ABSTRACT POSTER NUMBER: 1

Observational study in adults with spinal muscular atrophy treated with Risdiplam

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INTRODUCTION:

Risdiplam was approved for SMA by Health Canada in 2021. Quebec is the only province to offer full access (all ages and types).

The aim of this study is to monitor long-term outcomes and safety profiles in SMA adults receiving Risdiplam at IRDPQ, Quebec.

METHODS:

Baseline characteristics (N= 11):

- Age at initiation : 17-53 years
- Type:
  - 2: seven
  - 3: three
  - 4: one
- Actual functional status:
  - Non sitter: eight
  - Sitter: one
  - Walker: two

Follow-up: 0.12, 24, 36 months

Assessment tools:

- Motor function: RULM, ATEND, HFMSE, 6MWT
- Pulmonary function: FVC, PCF
- PROM: SMAFRS, SMAIS

RESULTS :

Safety profile:

- the majority of patients had minor digestive symptoms at the start of treatment, but all disappeared after the first weeks.
- three patients experienced severe and disabling upper limb weakness during the first weeks. This weakness resolved on its own within the first six months for two patients. Treatment had to be interrupted for the third.
- three patients showed significant weight gain between 20 and 40 kg. Treatment was interrupted for one of them. The causal link has not yet been established.

Since the approval in 2021,

- 8/11 patients demonstrated stability or improvement in motor function.
- 7/11 patients demonstrated stability or improvement in their PROM.
- the majority of patients demonstrated stable lung function.
- 2 patients stopped the treatment because of disabling weakness in the upper limbs or significant weight gain.

CONCLUSION:

Risdiplam is generally well tolerated. Monitoring for adverse events is recommended to anticipate the appearance of upper limb weakness and weight gain, particularly in non-walkers.

ABSTRACT POSTER NUMBER: 2

A Five-Year Update on a Real-World Study of Canadian Adults with Spinal Muscular Atrophy Type 2 and 3 Treated with Nusinersen

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In 2018, nusinersen was approved in the province of Quebec for adult and pediatric patients with SMA as the first disease-modifying agent. Although more thoroughly studied in pediatric settings, long-term effects in the adult population have yet to be well explored.

Annual clinical assessments include: HMFSE, RULM, 6MWT, ATEND 3.0, SMAIS, PCF, FVC and PROM.

RESULTS:

Longitudinal data were collected from 19 adult patients, mainly type 3 (N=14), and included 8 walkers, 6 sitters, and 5 non-sitters. One patient had to discontinue treatment due to debilitating sciatalgia.

Regarding motor function tests, 13 out of 18 patients showed stabilization or improvement. Stability of lung function was observed. As for self-reported subjective changes, 16 patients reported improvement in at least one category; strength and energy being the two most frequent areas of positive progress. Only SMAFRS reached statistical significance.

CONCLUSIONS:

- After up to five years of treatment, 72% of patients experienced stabilization or improvement of motor function.
- Stabilization of lung capacity was observed.
- 89% of patients reported subjective improvement in their symptoms, mainly in terms of strength and energy levels.

Given these results, further research on bulbar function assessment tools would be crucial.

Due to the floor effect, ATEND 3.0 and SMAIS appeared more suitable for patients with more severe phenotypes (non-walkers).

ABSTRACT POSTER NUMBER: 3

Positive predictive value of myositis antibody line blot testing in patients with suspected idiopathic inflammatory myopathy

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INTRODUCTION/AIMS:

Line blot (LB) is in widespread use for myositis antibody detection. Yet, studies of its positive predictive value (PPV) in patients with suspected idiopathic inflammatory myopathy (IIM), which would be of particular relevance to neuromuscular clinicians, are lacking. We aimed to determine the PPV of myositis antibody LB testing in patients with suspected IIM, and examine whether PPV was significantly impacted by intensity of antibody positivity.

METHODS:

This was a retrospective study of patients who underwent myositis antibody LB testing for suspected IIM between March 2019 and August 2022.

RESULTS:

Of 70 patients who underwent testing for suspected IIM and had positive myositis antibody LB results, 43 (61%) were female and the median age was 61 years (range: 10-83 years). Forty-four were classified as true-positives, yielding a PPV of 63%. The PPV of patients with weak-positive myositis antibody results (14/30, 47%) was significantly lower than the PPV of patients with moderate-positive or strong-positive myositis antibody results (30/40, 75%) ($p = .02$).

DISCUSSION:

Our study found that myositis antibody LB testing in patients with suspected IIM had a modest PPV, underscoring the need for antibody interpretation in the context of all available clinical and ancillary test data to avoid misdiagnosis. The significantly lower PPV in patients with weak-positive results emphasizes the particular importance of clinical correlation in such patients. Further study into the diagnostic performance of various LBs for myositis antibody detection is needed to inform their interpretation in clinical practice.

ABSTRACT POSTER NUMBER: 4

NADMED: Targeted REDOX profiling for clinical and research use

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Nicotinamide adenine dinucleotide (NAD) and its derivatives are drivers of cellular metabolism, connecting hundreds of metabolic reactions and maintaining their direction and rate. NADs are bodily forms of vitamin B3 and are recognized for their housekeeping functions. They are shown to be implicated in various pathologies and viewed as actionable targets for intervention. Accurate detection of these metabolites is critical for establishing significance of NAD molecules in health and disease and for implementation into clinical use.

Here we present a standardized high-throughput REDOX profiling technology for measurement of NAD^+, NADH, $NADP^+$, NADPH, and both oxidized and reduced glutathiones (GSH and GSSG) in various biological matrices including fresh or frozen whole blood.

Using the NADMED methodology, we analyzed 300 whole blood samples and established reference ranges in a healthy population. For clinical proof-of-concept, we applied NADMED technology to perform blood REDOX profiling in patients with PEO mitochondrial myopathy—a progressive disorder characterized by impaired NAD metabolism. These patients exhibit reduced NAD^+ levels in skeletal muscle, and importantly, this decline is mirrored in blood NAD^+ levels. This proof-of-concept supports the broader potential of the test as a clinical tool to probe REDOX components in various pathologies, offering a pathway to leverage targetable metabolites for disease modification.

