations as a potential biomarker of cognitive dysfunction in Myotonic Dystrophy Type 1 (DM1). By integrating neuropsychological assessments, multimodal neuroimaging, and polysomnography (PSG), this study explores the associations between theta-related cognitive processes, white matter integrity, and sleep disturbances, aiming to improve biomarker discovery and therapeutic strategies.

Methodology: A cross-sectional study was conducted on a cohort of genetically confirmed DM1 patients and age-matched healthy controls. Participants underwent comprehensive neuropsychological testing, with a focus on theta-related cognitive tasks such as verbal and visual working memory. Structural neuroimaging data were collected, including Diffusion Tensor Imaging (DTI) and diffusion-derived T1-contrast imaging. DTI-derived features were analyzed in key white matter tracts associated with cognitive processing, including the cingulum, corpus callosum, and superior longitudinal fasciculus. Additionally, PSG-derived sleep metrics (e.g., sleep architecture, slow-wave sleep, REM sleep) were analyzed to investigate the relationship between sleep disturbances, theta-mediated cognitive function, and white matter microstructure.

Results: DM1 patients exhibited significant impairments in executive function, working memory, and cognitive flexibility, particularly in tasks reliant on theta-driven processing. PSG findings demonstrated reduced sleep efficiency, prolonged REM latency, and altered slow-wave activity, which were associated with poorer neuropsychological performance.

Conclusions: These findings suggest that theta-related cognitive impairments in DM1 are strongly associated with disruptions in white matter integrity and sleep disturbances. The strong correlations between theta-mediated cognitive tasks, white matter integrity, and sleep disturbances highlight the potential of theta activity as a biomarker for CNS involvement in DM1.

Practical Implications: Theta-mediated cognitive dysfunction could serve as a non-invasive biomarker for monitoring disease progression and assessing intervention efficacy in DM1. The integration of neuropsychological testing, neuroimaging, and sleep metrics provides a comprehensive framework for tracking cognitive decline and informing patient-specific treatment strategies. Future research should explore longitudinal changes in theta-associated cognitive performance and the potential role of sleep-based interventions to mitigate cognitive decline in DM1.

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Young Investigator!

Poster # 78

The Role of Hsp90 Pathway in Rescuing Mis-Splicing in Myotonic Dystrophy

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Myotonic dystrophy (DM), the most common form of adult-onset muscular dystrophy, is caused by expanded CUG (type 1, DM1) or CCUG (type 2, DM2) repeat RNAs. These abnormal expansion RNAs trap the Muscleblind (MBNL) family of RNA-binding proteins, leading to dysregulation of alternative splicing and numerous downstream health issues. Given that multiple specific splicing events are directly connected to disease symptoms, correcting this faulty splicing, or spliceopathy, presents a promising treatment strategy for DM. To discover new therapeutic compounds, I developed a medium-throughput screening system based on monitoring splicing changes in patient-derived fibroblasts from DM1 patients. Using this screening system, I tested ~1600 compounds and identified macbecin, a small molecule that inhibits heat shock protein 90 (HSP90), as a potential corrective agent for DM-related splicing errors. HSP90 inhibitors are currently being tested in cancer trials for their role in protein chaperoning, but recent studies indicate that they can also influence RNA splicing. Our preliminary results show that both macbecin, and another structurally distinct HSP90 inhibitor, improve splicing in DM1 and DM2 patient-derived cell lines. My central hypothesis for this proposal is that the HSP90 pathway plays a role in the DM spliceopathy, such that modulating this pathway may have therapeutic potential for the treatment of DM. Herein I will focus on characterizing the role of HSP90 in DM-related splicing issues and examine how targeting HSP90 could correct various aspects of the disease. This work will entail determining how a panel of chemically distinct HSP90 inhibitors and activators affect the spliceopathy in DM1 patient-derived cells. Next, I will test splicing rescue, bioavailability and safety of the HSP90 inhibitor macbecin and other leads HSP90 modulators in a DM1 mouse model to determine therapeutic potential. Lastly to address the limited therapeutic focus on DM2, I will characterize the potential of the HSP90 pathway to address DM2 spliceopathy using DM2 patient-derived cells. Taken together these findings will enhance our understanding of the pathogenesis of DM, the potential role of HSP90 in disease biology, and bring to focus a potentially new promising target for therapeutic treatments for both myotonic dystrophy type 1 and type 2.

Young Investigator!

Poster # 105

The effects of repeat expanded CUG RNA in the esophagus of a DM1 mouse model

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Objective: Myotonic dystrophy type 1 (DM1) is neuromuscular disease with multisystemic symptoms affecting muscle, heart, brain, and the gastrointestinal (GI) tract1. GI related symptoms in DM1 impact the entire GI tract2, yet little is known about the mechanisms of GI dysfunction. DM1 is caused by expanded CUG repeat RNA (CUGexp) in DMPK transcripts that sequester muscleblind-like (MBNL) proteins which regulate multiple facets of mRNA processing including alternative splicing3. MBNL sequestration causes changes in alternative splicing which have been linked to DM1 symptoms. Our aim

is to understand how CUGexp RNA expression affects the GI tract; the specific focus of this project is the impact of CUGexp RNA on the esophagus.
Methodology: We used the inducible bitransgenic mouse model (CUG960) homozygous for the tetracycline-inducible TREDT960I transgene containing human DMPK exon 15 with 960 interrupted CTG repeats and hemizygous for the muscle specific HSA-rtTA transgene. Seven-week-old CUG960 mice were fed doxycycline chow for 32 days to induce CUGexp RNA in muscle. We dissected esophagus from induced CUG960 and control mice and cut it into four sections along proximal to distal axis. RT-qPCR and PCR on RNA isolated from all sections was used to assess CUGexp RNA expression and alternative splicing changes observed in DM1.
Results: We found CUGexp RNA expression and dramatic changes in alternative splicing throughout the esophagus. The levels of CUGexp RNA and extent of splicing changes were the same in all sections of the esophagus.
Conclusions: Changes in alternative splicing could be a driver of GI symptoms in the esophagus. We will next test if CUGexp RNA can cause esophageal phenotypes and identify which splicing changes contribute to GI symptoms.
Practical implications: Analysis of esophageal tissue in the CUG960 mouse model can reveal pathways affected by CUGexp RNA in the esophagus and potential therapeutic targets to alleviate GI symptoms in DM1 patients.

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Poster # 119

Reversal of miRNA expression and disease hallmarks in myotonic dystrophy by a small-molecule inhibitor

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Sequestration of MBNL1 by expanded CUG or CCUG repeats causes myotonic dystrophy (DM) [1-3]. Besides its activity as a splicing factor, MBNL1 binds to a UGC motif located within the terminal loop region of pre-miR-1 and prevents access to the RNA from LIN28, an RNA binding protein which mediates uridylation and subsequent degradation of miRNA precursors by TUT4 [4]. Several groups are investigating peptides or small molecules as possible inhibitors of the MBNL1.CUGexp interaction as potential therapeutic approaches for treatment of DM [5-6]. However, targeting of MBNL1 might also interfere with non-pathological MBNL1-RNA interactions. Therefore, we are investigating an alternative high-risk approach by inhibition of LIN28. Cardiac defects and myotonic dystrophies in DM patients are directly associated with dysregulation of a small number of ion channels that are targets of miRNAs, e.g. miR-1, miR-9, miR-30, miR-107 and miR-181. We have shown that precursors of these miRNAs bind to MBNL1 and LIN28 in in vitro assays and furthermore are lowly expressed in myotubes from DM1 patients compared to those from healthy volunteers. Two prominent ion channels in cardiac defects are CACNA1C and KCNJ2 [4, 7], both of which have conserved predicted binding sites for some of these miRNAs in their 3'UTRs. Treatment of cells with a small molecule NTPA, increased levels of several of these miRNAs in DM1 myotubes, and reduced levels of KCNJ2 and other ion channels, possibly via altered biogenesis of miR-1 and miR-9, both of which are predicted to regulate KCNJ2. We are currently testing other small molecule as a RNA therapeutics strategy towards pathophysiology of myotonic dystrophies or cardiac defects.

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Poster # 130

The REACT Project: Building Real World Evidence (RWE) based Machine Learning models for Selection of Endpoints with Impact on Quality of Life (QoL) for SAR446268 program in Non-congenital DM1 patients

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Objective: To utilize real-world evidence (RWE) to support the Sanofi Gene Therapy Program for Non-congenital DM1 patients (SAR446268)

Methodology: The RWE Endpoints Advancement for Clinical Trials (REACT) project was established for identifying candidate endpoints (CE) that correlate with relevant outcomes in DM1 patients. Data collected from the DM1 natural progression study (END-DM1, NCT03981575) was used for generating patient-level machine learning (ML) predictive models of long-term outcomes to determine relationships of CEs with QoL/ patient-reported outcome measures (PROM) according to the following steps: 1) PROs and CEs were prioritized for consideration in the ML approach based on registry availability, data quality checks, and the complementary shortlist from Sanofi Clinical team for preferred CEs based on clinical rationale. 2) Potential confounders were defined based on data availability, quality and predictive power per confounder, including demographics , medications and assistive devices. 3) Machine learning models (e.g., Random Forest, XGBoost, GAM) were trained to predict PROMs based on data sets containing values for one CE as well as all selected baseline confounders. Model classes were selected based on their explainability and predictive accuracy. Two different analyses predicting PROMs were performed, a cross-sectional, and a delta analysis. Model performance was evaluated by scoring correlation of predicted vs actual PROM values and correlation metrics, e.g. R2 or Spearman's correlation.

Practical Implications: The REACT project allowed for generation of RWE for the identification of CE with the strongest association with QoL measures for SAR446268, a single dose novel AAV-based muscle-targeting gene transfer therapy that aims to deliver persistent DMPK mRNA downregulation and restore normal splicing function in the transduced cells.

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Young Investigator!

Poster # 168

Assessing Disease Severity in Myotonic Dystrophy: Insights from Video Hand Opening Time, Grip Strength and Timed Function Tests

Authors: Sarah Ismail, Lin Karman, Constance de Monts, Shelby Vogt-Domke, Sanchalee Khonde, Nóirín Ní Ghiollagáin, Paxton Ataide, Melissa McIntyre, Whitney Tang, Tina Duong
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Introduction: Understanding functional impairments in Myotonic Dystrophy (DM) is essential for assessing disease health outcomes. Clinical endpoints for DM include Video Hand Opening Time (VHOT), grip strength and timed function tests (TFTs). This pilot study evaluates the association between disease severity, myotonia, and functional performance in DM patients.
Methods: Data were collected at Myotonic Dystrophy Foundation events in 2022 and 2023. Participants underwent standardized assessments, including VHOT, three grip strength trials per hand using the Microfet dynamometer, and TFTs including Timed Up and Go (TUG), 10-Metre Walk/Run Test (10MWRT) and Five-Time Sit to Stand (5xSTS). VHOT scores were independently rated by five blinded over-raters to ensure reliability. Peak values were analyzed to capture maximal performance, and intraclass correlations assessed the relationships among VHOT, grip measures, and TFTs.
Results: Twenty-nine ambulatory DM participants aged 14-60 years, with a median (sd) of 41 (10.25) years and 550 (326.27) CTG repeats, completed standardized assessments. Significant correlations included maximum left-hand grip strength with 10MWT (p=0.02) and between left and right hand grip strength (p=0.004). Left hand grip strength showed non-significant correlations with TUG (p=0.17), 10MRT (p=0.09), 5XSTS (p=0.08), and left-handed VHOT (p=0.97), as did right hand grip strength with all TFTs and right-handed VHOT (p>0.05). Non-significant correlations were found between both right and left-handed VHOT to all individual TFTs (p>0.05).
Conclusions: These findings suggest that grip strength may serve as a useful complementary measure to functional assessments in DM. Further investigation is needed to determine whether muscle weakness asymmetry is characteristic of DM or reflects individual variability. Larger, longitudinal studies are needed to understand the sensitivity of these measures and their implications for individualized clinical management and outcome measure refinement.

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Young Investigator!

Poster #188

RNA quality control pathway of mRNAs with exposed GC-rich sequences

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Objective: Spatially organized messenger RNAs (mRNAs) influence protein distribution within the cytoplasm, shaping cellular morphology and determining cell lineages across metazoans1,2. However, dysregulation of this process leads to disease, including myotonic dystrophy 1 (DM1)3-6. In DM1, long stretches of exposed CUG repeats located within the Myotonic dystrophy protein kinase (DMPK) gene

promote the formation of toxic nuclear RNA foci. These repeats base pair with each other within the foci, sequestering MDPK mRNAs in the nucleus and preventing their release into the cytosol3-8.
Methodology: We use genetic analysis, quantitative microscopy, RNA structure mapping and in vitro RNA phase separation and dimerization assays to investigate how intermolecular RNA-RNA interactions drive the formation of RNA foci in Drosophila and human cell lines.
Results: Introducing four exposed GC-rich sequences into an essential Drosophila mRNA promotes its intermolecular base pairing in vitro and in vivo9. These sequences also trigger the formation of RNA foci in the nucleus at the site of gene transcription, reduce cytosolic mRNA and protein levels and prevent fly development. Treatment with a transcriptional inhibitor revealed that mRNAs are retained within RNA foci rather than being released into the cytosol. These observations are specific to exposed GC-rich sequences, as sequences with mixed nucleotide composition do not elicit these phenotypes.
Conclusion: Our findings suggest the existence of an RNA quality control pathway that detects and retains mRNAs with exposed GC-rich sequences at their site of transcription and reduce their levels in the cytosol.
Practical Implication: As many repeat mRNAs contain GC-rich regions3-6, they may be natural targets for the RNA quality control we identified. Given the limited understanding of the regulation of repeat mRNAs, our research uncovers a potential RNA quality control mechanism that may regulate repeat mRNAs, offering new insights into DM1 pathology.

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Poster #227

Nonclinical Data for PGN-EDODM1 Demonstrated Mechanistic and Meaningful Activity and Safety for the Potential Treatment of DM1

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Objective: PepGen's novel enhanced delivery oligonucleotide (EDO) cell-penetrating peptide technology is engineered to optimize tissue delivery and nuclear uptake of therapeutic oligonucleotides. Investigational drug PGN-EDODM1 comprises an EDO peptide conjugated to a phosphorodiamidate morpholino oligonucleotide (PMO) and is being evaluated for the treatment of myotonic dystrophy type 1 (DM1). PGN-EDODM1 binds to the pathogenic trinucleotide CUG repeat expansion in DMPK mRNA, thereby liberating MBNL1 protein through steric blocking without degrading DMPK transcripts. Liberation of sequestered MBNL1 is hypothesized to restore splicing profiles of multiple downstream transcripts; a central cause of DM1 pathology.
Methodology: Cellular and animal evaluations of PGN-EDODM1 activity and safety.

Results: Treatment of DM1 patient muscle cells with PGN-EDODM1 resulted in high levels of delivery to the nucleus when compared to an unconjugated oligonucleotide. Treated DM1 patient muscle cells demonstrated a reduction of pathogenic myonuclear foci, liberation of MBNL1 from foci, and correction of mis-splicing without DMPK RNA degradation. A single dose of PGN-EDODM1 to HSALR mice resulted in correction of mis-splicing and improvement in myotonia. Following repeat dosing of PGN-EDODM1, near complete resolution of DM1 pathology, including correction of mis-splicing and resolution of myotonia, was observed. Toxicology studies in non-human primates indicate repeat dosing with PGN-EDODM1 was generally well tolerated. No persistent elevation of kidney biomarkers was observed at doses through 60 mg/kg. Additionally, there were no adverse findings in the kidney after 4 monthly doses of 60 mg/kg, nor notable hematologic or hepatic or cardiovascular effects.
Conclusions/Practical Implications: There are no approved therapies for DM1. Nonclinical pharmacology which addresses the central cause of the condition and safety studies with PGN-EDODM1, showed meaningful therapeutic potential on splicing correction and myotonia correction. Combined, these studies support the ongoing Phase 1 single ascending dose study FREEDOM-DM1 and Phase 2 multiple ascending dose study FREEDOM2-DM1 in adults with DM1.