We present an efficient technology for measuring REDOX metabolites, suitable for clinical and research use. This tool enables improved understanding and monitoring of conditions involving mitochondrial dysfunction, neurodegeneration, and metabolic imbalance—advancing the potential for personalized medicine in REDOX-related disorders.

ABSTRACT POSTER NUMBER: 5

Rewiring myogenic cell identity: Comparative analysis of PAX7 and PAX7-FOXO1 in Rhabdomyosarcoma and muscle progenitors

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Alveolar rhabdomyosarcoma (ARMS) is an aggressive pediatric cancer originating from skeletal muscle progenitor cells, vital for muscle growth and regeneration. These myogenic cells rely on tightly regulated transcriptional programs, governed by factors like PAX7, to coordinate their proliferation and differentiation. In ARMS, chromosomal translocations fuse PAX7 with FOXO1, generating the chimeric transcription factor PAX7-FOXO1, which disrupts these programs and reprograms muscle progenitors toward malignancy.

Our research investigates how PAX7-FOXO1 alters muscle progenitor function by comparing its transcriptional and protein interaction landscapes to those of native PAX7. Using human myoblasts, which model skeletal muscle progenitors, we ectopically express PAX7-FOXO1 to identify fusion-specific versus conserved gene signatures. concurrently, we employ siRNA knockdown in PAX7–FOXO1–positive RMS cells to assess the impact of silencing the fusion on both myogenic and oncogenic transcriptional networks.

To explore changes in protein interactions, we apply BioID2 proximity labeling to map the interactomes of PAX7 and PAX7-FOXO1 directly in rhabdomyosarcoma cells. Comparative analysis will highlight cofactors that are either recruited or lost upon fusion, revealing mechanisms that may drive proliferation and block differentiation. Functional assays, which examine proliferation, migration, and differentiation, will help determine the phenotypic outcomes of these molecular alterations.

This study positions PAX7-FOXO1 as an oncogene and a rogue regulator of muscle progenitor fate. By contrasting its activity with that of wild-type PAX7, we aim to identify fusion-specific vulnerabilities and uncover opportunities to restore normal regenerative pathways, offering novel therapeutic insights at the intersection of cancer biology and regenerative medicine.

ABSTRACT POSTER NUMBER: 6

Thoracic Electric Impedance Tomography Detects Lung Volume Changes in Amyotrophic Lateral Sclerosis

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INTRODUCTION/AIMS:

Spirometry is the conventional means to measure lung function in amyotrophic lateral sclerosis (ALS), but is dependent on patient effort and bulbar strength. We aimed to use electric impedance tomography (EIT), an emerging non-invasive imaging modality, to measure dynamic lung volume changes.

METHODS:

Twenty-one patients with ALS underwent sitting and supine spirometry for forced vital capacity (FVC), and sitting and supine EIT. There were 13 patients in the high FVC group (FVC ≥80% predicted) and 8 in the low FVC group (FVC <80% predicted). Additional demographic and clinical data were collected from clinical records.

RESULTS:

Only the low FVC group had significant loss of lung volumes in the supine position ($R^2 = 0.89$ and $p < 0.001$). The supine volume loss measurement at 10 minutes correlated with sitting ($r^2 = 0.47$) and supine FVC ($r^2 = 0.36$), maximum inspiratory ($r^2 = -0.44$) and expiratory pressures ($r^2 = 0.36$) (MIP, MEP), and the ALS Functional Rating Scale-Revised (ALSFRS-R) dyspnea subscore ($r^2 = 0.36$).

DISCUSSION:

EIT is an emerging alternative to existing measures of lung function in ALS, but without need for patient effort or bulbar strength. Significant losses in lung volume are seen on supine compared to upright position in patients with respiratory dysfunction. Further study is needed to determine relationships to existing clinical measures.

ABSTRACT POSTER NUMBER: 7

Best practice recommendations for the clinical care of spinal bulbar muscular atrophy

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BACKGROUND:

While rare in the general population, spinal bulbar muscular atrophy (SBMA) is a neuromuscular condition highly prevalent in people identifying as Indigenous in western Canada.

METHODS:

A needs assessment survey aided in development of topic questions, followed by a literature search, evidence review by interdisciplinary working group members, and external review by health practitioners and persons with lived experience. We followed the ADAPTE framework to evaluate the only pre-existing SBMA guideline (2020 French protocol) for appropriateness of adaptation. Our process adhered to the Appraisal of Guidelines for Research and Evaluation (AGREE II) tool, utilized the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach, and followed the McMaster checklist for guideline development. Indigenous community engagement was led by the Pewaseskwan Indigenous Research Group who participated in guideline development.

RECOMMENDATIONS:

Forty-one recommendations were developed to address the continuum of care in SBMA including diagnosis; multidisciplinary teams; management of limb and bulbar symptoms, respiratory and cardiac complications, and multisystem symptoms; female carriers; emotional supports; and considerations for Indigenous people. SBMA is best managed by multidisciplinary teams that can address both the motor and non-motor manifestations of the condition including cardiac involvement, sensory symptoms, and metabolic dysfunction. Concerns for female carriers may include symptom management and genetic counselling. Providers should ensure culturally appropriate care for Indigenous people.

INTERPRETATION:

This guideline is meant to raise awareness and provide health care professionals with a culturally responsive standard of care which we hope will translate into improved quality of life for persons affected by SBMA.

Longitudinal analysis of glymphatic function in amyotrophic lateral sclerosis and primary lateral sclerosis

Sharkey RJ[1], Cortese F[1], Goodyear BG[1], Korngut LW[1], Jacob SM[1], Sharkey KA[1], Kalra S[1], Nguyen MD[1], Frayne R[1], Pfeffer G[1]. **Presented by: Soule, T.**
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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder of motor neurons in the brain and spinal cord. Accumulation of misfolded proteins is central to the pathogenesis of ALS and the glymphatic system is emerging as a potential therapeutic target to reduce proteinopathy. Using diffusion tensor imaging analysis along the perivascular spaces (DTI-ALPS) to assess glymphatic function, we performed a longitudinal analysis of glymphatic function in ALS and compared it to a disorder in the motor neuron disease spectrum, primary lateral sclerosis (PLS). From a cohort of 45 participants from the Calgary site in the CALSNIC study (Canadian ALS Neuroimaging Consortium), including 18 ALS, 5 PLS and 22 control participants, DTI-ALPS was analysed and correlated to clinical features (age, sex, disease presentation, disease severity and progression rate) and white matter hyperintensity burden. This included longitudinal measurements at three time points, 4 months apart. The DTI-ALPS index was reduced in ALS participants compared with PLS and control participants across all three time points. There was no association with clinical factors; however, the index tended to decline with advancing age. Our study suggests heterogeneity in glymphatic dysfunction in motor neuron diseases that may be related to the underlying pathogenesis.

ABSTRACT POSTER NUMBER: 9

Higher than expected incident cases of spinal bulbar muscular atrophy in western Canada

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Spinal bulbar muscular atrophy (SBMA, or Kennedy disease), is an X-linked, genetic neuromuscular disorder. The mutation is a CAG expansion in exon 1 of AR, which results in a toxic effect of the protein upon exposure to androgens. Hemizygous males develop disease affecting multiple systems including a progressive motor neuron disease, endocrine dysfunction, androgen insensitivity, and cardiac disease among others. We have previously described a high prevalence of SBMA in Saskatchewan due to a founder effect, and followed up this work with a survey of molecular diagnostic lab results in Calgary, which performs testing for SBMA in Alberta, Saskatchewan, and Northwest Territories. After surveying results over 5.5 years since Calgary began performing this testing (January 2018-July 2023), the incident case rate was 0.89/100,000 population in Alberta, 1.62/100,000 population in Saskatchewan, and 2.23/100,000 in Northwest Territories. Incidence case rates are not available for other populations, but when comparing total prevalence, this has typically been estimated at 1-2/100,000. As presented the data suggest the total prevalence in our regions must be much higher than this, and ongoing research will attempt a more comprehensive ascertainment of this.

The oral microbiome in ALS shows differentially abundant organisms in limb versus bulbar onset disease: a binational study

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of upper and lower motor neurons leading to progressive disability and death. Approximately 10% of cases are caused by single-gene disorders with the remaining 90% of cases presumed to be caused by a combination of environmental and genetic factors. The microbiome (the ensemble of microorganisms which colonize body surfaces and organs) was recently identified for its importance in the pathogenesis of ALS. In this study, we recruited 100 participants from two ethnically and geographically distinct sites (71 from Calgary, Canada, and 29 from Seoul, Republic of Korea) which included 59 ALS participants and 41 controls. All participants provided saliva samples for oral microbial analysis using 16S rRNA sequencing. Basic demographic information was collected from all participants, and ALS participants provided additional clinical information including site of disease onset, disease duration, and ALSFRS-R score. Significant differences in beta diversity of the oral microbiomes were seen between limb- and bulbar-onset ALS participants. Three bacterial species were differentially abundant between these groups, Bifidobacteriaceae Bifidobacterium was enriched in bulbar-onset cases, while Pasteurellaceae Haemophilus and Vagococcaceae Vagococcus were enriched in limb-onset cases. No significant differences were found between ALS participants and controls, but there were significant differences when comparing participants from different sites of recruitment. Amongst household pairs (n = 35 pairs), ALS participants differed from control participants at the Seoul site. Despite the cohort and household effects, our study identified differentially abundant organisms that may be important to the phenotypic variability of ALS and should be considered for future study. Our study provides novel insights into design for future multi-site microbiome research in ALS.

Results of VBP15-006: A Phase 2, Open-Label, Multiple Dose Study of Vamorolone in Boys With DMD Aged 2 to <4 and 7 to <18 Years

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Vamorolone, a novel corticosteroid (CS), is approved to treat boys with Duchenne muscular dystrophy (DMD) in the US, Europe, and UK.

VBP15-006 was a phase 2, open-label, multiple-dose study in 54 DMD patients. Those aged 2 to <4 years were CS-naïve and received vamorolone 2 mg/kg/day (d) (n=10) or 6 mg/kg/d (n=10). Those aged 7 to <18 years were either CS-naïve and received vamorolone 2 mg/kg/d (n=6) or 6 mg/kg/d (n=6), or CS-treated and received vamorolone 2 mg/kg/d (n=6) or 6 mg/kg/d (n=16).

All participants completed the study. Median prior CS exposure for age 7 to <18 years CS-treated boys was 54.1 months (vamorolone 2 mg/kg/d) and 93.1 months (vamorolone 6 mg/kg/d). Baseline comorbidities in CS-treated boys included fractures, cataracts, psychiatric and endocrine disorders. At baseline, severe growth delay (median height percentile 1.6 [0.2; 16.8]) was reported for the age 7 to <18 years CS-treated boys allocated to receive vamorolone 6 mg/kg/d. Vamorolone showed dose-dependent pharmacokinetics at doses of 2–6 mg/kg/d, with maximum concentration reached within 2–4 hours and a half-life of 2 hours. Exposures were consistent with moderate variability at both doses after single and multiple dosing in both age groups. Dose-dependent increases in adverse events were seen across all age groups; none led to study discontinuation or death. These results support the potential use of vamorolone across a broad age range in boys with DMD.

ABSTRACT POSTER NUMBER: 12

Modeling Fetal Acetylcholine Receptor Inactivation Syndrome (FARIS) Using Patient-Derived iPSCs to Elucidate Pathogenesis and Identify Therapeutic Targets.

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Fetal Acetylcholine Receptor Inactivation Syndrome (FARIS) is a rare neuromuscular disorder caused by maternal antibodies targeting the fetal γ subunit of the acetylcholine receptor (AChR), potentially leading to severe congenital muscle weakness despite maternal asymptomatic status. Current knowledge of FARIS is limited, and no established model exists to study its pathogenesis or explore therapeutic strategies. We propose a novel approach to model FARIS using a combination of serological, cellular, and transcriptomic methods. Serum from a mother of a FARIS-affected infant will be analyzed for AChR antibodies using radioimmunoassay, followed by immunofluorescence on HEK cells transfected with fetal or adult AChR isoforms to determine antigen specificity. To investigate the functional impact on neuromuscular development, we generated induced pluripotent stem cells (iPSCs) from patient-derived mononuclear blood cells. We differentiated them into myogenic and neural lineages to form neuromuscular junctions (NMJs). Immunostaining reveals that this model specifically expresses the fetal γ subunit of the AChR. Exposure of these cultures to serum antibodies will allow assessment of NMJ integrity and myofiber formation using immunofluorescence and automated image analysis. Co-culture with Jurkat T cells will test for immune-mediated cytotoxicity, while purified myogenic populations will determine direct antibody effects. Single-cell RNA sequencing will elucidate antibody-induced transcriptional changes in muscle and neuronal cells, identifying disrupted signaling pathways and novel therapeutic targets. This study will establish the first in vitro model of FARIS and provide critical mechanistic insights, paving the way for the development of targeted treatments.