Young Investigator!
Poster # 257
Multisystem involvement and disability status in adult-onset myotonic dystrophy type 1: A Chinese single-centered study
Authors: Wenhan Zhang, Huahua Zhong, Sushan Luo, Chongbo Zhao
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Background: Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy characterized by progressive proximal muscle weakness and multisystem involvement, leading to early disability and increased mortality. Currently the clinical spectrum and disability-causing events have not been well documented in Chinese patients with DM1.
Methods: A total of 71 adult-onset patients with genetically confirmed DM1 were enrolled through questionnaire in this single-centered study by Huashan Hospital Fudan University. The baseline clinical information, DMPK CTG repeats, Epworth Sleepiness Scale and Fatigue Severity Scale, and the disability-causing events, including surgical intervention due to cataracts and cardiac abnormalities, use of the nasogastric tube, mechanical ventilation, accessory demand for walking and the use of wheelchair, were retrospectively collected and analyzed.
Results: This cohort is comprised of 41 male and 30 female patients with an average diagnostic age of 40 years (17~67) and an onset age of 28 years (5~59). The CTG repeats in the DMPK gene is 591 (95~1218). The most common symptom was fatigue (88%), followed by myotonia (64%) and daytime sleepiness (64%). In addition, 91% of the cohort reported multisystemic involvement such as gastrointestinal distress (52%), slurred speech (54%) and dysphagia (46%). For the cardiac involvement, premature beats (84%), and conduction block (26%) were the most prevalent presentations in Holter monitoring. The CTG repeats in the DMPK gene exhibited a significant negative correlation with respiratory impairment, including FVC ($P < 0.05$) and FEV1.0 ($P < 0.05$). Disability-causing events are present in 31% of the DM1 cohort. Among them, 45% of patients were wheelchair-bound, while 36% required accessories for walking other than wheelchairs. Further, 36% of patients had a history of cataract surgery, 18% required mechanical ventilation, and 14% had cardiac surgery. The concurrence of disability-causing events was significantly associated with age ($P < 0.05$) and the disease duration ($P < 0.05$).

Conclusion: Multisystem involvement is common in Chinese adult-onset DM1 patients. Disability-causing events are present in 31% of the cohort, which implies that future natural history studies are required to better delineate the disease trajectories of DM1.

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Poster # 310

Myotonic Dystrophy Family Registry. Relationship between DM type, disease severity, age of onset, and repeat length

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Introduction: Patient registries collect data on populations with specific diseases, offering crucial insights into disease progression, outcomes, and research opportunities. This is especially vital for rare diseases like myotonic dystrophy (DM), which is characterized by diverse and geographically dispersed populations and highly variable clinical presentations. The Myotonic Dystrophy Family Registry (MDFR) is an online, patient-reported database aimed at understanding the impact and scope of DM, with potential to guide clinical trial designs.

Objectives: This study aimed to analyze registry participation, describe participant demographics, and explore relationships between disease type, severity, age of onset, and repeat length.

Methods: Data from registry entries between 2013 and July 2024 were analyzed. Two subsets were created: Subset 1 included participants with a correctly reported diagnosis and matching age of onset; Subset 2 included those with a correct diagnosis, matching age of onset, and diagnostic test results with repeat length information.

Results: 1680 respondents (61% of total) met Subset 1 criteria, while 645 (23.5%) met Subset 2. In Subset 1, disease subtypes were 12.4% CDM, 19.6% JOA, 48.2% DM1, and 19.8% DM2. Subset 2 comprised 21.5% CDM, 5.1% JOA, 64.8% DM1, and 8.6% DM2. The analysis revealed significant disease burden with age, including difficulty walking, myotonia, daytime sleepiness, pain, and fatigue in both DM1 and DM2. Additionally, Subset 2 showed correlations between repeat length, age of onset, and symptom severity.

Conclusions: Despite concerns about self-reported data quality, well-curated registry data can reveal significant relationships between DM type, severity, age of onset, and repeat length. The findings highlight the substantial disease burden on a large proportion of DM patients.

Young Investigator!

Poster # 355

Investigating the molecular mechanisms of liver dysfunctions in myotonic dystrophy

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Objective: Myotonic Dystrophy Type 1 (DM1) is the most common form of adult-onset muscular dystrophy in the United States. Studies indicate that the liver is negatively affected by DM1; patients have abnormally high sensitivity to anesthesia and muscle relaxants, as well as an increased risk of metabolic dysfunction-associated steatotic liver disease (MASLD). These data suggest a disruption of normal liver function and predisposition to liver damage in DM1; however, the underlying pathological mechanisms have not been thoroughly investigated. We have recently generated a liver-specific DM1 mouse model that reproduces the molecular and pathological features of the disease, including

susceptibility to steatotic liver disease and impaired metabolism of analgesics and muscle relaxants. Our goal is to elucidate the precise mechanisms driving DM1-associated liver pathology.
Methodology & Expected Results: We will study the interplay of MBNL proteins in DM1 liver pathogenesis. Notably, Mbnl1 knockout (KO) mice do not phenocopy the liver symptoms in DM1 liver model, possibly due to compensatory upregulation of MBNL2. To investigate this, we generated a mouse model with compound loss of MBNL1 and MBNL2 in the liver. By enhancing our understanding of MBNL protein targets in the liver, as well as of pathways that are specifically affected in the compound depletion model, we aim to uncover new therapeutic targets for DM1 liver. Additionally, we will examine the mechanisms of lipid accumulation and altered drug metabolism in DM1 liver using hepatocyte cell lines that express toxic RNA repeats.
Practical Implications: As the liver is the primary organ for drug metabolism, an accurate understanding of liver dysfunction is critical for therapeutic development in DM1. Our research will help identify molecular targets to reverse the DM1 liver symptoms and avoid potential toxicity of DM1 treatments in the liver, ultimately advancing safer and more effective therapeutic strategies.

Young Investigator!
Poster # 377
Establishing Consensus on Key Symptoms for Dysphagia Assessment in Myotonic Dystrophy Type 1: A Delphi Study
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Objective: This study aimed to establish expert consensus on the key symptoms essential for dysphagia assessment in adults with DM1 as part of a broader initiative to select and adapt a patient-reported outcome measure (PROM).
Methodology: A modified Delphi survey was conducted, starting with a symptom list derived from a literature review. Forty-one experts, including healthcare clinicians and researchers experienced in DM1, rated each symptom based on its 1) relevance to DM1, 2) potential to change, and 3) extent to which patients or capable of assessing this symptom, as used by Murphy et al. (2016). In each round, symptoms meeting or failing the 60% agreement threshold were accepted or excluded, while those without consensus proceeded to the next round for further evaluation.
Results: While some symptoms were consistently recognized across professions, others exhibited considerable variability, suggesting that professional perspectives can influence symptom assessment. This variability was accounted for in the analysis by considering each profession separately and adjusting the threshold based on the number of groups endorsing each symptom. The final set of symptoms encompassed oral and pharyngeal function, highlighting key clinical markers for dysphagia assessment in DM1.
Conclusions: This study identified expert-validated symptoms critical for dysphagia assessment in DM1. These findings will support the selection and adaptation of a PROM tailored to this population.
Practical Implications: The validated symptom set will serve as the foundation for the next step of the project, involving qualitative interviews with patients and family members to explore how these symptoms manifest in daily life. This step will ensure that the PROM reflects both patient experiences and clinical priorities.
Murphy M, Hollinghurst S, Salisbury C. Agreeing the content of a patient-reported outcome measure for primary care: a Delphi consensus study. Health Expect. 2017 Apr;20(2):335-348. doi: 10.1111/hex.12462. Epub 2016 Apr 28. PMID: 27123987; PMCID: PMC5354062.

Myotonic Dystrophy FOUNDATION

Young Investigator!

Poster # 388

MBNL Multivalency Drives RNA Foci Formation & Tethers RNAs to Foci

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MBNL proteins are a key driver of RNA foci formation in DM1, however, the mechanisms underpinning this phenomenon are poorly understood. MBNL proteins have two pairs of zinc fingers (ZnFs) capable of binding RNAs independently and a C-terminal homodimerization domain. We hypothesize that MBNL drives foci formation by crosslinking RNAs through independent binding by each ZnF pair of a single MBNL molecule, or by homodimerization of two MBNL molecules. We took MBNL proteins with ZnF mutations that abolish RNA binding and/or removed the C-terminal domain and introduced them into mouse embryonic fibroblasts expressing 480 CUG repeats and lacking endogenous MBNL1/2. RNA FISH was used to quantitate the number and size of RNA foci. To assess the possibility that other MBNL target RNAs are tethered to RNA foci through this crosslinking mechanism, we employed O-MAP proximity labeling to identify RNAs near the expanded DMPK mRNA in primary DM1 myoblasts, frontal cortex, heart tissue, and skeletal muscle. We observed a significant decrease in RNA foci in cells expressing ZnF mutants and truncated MBNL proteins and noted that ZnF mutants only exhibited robust RNA foci formation in cells expressing the mutant protein at high levels with the C-terminal domain included. O-MAP identified RNAs enriched at foci, and we confirmed this localization for several key targets using multiplexed RNA FISH against the foci and tethered RNA. These results suggest that MBNL drives RNA foci formation in DM1 through two distinct RNA crosslinking mechanisms: independent binding by each ZnF pair and homodimerization of two MBNL molecules. This model raises the possibility that other MBNL target RNAs may be tethered to RNA foci impacting their downstream function. O-MAP and RNA FISH confirmed enrichment of certain RNAs at RNA foci, and work is ongoing to determine how this may contribute to DM1 pathology.

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Young Investigator!

Poster # 421

Systems level transcriptomics in DM-model zebrafish at single-cell resolution

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Myotonic dystrophy is a complex multisystemic disorder that disrupts gene expression across many organs. While the genetic basis of DM1 has been well described, determining the effects of CUG repeat expression and MBNL depletion on gene expression and cell fate during development requires system-level analysis. Our goal is to identify changes in transcriptomic profile in DM1 at multiple levels: organs, cell-types and gene-regulatory networks. We integrate this information to make connections between diseased cell-types and symptoms. Identifying disease-specific cell-types, and their molecular identity, is an essential guide for targeted therapeutic delivery. To this end, we recently generated organism-scale single-cell RNA sequencing (scRNA-seq) datasets using DM1-model zebrafish to identify transcriptionally unique cell types and disease-specific gene expression patterns during organogenesis. Our ongoing work tests the hypothesis that gut disorders present in both DM1 patients and DM1-model zebrafish are

linked to disruptions in circadian gene expression and sleep patterns. We have found differential gene expression in the circadian genes including, dbpa and per1b, and cell-type specific changes in gene expression within blood, liver, immune and neural cells. We will also discuss our recent transgenic approach to creating new zebrafish models of DM1.

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Young Investigator!

Poster # 433

Development of a Patient-Reported Outcome Measure to Assess Fatigue, Sleepiness, and Apathy in Myotonic Dystrophy Type 1

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Objective: This study fills a critical gap by developing a patient-reported outcome (PRO) to assess fatigue, sleepiness, and apathy in DM1, key symptoms affecting daily life.

Method: A mixed-methods approach was used. A scoping review identified existing scales, generating potential items. Interviews with patients and caregivers provided additional insights, followed by an expert focus group for clinical relevance. An online survey with numerous items will be completed by 250 patients. Rasch measurement theory will refine item selection, ensuring strong psychometric properties.

Results: Items were identified by screening 15 apathy, 52 fatigue, and 25 sleepiness scales, plus 50 with sleepiness as a subscale. Patients highlighted adaptive strategies that could mask symptoms. Divergent views emerged between patients and experts, particularly regarding naps as a fatigue-coping strategy vs. a sleepiness symptom. Rasch analysis will optimize item fit, response scale functionality, and measurement precision. The final PRO will comprehensively assess DM1-related fatigue, sleepiness, and apathy.

Conclusions: By incorporating patient inputs and expert validation, this tool will ensure a more accurate and meaningful evaluation of behavioral symptoms. Its strong psychometric properties will support clinical care and research, improving patient management and advancing DM1 therapies.

Practical Implications: This PRO will aid clinicians and researchers in monitoring symptoms and evaluating treatment efficacy, strengthening both clinical practice and trial readiness.

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Young Investigator!

Poster # 490

MBNL overexpression rescues cardiac phenotypes in a myotonic dystrophy type 1 heart mouse model

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Objective: Over half of individuals affected by DM1 have cardiac involvement including conduction defects and arrhythmias, which can lead to sudden cardiac death, the second leading cause of death in DM1. The expanded CUG repeat (CUGexp) RNA transcribed from the DMPK mutant allele sequesters the muscleblind-like (MBNL) family of RNA-binding proteins, causing their loss of function and disrupting regulated pre-mRNA processing, which has been shown to cause the DM1 skeletal muscle pathology. However, their contribution to the manifestation of DM1 cardiac abnormalities remains unclear.
Methodology: We used a DM1 heart mouse model (CUG960) that inducibly expresses CUGexp RNA to test the contribution of MBNL loss to DM1 cardiac abnormalities and explore MBNL restoration as a potential therapy through AAV9-mediated heart-specific expression of MBNL1 and/or MBNL2 proteins. The degree of rescue by MBNL was compared side by side to the maximum level of rescue observed by turning off the transgene.
Results: Exogenous MBNL1 and MBNL2 proteins were overexpressed throughout ventricles and atria of CUG960 +dox mice and colocalized with CUGexp RNA foci. Both MBNL proteins significantly rescued DM1 physiological and structural abnormalities induced by CUGexp RNA including conduction delays, reduced cardiac contractility and cardiac hypertrophy. RNA-seq analysis revealed rescue of mis-regulated alternative splicing and differential gene expression events, especially those linked to disease-associated cardiac function and morphology. While robust, the rescue was partial compared to reduced CUGexp RNA and plateaued with increased exogenous MBNL expression.
Conclusions: Overall, the heart-specific overexpression of MBNL proteins in CUG960 +dox mice significantly but partially rescued multiple physiological and molecular aspects of cardiac phenotypes caused by CUGexp RNA expression.
Practical Implications: These findings demonstrate that MBNL loss is a major contributor to DM1 cardiac manifestations and are worth pursuing for therapeutic interventions, but also suggest that additional mechanisms participate in cardiac pathogenesis induced by CUGexp RNA.

Rao, A.N., Campbell, H.W., Guan, X, Word, T.A., Wehrens, X.H.T, Xia, Z. and Cooper, T.A. (2021) Reversible cardiac disease features in an inducible CUG-repeat RNA expressing mouse model of myotonic dystrophy. J. Clin. Invest. Insight. 8, 143465.

Young Investigator!