ABSTRACT POSTER NUMBER: 13

Sustained functional improvement with DYNE-251 in males with DMD mutations amenable to exon 51 skipping enrolled in the Phase 1/2 DELIVER trial

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In DMD, absence of functional dystrophin leads to progressive functional decline. DYNE-251, an investigational therapeutic, leverages the transferrin receptor 1 to deliver an exon 51-skipping PMO to affected tissues, with the goal of achieving functional improvement. The safety and efficacy of DYNE-251 are studied in the Phase 1/2 DELIVER trial (NCT05524883) in 4-16 year-olds. The multiple ascending dose (MAD) portion is completed; participants are currently receiving 20 mg/kg DYNE-251 in the OLE/LTE. 20 mg/kg Q4W was selected as the dose regimen for the DELIVER registrational expansion cohort, which is fully enrolled.

In participants who received 20 mg/kg Q4W in the MAD portion, DYNE-251 led to robust expression of near full-length dystrophin at Month 6. Functional improvement vs baseline in SV95C was evident by Month 6 and sustained through Month 12 in participants treated with 20 mg/kg Q4W DYNE-251 (n=6) and through Month 18 in participants who enrolled at 10 mg/kg Q4W and transitioned to 20 mg/kg Q4W DYNE-251 in the OLE/LTE (n=6). Early and sustained functional improvement vs baseline was seen in both cohorts for other motor function endpoints, including NSAA, TTR, and 10MWR. As of February 7, 2025, DYNE-251 had a favorable safety profile, with up to ~2.5 years of follow-up in some participants.

DYNE-251 has a favorable safety profile and has shown consistent early and sustained functional improvement vs baseline across multiple clinical and real-world functional assessments.

Novel prodrug improves locomotor disfunction in gne deficient zebrafish

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The *GNE* gene encodes a bifunctional enzyme critical in the sialic acid biosynthesis pathway. This enzyme has two key catalytic domains: UDP-GlcNAc 2-epimerase and ManNAc kinase which catalyze the first two steps in the production of N-acetylneuraminic acid (Neu5Ac). Mutations in *GNE* reduce sialic acid levels, impairing normal muscle function and lead to GNE myopathy (GNEM). Neu5Ac and its precursors, such as ManNAc - which bypass the catalytic step performed by UDP-GlcNAc 2-epimerase, have been targeted as therapeutic options for patients with GNEM. In this study, we utilized the zebrafish morpholino knockdown and CRISPR/Cas9-generated knockout models of GNEM (*gne* KO) to verify a pre-clinical prodrug approach aiming to efficiently restore locomotor disfunction in these models. We found immediate, severe morphological deformities in addition to locomotor defects in the morphant model. The *gne* KO has les pronounced morphological changes but shows locomotor phenotype from 12 days post fertilization (dpf) and eventually dies around 15 dpf. Daily application of the selected prodrug candidate partially restores severe morphological deformities in the *gne* morphant and partially restores locomotor dysfunction in *gne* KO zebrafish. These are the first data demonstrating significant rescue of the locomotor phenotype in an *in vivo* model of GNEM and suggest our prodrug approach has efficacy. Further refinement of the treatment regime should prove a valuable contribution towards completing pre-clinical validation of this prodrug.

ABSTRACT POSTER NUMBER: 15

Galactose treatment rescues neuromuscular junction transmission in glutamine-fructose-6-phosphate transaminase 1 (Gfpt1) deficient mice

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Congenital myasthenic syndromes (CMS) arise from mutations to proteins involved in neuromuscular junction (NMJ) development, maintenance, and neurotransmission. To date, mutations in more than 35 genes have been linked to CMS. Glutamine fructose-6-phosphate transaminase 1 (*GFPT1/Gfpt1)* serves as the rate-limiting enzyme of the hexosamine biosynthetic pathway (HBP), producing the byproduct (UDP-GlcNAc) necessary for protein glycosylation. Gfpt1-deficient models have impaired protein glycosylation, impacting key proteins at the NMJ. Previously, we demonstrated that Gfpt1-deficient expression resulted in the hypoglycosylation of delta (δ) subunit of the acetylcholine receptor (AChRδ) at the NMJ. The Leloir pathway is a galactose metabolizing pathway which produces UDP-GalNAc as its final product. The enzyme UDP-GalNAc Epimerase (*GALE)* can also convert excess UDP-GalNAc into UDP-GlcNAc, the byproduct of the HBP. We hypothesized that treatment with galactose both *in vitro* and *in vivo* in Gfpt1-deficient models would rescue impaired protein O-GlcNAcylation and reverse the glycosylation status of key NMJ-associated proteins. We show that galactose treatment *in vitro* activated the Leloir pathway and rescued protein O-GlcNAcylation in Gfpt1-deficient C2C12 myoblasts. In addition, we demonstrated that galactose rescued neuromuscular deficits, improved muscle fatigue and restored NMJ morphology in a skeletal muscle-specific Gfpt1 knockout mouse model. Lastly, we showed that galactose treatment rescued protein O-GlcNAcylation in skeletal muscle, preserving the glycosylation status of the AChRδ. Taken together, we suggest that galactose supplementation can be further explored as a therapy for *GFPT1*-CMS patients.

ABSTRACT POSTER NUMBER: 16

Unraveling the Function of MLIP in Myogenic Cells: Toward Understanding Its Role in Rare Myopathy

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Muscle LMNA-Interacting Protein (MLIP)-related myopathy is an exceptionally rare neuromuscular disorder. Although most cases manifest in childhood, recent adult-onset reports suggest a broader phenotypic spectrum than previously recognised. Experimental data link MLIP deficiency to defective myogenic differentiation and disturbed cardiac metabolism. At the molecular level, MLIP binds lamin A/C and is thought to bind DNA, implying a role in transcriptional regulation; nevertheless, its precise function remains undefined.