Poster # 507

Development of small molecule drugs: potential therapies for DM1 and DM2

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Background: Myotonic dystrophy (DM) currently lacks approved treatments that target the expanded CTG/CCTG DNA and CUG/CCUG RNA repeats that cause DM, type 1 (DM1) and type 2 (DM2). We have identified a class of small molecules, modified polycyclic compounds or MPCs, that interact with the repeats and have shown that several MPCs rescue splicing in DM1 and DM2 patient derived cell models.
Objective: The aim of the project is to design and synthesize a library of 2nd generation MPCs to better understand the structure-activity relationship (SAR) of these molecules. MPCs with improved drug-like properties that rescue splicing in the low nanomolar concentration in DM1 and DM2 patient derived cell models will be moved forward and tested in DM mouse models for splicing rescue. Pharmacokinetic and pharmacodynamic (PK/PD) measurements will also be performed and incorporated in an iterative design process.
Methodology: Synthesis of MPC compounds will probe the importance of shape and chemical properties for splicing rescue in DM1 and DM2 cells. Splicing rescue was studied via RT-PCR with RNA-seq used to broadly investigate splicing, gene expression rescue and off-target effects. Biophysical assays will measure binding affinity to repeats and computational modeling to understand binding of MPCs to the repeats and inform design of new MPC compounds.
Results: Our data show in multiple DM1 cell models and a DM2 myoblast cell model that MPCs rescue splicing at nanomolar concentrations with little to no toxicity and modest off-target effects.
Conclusions: MPCs are a promising new class of small molecules that have long-term potential to be developed into drugs that can reach all tissues in DM patients.
Practical implications: Although there are many promising approaches in development and in the clinical trials for DM1, there is a need for compounds that target both DM1 and DM2 and can be delivered systemically.

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Young Investigator!

Poster # 522

Bottlebrush polymer-antisense oligonucleotide conjugate therapeutics for myotonic dystrophy type 1

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Objective: Myotonic dystrophy type 1 (DM1) is the most common adult-onset muscular dystrophy, yet no curative drug exists. Poor biodistribution and cellular uptake have hindered the development of antisense therapeutics targeting pathogenic repeat RNA (CUGexp). Conjugation of oligonucleotides to high molecular weight (~300 kDa) polyethylene glycol-based brush polymers ("pacDNA") has been shown to limit oligo-protein interactions, enhance pharmacokinetics, lower renal/hepatic clearance, and boost tissue accumulation ~100-fold without affecting hybridization. The pacDNA also promotes cell uptake via membrane adsorption and macropinocytosis.
Methodology: HSALR DM1 mice were injected by tail vein with low nanomole doses of a pacDNA antisense oligonucleotide steric blocker of CUGexp RNA.
Results: Two weeks post-injection, mice displayed up to ~67% correction of DM1-associated mis-splicing and reduced levels of CUGexp RNA. Repeated injections improved myotonia, body weight, and grip strength while restoring a large fraction of DM1-related gene dysregulation, including genes involved in muscle function, cytoskeleton organization, and energy storage and metabolism. Unlike linear PEG conjugates, pacDNA avoids triggering adaptive immunity and maintains safe cytokine and blood marker levels with repeated dosing.
Conclusions: These bottlebrush polymer bioconjugates may bridge the gaps impeding traditional antisense drugs, representing a potentially more potent, durable, and cost-efficient DM1 therapy.

Young Investigator!

Poster # 526

Impact of long-term CUGexp RNA expression on skeletal muscle pathogenesis and rescue

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Objective: Progressive skeletal muscle disease in Myotonic Dystrophy Type I (DM1), drives worsening weakness and atrophy, reducing strength, mobility, and increasing mortality1,2. I will evaluate the impact of long-term expression of expanded CUG repeat (CUGexp) RNA on skeletal muscle disease progression. Additionally, I will assess muscle tissue recovery following the removal of toxic CUGexp RNA after prolonged exposure.
Methodology: The study will use CUG960 mice3 homozygous for a stable 960 CTG repeat transgene (TREDT960I) and hemizygous for the HSArtTA transgene, which drives skeletal muscle-specific rtTA and doxycycline (dox) inducible CUGexp RNA expression. CUGexp RNA will be induced at 7 weeks of age for 2, 6, 12, and 20 months for prospective longitudinal skeletal muscle phenotyping. To assess phenotype rescue, dox will be withdrawn followed by subsequent assessments 2 months later (at 4, 8, 14, and 22 months). Extensive skeletal muscle phenotyping will include in vivo muscle function analysis, assessment of muscle wasting, myotonia, and histology. Ex vivo analyses will focus on fatigue, force-frequency characteristics, and calcium handling. At the molecular level, alterations in MBNL and CELF1 levels, RNA foci formation with MBNL colocalization, and alternative splicing defects will be assessed.
Results: Our preliminary data show that CUG960 mice exhibit muscle wasting and a strong reversal to fetal splicing patterns in the quadriceps muscle. They also display myotonia and reduced ex vivo force generation in both fast- and slow-twitch muscles.

Conclusions: This study will track DM1 disease progression independent of somatic mosaicism and, through phenotype rescue after varying durations of CUGexp RNA expression, pinpoint critical windows for disease reversibility and optimal intervention times in an age-dependent manner.
Practical Implications: This study will define the trajectory of muscle disease progression and its correlation with functional decline, informing therapeutic strategies by identifying critical intervention windows for reversing muscle dysfunction.

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Young Investigator!

Poster # 563

Timing is everything: Understanding hypersomnolence in myotonic dystrophy

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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 hypersomnolence or excessive daytime sleepiness and sleep dysregulation. As defects in the circadian system can contribute to these symptoms, we are investigating whether circadian rhythms are disrupted in animal and cell culture models of DM1 as well as in DM1 patients. Circadian activity analyses of a CTG250 expressing Drosophila model displays lengthening of the circadian period caused by perturbations to core circadian clock proteins. Interestingly, these effects are not mediated through loss of MUSCLEBLIND function. To determine whether expanded CTG repeat expression perturb mammalian circadian rhythms, we analyzed the behavior of the DmpkCTG480 knock-in mouse model. We find that these mice also display changes in the period of circadian activity. 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 protein-luciferase fusion reporters. To determine whether the circadian system is affected at cellular and physiological levels in DM1 patients, we are now examining the oscillations of clock proteins in human cells expressing expanded CTG repeats and of the urinary circadian biomarker, 6-sulfatoxymelatonin, respectively. Taken together, our data show that circadian rhythms are disrupted in DM1 models and future studies will provide key insights into how circadian disruption contributes to disease pathology in DM and possibly other repeat expansion diseases.

Poster # 564

Safety and Efficacy of DYNE-101 in Adults with DM1 in the Phase 1/2 ACHIEVE Trial

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Objective: DM1 is a rare, progressive disease in which dysregulated alternative splicing leads to multisystem clinical manifestations. Individuals with DM1 experience a wide range of symptoms related to skeletal and non-skeletal muscle involvement and CNS-related symptoms. DYNE-101 is an investigational therapeutic for the treatment of DM1.
Methodology: The safety and efficacy of DYNE-101 in adults with DM1 are being investigated in the Phase 1/2 ACHIEVE trial (NCT05481879). In the 24-week placebo-controlled multiple ascending dose portion of ACHIEVE, 56 participants received one of 5 dose/dose regimens of DYNE-101.
Results: At the dose selected for the registrational expansion cohort of ACHIEVE (6.8 mg/kg Q8W), DYNE-101 demonstrated robust total DMPK knockdown and splicing correction at 3 months. Correction in splicing was associated with early and robust functional benefit across multiple measures of muscle strength and function at 6 months, including video hand opening time, Quantitative Muscle Testing total score, 10-meter walk/run test, and 5 times sit-to-stand. Importantly, regression analyses show that CASI improvement at 3 months was well correlated with functional improvements at 6 months, suggesting that CASI is a reliable biomarker predictive of functional outcomes. Trends in improvement with 6.8 mg/kg Q8W DYNE-101 were also noted in the myotonic dystrophy health index (MDHI) total score, a patient reported outcome, including in the CNS-related subscales of fatigue, cognitive impairment, sleep, communication, emotional issues, and pain. DYNE-101 had a favorable safety profile as of the data cut date, with mostly mild/moderate TEAEs, and no serious related TEAEs.
Conclusions: Data from the ACHIEVE trial suggest that DYNE-101 has a favorable safety profile and results in improvement in molecular biomarkers, such as CASI, that may have the ability to predict clinical outcomes in DM1.
Practical Implications: In the absence of disease-modifying therapies, individuals with DM1 have a profound unmet medical need. DYNE-101 is being investigated as a potential treatment for DM1.

Poster # 579

Video-Based Biomechanical Analysis Captures Disease-Specific Movement Signatures of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy

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Objective: Assessing human movement and function is essential for diagnosing and monitoring movement-related conditions. Timed function tests (TFTs) are widely used in neuromuscular clinics to monitor disease progression because of their simplicity and efficiency. However, TFTs cannot capture disease-specific compensations which may be more sensitive to change by providing an individualized phenotypical signature of muscle weakness across heterogenous neuromuscular diseases. Advances in smartphone video-based biomechanical analysis, such as OpenCap, allow detailed movement quantification with the ease and speed required for clinical settings.
Methodology: To compare video-based analysis against TFTs, we collected data from 129 individuals: 58 with myotonic dystrophy (DM), 28 with facioscapulohumeral muscular dystrophy (FSHD), and 43 with

typical movement. Participants performed traditional TFTs such as: 10 meter run/walk, timed up and go, and 5 time-sit-to-stand, along with the Brooke Upper Extremity Scale, with data collection taking approximately 15 minutes each. Thirty-one of these participants completed repeat data collection.
Results: Video metrics reproduced all 4 clinician reported TFTs ($r > 0.98$) and outperformed TFTs at disease classification ($p < 0.001$). Video-based analysis identified differences between disease populations in arm range of motion ($p=0.027$), stride length ($p=0.003$), and ankle height ($p=0.037$). Our analyses also reproduced Brooke upper extremity scores (UE) ($r=0.795$), and identified disease specific differences among participants with the same Brooke score. The key features for differentiating FSHD from DM were upper-limb activities while lower-limb activities differentiated DM, aligning with the clinical presentation of FSHD and DM.
Conclusions/ Practical Implications: Video-based biomechanical analysis complement existing functional movement assessments and capture more sensitive, disease-specific variables from human movement. These findings underscore the value of incorporating OpenCap into clinical trials and practice to enhance precision, reduce variability and improve individualized clinical outcome measures.

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Poster # 587

Fatty Acid Mediated Oligonucleotide Uptake System (FAMOUS) enables antimiR-23b mediated functional and molecular rescue of cognitive dysfunction in DMSXL mice

Authors: Diego Piqueras-Losilla[1], Andrea Garcia Rey[1], Aline Huguet-Lachon[2], Isabel Campillo[1], Melanie Nufer[1], Mouli Chakraborty[1], Nuria Barquero[1], Geneviève Gourdon[2], Ruben Artero[3,4], Beatriz Llamusí[1], Estefanía Cerro-Herreros[1].
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Background: Myotonic dystrophy type 1 (DM1) is a severe neuromuscular disorder, with the congenital form (CDM1) being particularly aggressive. CDM1 affects the muscles and brain, leading to cognitive and intellectual deficits. It is characterized by large CTG repeat expansions in the DMPK gene, resulting in toxic RNA foci that sequester essential splicing regulators such as MBNL1/2. Previous studies identified a link between miR-23b and DM1 development, suggesting the therapeutic potential of miR-23b inhibition.
Objective/Methods: This study demonstrates fatty acid-conjugated AntimiR-23b delivery to the CNS and validates its effectiveness in treating CDM1 using DMSXL transgenic mice (>1000 CTG repeats). The delivery to brain tissues of AntimiR-23b administered intravenously was confirmed using fluorescence in situ hybridization imaging. Molecular efficacy was assessed by measuring MBNL1/2 protein levels (Western blot), hDMPK mRNA levels (RT-qPCR), and splicing correction (semiquantitative PCR) in various brain regions. Functional improvements were evaluated using an open-field test to monitor behavioral abnormalities.
Results: Systemic administration of AntimiR-23b reached mouse brain tissue, crossing the blood-brain barrier and remaining in the CNS. This treatment led to a significant increase in MBNL1/2 expression and a reduction of hDMPK mRNA in the brain, together with splicing correction across regions, including complete rescue of splicing defects in the temporal and frontal cortices. Functionally, behavioral defects in the open-field assay were completely rescued in treated mice.
Conclusion: AntimiR-23b effectively crosses the BBB and restores key molecular deficits in a large CTG repeat expansion mouse model, leading to improvements in both splicing regulation and behavioral outcomes. Our findings support the potential of systemic AntimiR-23b therapy as a promising approach for addressing cognitive impairments associated with CDM1, and confirms that, inhibition of miR-23b acts through a dual mechanism of action (MBNL upregulation and DMPK depletion) in the brain, similarly to what we have previously shown in DM1-related muscle dysfunction models.

Young Investigator!
Poster # 606
Treatment of a severe DM1 mouse model with verapamil, amlodipine, and ranolazine.
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Objective: Our lab showed the benefit of verapamil, amlodipine, or ranolazine in a bi-channelopathy mouse model for myotonic dystrophy type 1 (DM1). All three treatments improved reduced survival, reduced muscle strength, prolonged time of righting and myotonia, and severe transient weakness. We were then interested in testing if verapamil, amlodipine, or ranolazine treatment could improve survival and health in DM1 mouse models that exhibit the full panoply of altered splicing and myopathic features.
Method: We chose to treat the HSALR/Mbnl1-/- mouse model with verapamil, amlodipine, or ranolazine to see if there was similar benefit to what was seen in the bi-channelopathy mouse. The HSALR/Mbnl1-/- mouse combines CTG-repeat expansion with loss of MBNL1, which are both seen in DM1. We measured muscle force generation, respiratory function, body weight, time of righting reflex, and overall survival.
Results: Treatment with verapamil or amlodipine allowed us to investigate if blocking Ca2+ entry via CaV1.1 alone is able to increase lifespan and overall health in HSALR/Mbnl1-/- mice. Targeting myotonia with ranolazine allowed us to determine if reducing a dominant skeletal muscle feature improves survival and muscle function. We found that treating with verapamil, amlodipine, or ranolazine

improved survival, increase muscle strength, improved respiratory function, increased body weight, and reduced time or righting in the HSALR/Mbnl1-/- model.

Conclusion and Practical Implications: These results suggest that targeting CaV1.1 conduction with verapamil or amlodipine has therapeutic benefit, even in a complex model for DM1. Further, long term treatment with ranolazine improved survival and health in the HSALR/Mbnl1-/- mice. The next step will be to test if there is a synergistic impact of combining a calcium channel blocker with an anti-myotonic. Since these drugs are FDA approved, rapid translation to the clinic would be expected.

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Poster # 609

New insights into DM1 mechanism of pathogenesis: DMPK, MBNL and miR-23b balance as the master key of DM1 pathology

Authors: Andrea García-Rey[1], Diego Piqueras-Losilla[1], Argimiro Mayoral-Olmos[1], Ana Díaz-Maqueda[1], Nuria Barquero[1], Rubén Artero[2,3], Beatriz Llamusí[1], Estefanía Cerro-Herreros[1].

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Background: Myotonic dystrophy type 1 (DM1) is a severe genetic disorder caused by the expansion of CTG repeats in the *DMPK* gene. This expansion leads to the accumulation of toxic RNA in ribonuclear foci, where it sequesters the splicing regulator muscleblind-like (MBNL) producing missplicing. Beyond sequestration, downregulation of MBNL levels has been recently confirmed to contribute to the loss of function of MBNL producing the symptoms in DM1(Aoki et al. 2024). ATX-01 is a novel oligonucleotide therapeutic currently in clinical development for DM1. It inhibits *miR-23b*, a negative regulator of *MBNL1* translation, the expression of which also affects DMPK mRNA levels.

Objective/Methods: This study aims to provide a wider view on the DM1 pathogenetic mechanism by investigating how *miR-23b* downregulation contributes to the reduction of mutant *DMPK* transcripts. *In vitro* studies were conducted to assess *DMPK* transcription levels, transcript stability, and nuclear export following ATX-01 treatment. Additionally, ATX-01's effects were compared with a DMPK-targeting oligonucleotide in order to compare the potency of these two therapeutic approaches in DM.

Results: Our findings suggest that the reduction of mutant *DMPK* transcripts does not occur at the transcriptional level but rather during nuclear RNA processing. ATX-01 allows correction of MBNLexon5 splicing, which produces a shift towards preferentially cytoplasmic MBNL isoforms, which facilitates the nuclear export of *DMPK* transcripts to the cytoplasm, where they undergo rapid degradation. Furthermore, the mechanism of action of ATX-01 demonstrates superior therapeutic effects on the DM1 pathogenic cascade compared to other oligonucleotide approaches.

Conclusion: The ability of ATX-01 to enhance the processing of mutant *DMPK* transcripts, in addition to *MBNL1* upregulation, provides a therapeutic advantage by targeting multiple aspects of DM1 pathogenesis. This dual mechanism of action positions ATX-01 as a first-in-class therapeutic compound, offering a promising upstream regulatory strategy for DM1 treatment.

Young Investigator!

Poster # 614

Identifying key domains in the muscleblind-like 1 (MBNL1) protein essential to regulate alternative splicing

Authors: Saumya Gupta, Carl Shotwell, John Cleary, J. Andrew Berglund
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Objective: The MBNL1 RNA-binding protein is an important regulator of alternative splicing (AS), a process that generates multiple isoforms of a single pre-mRNA transcript. MBNL1 sequestration in myotonic dystrophy (DM) leads to aberrant splicing and disease phenotypes. MBNL1 is composed of four CCCH zinc fingers (ZF) tandemly arranged into 2 domains, ZF1-2 and ZF3-4 separated by a disordered 76 amino acid linker. The ZF domains bind to YGCY (Y= C or U) RNA motifs. ZF2 and ZF4 contain primary RNA binding sites whereas ZF1 and ZF3 serve as stabilizing domains1. Previous studies have predicted the linker plays a role in splicing regulation2,3, however the function of the linker and its relationship with the ZFs are not well understood. We hypothesize that the linker region separating ZF1-2 and ZF3-4 plays an important role in regulating AS, with different functions for negative and positively regulated AS events.

Methodology: To address this hypothesis, we designed synthetic MBNL1 constructs with mutations in the linker region and transfected them at various concentrations into HEK293 cells that have low endogenous MBNL1 expression and calculated percent spliced in values.

Result: MBNL1 overexpression in this system shifts splicing significantly for multiple events in a concentration dependent manner.

Conclusion: Comparing wildtype MBNL1 to a series of mutant MBNL1 revealed that the linker is a critical component of MBNL1 protein in regulating splicing, functions independently of the ZF3-4 domain and regulated splicing of positive and negative events differently.

Practical Implication: The overall goal of the project is to identify the regions and amino acids within the linker that modulate MBNL1 mediated splicing activity, and explore if these modifications can complement the current therapeutics strategies to alleviate MBNL sequestration in DM.

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Poster # 623

Fatty Acid Mediated Uptake System (FAMOUS) enables improved oligo delivery to muscle and brain

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Background: Oligonucleotide-therapeutics represent an emerging drug discovery platform designed to modulate gene expression targeting DNA or RNA in a sequence-specific manner. The development of these molecules involves improved chemistry and conjugates to overcome extracellular and intracellular obstacles and efficiently reach their targets. However, delivery to skeletal muscle and central nervous system (CNS) remains a challenge. To enhance biodistribution, efficacy, and safety in a clinically acceptable manner, we investigated the effects of oleic acid conjugation on a defined chemical antimiR-23b scaffold (ATX-01) as a potential therapy for Myotonic Dystrophy type 1 (DM1).

Methodology: The therapeutic potential of ATX-01 was tested in vivo in HSA^{LR} mice, a widely used DM1 model. Mice were treated intravenously with either Naked-antimiR-23b or ATX-01 at different doses. Efficacy was assessed by measuring MBNL1 levels as a miR-23b-target involved in the pathogenesis of DM1. Additionally, mice were treated intrathecally to evaluate the pharmacokinetics and compare the biodistribution with intravenous injection. Biodistribution was measured with a LNA-probe in DM1-relevant tissues. Preliminary toxicity profile and biodistribution of ATX-01 were also assessed in a non-human primate model (NHP).
Results: In mice, ATX-01 displayed more efficiency than Naked-antimiR-23b in rescuing DM1 phenotypes whilst maintaining a favorable safety profile. Biodistribution studies demonstrated that ATX-01 not only outperformed the Naked version of the oligonucleotide in skeletal muscle, but also crossed the blood-brain-barrier reaching the brain. After intrathecal administration, higher ATX-01 levels were detected compared to the Naked oligonucleotide, indicating enhanced stability and retention due to the oleic acid conjugation. In NHPs, ATX-01 efficiently targeted muscle and brain, consistent with the results observed in mice.
Conclusion: This work highlights oleic acid conjugation as a strategy to enhance AntimiR delivery to muscles and brain with a favorable safety profile. These findings provide insights into overcoming CNS delivery and support further studies for exploring ATX-01's therapeutic potential in neurological conditions.

Poster # 630
Changes in RNA Splicing as a Surrogate Endpoint for Myotonic Dystrophy Type 1 (DM1) Clinical Trials
Authors: J. Andrew Berglund[1], Aaron Novack[2], Iva Ivanovska Holder[2], Soma Ray[2], Nicholas E. Johnson[3,4]
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4. Center for Inherited Myology Research, Virginia Commonwealth University, Richmond, VA
Objective: DM1 is a slowly progressive, multi-systemic disorder with phenotypes that vary by age of onset and severity of symptoms. Abnormal regulation of alternative splicing is universal across affected individuals and phenotypes and drives the clinical manifestations of DM1, including a diverse array of signs and symptoms affecting most organ systems. Heterogeneity in the developmental and degenerative features and patterns of DM1 complicates the stratification, powering, and execution of interventional clinical trials in a reasonable timeframe. In this review, we summarize evidence supporting the use of splicing change as a surrogate endpoint in DM1 clinical trials.
Methodology: Published articles related to splicing in DM1 were reviewed and findings were summarized.
Results: The use of splicing change as a surrogate endpoint in DM1 is based on the principle that the degree of DM1-affected exons reflects the level of functional MBNL activity in muscle cell nuclei. Surrogate endpoints based on PCR-based panels of mis-splicing events reflecting the underlying DM1 molecular mechanism are reasonably likely to predict clinical benefit in a timely fashion, thus enabling accelerated clinical development of therapies that address unmet needs in DM1. Natural history data from the DM1 population support a strong correlation between dysregulated splicing and muscle function and point to the utility of a composite splicing index as a surrogate endpoint to predict future functional benefit, particularly in clinical trials of reasonable duration.
Conclusions: Ongoing and future clinical trials will establish the validity of surrogate endpoints based on measuring changes in splicing and whether the correction of spliceopathy correlates with meaningful clinical outcome assessments in individuals with DM1.

Practical Implications: The use of splicing correction as a surrogate endpoint able to predict functional outcomes will enable accelerated clinical development of therapies that address unmet needs in DM1.

Poster # 634

Characteristics of Patients with Myotonic Dystrophy Type 1 with Complex Care Needs: Results from the Real-World IMPaCT Study

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Objective: Myotonic dystrophy type 1 (DM1) is a rare, progressive, genetic disorder that affects multiple organ systems and leads to significant clinical and economic burden. Given the heterogeneous nature of the disease, this study evaluated the clinical factors most strongly associated with being an individual with DM1 incurring high cost of care (HCC).

Methodology: Records from 01/01/2015 to 08/25/2023 in Clarivate Real-World Database, an integrated insurance claims and electronic health record database, were utilized. Eligibility criteria included: DM1 diagnosis (index date), data activity during =6 months pre- and =12 months post-index, and age =12 years at index date, excluding congenital DM. HCC individuals were defined as those in the top 25th percentile of total costs in the 12-month post-index period. Baseline demographic and clinical characteristics associated with being a HCC individual with DM1 were evaluated.

Results: The study included 301 HCC and 900 non-HCC individuals, with females comprising 56.1% and 56.5% of each group, respectively. HCC individuals were older (mean age 48.8 vs. 46.6 years, p=.047), and had higher Charlson Comorbidity Index scores (1.7 vs 0.7, p

Poster # 651

Health Insurance Literacy in DM1

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DM1, is a progressive, inherited disorder, with multisystem clinical manifestations caused by mis-splicing of key genes (i.e., a spliceopathy) resulting in loss of muscle function and CNS symptoms (e.g., fatigue, excessive sleepiness). There are no approved treatments for DM1, however new therapies are in development[1]. To optimize access, the DM1 community has an urgent need to understand their health insurance benefits. Dyne Therapeutics and the Myotonic Dystrophy Foundation are conducting a study to examine health insurance literacy in DM1. Interim results from 73 affected community members who have participated in the study as of March 13, 2025 are presented. Fifty-eight were diagnosed individuals who managed their own insurance, 8 were diagnosed individuals who did not, and 7 were caregivers who managed the insurance. To assess health insurance literacy, this study utilized the questionnaire used by Kaiser Family Foundation (KFF), *Assessing Americans' Familiarity with Health Insurance Terms and Concepts* October, 2014. Our DM1 population scored better than the general population studied by KFF. Though small sample sizes, diagnosed individuals who managed their own insurance did better on 90% of questions than those who did not; and caregivers who managed insurance did better on all questions than diagnosed individuals. Respondents scored best on questions

pertaining to health insurance terminology. The two most frequently missed questions were about calculating out of pocket costs and health insurance formularies. This knowledge gap may reflect lack of experience calculating costs, and the fact that there are not yet DM1-specific therapies on formulary. It will be critical that the DM1 community better understands health insurance concepts to access new therapies / care. This study highlights an opportunity for industry and support organizations to prepare the community for issues such as transitioning to managing their own insurance, and understanding how to determine their overall costs.

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Young Investigator!

Poster # 654

Using miRNA as a non-invasive oral biomarker for Myotonic Dystrophy Type 1 (DM1)

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Objective: To evaluate the potential of miRNAs, specifically miR-206, as a valuable biomarker for DM1 using non-invasive sampling techniques.

Methodology: To validate miR-206 as a potential biomarker as previously published(1,2&3), we first measured the level of miR-206 in DM1 and control cell lines. These lines included DM1-MyoD-converted fibroblasts, primary myoblast lines with either 1,317 (DM1-A) or 2,900 (DM1-B) CTG repeats, and control cell lines. To understand the response of miR-206 to therapeutics, we also treated these lines with Vorinostat or novel modified polycyclic compounds (MPCs), which are known to rescue alternative splicing. Total RNA was extracted from these lines along with six buccal swab samples (four from DM1 subjects and two from controls). DM-specific splicing events were measured utilizing reverse transcription polymerase chain reaction (RT-PCR) and miR-206 expression was measured via digital droplet PCR.

Results: In the DM1 cells the levels of miR-206 expression were elevated compared to control cells, aligning with previously published results(4). Both DM1 dysregulated splicing events and miR-206 expression levels were rescued in treated DM1 cells. In our limited sampling of buccal swabs, preliminary results suggest that while miR-206 levels were minimally detectable, they appeared indistinguishable between DM1 patients and healthy controls. Further analysis of miR-206 and other miRNA is ongoing.

Conclusion: While both slicing defects and miR-206 were rescued by therapeutic treatment in DM1 patient-derived cells, further research into miR-206 is required in patient buccal samples. To facilitate this research, we will use small RNA sequencing to identify unique miRNAs in the biofluids of DM1 patients and healthy controls and their correlation with disease variables including severity and progression.

Practical Implications: The development of non-invasive oral biomarker based on the stability and accessibility of miRNAs in bodily fluids will facilitate early diagnosis and monitoring of DM1.

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Poster # 671

Benefit of Verapamil, Amlodipine, and Ranolazine on Myotonic Dystrophy Bi-Channelopathy

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Objective: We recently found that aberrant splicing of the skeletal muscle voltage-gated calcium channel (CaV1.1) and chloride channel (ClC-1) together contributes to severe muscle weakness, respiratory defects and reduced survival in mice. As a therapeutic strategy, we wanted to determine if existing drugs that target the CaV1.1 and myotonia improve muscle function and survival in longitudinal studies.

Method: We generated mice with forced DM1 splicing of CaV1.1 (CaV1.1?e29) and loss of ClC-1 channel function to study their combinatorial impact on muscle function. Further, to determine the effects of oral feeding of verapamil and amlodipine (calcium channel blockers), and ranolazine (anti-myotonic), we measured body weight, respiratory function, ex vivo muscle force generation, myotonia, time of righting reflex (TRR) and overall survival.

Results: Daily treatment with verapamil, amlodipine, and ranolazine, significantly improved survival, weight gain, and reduced time of righting reflex. Further, we found that verapamil, amlodipine, and ranolazine improved transient weakness and myotonia in in vitro muscle contraction experiments. Verapamil and ranolazine were able to significantly improve myotonia both in vitro and in vivo as well.

Conclusion and Practical Implications: Results demonstrate that targeting CaV1.1 ?e29 conductance and myotonia has therapeutic potential for people with DM1 and DM2.

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Young Investigator!

Poster # 691

Behavioral and Molecular Changes in Brain Specific Models of Myotonic Dystrophy Type 1 (DM1)

Authors: Bethlehem A. Bekele, Juan Arboleda, Liang Shi, Jingsheng Gu, Eric T. Wang, Jie Jiang, Gary J. Bassell
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Myotonic dystrophy type 1 (DM1) is a multisystemic disorder affecting muscle, the central nervous system (CNS), and other organs. It is caused by an expansion of cytosine-thymine-guanine (CTG) repeats in the DMPK gene, leading to the formation of toxic RNA foci that trap essential RNA-binding proteins like the Muscleblind-like (MBNL) family. This disruption affects the normal processing of many genes, contributing to symptoms such as muscle weakness, cognitive impairment, and sleep disturbances. While DM1 has been widely studied in muscle, its impact on the brain remains underexplored. To better understand how DM1 affects the central nervous system (CNS), we developed a transgenic mouse model that expresses toxic CUG-repeat RNA specifically in neurons. We found that these mice accumulate RNA foci in the brain, leading to a loss of functional MBNL proteins and widespread mis-splicing of key neuronal genes. One of the most affected genes is GABRG2, which encodes the gamma2 subunit of the GABAA receptor, a critical component in the brain's inhibitory signaling system. Normally, GABRG2 produces two isoforms: gamma2L (synaptic) and gamma2S (extrasynaptic). In DM1, mis-splicing shifts the balance toward gamma2S, potentially increasing extrasynaptic GABAA receptor activity and contributing to excessive inhibition in the brain. This may underlie sleep problems and cognitive symptoms seen in DM1 patients. Our study further shows that by controlling the expression of

toxic CUG repeats, we can partially reverse GABRG2 mis-splicing, suggesting a direct link between RNA toxicity and altered brain function. Behaviorally, DM1 mice exhibit normal motor function but show learning and sleep impairments, mirroring cognitive and emotional symptoms reported by patients. These findings provide new insights into how DM1 affects brain function at both the molecular and behavioral levels and identify a potential therapeutic target for sleep disorders and other behavioral manifestations of DM1.