To address this gap, our project pursues two objectives. 1- We determined the expression pattern of MLIP during myogenesis. Our results indicate a progressive rise in MLIP transcript and protein level during myogenic differentiation. We also employed immunofluorescence to establish MLIP's subcellular distribution and co-localization with lamin A/C. Strikingly, we observed that MLIP expression is not restricted to the nuclear compartment as previously anticipated, but is also present in the cytoplasm during myogenesis. 2- We aim to map the interactome of MLIP in myogenic cells. **To this end, a** BioID2-MLIP fusion is being engineered to biotinylate neighbouring proteins in myogenic cells. High-confidence interactors identified by LC-MS/MS will be confirmed by co-immunoprecipitation. In parallel, AlphaFold3 structure predictions will prioritise candidate interfaces and generate mechanistic hypotheses for functional testing.

By integrating transcriptional, proteomic, and structural datasets, the study will yield the first comprehensive view of MLIP's functional network in skeletal muscle. These insights will illuminate the molecular pathogenesis of MLIP-associated distal myopathy and uncover tractable targets for therapy across a wider range of neuromuscular diseases.

ABSTRACT POSTER NUMBER: 17

Correcting mutations responsible for LGMD R2/2B (dysferlinopathy) with Prime editing

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Dysferlinopathy is a recessive Limb-gridle muscular dystrophy (LGMD) caused by mutations in the *DYSF* gene. My PhD project aims to correct point mutations in *DYSF* using Prime editing (PE). This technique uses a Cas9 nickase fused to a reverse transcriptase and a pegRNA encoding the desired edit.

I designed pegRNAs to correct patient mutations in the *DYSF* gene and I achieved up to 31% editing in HEK293T cells and 11% in patient-derived myoblasts after one treatment. This is promising since a healthy carrier of a recessive LGMD only has 50% of the correct DNA and research shows that reaching 20% expression could remove the symptoms.

I also noticed that for correcting different myopathies in vitro, using myoblasts instead of fibroblasts leads to more editing. I also verify that my treatment will not modify other genes.

The next step is to use a new *DYSF*-R1925X mouse model. I validated the *DYSF* mutation and protein absence and I verify if the symptoms of the R1925X mice are comparable to the patients' symptoms to then test the Prime editing treatment on the mouse and see its impacts on the symptoms.

In collaboration with clinicians in the Province of Quebec, I conduct a census of Quebec LGMD mutations. I already noticed a founder effect for specific mutations and it coincides with the population movements through history.

In conclusion, I developed PhD project to learn more about the rare disease affecting my family and hope to help many other families.

ABSTRACT POSTER NUMBER: 18

Muscle-specific allelic imbalance of IARS1 unmasks a novel compound heterozygous mechanism in congenital myopathy

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Congenital myopathies are diagnostically challenging and genetically heterogeneous disorders. We investigated a familial case comprising of three affected siblings born to unaffected parents. Clinical features included generalized hypotonia, while muscle biopsies revealed fiber atrophy with centralized nuclei.

A combination of whole-exome and muscle RNA sequencing enabled the identification of a tissue-specific allelic imbalance of the isoleucyl-tRNA synthetase (IARS1) gene. Variant analysis revealed a compound heterozygous inheritance pattern in IARS1, whereas the mother carried a heterozygous missense variant (p.R661H) while an intronic variant near exon 25, a known methylation region, was uncovered in the father through whole-genome sequencing. In the affected children, transcriptomic data revealed a near-exclusive expression of the maternal pathogenic allele in muscles, suggesting a tissue-specific silencing of the paternal allele. The resulting pseudo-homozygous state explains the congenital myopathy in the children despite the asymptomatic carrier status of the mother and the absence of additional de novo variants.

To validate IARS1's role in muscle function, we showed that muscle-specific knockdown of its ortholog in C. elegans causes progressive sarcomeric disorganization. To dissect the cellular consequences of the variants, we established human cell models of IARS1-knockout myoblasts and myotubes transdifferentiated from patient fibroblasts for mechanistic studies. We performed functional assays to determine the downstream effects of impaired IARS1 function, including cellular metabolism (WST-1), proliferation (IncuCyte), and enzyme kinetics (aminoacylation).

This work highlights a novel muscle-specific, epigenetically modulated compound heterozygous inheritance mechanism for an AARS-related myopathy and provides insights into how disruptions in protein synthesis lead to a specific muscle pathology.

ABSTRACT POSTER NUMBER: 19

Systemic CRISPR/SaCas9-mediated exon skipping achieves long-term benefit in canine Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a severe X-linked disorder caused by mutations in the *DMD* gene, leading to absence of functional dystrophin. Genome editing offers a promising therapeutic approach, particularly exon skipping to produce truncated, functional dystrophin. While efficacy has been demonstrated in animal models, long-term outcomes remain unclear.

We examined the effects of genome editing in Canine X-linked Muscular Dystrophy (CXMD) with a point mutation at the splice acceptor site of exon 7, located in a hotspot N-terminal region of the DMD gene. We employed the CRISPR/Staphylococcus aureus Cas9 (SaCas9) system to excise exons 6-8 of the DMD gene, aiming to induce exon skipping and produce truncated dystrophin protein. An all-in-one AAV9 vector expressing SaCas9 under a muscle-specific promoter and dual guide RNAs targeting exons 6–8 was systemically administered to 4-week-old CXMD dogs.

One year post-treatment, persistent exon skipping and dystrophin expression were observed in skeletal and cardiac muscles. RT-PCR revealed exon skipping efficiencies of 1–22% in skeletal muscle and 5–33% in the heart. Western blot showed 1–5% dystrophin restoration in skeletal muscle and 2–17% in the heart. Histological analysis showed improved muscle structure with reduced fibrosis. Functional assessments indicated trends toward improved motor performance. No serious adverse effects were noted, although transient, mild elevations in liver enzymes occurred.

This study demonstrates that systemic CRISPR/SaCas9 delivery enables long-term dystrophin restoration and functional benefit in a canine model of DMD, supporting the potential of gene editing strategies for future clinical application.