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Young Investigator!

Poster # 693

RNA mis-splicing in children with congenital myotonic dystrophy is associated with physical function

Authors: Julia M. Hartman, Kobe Ikegami, Marina Provenzano, Kameron Bates, Amanda Butler, Aileen S. Jones, Kiera N. Berggren, Jeanne Dekdebrun, Marnee J. McKay, Jennifer N. Baldwin, Kayla M. D. Cornett, Joshua Burns, Michael Kiefer, Nicholas E. Johnson, Melissa A. Hale on behalf of the DMCRN Consortium. Virginia Commonwealth University, Richmond, VA, United States

Objectives: Dysregulated RNA alternative splicing is the hallmark of myotonic dystrophy type 1 (DM1). However, the association between RNA mis-splicing and physical function in children with the most severe form of the disease, congenital myotonic dystrophy (CDM), is unknown.

Methods: Eighty-two participants (42 adults with DM1 and 40 children with CDM) with muscle biopsies and measures of myotonia, motor function, and strength were combined from five observational studies. Data were normalized and correlated with an aggregate measure of alternative splicing dysregulation, [MBNL]inferred, in skeletal muscle biopsies. Multiple linear regression analysis was performed to predict [MBNL]inferred using clinical outcome measures alone. Lastly, we developed regression models to predict 12-month physical function using baseline performance and baseline [MBNL]inferred values.

Results: Myotonia, measured via video of hand opening time (vHOT), was significantly correlated with RNA mis-splicing in our cross-sectional population of all DM1 individuals; CDM participants alone displayed no myotonia despite a similar range of RNA mis-splicing. Measures of motor performance and muscle strength were significantly associated with [MBNL]inferred in our cohort of all DM1 individuals and when assessing children with CDM independently. Multiple linear regression analyses yielded two models capable of predicting [MBNL]inferred from select clinical outcome assessments alone in all subjects (adjusted r^2 = 0.6723) or exclusively in children with CDM (adjusted r^2 = 0.5875). [MBNL]inferred contributed significantly to the predictive power of our 12-month models for stair climbing speed. In our cross-sectional cohort of adults and children, baseline stair climbing speed and baseline [MBNL]inferred were able to accurately predict 12-month performance (adjusted r^2 = 0.8057).
Conclusions: Our findings establish significant correlations between skeletal muscle performance and a measure of alternative splicing dysregulation, [MBNL]inferred, in DM1.
Practical Implications: The strength of these correlations and the development of predictive models will assist in designing efficacious clinical trials for individuals with DM1, particularly CDM.

Hartman, J.M., Ikegami, K., Provenzano, M., Bates, K., Butler, A., Jones, A.S., Berggren, K.N., Dekdebrun, J., McKay, M.J., Baldwin, J.N., Cornett, K.M.D., Burns, J., Kiefer, M., Johnson, N.E., Hale, M.A. and (2024), RNA mis-splicing in children with congenital myotonic dystrophy is associated with physical function. Ann Clin Transl Neurol, 11: 3175 -3191. https://doi.org/10.1002/acn3.52224

Young Investigator!

Poster # 724

Telerehabilitation-based Respiratory Muscle Training in Myotonic Dystrophy Type 1: A Pilot Study

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Objective: To document the feasibility and acceptability of a respiratory muscle training (RMT) program delivered via telerehabilitation in individuals with myotonic dystrophy type 1 (DM1) and explore its potential benefits to support a larger-scale study.
Methodology: Twelve individuals with DM1 took part in the study. A one-week in-person familiarization phase preceded the 7-week telerehabilitation-based RMT program (one weekly synchronous and one daily asynchronous session) in groups of 2 to 3. Training load (TL) was determined starting at 50% of the maximal inspiratory pressure and increasing by 10%, maintaining a respiratory rate of 12 breaths per minute for at least one minute until the participant was unable to sustain this cadence, requested to stop, or reported a Borg score above 7/10. The tolerance period (TP) was determined as the total tolerated time at the TL. The training sessions consisted of 3 sets of one-third of the TP at the TL interspersed with a 2-minute rest period using the Powerbreath device. Feasibility outcomes include recruitment, retention, adherence, and protocol fidelity, alongside patient-reported acceptability measures. Assessments were conducted at baseline, post-intervention, and after a 12-week unsupervised period, evaluating respiratory muscle strength and endurance, pulmonary function, the 6-minute walk test, heart rate variability, fatigue, sleepiness, quality of life, and physical activity levels.
Results: The feasibility and acceptability of the telerehabilitation-based RMT program yielded favorable results, with satisfactory recruitment, retention and adherence rates, and protocol fidelity score. Participants reported satisfaction with the intervention, and significant improvements were observed in respiratory muscle strength, endurance, pulmonary function, and functional capacity. More detailed results will be presented at the conference.
Conclusions: This study provided critical insights for implementing a large-scale clinical trial and integrating telerehabilitation-based RMT. By addressing the need for accessible, technology-driven rehabilitation strategies, this project holds the potential to transform care for individuals with DM1.

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Young Investigator!

Poster # 726

Uncovering regional and cell-type specific transcriptomic signatures in the DM1 brain

Authors: Emily E. Davey[1,2], Juan D. Arboleda[1], Kendra McKee[1], Katharine A. Hagerman[3], Jacinda B. Sampson[3], John W. Day[3], Eric T. Wang[1]

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3. Department of Neurology, Stanford University, Palo Alto, CA, USA.

Objective: To understand how specific cell types contribute to CNS disease mechanisms by utilizing high-throughput sequencing tools such as bulk and single nuclei RNA-seq (snRNA-seq) to identify regional and cell type-specific transcriptomic changes.

Methodology: We performed bulk RNA-seq using postmortem tissue from up to 11 brain regions of DM1 patients and quantitated splicing dysregulation. We are also obtaining unaffected control tissue from all 11 regions; however, we have initially 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. Furthermore, we performed snRNA-seq using postmortem frontal cortex tissue from DM1 patients and controls to investigate cell type-specific dysregulation.

Results: We observed widespread transcriptomic dysregulation across all five regions and identified numerous shared mis-splicing signatures, including many mis-splicing events that have been identified across other tissues in DM1. Using snRNA-seq to quantify cell type-specific dysregulation, we found an increase in microglial and endothelial cell composition in DM1 patients and the highest number of differentially expressed genes in microglia. Interestingly, we observed a correlation between endothelial cell proportion and severity of overall splicing and gene expression dysregulation.

Conclusions: We have identified regional and cell type-specific transcriptomic changes, as well as changes in cell type composition in the DM1 CNS. In future studies, we will perform HCR RNA-FISH against cell type-specific transcripts, followed by fluorescence-activated nuclei sorting and bulk RNA-seq to identify cell type-specific splicing dysregulation. Additionally, we will isolate genomic DNA from each of these cell populations to obtain cell type-specific repeat length measurements.

Practical Implications: As therapeutic molecules currently in clinical trials for DM1 have been reported to enter the CNS, it is crucial to establish which brain regions, cell types, and molecular pathways are affected in the DM1 CNS to identify biomarkers that can be used to monitor CNS disease progression.

Otero, B. A., Poukalov, K., Hildebrandt, R. P., Thornton, C. A., Jinnai, K., Fujimura, H., Kimura, T., Hagerman, K. A., Sampson, J. B., Day, J. W., & Wang, E. T. (2021). Transcriptome alterations in myotonic dystrophy frontal cortex. *Cell reports*, *34*(3), 108634. https://doi.org/10.1016/j.celrep.2020.108634.

Poster # 735

Pharmacokinetics Profile of NaMuscla® Prolonged Release QD is comparable to NaMuscla® Immediate Release TD

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Objective: The pharmacokinetics (PK) profile of a new mexiletine formulation, NaMuscla® Prolonged Release (PR; 500mg granules for oral suspension in sachet, QD) was compared with NaMuscla Immediate Release (IR; 167mg hard capsules, TID).
Methodology: Three open-label, randomized PK/safety/tolerability studies were conducted in healthy adult males: single-dose study; food-effect study (fasting and fed [standard or high-fat]); 5-day multiple-dosing steady state study. NaMuscla PR palatability was evaluated via a questionnaire. Descriptive statistical analyses were undertaken.
Results: Subjects: single-dose study, N=20; food-effect study, N=24; multiple-dosing study, N=22; taste/palatability assessments, N=226. No serious or life-threatening adverse events were reported. Relative bioavailability for NaMuscla PR QD was comparable to IR capsule TID, for most PK parameters. A positive food effect was observed for NaMuscla PR under the fed state versus fasting. Cmax and AUC increased 39% and 17% respectively; effect on Cmax was statistically significant. The high-fat meal did not enhance the magnitude of the positive food effect. Accumulation index was 1.67 for NaMuscla PR and 3.23 for NaMuscla IR, suggesting no notable accumulation of mexiletine after repeat administration. Fluctuation% through steady state: NaMuscla PR QD, 85.24%; NaMuscla IR TID, 42.67%. Ratio of NaMuscla PR/NaMuscla IR at steady state, 81.71% (90% CI: 77.19–86.49). Average Cmin steady state value derived for NaMuscla PR was maintained above 0.5µg/mL for 21/22 (95%) of subjects (i.e. within the therapeutic window). 90% of subjects rated the taste of NaMuscla PR as good to very good; 86% rated its mouth feel as good or very good.
Conclusions: PK studies demonstrate comparable bioavailability, safety, tolerability and palatability profiles for NaMuscla PR 500mg oral suspension QD and NaMuscla IR 167mg hard capsules.
Practical implications: NaMuscla PR is a new once-daily oral liquid formulation that is convenient for patients who require mexiletine treatment for myotonia.

Young Investigator!

Poster # 749

Muscular and mitochondrial adaptations to strength training in women with Myotonic Dystrophy type 1

Authors: Laura Girard-Côté*, Vincent Marcangeli*, Valeria Di Leo, Marie-Pier Roussel, Conor Lawless, Olivier Charest, Anteneh Argaw, Maude Dulac, Guy Hajj-Boutros, José A. Morais, Amy Vincent, Gilles Gouspillou, Jean-Philippe Leduc-Gaudet#, Elise Duchesne# * These authors contributed equally to this work as first author. # These authors contributed equally to this work as last author.
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Myotonic dystrophy type 1 (DM1) is a hereditary disease characterized by progressive muscle weakness, atrophy, and physical limitations. Strength training has shown positive effects in individuals with DM1,

improving muscle function as well as mitochondrial content (mass and complexes I and IV protein levels). This study aimed to evaluate the impact of 12 weeks of strength training on mitochondrial bioenergetics in women with DM1, compared to non-affected individuals. Muscle biopsies were taken from the vastus lateralis of 9 women with DM1, before and after training, and 9 age-matched controls. Mitochondrial respiration and H2O2 emission were measured using high-resolution respirofluorometry (Oroboros O2K) and were normalized to the content of representative subunits of the oxidative phosphorylation measured via immunoblotting. Key respiratory chain subunits of I and IV, and markers of mitochondrial mass (VDAC1) were analysed by quadruple immunofluorescence (QIF). NCAM-dystrophin and Laminin-
DAPI, histological markers of muscle integrity, were assessed on muscle cross-sections. Strength training increased mitochondrial respiration ($p<0.01$) and restored it to control levels. Mitochondrial mass markers were significantly decreased ($p<0.01$) and the proportion of myofibers showing low levels of complex I and IV mitochondrial subunits were reduced as well in most participants (five out of nine). Additionally, markers of muscle integrity (i.e. fibers with damaged laminin and presence of nuclear clumps) were improved by strength training ($p=0.008$ and $p=0.039$, respectively). These findings, combined with previous studies showing clinical
improvements, underscore the potential of strength training to improve histological markers of muscle integrity and mitochondrial bioenergetics in DM1, potentially slowing disease progression.

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Poster # 762

The landscape of myotonic dystrophy clinical trials: a 2025 expansion to the TREAT-NMD myotonic dystrophy dataset

Authors: Anne-Berit Ekström[1], Poll A[1], Masic D[1], Ashley E-J[1], Esparis B[1], Rodrigues M[1], Guglieri M[1], Ambrosini A[1], Peric S[1], Campbell C[1]

1. On behalf of the TREAT-NMD Global Registry Myotonic Dystrophy Subgroup

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The TREAT-NMD Global Registry Network is an international network of independent patient registries aiming to accelerate treatment to patients, including myotonic dystrophy (DM1/DM2). The network contains 29 DM registries, collecting an agreed minimum dataset. The dataset has 30 unique items that capture essential components of DM. As ongoing clinical trials involving DM progress towards marketable treatments, the current dataset no longer fully meets stakeholders needs. We have reviewed a list of registered clinical trials, focusing on location, eligibility criteria & primary/secondary outcome measures. We compared our findings to data currently being collected by the TREAT-NMD registries in the minimum dataset. First, we highlighted the location overlap between DM clinical trials & TREAT-NMD registries. Second, we explored the most common inclusion/exclusion criteria described in trials & compared how these fit with the most recent available snapshot of demographic patient data in TREAT-NMD registries. Finally, we highlighted critical differences between clinical trial eligibility criteria and outcome measures, & the current TREAT-NMD DM dataset. We were able to identify key areas in which the DM dataset wouldn't be able to capture necessary information required in clinical trials. Our study points towards a need for an expanded DM dataset to allow registry data to be utilised in future

post-marketing surveillance studies. We go on to suggest new data items that may work towards bridging the gap between the data already being collected, and future data needs. Data collection and analysis is currently in progress, and the full results will be available to view on the poster. TREAT-NMD, will now work towards delivering a DM dataset able to meet the needs of multiple stakeholders. This will be conducted in conjunction with KOLs in the field of DM, including the TREAT-NMD DM registries, patient advocacy groups, academics, clinicians, and representatives from industry.

Young Investigator!

Poster # 763

DM1 Neuropathology: From Neuronal Morphology and Axonal Transport to the Reversion of Brain Disease

Authors: Louison Daussy, Louison Lallemant, Johanna Cormenier, Aline Huguet-Lachon, Frederic Saudou, Genevieve Gourdon, Mario Gomes-Pereira
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Brain function relies on the intricate interplay of highly ramified and polarized neurons, which function as the messengers of electric and molecular signals, essential for cognition, behavior and sleep regulation, all affected in DM1 [1, 2]. While it is established that expanded CUG RNA accumulates in the nucleus of DM1 neurons, our understanding of the molecular and cellular pathways involved in brain pathology remain largely unknown. Moreover, we do not know the extent to which neuronal phenotypes are reversible, a crucial question to determine if future therapies can effectively alleviate the neuropsychological symptoms of DM1. To address these knowledge gaps, we have investigated the neuronal impact of RNA toxicity in a transgenic mouse model of DM1. DMSXL mice show spatiotemporal expression of an expanded DMPK transgene [3, 4], and exhibit behavioral, electrophysiological and neurochemical abnormalities [5, 6]. Our work in primary DMSXL neurons has revealed impaired neuritogenesis, abnormal axonogenesis with distal axon initial segment (AIS) positioning, and reduced axonal vesicle transport, without pronounced splicing changes. These findings suggest that CUG RNA may impact neuronal morphology and function by mechanisms beyond missplicing. Interestingly, global analyses indicated widespread protein hyperphosphorylation in DMSXL primary neurons, pointing to the involvement of underexplored signaling pathways. This project will focus on refining the characterization of neuronal morphology and axonal transport defects in DM1, while exploring the underlying molecular mechanisms, as well as the implications for the morphological plasticity and function of neurons. Importantly, we will assess the reversibility of these phenotypes using a new inducible DM1 mouse model, which allows for precise neuron-specific control of toxic RNA expression. By identifying potential new therapeutic targets and providing a proof of concept for neuropathology correction, our research will determine whether DM1 brain symptoms are reversible or reflect permanent neuronal damage. Ultimately, our findings will help shape future CNS-focused treatment strategies and patient prognosis.