ABSTRACT POSTER NUMBER: 20

Maternal transfer of disease modifying therapies for spinal muscular atrophy: a novel, minimally invasive approach to deliver SMN therapy prenatally

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Spinal muscular atrophy (SMA) is a neuromuscular disorder characterised by alpha motor neuron loss and muscle atrophy. Loss of the survival motor neuron 1 gene (*SMN1*) leads to depletion in survival motor neuron (SMN) protein. In patients treated with a disease modifying therapy (DMT), those treated early had the greatest therapeutic outcomes. While promising, this early post-natal treatment does not cure the disease. Previous research has found that *in utero* injections in the SMN **Δ**7 mouse model improve survival. While risdiplam has been administered to one human fetus with SMA demonstrating an ameliorating effect on the disorder. We aim to investigate two novel strategies (maternal transfer and *in utero* administration) to deliver DMTs prenatally using SMA mouse models (*Smn*$^{2B/-}$ and SMN**Δ**7). Our results demonstrate oral gavage of risdiplam to the dam during pregnancy does not impact dam weight. SMN**Δ**7 weight, motor function and survival are significantly increased following maternal transfer of risdiplam, with no treatment post birth. Additionally, we show AAV9-GFP can be maternally transferred from dam to pup. Live imaging, immunohistochemistry and immunoblot analysis demonstrate expression of GFP in dam liver, uterine horns and mammary glands. We also demonstrate pup survival following *in utero* surgery and embryonic intracerebroventricular injections. Overall, fetal administration of risdiplam, prolongs survival and improves motor function in SMN**Δ**7 mice. We conclude treatment administered within this narrow therapeutic time window, by maternal transfer or *in utero* injections, may be necessary for optimal therapeutic outcome.

ABSTRACT POSTER NUMBER: 21

Creating an open-access SOP resource for neuromuscular disease researchers

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The Neuromuscular Disease Network for Canada (NMD4C) pre-clinical science pillar supports fundamental neuromuscular disease (NMD) research across Canada, including research involving cell and animal models. A major initiative of this pillar is the development of an open-access database to share standard operating procedures (SOPs) related to neuromuscular disease research. We aim to cover a broad range of techniques related to molecular and cellular techniques, cell culture, animal models, bioinformatics, and human biopsies. These SOPs are curated by the NMD4C Basic Science Trainee Committee and reviewed by appropriate experts in the NMD field. Initial efforts include two widely used NMD techniques that were highlighted at our recent 2025 NMD4C Basic Research Summer School: single myofiber isolation and analysis and rodent muscle function assessment. As we develop this initiative, we look forward to feedback from the NMD4C community. By creating this SOP resource, our ultimate goal is to support open-access science, sharing of expertise, and reproducibility in NMD research.

A Canadian Perspective on the Diagnosis and Management of Mixed Phenotype Hereditary Transthyretin Amyloidosis1

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BACKGROUND:

Hereditary transthyretin amyloidosis (ATTRv amyloidosis) is caused by the aggregation of transthyretin (TTR) protein in tissues, most frequently in the heart and/or nervous system. As the presentation of ATTRv amyloidosis varies widely, diagnosis is challenging and frequently delayed. Furthermore, current guidelines lack specific guidance on the management of mixed-phenotype disease.

METHODS:

A pan-Canadian panel of six experts (three cardiologists and three neurologists) specializing in ATTRv amyloidosis was assembled to develop a practical guide for general and specialized cardiologists and neurologists on the management of mixed-phenotype disease. Three clinical questions were addressed: (1) Which patients should be screened for mixed phenotype ATTRv amyloidosis?; (2) How should patients with ATTRv amyloidosis with mixed phenotype be treated?; and (3) How should patients with mixed phenotype ATTRv amyloidosis be monitored pre- and post-treatment? Oral feedback from panelists were used to guide a literature search and develop a review.

RESULTS:

Key takeaways included the need for: (i) all patients with ATTRv amyloidosis to be screened for mixed phenotype disease through early multidisciplinary referral; (ii) prompt therapy selection and initiation, based on multidisciplinary collaboration; and (iii) establishment of a disease monitoring schedule pre- and post-treatment. Case studies were used to illustrate the nuances in the diagnosis, treatment, and monitoring of mixed phenotype ATTRv amyloidosis.

CONCLUSION:

To improve early diagnosis and management of mixed phenotype ATTRv amyloidosis, multiple specialties must be aware of the signs, symptoms, and treatment options for this disease.

ABSTRACT POSTER NUMBER: 23

Autosomal dominant rhabdomyolysis is associated with a missense variant in the ATP2A2 reducing SERCA2 calcium pump function in skeletal muscle.

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Rhabdomyolysis is a severe condition marked by skeletal muscle breakdown and the release of intracellular contents into the bloodstream, leading to acute myalgia and weakness. Among its various causes are genetic disorders, including metabolic and neuromuscular diseases (NMDs). We identified 14 affected individuals from three unrelated families, all carrying the same heterozygous missense variant in the *ATP2A2* gene (c.1583G>A; p.R528Q). Patients experienced recurrent rhabdomyolysis episodes, typically triggered by viral infections, fever, or intense physical activity. *ATP2A2* encodes SERCA2, a calcium pump crucial for intracellular Ca^{2+} homeostasis. In striated muscle, the SERCA2a isoform mediates Ca^{2+} reuptake into the sarcoplasmic reticulum (SR) during relaxation. We hypothesised that the p.R528Q variant impairs SERCA2a function, causing cytosolic Ca^{2+} overload and subsequent rhabdomyolysis. Functional studies were performed on patient-derived myotubes and zebrafish models (atp2a2a knockdown and CRISPR/Cas9 R528Q larvae). In vitro, patient myotubes showed delayed SERCA-mediated Ca^{2+} reuptake upon KCl stimulation. In vivo, atp2a2a morphants displayed abnormal morphology and impaired swimming. R528Q zebrafish showed no morphological abnormalities but exhibited consistently reduced locomotor performance. In slow muscle fibres of R528Q larvae, live calcium imaging revealed reduced Ca^{2+} transients during repeated electrical stimulation. Our findings demonstrate that the p.R528Q variant alters SERCA2a function and calcium homeostasis in skeletal muscle, predisposing to rhabdomyolysis. While heterozygous *ATP2A2* variants were previously linked only to autosomal dominant skin disorders, our study broadens the phenotypic spectrum by including a novel neuromuscular condition and provides a definitive diagnosis for the affected families.

ABSTRACT POSTER NUMBER: 24

Developing and optimizing a structured therapeutic platform for individualized gene editing therapies in DMD: Integrating therapeutic innovation and ethical implementation

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Individualized therapies offer new hope to patients with genetic and acquired conditions who previously had few or no treatment options. However, these advances bring profound technical, ethical and regulatory challenges, and it is essential to establish scientific and ethical frameworks that ensure these treatments reach clinical settings and meet patients' needs quickly and responsibly. This project aims to contribute to both scientific and ethical frameworks, recognizing these dimensions are intrinsically linked.

A new approach to individualized therapies envisions a therapeutic platform as a modular system where most components of the therapy remain constant across patients, while a few key elements are tailored to each individual. This framework has the potential to streamline development, accelerate regulatory approval, and improve patient access.

Our first focus is, therefore, to develop and evaluate such a therapeutic platform for patients with Duchenne Muscular Dystrophy (DMD), a rare neuromuscular disorder for which individualized therapeutics are rapidly advancing. Here, the objectives are to optimize a CRISPR/Cas9 system to correct DMD duplications and restore full-length dystrophin, evaluate this system in a mouse model recapitulating a patient duplication and optimize the therapy by combining gene editing and anti-inflammatories.

From a bioethics perspective, we first explore where personalized interventions fall on the research-care continuum and examine the ethical, regulatory and institutional implications when bespoke therapies are classified as research, care, or a mix of both. Further work then addresses the challenge of equitable access to these high-cost therapies, considering both institutional settings and barriers in low- and middle-income countries.

ABSTRACT POSTER NUMBER: 25

Systematic Reanalysis of NGS Data in 103 NMD Families Enhances Diagnostic Yield, Reveals Deep Intronic Variants, and Identifies ATP2A2 as a Novel Neuromuscular Disease Gene

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BACKGROUND: Neuromuscular disorders (NMDs) affect ~1 in 1000 individuals and exhibit clinical and genetic heterogeneity. Despite diagnostic advances, many cases remain unsolved after initial genetic testing. Bioinformatic reanalysis of preexisting data helps identify missed diagnoses and uncover novel disease genes.

METHODS: Next-generation sequencing (NGS) data from 103 undiagnosed NMD families was reanalyzed using the RD-Connect Genome-Phenome Analysis Platform (GPAP). The dataset included clinical or whole exome sequencing (CES/WES; n=76) and whole genome sequencing (WGS; n=27). Variant prioritization incorporated population frequency, *in silico* predictions and reverse phenotype correlation.

RESULTS: Reanalysis identified putative disease-causing variants in 20 of 103 previously unsolved cases (19.41% diagnostic yield). Of these, eight harbored coding variants (*RYR1, AGRN, SCN4A, TTN, MYH2, GOLGA2*) established by reverse phenotype correlation. In six cases, intronic variants with predicted splicing impact affecting known NMD genes (*COL13A1, COL6A3, DOK7, DYSF, CHRND, SGCA*) were considered significant. Two cases had extended phenotypes (*KLHL40, PTPN11*), one had presumed double-trouble disease (*MYH2, KIF21A*), and another had a two-exon deletion (*DOK7*). A novel variant in *ATP2A2* was identified in two unrelated families with autosomal dominant rhabdomyolysis, establishing *ATP2A2* as a new NMD gene. A further 12 cases had significant heterozygous variants in recessive NMD genes, while 71 remained without strong candidates.

CONCLUSION: Reanalysis improved diagnostic yield and provided long-sought answers for families with unsolved NMDs. This study reinforced the utility of reevaluating cases for previously missed non-coding variants as well as reverse phenotype correlation to identify both known and novel genotype-phenotype associations in NMDs.

ABSTRACT POSTER NUMBER: 26

The Impact of Premature Cellular Senescence in Muscle Regeneration in Myotonic Dystrophy type 1.

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Myotonic dystrophy type 1 (DM1) is the most common adult-onset inherited myopathy, caused by an abnormal expansion of CTG repeats in the *dystrophia myotonica protein kinase (DMPK)* gene. The disease affects the whole-body system, but particularly the skeletal muscle leading to myotonia, weakness, and atrophy. However, limited comprehension on the molecular mechanisms of DM1 pathogenesis has impeded the development of DM1 treatment.

To address this gap, we utilized DMSXL mice, a preclinical model for DM1, and confirmed significant skeletal muscle phenotypic defects, including fiber atrophy and myotonia. The qPCR and immunostaining data indicated the sign of cellular senescence in DMSXL, characterized by elevated expression of cell cycle arrest markers and components of the senescence-associated secretory phenotype (SASP). These features were also observed in myoblasts isolated from DM1 patients, which exhibited impaired proliferation and differentiation capacities, particularly influenced by senescence-associated secretory phenotype (SASP) factors, as confirmed through conditioned-medium assays. Consistent with these *in vitro* findings, DMSXL mice exhibited delayed muscle regeneration following injury, characterized by a lag in new fiber formation, indicating dysfunction in muscle stem cell-mediated repair processes.

In conclusion, our study identifies cellular senescence as a critical factor contributing to skeletal muscle weakness in DM1. These findings would provide new insights into DM1 pathogenesis and propose a potential therapeutic target aimed at mitigating senescence-associated dysfunction.