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Poster # 766

Design of the first in human ArthemiR study, of a novel drug, ATX-01, for the treatment of DM1

Authors: Judith Walker, Julia Presanis, Nikki McIntyre, Georgina Butler, Lilian Chow, Nuria Barquero, Beatriz Llamusi
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Objectives: ATX-01 is a novel anti-microRNA in clinical development for the treatment of Myotonic Dystrophy. The objective of the current project was to design an efficient and feasible study to assess the safety, tolerability, pharmacokinetics (PK), pharmacodynamics (PD), and preliminary clinical efficacy of ascending single and multiple doses of ATX-01 in participants with adult-onset Myotonic Dystrophy Type 1 (DM1).

Methods: The Arthex drug development team proposed an initial design based on non-clinical data, regulatory guidance, and latest knowledge of DM1 clinical trial design.

The design was reviewed and modified based on feedback from patient groups, key opinion leaders in DM1, clinical study site staff, technical specialists, FDA, EMA, MHRA and Spanish National Regulatory feedback, and external drug development experts.

Learnings from other trials which preceded and are ongoing were incorporated.

Results: The ArthemiR Trial Design was created and the study protocol was approved in at least 6 countries including the USA, Canada, UK, Spain, France and Italy.

The design ensures that risks to participants are minimised, whilst optimising the design efficiency, to allow maximal knowledge building on the potential for ATX-01 to become a leading therapeutic in the DM space. This was balanced with feasibility of conducting the study from various stakeholder viewpoints

Conclusions and practical implications: Best practices in study design include considerations of patient safety, data integrity and feasibility. It is critical to obtain and evaluate inputs from different sources before deciding on a final study design. These best practices provide a framework for future trials targeting rare diseases like DM1. Future phases will explore long-term efficacy and broader applications of ATX-01 in related conditions

Poster # 784

FREEDOM-DM1: A Phase 1, placebo-controlled single ascending dose study to evaluate PGN-EDODM1 in people with myotonic dystrophy type 1 (DM1)

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Objective: PGN-EDODM1 is an investigational peptide-conjugated oligonucleotide (PPMO) being evaluated for the treatment of myotonic dystrophy type 1 (DM1), based on PepGen's enhanced delivery oligonucleotide (EDO) cell-penetrating peptide technology. EDOs are engineered to optimize tissue delivery and nuclear uptake of therapeutic oligonucleotides. PGN-EDODM1 binds to pathogenic CUG trinucleotide repeat expansions in DMPK mRNA, thereby liberating MBNL1 protein through steric blocking without degrading DMPK transcripts. Liberation of sequestered MBNL1 is expected to restore

splicing profiles of multiple downstream transcripts; a central cause of DM1 pathology. The PGN-EDODM1 clinical development program is designed to evaluate the safety and tolerability of PGN-EDODM1 in people with DM1, mis-splicing pharmacology and to assess potential clinical benefit.
Methodology: The clinical development program includes FREEDOM-DM1, a randomized, double-blind, placebo-controlled single ascending dose study and FREEDOM2-DM1, a randomized, double-blind, placebo-controlled multiple ascending dose study (NCT06204809 and NCT06667453, respectively). Both studies are ongoing. FREEDOM-DM1 is designed to evaluate safety and tolerability; the secondary objective is plasma pharmacokinetics following a single dose of PGN-EDODM1. Exploratory measurements include concentration of PGN-EDODM1 in skeletal muscle, pharmacodynamics (changes in splicing of affected transcripts), patient-reported outcomes (PROs), and functional assessments. Key eligibility criteria include genetic diagnosis of DM1, presence of myotonia, and age 18 to 50 years. This study consists of four dose-ascending cohorts of participants (N=8), each randomized 3:1 PGN-EDODM1 to placebo. Muscle needle biopsies are being performed at Baseline, Week 4, and Week 16 to measure tissue drug concentrations and splicing of selected transcripts.
Results/Conclusions: Interim results from the 5 and 10 mg/kg cohorts of FREEDOM-DM1 will be presented.
Practical Implications: There are currently no effective therapies that treat DM1. PGN-EDODM1 is designed to address the central cause of the condition. FREEDOM-DM1 data provide initial support for the continued clinical development of PGN-EDODM1 for the treatment of DM1.

Young Investigator!
Poster # 791
TranSTIRomics for DM1: a muscle MRI-based transcriptome study in Myotonic Dystrophy type 1 (DM1)
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Objective: In muscular dystrophies, and also in DM1, muscle degeneration is preceded by a period of STIR positivity at muscle MRI. The aim of this project is to investigate the biological processes occurring in STIR-positive muscles before fat replacement in DM1 by histopathological and transcriptome studies.
Methodology: we collected two MRI-matched muscle biopsies collected from distal (STIR positive) and compared them to proximal (STIR-negative) regions of the same unreplaced (T1-negative) muscle (vastus lateralis) in 10 DM1 patients, based on evidence obtained from previous muscle MRI studies.
Results: Interim analysis obtained from biopsies from 4 patients revealed that distal biopsies show 1) mild histopathological alterations in the distal biopsies consistent with DM1 typical histopathological alterations (fiber size variability, nuclei internalization, nuclear clumps), whereas proximal biopsies showed normal findings; 2) bulk transcriptome profile showed that the distal region, compared to the proximal one, exhibits upregulation of genes involved in cell-cell adhesion, extracellular matrix organization, ion transport, and ligand-receptor activity, and downregulation of genes involved in metabolic pathways (mitochondrial activity, cellular respiration, and oxidative phosphorylation activity).
Conclusions: Preliminary data showed a significative difference in histopathological and transcriptome profile between the early affected (distal, STIR+) and spared (proximal STIR-) regions of the same muscle in DM1, suggesting the earliest pathophysiological mechanisms underlying muscle degeneration and fat replacement in DM1.
Practical Implications: This project will contribute to identifying novel biomarkers and potential therapeutic targets for the earliest stage of disease, anticipating fat replacement and muscle weakness.

Young Investigator!

Poster # 797

ASO-Modified RiboTAC degrades CUG RNA and rescues mis-splicing in HeLa-based DM1 Cell Model

Authors: Amy Mascorro, Yu Sheng Chen, Angela White, Lucas Hooker, Shemari Blackwood, Sanjida Ahmed, Dr. Andrew Berglund*, Dr. Jia Sheng*
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Myotonic Dystrophy Type 1 (DM1) is a repeat expansion disease that is caused by the overexpansion of CTG repeats in the 3'UTR of the DMPK gene. This over expansion results in the transcription of this CTG DNA into gain-of-function CUG RNA which forms a hairpin loop that is capable of sequestering MBNL family proteins in a YGCY motif causing a cascade of transcriptomic failures further in the alternative splicing process. This disease causes multisystemic symptoms that can vary from patient to patient which include but are not limited to insulin resistance, apathy and cognitive deficiencies, muscle wasting and myotonia, etc. While some therapeutics are making their way to clinical trials and have been given special FDA recognition, there have been no FDA approved therapeutics to treat DM1. Using a previously developed CTG expansion HeLa cell line, a novel therapeutic was tested for its ability to cleave CUG RNA repeat expansions by hijacking endogenous RNase L. Below we show results from many studies to show that this novel system is capable of cleaving CUG RNA repeats, allowing MBNL1 proteins to release from these hairpin loops and perform their native function.

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Young Investigator!

Poster # 803

Transcriptomic effects of strength training on vastus lateralis muscle in DM1 patients

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Objective: To validate how splicing biomarkers in vastus lateralis relate to clinical improvements following strength training in DM1 patients.

Methodology: 9 males and 8 females DM1 patients underwent a 12-week strength training program(leg extension, leg press, squat and hip abduction) with vastus lateralis biopsies obtained before and after the strength training. RNA extracted from the samples was sequenced along with RNA from vastus lateralis biopsies taken from 8 healthy females and 5 healthy male participants that did not participate in the training program. Reads were aligned to the hg38 genome with Star. Alternative splicing analysis was done with rMATS. A splicing index(SI) score was computed with data from RNA sequencing using the 95th DM1 percentile and median value for control from the tibialis anterior as described in (1).
Results: All participants showed clinical improvements from strength training, as described previously(2,3). Alternative splicing and SI score varied considerably across patients, with 8 out of 17 patients having SI scores similar to healthy participants. The latter patients were primarily DM patients with late-onset phenotype or less than 300 CTG repeats measured in blood. Age also appeared to have an effect on SI scores as patients younger than 40 years had lower SI scores than older patients. While these analyses are still ongoing, strength training appears to have a variable effect on SI score.
Conclusion: While strength training shows consistent clinical improvements, response of splicing is variable and requires further analyses, including validating the effects of age, CTG repeat, and phenotype. It will also be interesting to see if other splicing events beyond the SI panel are more representative of the splicing dysregulation in vastus lateralis.
Practical implications: Strength training is a good therapeutic strategy for men and women with DM1 and alternative splicing events may be useful as response biomarkers for exercise.

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Virtual Only!

Poster # 806

Expanding the UK Myotonic Dystrophy Patient Registry Dataset - Improving Data Collection and Amplifying 'Patient Voice'

Authors: Helen Walker[1], Lucy Hickson[1], Jassi Sodhi[1], Daren Monckton[2], Emma-Jayne Ashley[3], Chiara Marini-Bettolo[1]

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Since 2012, the UK DM Patient Registry collected the TREAT-NMD Core Dataset for DM, a minimal list of data items agreed at a 2009 ENMC workshop. The dual-reported registry collected both patient-entered and clinical data using this dataset, creating a valuable repository of longitudinal data on their national patient cohort. The 2009 dataset contains mandatory patient-entered questions on diagnosis, family history and ethnicity, and mandatory longitudinal questions on motor function, wheelchair use, symptoms and medication. Optional questions on pregnancy history and children are also included. The clinician-entered section can be completed by patients' healthcare professionals and includes cardiac information, ECG and Echo results, pulmonary function testing and ventilation support, feeding tube use, cataract surgery, age of symptom onset, and some genetic test results (if available). In 2023, the UK DM Registry identified the need to expand and refine the questionnaires within the registry dataset. A

Working Group was established comprising academic, genetic, physiotherapy, clinician and patient advocate experts from the registry's Steering Committee to review the dataset and ensure data collection remains relevant and appropriate to the UK's patient and research communities. As the number of clinical trials arriving in the UK increases, the ability to collect data on potential treatments or disease modifying therapies becomes crucial. The expanded dataset has been developed to allow the registry system to run studies to provide real-world data to support post-marketing surveillance activities. It also enables the creation of Privacy Preserving Record Linkage keys to enable patient-level data to be anonymously shared and linked with other data sources, greatly increasing the usability and value of the data for research. The expanded dataset is available to view on the UK DM Registry website and can be adopted by other national DM registries and data collection initiatives to ensure harmonisation and alignment of data collection.

Poster # 807

The Practicality of Conducting Remote Assessments in Myotonic Dystrophy Type 1 (DM1)

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Objective: To assess the feasibility of conducting remote research and its accessibility for participants and families with DM1.

Methodology: Subjects receive necessary equipment in toolkits via mail. Remote study visits (RSV) include an interview, strength and functional assessments via videoconferencing, independent completion of questionnaires, and wearing activity monitors for 7 days. Subjects and the study team complete a satisfaction questionnaire. Subjects can elect to participate in future RSVs. Subjects ship their blood for CTG repeat analysis and receive the results in return.

Results: We have conducted 141 RSVs among 73 subjects, including 21 families. The majority (74%) live outside of New York State. 37% of subjects were research naïve prior to participation. 80% volunteered for more than one RSV. Over 90% of the assessments were completed. One toolkit was lost. 24% of subjects required assistance from another person. Questionnaires were completed by 88% of subjects. 85% of subjects wore activity monitors for 7 days. We received blood samples from 93% of the subjects, with adequate quality in 92%. 38 subjects received their genetic test results via mail. 59 subjects who completed the feedback survey indicated high satisfaction with remote research. When asked if the subject was more likely to participate in RSVs or on-site visits, 94% of subjects preferred RSVs or either, equally.

Conclusions: Conducting remote assessments is feasible in DM1, including in people with no prior research experience, and is well-perceived.

Practical Implications: Remote research provides an opportunity to engage a broader DM1 community and increase research participation across the United States while reducing burden on individuals and families.

Young Investigator!

Poster # 810

Intellectual Disability and Motor Function in Congenital Myotonic Dystrophy: Implications for Clinical Assessment and Trial Design

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Background: Congenital myotonic dystrophy (CDM) is characterized by cognitive and motor impairments, yet their interplay in shaping developmental trajectories remains poorly defined. Accurate physical function assessment is critical for clinical monitoring and trial readiness, particularly given the confounding effects of intellectual disability (ID).
Objective: This study examines the impact of ID on motor performance in children with CDM and explores factors that contribute to variability in functional motor outcomes to enhance clinical assessment strategies.
Methods: A cohort of 36 children with CDM (20 with ID, 16 without ID; age range: 2.9–13.9 years) was assessed using univariable and multivariable linear regression models to examine the effects of cognitive ability and years of ambulation on six standardized physical function assessments, including timed walking, running, stair navigation, and endurance measures. Participants were stratified by ID status to assess differential effects.
Results: Years of ambulation emerged as the strongest predictor of motor performance across all functional tasks ($p < 0.05$). While cognitive ability was not a significant predictor for most measures, children with ID exhibited significantly slower times on the 10m Run task ($p = 0.033$), suggesting that cognitive-motor integration may play a role in tasks requiring rapid motor execution. Additionally, children with ID had lower adaptive behavior scores and greater CTG repeat expansions ($p = 0.010$), reinforcing the genetic underpinnings of neurodevelopmental variability in CDM.
Conclusions: Findings indicate that mobility experience is the primary determinant of motor function in CDM, with cognitive ability exerting a more limited influence, except in tasks requiring higher-speed coordination. These results highlight the need for developmentally sensitive neuropsychological models that account for interactions between executive function, motor learning, and adaptive behavior. An integrated approach to neuropsychological and motor assessments will enhance the precision of longitudinal outcome measures, intervention strategies, and trial design in CDM.

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Young Investigator!

Poster # 818

Differential pathology and susceptibility to MBNL loss across mouse muscles in a myotonic dystrophy model

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There are two subtypes of Myotonic Dystrophy (DM): DM1 caused by a CTG repeat expansion in the 3'UTR of the DMPK gene and DM2 caused by a CCTG repeat expansion in intron 1 of the CNBP gene. The leading DM pathogenic mechanism is RNA mediated toxicity whereby (C)CUG expansions lead to sequestration of the muscleblind-like (MBNL) family of RNA binding proteins. A key difference between DM1 and DM2 is the muscle groups affected with distal muscles more severely affected in DM1 and proximal muscles more affected in DM2. DM1 is also characterized by type I fiber atrophy, while type II atrophy is more common in DM2. The cause of these disparities in affected muscles is unknown, and it is

currently unclear if DM mouse models recapitulate these differences. Further, it is important to clarify which mouse muscles are more susceptible to disease pathogenesis for the purpose of therapeutic development. To address these issues, we collected a series of muscles from Mbnl knockout mice and evaluated them for characteristic histologic and molecular features of DM pathology. Our results indicate that Mbnl loss discordantly affects muscles; however, it does not recapitulate the distal-proximal or proximal-distal gradient observed in either DM1 or DM2. Instead, some mouse muscles, such as the TA, are more susceptible to MBNL loss and develop more histological features resembling DM. This is despite increased sensitivity to some missplicing events in other muscles. Additionally, in muscles such as the EDL and diaphragm, Mbnl loss results in a fiber atrophy profile more like DM1 than DM2. These findings begin to explain the affected muscle disparity in DM and have important implications for the muscle of choice when performing analyses in new mouse models as well as evaluating new therapeutic modalities and biomarkers.

Young Investigator!

Poster # 831

Differential expression of core spliceosome proteins modulates the spliceopathy in myotonic dystrophy type 1

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Objective: Myotonic dystrophy type 1 (DM1) is a heterogeneous multisystemic disorder caused by a CTG repeat expansion in DMPK. Transcription of the expanded repeats produces toxic CUG RNA, leading to aberrant cellular functions including the sequestration of MBNL proteins which triggers a global spliceopathy that has been linked to several DM1 symptoms. The CTG repeat length alone is not sufficient to account for the molecular and clinical heterogeneity in DM1, supporting the existence of additional modifiers.

Methods: To identify novel factors that modify toxic CUG RNA outcomes, a genome-scale siRNA screen of over 16,000 genetic targets was performed using a previously developed DM1 HeLa CTG repeat-selective screening cell line. Top hits from our screen were validated in primary DM1 patient-derived fibroblasts and myoblasts using siRNA-mediated knockdown followed by an analysis of splicing (RT-PCR), DMPK expression (RT-qPCR) and ribonuclear foci (fluorescence in situ hybridization). Global transcriptomic effects were analyzed using RNA sequencing.

Results: Our genome-scale screen identified core spliceosomal proteins as a new class of genetic modifiers of toxic CUG RNA levels and the MBNL-mediated spliceopathy in DM1. Analysis of DM1 patient tibialis anterior muscle biopsy RNA sequencing data revealed a significant positive correlation between expression of our top hits including SNRPD2, SNRNP200 and SNRPF and DMPK levels and a negative correlation with ankle dorsiflexion strength. Modest knockdown of SNRPD2, SNRNP200 and SNRPF in DM1 patient cells significantly reduces DMPK expression and rescues MBNL-mediated mis-splicing without inducing general splicing failure. RNA sequencing analysis identified altered DMPK splicing, predicted to accelerate transcript turn-over, and increased MBNL1 expression as relevant mechanisms. Expression levels of SNRPF also modulates splicing rescue of antisense oligonucleotides (ASOs), supporting a potential influence on therapeutic response.

Conclusions: Our study identified that the expression levels of select core spliceosome proteins modulate the molecular hallmarks in DM1.
Practical Implication: Our findings could have implications for the clinical heterogeneity and the variable therapeutic response to ASOs observed in DM1 patients.

Virtual Only!
Poster # 832
The UK Myotonic Dystrophy Patient Registry - Empowering Clinical Research and Patient Voice with an Effective Translational Research Tool
Authors: Lucy Hickson [1], Helen Walker [1], Jassi Sodhi [1], Chris Turner [2], Daren Monckton [3], Channa Hewamadduma [4], Mark Hamilton [5], Emma-Jayne Ashley [6], Michael Walker [7], Kate Adcock [8], Chiara Marini-Bettolo [1]

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The UK Myotonic Dystrophy Patient Registry is a patient self-enrolling online database collecting clinical and genetic information about myotonic dystrophy type 1 (DM1) and type 2 (DM2). The registry was established in May 2012 by Newcastle University and is supported by Muscular Dystrophy UK, Cure-DM and the Myotonic Dystrophy Support Group. The registry facilitates academic and clinical research, enables better characterisation and understanding of DM, and disseminates information relating to upcoming studies and research advancements to participants. The registry is used to capture longitudinal, self-reported data through an online portal available to patients and clinicians. Where specialised clinical or genetic information is available, the neuromuscular specialist involved in the patient's care can provide some additional data. The registry is a Core Member of the TREAT-NMD Global Registries Network for DM1, collecting the standardised dataset and contributing to global data enquiries. The registry questionnaires have recently been expanded to improve the detail and scope of patient data, including the addition of PROMs, questions on access to care and trial preferences, and more detail on medication and device use. The registry has successfully assisted with recruitment to clinical trials and has supported over 30 research enquiries to date. These include anonymised data reports to industry, and academic research surveys into topics including COVID-19, dysphagia, pregnancy, patient preferences for future treatments and the patient/caregiver experience. The registry continues to be a versatile, cost-effective research tool to facilitate and advance a range of DM research. Additional work continues to be done to improve reporting of genetic information on the registry, and to overcome perceived boundaries to registration and participation.

Young Investigator!
Poster # 834
Therapeutic potential of targeting defective muscle stem cells as a treatment for DM1

Authors: Tatiana Koike[1,2]; Taeyeon Kim[1,2]; Ines Mokhtari[1,2]; Talita Conte[1,2]; Elise Duchesne[3]; Nicolas Dumont[2,1]

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Myotonic dystrophy type 1 is a hypervariable multisystemic disease affecting muscle mass and function. Previous findings from our lab showed that DM1 muscle stem cells (MuSCs) exhibits signs of premature senescence, which affects their ability to repair and grow muscle tissue. Therefore, we aim to evaluate the effectiveness of senolytic therapy and physical exercise in reducing senescence markers and restoring muscle function in DM1. Our spatial transcriptomics analysis of TA muscles from WT and DMSXL mice (DM1 model) showed a senescence signature in the DMSXL mice. Isolated MuSCs from DMSXL have signs of senescence indicated by higher p21 expression and reduced proliferation (Ki-67). These markers are reduced by a 6 weeks exercise program. Moreover, the myofiber size increased compared to non-exercised DMSXL, especially for IIA and IIB fibers. DMSXL exercise demonstrated improvements in muscle strength (in vivo, grip strength test) and reduction of the time for muscle relaxation. Muscle regenerative capacity was also enhanced by the exercise program. Regenerating exercised DMSXL mice had a higher number of MuSCs in proliferation (MyoD+) and in MuSCs undergoing differentiation (Myog+), along with an increase in the size of newly-formed fiber (eMyHC+) compared to DMSXL non-exercised. We also demonstrated that the senolytic BCL-XL inhibitor (A1155463) removes senescent DM1 myoblasts, reduces senescence associated secretory phenotype expression, and restores myogenesis. Our new findings on cellular senescence in DM1 open new therapeutic avenues, including the potential of senolytics and physical training. Combining these approaches could set the foundation for future treatment developments in DM1.

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Young Investigator!

Poster # 838

Inflammatory and Interferon Signatures in Myotonic Dystrophy Type 1

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Myotonic dystrophy type 1 (DM1) is a progressive, debilitating disease that affects up to 1 in 8,000 people worldwide. Although the cause of DM1 is known, many symptoms are still difficult to account for. The goal of my research is to determine whether the activity of the immune system in DM1 contributes to the pathology of the disease. Intracellular accumulation of double-stranded RNA is known to provoke an immune response in the context of viral infection, and DM1 is associated with the intracellular accumulation of CUG-repeat RNA, which forms semi-stable hairpin structures. In order to determine whether patients with DM1 demonstrated evidence of an anti-viral type immune response, I analyzed gene expression datasets from DM1 patients across multiple tissue types and patient-derived cell lines. These sequencing datasets revealed evidence of a largely unexplored immunological component to myotonic dystrophy. In many datasets, gene ontology analysis showed that the most significantly upregulated pathways are related to the immune response. These pathways contain genes involved in inflammation and the innate response to interferons – the main signaling molecules in viral infections. The results are especially striking in neurological tissues such as the brain and eye.

Preliminary results from our in-vitro experiments show that fibroblasts transfected with the CUG repeat RNA also upregulate interferon-stimulated genes. In order to determine if the immune response we observed is contributing to the symptoms and progression of DM1 in patients, it is vital to have an appropriate model. In ongoing studies, I am investigating the immune response in brain tissue from several mouse models of myotonic dystrophy, using bulk, single-nuclei, and spatial transcriptomics, to see if it mirrors what has been observed in humans. This work has the potential to broaden our understanding of the pathology of DM1, and open new avenues for alleviating its symptoms.

Young Investigator!

Poster # 859

REPEAT EXPANSION IN MYOTONIC DYSTROPHY: Exploration of Genetic Modifiers of Repeat Instability

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OBJECTIVE: Unstable CTG repeat expansions in Myotonic Dystrophy Type 1 (DM1) contribute to genetic anticipation, with significant intergenerational expansions often observed from mothers passing the repeat expansion to offspring with congenital myotonic dystrophy (CDM). Disruptions in DNA repair pathways are suspected drivers of this rapid intergenerational expansion, yet the molecular pathogenesis remains elusive. This study aims to identify genetic modifiers of repeat instability in a population of DM1 families enriched for genetic anticipation.
METHODOLOGY: Whole exome sequencing (WES) of 57 CDM patients and 18 families with large intergenerational expansion with maternal inheritance was performed, thereby creating the largest CDM WES cohort to date. Variant analysis for this cohort employs a top-down approach, starting with a broad evaluation of variants across the entire CDM cohort and progressively focusing on specific kindreds. Predicted deleterious variant effects will be functionally defined through in vitro biochemical assessments.
RESULTS: Current findings show an enrichment of mismatch repair and muscle integrity genes in the CDM cohort. Family based analysis looking at intergenerational variants has started and revealed an MSH3 rare and potentially deleterious variant (MAF0.9) segregating intergenerationally along the affected DMPK allele. This variant resulted in decreased global DNA repair ability of MMR deficient LoVo cells measured by mean percent tail DNA via Comet Assay.
CONCLUSION: This project investigates genetic modifiers of repeat instability in a cohort of children with CDM, thus enriched for genetic anticipation. These data are enriched for mismatch repair variants, consistent with the potential role of DNA repair mechanisms as modifiers of DM1 repeat instability.
PRACTICAL IMPLICATIONS: This cohort offers an ability to explore genetic modifiers in CDM, potentially providing insight into the role of repeat instability in disease progression.

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Young Investigator!

Poster # 865

Multidrug Therapeutic Strategies Improve Spliceopathy Correction in Myotonic Dystrophy Type 1

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Objective: Myotonic Dystrophy Type 1 (DM1) is a genetic disorder caused by a CTG repeat expansion in the DMPK gene, leading to the sequestration of muscle-blind-like (MBNL) proteins and RNA splicing disruption[1]. Correcting mis-splicing by reducing or blocking toxic RNA to restore MBNL function is a promising therapeutic approach[2]. Antisense oligonucleotides (ASOs) and small molecules (SMs) exhibited encouraging pre-clinical results in DM1 cells and mouse models, with ASOs recently demonstrating partial splicing rescue in clinical trials[3]. Our research aims to determine the therapeutic potential of ASO-SM and SM-SM combinations in correcting the spliceopathy in DM1.
Methodology: Our group has previously demonstrated the efficacy of a novel series of small molecules, termed modified polycyclic compounds (MPCs), in correcting mis-splicing in DM1 cell and mouse models. In this study, we have investigated the effects of combination therapies using ASOs with the small molecule histone deacetylase inhibitor, vorinostat, as well as combinations of MPCs and vorinostat, in patient-derived cell lines via RT-PCR[4]. Currently, we are performing RNA-sequencing to evaluate the efficacy and off-target effects of combination treatments compared to single-agent therapies and extending our study to additional small molecules. The most effective combinations will be further tested in DM1 mouse models to validate their therapeutic potential.
Results: Our data indicate these combinations may produce additive or synergistic effects at lower doses, significantly enhancing splicing correction and reducing toxic RNA levels compared to single-agent therapies. ASO-SM combinations and SM-SM combinations led to over 85% splicing correction in DM1 myoblasts and over 90% correction in myotubes following 72-hour and 96-hour treatments, respectively.
Conclusions: Combinatorial therapies show significant promise in correcting spliceopathy while allowing dose reduction, enhancing therapeutic efficacy, and potentially minimizing off-target effects and toxicity.
Practical Implications: This approach could significantly advance existing DM1 treatments by offering a more effective and safer intervention for patients.

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Young Investigator!

Poster # 903

Proteomic Profiling of Cerebrospinal Fluid in Myotonic Dystrophy Type 1 (DM1): Mapping Spectra to Splice Junction Peptides as Potential Biomarkers

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Objective: To identify potential biomarkers and novel splice-junction peptides in Myotonic Dystrophy Type 1 (DM1) by integrating mass spectrometry from cerebrospinal fluid (CSF) and RNA sequencing of post-mortem brain from DM1 patients.

Methodology: Cerebrospinal fluid (CSF) samples from DM1 patients (n=5) and healthy controls (n=5) were analyzed using mass spectrometry on the TIMS-TOF Ultra system coupled with the nanoElute 2. Proteomic analysis was conducted for proteins and high-resolution peptide identification. Proteomics data were processed with Spectronaut for spectral analysis, and enrichment analyses were performed using KEGG and Gene Ontology (GO) databases to identify altered pathways. A bioinformatics workflow was developed to investigate whether mis-splicing events previously identified in the DM1 post-mortem brain could be detected in peptide sequences present in CSF.

Results: A total of 2,056 proteins were quantified, of which 52 exhibited differential expression in DM1 patients compared to healthy controls. Among these, 13 proteins were upregulated and 30 were downregulated, including key proteins such as RPS7, SNED1, NCAM1, FN1, PBXIP1, GNS, ICAM1, LAMP5, and THSD4. Notably, dysregulated pathways and processes were identified, including the NF-kappa B signaling pathway, cytoplasmic translation, cell adhesion, axon guidance, collagen-containing extracellular matrix, and integrin binding. These alterations suggest that common pathways implicated in other neuromuscular disorders may also contribute to the pathophysiology of DM1. Further analysis of splice-junction peptides is ongoing, intending to map the unique peptides to already identified novel splice variants, which will enhance our understanding of the molecular mechanisms underlying DM1.

Conclusions: This is one of the first studies integrating RNA-Seq and mass spectrometry [1,2,3,4] to identify proteins and splice junction peptides altered in DM1. These peptides, resulting from splicing events such as skipped exons and alternative splice sites, present new opportunities for biomarker discovery and deepen our understanding of molecular mechanisms in DM1.

Practical Implications: The identification of these novel peptides offers new insights into disease-specific proteomic alterations, which can aid in early diagnosis, disease monitoring, and the development of targeted therapies for DM1 and other neuromuscular disorders.

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Poster # 912

Identification of Genetic Modifiers of Clinical Severity in DM1 through Integrated Cohort Analysis and Functional Genomics

Authors: Samuel Carrell, Michael Kiefer, Gowon McMichael, Man Hung, Beverly Davidson, Nicholas Johnson, the Myotonic Dystrophy Clinical Research Network (DMCRN).
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Objective: Myotonic dystrophy type 1 (DM1) is a highly variable monogenic disorder with severity ranging from severe, congenital onset disease to a late onset disease with mild symptoms, and

individual patients develop variable multisystemic involvement (e.g., cardiac arrhythmia, GI disturbances). We hypothesize that trans-acting genetic variants in components of the molecular cascade of DM1 contribute to this variability.
Methodology: To identify these variants, we will take a two-pronged translational approach. First, we will combine data from six large natural history studies in DM1 to generate the largest known cohort (n?1600) with combined quantitative clinical outcomes data (e.g., age-of-onset, 6-minute walk test, EKG) and blood DNA samples. We will measure CTG repeat lengths to model expected severity for individuals, then calculate the difference between the actual and expected outcomes and correlate this metric with genetic variants identified in the study population. Second, we will perform a genome-wide CRISPRi screen in DM1 iPSC-derived muscle cells using a reporter of MBNL activity, a downstream molecular biomarker of DM1 pathology, as an outcome variable. This approach overcomes the limitation of natural genetic variation in our population.
Results: Preliminary analysis of a subset of participants showed high repeatability of reported age-of-onset (AOO) over time (ICC = 0.992), and that CTG repeat length explains a relatively small fraction of this clinical outcome (n = 144; R2 = 0.24). Both 6-minute walk distance and normalized grip strength correlate moderately with years since onset (r = -0.339 and -0.282, respectively) and log(CTG) repeat length (r = -0.426 and -0.527). Introduction of the MBNL activity reporter in iPSC-derived myotubes shows clear distinction between DM1 and isogenic control lines.
Conclusions: We aim to combine clinical and molecular approaches to identify modifying genetic variants impacting clinical severity in DM1.

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Poster # 929
The BrAAVe Study: An open label, single arm Phase 1/2 study to investigate the safety, tolerability, and efficacy of SAR446268, an adeno-associated viral vector-mediated gene therapy in non-congenital
Authors: Isobelle Galeon, Didier Rouy, Pablo Rendo
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Objective: To evaluate the safety, tolerability, and efficacy of SAR446268, an adeno-associated viral vector-mediated gene therapy, in downregulating DMPK mRNA levels in participants 10 to 50 years old with non-congenital DM1.
Methodology: This Phase 1/Phase 2 study is divided into dose escalation (Part A) and dose expansion (Part B) with up to approximately 32 total participants with non-congenital DM1. Each participant meeting the eligibility criteria for each of the study parts will receive a single infusion of SAR446268. In Part A, single ascending doses of SAR446268 will be evaluated in 3 distinct cohorts of participants aged 18 to 50 years old with approximately 3 participants per cohort. Once the dose for the expansion phase is chosen, approximately 20 eligible participants aged 10-50 years old will receive SAR446268 in Part B. Clinical and laboratory assessments will be collected during the screening/pre- and post-treatment visits to assess eligibility, as well as safety-related events and efficacy, respectively. The primary safety

endpoint for both Parts A and B is the incidence and severity of TEAEs following SAR446268 administration. Other study endpoints focusing on safety (i.e., vector shedding), efficacy, (i.e., DMPK knockdown levels, functional neuromuscular tests, changes in splicing patterns) and quality of life measures (i.e., sleep, hospitalization rates) will also be assessed.
Results: Statistical analyses will be performed as outlined in the statistical analysis plan.
Conclusions: SAR446268 is a novel AAV-based muscle-targeting gene transfer therapy that aims to deliver persistent DMPK mRNA downregulation and restore normal splicing function in the transduced cells, through a single dose infusion.
Practical Implications: There are currently no disease modifying therapies available for DM1 patients. If deemed safe and effective, SAR446268 can potentially deliver a one-time disease modifying therapy with long-term and durable benefits for non-congenital DM1 patients.

Young Investigator!
Poster # 941
Identification of enzymatically modified isoquercitrin (EMIQ) as a therapeutic candidate for myotonic dystrophy type 1 and type 2
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Background and Objective: Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are multisystemic disorders caused by CTG repeat expansion in the 3' UTR of the DMPK gene (DM1) and CCTG repeat expansion in the first intron of the CNBP gene (DM2). This expansion leads to the production of toxic CUG/CCUG repeat RNA, which sequesters MBNL proteins, resulting in widespread MBNL dependent mis-splicing, a hallmark of DMpathogenesis. Currently, no FDA-approved disease-targeting treatments are available for DM. This study identifies enzymatically modified isoquercitrin (EMIQ), a bioavailable derivative of quercetin, as a therapeutic candidate that selectively reduces toxic RNA production, leading to the rescue of MBNL dependent mis-splicing and improvement of myotonia, a phenotypic hallmark of DM.
Methods: A HeLa cell-based screening platform was used to identify compounds from the NCI Natural Product Library V that selectively reduce toxic r(CUG) RNA levels. Quercetin was tested in DM1 and DM2 patient-derived fibroblasts and DM1 myotubes for its effects on DMPK/CNBP transcript levels and MBNL-dependent mis-splicing. The in vivo efficacy of EMIQ, a bioavailable quercetin derivative, was assessed in HSALR DM1 mice by measuring toxic CUG RNA levels, mis-splicing rescue, and myotonia after oral treatment.
Results and conclusion: Primary and secondary screening in a DM1 HeLa cell line revealed the dietary flavonoid quercetin as a selective modulator of toxic CUG RNA levels without significantly affecting cellular viability. Quercetin treatment in DM1 and DM2 patient-derived fibroblasts significantly reduced DMPK transcript levels and rescued MBNL-dependent mis-splicing events while maintaining cell viability. Furthermore, treatment of DM1 patient-derived myotubes with quercetin resulted in a significant reduction in DMPK transcript levels and rescued MBNL dependent mis-spliced events without significant impacts on cell viability. Finally, in DM1 mice, oral treatment with EMIQ, a bioavailable form of quercetin, selectively reduced toxic CUG RNA levels, mitigated DM1-associated mis-splicing, and reduced myotonia. No adverse events were noted in these animals and quercetin was detected in both muscle and brain of treated animals aligning with EMIQ's well established safety and bioavailability

profile This data coupled with its in vivo efficacy, positions EMIQ as a promising candidate for future clinical evaluation as a disease-modifying therapy for DM.

Young Investigator!

Poster # 944

More than meets the PSI: transcript turnover impacts apparent splicing rescue following EEV-PMO treatment

Authors: Emma N. Shea, Derek R. Muscato, M. Carmen Valero, Leanne M. Adams, Xiulong Shen, Mahboubeh Kheirabadi, Mark Wysk, Matthew Streeter, Ziqing Qian, Eric T. Wang
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Steric-blocking antisense oligonucleotides (ASOs) rescue Myotonic Dystrophy type 1 (DM1) disease phenotypes in preclinical models and are currently being tested in Phase 1/2 clinical trials. In this study, we report that a cyclic cell-penetrating peptide that escapes endosomes (endosomal escape vehicle, EEV) enhances muscle delivery of oligonucleotides, specifically a CAG7 phosphorodiamidate morpholino oligomer (PMO) in the HSALR mouse model of DM1 and in differentiated DM1 myoblasts. In mice, a single intravenous administration rescued mis-splicing and eliminated myotonia one week after injection, with partial splicing rescue as early as one day after injection for some exons. We reanalyzed previously published datasets to define the relationship between [MBNL] and Ψ for each exon in mouse quadriceps and found that these dose response curves could not fully explain the extent to which each exon was rescued. Since transcripts present prior to EEV-PMO administration must be degraded in order to reveal full drug effect, we hypothesized that rate of replacement accounts for this discrepancy. We formulated a mathematical framework that accounts for the relationship between [MBNL] and Ψ and the rate of transcript replacement and used Bayesian inference to model how observed Ψ lags behind newly transcribed, or nascent Ψ, as a function of time; faster rates of replacement result in shorter lags. We validated our model with pharmacokinetic measurements and RNAseq of nascent transcripts. Overall, this fast-acting EEV-PMO rescued DM1 phenotypes and revealed how various factors, including transcript turnover, influence Ψ during periods of dynamic shifts in free [MBNL]. Careful selection of biomarkers based on these factors could allow for earlier biopsy or tissue collection, within days instead of weeks, expediting assessments of target engagement and efficacy. At later time points, monitoring of early-responding biomarkers could inform redosing strategies.

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Young Investigator!

Poster # 957

Metabolic defects as a promising therapeutic target in DM1

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Objectives: The CTG repeat expansion in the DMPK gene disrupts the regulation of RNA-binding proteins and alternative splicing in Type I Myotonic Dystrophy (DM1), accounting for the deregulation of many major cellular pathways, including AMPK signaling. AMPK is the main metabolic sensor and regulator

and plays a key role in maintaining muscle homeostasis and regenerative capacities. We hypothesize that the repression of AMPK signaling in DM1 affects the metabolic regulation of myogenesis. This study aims to characterize the metabolic defects in DM1 muscle cells and evaluate the potential therapeutic benefits of AMPK activators on their myogenic capacities.
Methodology: Human immortalized DM1 myoblasts (D. Furling, Myology Institute, Paris, France).
Results: DM1 muscle cells exhibited significant defects in differentiation and fusion, partially improved after pharmacological activation of AMPK by the allosteric activator Compound 991. AMPK activation capacity was assessed by Western Blot and the AMPKAR-EV biosensor. Additionally, we utilized innovative techniques such as Scenith (an anti-puromycin antibody-based flow cytometry method) and the iATPs metabolic biosensor to evaluate energy production. DM1 cells were unable to increase oxidative metabolism in a galactose medium, indicating a defect in energy production flexibility. Furthermore, the mitochondrial membrane potential was significantly deregulated. 3D reconstruction of the cellular mitochondrial network showed altered morphology in DM1 myoblasts, with preliminary evidence suggesting that AMPK activation partially restored the mitochondrial phenotype.
Conclusion: Human DM1 cells demonstrated impaired AMPK signaling and impaired myogenic capacities that were partially improved by pharmacological activation of AMPK. In addition, human DM1 muscle cells exhibit metabolic flexibility defects and changes in mitochondrial structure and function that most likely participate in their low myogenic and regenerative capacities.
Practical implications: Activating AMPK represents a promising strategy for addressing these metabolic defects and improving muscle regeneration in DM1.

Young Investigator!
Poster # 981
Feasibility of remote assessments in congenital and childhood myotonic dystrophy
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Objective: Assess the feasibility and reliability of remote assessments of disease manifestations in children with congenital (cDM) and childhood-onset DM1 (chDM).
Methods: Toolkits were mailed to subjects. Remote study visits (RSV) included an interview followed by motor, neuropsychologic, and multisystem assessments. Repeat RSV participation was voluntary. Feasibility was defined as >80% completion of tasks. Reliability was analyzed using intraclass correlation coefficients and Bland-Altman plots.
Results: 19 subjects (11 cDM, 8 chDM) have enrolled to date. 18 completed initial RSV, and 13 completed repeat RSV. Assessment completion depended on age and disease severity. Preliminary data analysis will be presented.
Conclusion: Performing remote assessments in cDM and chDM is feasible, as shown by successfully establishing a workflow to obtain remote consent, shipping toolkits containing necessary equipment, and conducting the RSV. The feasibility and reliability of specific remote assessments may depend on disease severity and caregiver support.
Implications: Myotonic dystrophy type 1 (DM1) is a highly variable genetic disease with a broad age of onset and multiorgan involvement. The adult DM1 phenotype is well described, but pediatric data are limited due to small studies and narrow phenotypic features evaluated. Mobility issues, systemic symptoms, and affected caregivers can make participation in research studies challenging for children with DM1. RSV can mitigate these barriers and enhance research participation.
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Poster # 996

A Phase 1/2 Trial (Galileo Study) of VX-670 in Adults with Myotonic Dystrophy Type 1 (DM1): Study Design

Authors: Hanns Lochmuller, Christopher Turner, Fares Nigim, Phillip Larimer, Asif Paker, Nicholas E Johnson

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Objective: The safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of VX 670, an investigational oligonucleotide (PMO) conjugated to a cyclic peptide, is being evaluated in an ongoing Phase 1/2 trial in adults with DM1.

Methodology: This 2-part, randomized, double-blind, placebo-controlled trial evaluates safety, tolerability, and PK of single ascending doses (SAD) of VX-670 or placebo in Part A and evaluates safety, tolerability, PK, and PD of multiple ascending doses (MAD) of VX-670 or placebo in Part B. Approximately 36 adults aged 18 to 64 years with confirmed diagnosis of DM1 will be enrolled. Eligible participants will have the opportunity to continue receiving VX 670 in an open-label follow-up study.

Results: Enrollment is completed for Part A (SAD) and is ongoing for Part B (MAD). Part B will provide data on safety, tolerability, PK, and splicing in muscle biopsies.

Conclusions: This trial will generate critical early data to support the development of VX-670 as a disease-modifying therapy for DM1.

Practical Implications: DM1 is a genetic disorder caused by expanded CTG repeats in the myotonic dystrophy protein kinase (DMPK) gene, leading to RNA toxicity and splicing defects. VX-670 is specifically designed to enhance cellular delivery and nuclear targeting to address delivery challenges associated with oligonucleotide therapies. By binding to mutant DMPK RNA, VX-670 releases sequestered splicing factors such as MBNL1, correcting RNA mis-splicing and hence has the potential to address the root cause of DM1.

Poster # 998

Discovery of Small Molecules that Bind CUG Repeats, Displace Muscleblind Protein, and Improve Pathogenesis of Myotonic Dystrophy Type 1

Authors: Sarah Mahoney, Emily Garcia Sega, Karthik Iye, Xuan Yang, Amy Andreucci, Sundar Thangapandian, Griffin Schroeder, Zach Boswell, Ewa Stypulkowski, Amit Shahar, Pallavi Awate, Wan Yee Leong, Alexandre Amlie-Wolf, Rishabh Narula, Timothy Demers, Pooja Joshi, Stephen Doris, Blessy Suresh, Jennifer Petter

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Myotonic dystrophy type 1 (DM1) is a form of muscular dystrophy and a genetic neuromuscular disease affecting at least 1 in 8,000 people worldwide, or approximately 45,000 people in the United States. It is a multi-system disease, affecting the skeletal muscle, heart, diaphragm, central nervous system, and gastrointestinal tract. DM1 is caused by a trinucleotide (CUG) repeat expansion of the RNA encoding myotonic dystrophy protein kinase (DMPK), resulting in the formation of nuclear aggregates that bind and sequester splicing factors such as Muscleblind-Like Splicing Regulator 1 (MBNL1). Depletion of critical splicing factors leads to global splicing abnormalities and widespread pathology. There are no approved disease-modifying treatments for DM1, but several muscle-targeted oligonucleotide therapies are in the early stages of clinical development. These therapies show evidence of addressing skeletal

muscle defects in patients but are unlikely to fully address systemic manifestations of the disease. Here we present preclinical data on RNA-targeted small molecules (rSM) that selectively bind the pathogenic CUG repeat RNA and release MBNL1 from nuclear aggregates. In DM1 donor cells with 2,600 CTG repeats, rSMs reduce nuclear aggregates by 90% and correct splicing defects in a dose-dependent manner. rSMs also modulate splicing in skeletal muscle and completely reverse myotonia in the HSALR mouse model. Although muscle pathology is a significant component of DM1, an orally bioavailable, broadly biodistributed rSM has the potential to address systemic manifestations of the disease beyond skeletal muscle and establish that rSMs represent a new class of small-molecule genetic medicines that directly address the genetic basis of disease.