Impact of Trained Immunity on Macrophage and Muscle Stem Cell Function in Dystrophic (mdx) Mice

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Duchenne muscular dystrophy (DMD) is a severe X-linked disorder caused by dystrophin deficiency, leading to progressive muscle degeneration and diaphragm failure. Muscle regeneration requires coordinated interaction between muscle stem cells (MuSCs) and macrophages. In the *mdx* mouse (DMD model), our lab has shown that bone marrow-derived macrophages (BMDMs) undergo metabolic and epigenetic reprogramming—referred to as "trained immunity"—which may promote dysregulated inflammation and impaired regeneration. We hypothesized that trained immunity in *mdx* BMDMs alters MuSC function. Bone marrow from 4–5-week-old *mdx* and wild-type (WT) mice was used to generate BMDMs, which were co-cultured with WT MuSCs. MuSC proliferation was assessed at 24 hours, and differentiation at 72 hours. Fibrinogen (a TLR4 ligand) was used to stimulate inflammation response of BMDMs. MuSCs co-cultured with *mdx* BMDMs showed increased proliferation but reduced differentiation, with lower fusion index, myotube length, and diameter compared to WT. This effect was also observed after 4-hours of fibrinogen stimulation. ChIP-sequencing revealed decreased H3K27me3 level (hence less gene repression) at autophagy-related genes in *mdx* BMDMs. Protein analysis showed significantly reduced expression of autophagy markers p62 in *mdx* BMDMs under basal conditions (-90%, p= 0.0166) and after 4 hours of fibrinogen stimulation (-58%, p= 0.030) and increased LC3-II/LC3B-I ratio compared to WT BMDMs. Trained immunity in *mdx* macrophages appears to alter MuSC proliferation and differentiation. Dysregulated autophagy in *mdx* BMDMs may contribute to these effects. Further investigation will explore autophagy's role in trained immunity and its impact on regenerative dysfunction in DMD.

ABSTRACT POSTER NUMBER: 28

Inhibition of translation initiation factor eIF4A in dystrophic muscle stem cells drives cell fate towards differentiation

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The regulation of muscle stem cell (MuSC) fate during homeostasis and regeneration is tightly controlled by complex regulatory networks. Post-transcriptional mechanisms refine the translatome of MuSCs to support distinct protein signatures associated with quiescence or differentiation. Central to this is the control of translation initiation by the eukaryotic initiation factor 4F complex (eIF4F), which harbours the RNA helicase eIF4A.

Isoforms of eIF4A in MuSCs are reciprocally expressed during differentiation: eIF4A1 levels are highest in undifferentiated progenitors and decrease while eIF4A2 accumulates. We demonstrate that cells isolated from the *mdx* mouse model of Duchenne muscular dystrophy (DMD) exhibit dysregulated expression of eIF4A. We therefore hypothesized that differential expression of these isoforms and their selectivity towards specific mRNAs may serve as a molecular switch between MuSC quiescence (eIF4A1-high) and differentiation (eIF4A2-high), which is disrupted in DMD.

We find that treatment of DMD MuSCs with a pharmacological inhibitor of eIF4A, Hippuristanol, enhances the differentiation of DMD MuSCs. Interestingly, treatment with Hippuristanol reduces expression of the quiescence marker PAX7. Moreover, we find that Hippuristanol-treated cells accumulate P-bodies, sites of RNA degradation and storage. Thus, we predict that select mRNA transcripts that are sensitive to eIF4A inhibition accumulate in these P-bodies, thereby inhibiting their translation.

Our findings reveal a pharmacologically targetable pathway that modulates the translation of transcripts influencing the transition of MuSCs from quiescence to differentiation. Our ongoing *in vivo* experiments will address eIF4A as a therapeutic target to enhance myogenic capacity of DMD MuSCs, filling a gap in DMD therapeutic strategies.

ABSTRACT POSTER NUMBER: 29

Exploring cardiac and skeletal muscle dysfunction in Myotonic Dystrophy Type 1 (DM1) with patient-derived iPSCs

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Myotonic Dystrophy Type 1 (DM1) is the most common adult-onset muscular dystrophy. While muscle weakness and wasting are hallmark symptoms, DM1 is a multisystemic disease. Notably, cardiac abnormalities occur in 80% of patients and are the second leading cause of death. DM1 is caused by a trinucleotide repeat expansion in Dystrophia Myotonica Protein Kinase *(DMPK)*, with repeat length correlating to disease severity. Mutant *DMPK* mRNA forms secondary structures which accumulate in the nucleus as toxic nuclear foci, disrupting RNA splicing, and leading to global spliceopathy. No disease-modifying treatments currently exist, underscoring the need for tissue-specific drug targets. Using patient-derived induced pluripotent stem cells (iPSCs), I will characterize cardiac and skeletal muscle in DM1 to uncover cell-specific mechanisms and guide therapy development. I will establish pathological features in iPSC-derived cardiomyocytes and myofibers, quantifying repeat length and nuclear foci to explore tissue-specific repeat instability. Clinical data will be used to correlate phenotypes with disease severity. In muscle, mitochondrial function and calcium signaling are critical but poorly understood in DM1. Live-cell imaging will assess mitochondrial membrane potential and morphology, and calcium activity. Electrophysiological profiling will evaluate the contribution of cardiomyocyte dysfunction to arrhythmias. To identify molecular targets, transcriptomics, proteomics, and metabolomics will be applied. RNA sequencing will reveal tissue-specific splicing defects, while mass spectrometry will identify therapeutic pathways and biomarkers. Finally, high-throughput drug screens using FDA-approved libraries will identify compounds that reduce nuclear foci and rescue disease phenotypes. Validated hits will hopefully advance to clinical trials, improving outcomes for DM1 patients.

ABSTRACT POSTER NUMBER: 30

Understanding myotonic dystrophy type 1 using iPSC-derived neurons

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Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy in adults with a prevalence as high as 1:550. People with DM1 can experience a range of symptoms, including excessive daytime sleepiness and cognitive dysfunction, which are reported to have the greatest impact on quality of life. DM1 is caused by a trinucleotide repeat expansion in the *DMPK* gene, where disease manifestation is associated with over 50 CTG repeats. This repeat expansion leads to the formation of double-stranded CUG hairpins within *DMPK* mRNA, which then accumulate as nuclear foci and sequester key RNA-binding proteins. Altogether, this leads to global missplicing and altered DMPK function, ultimately eliciting toxic cellular effects in muscle, cardiac, and neuronal cells. Although the pathomechanism of disease is known, there remain no disease-modifying treatments for DM1. I hypothesize that garnering a better understanding of the relationship and intracellular milieu of muscle and brain cells will aid in the successful development of biomarkers and the identification of druggable targets. I generated induced pluripotent stem cell (iPSC) derived glutamatergic neurons from people with varying CTG repeat lengths and began general characterizations of neuronal morphology, mitochondrial membrane potential, and cellular reactive oxygen species. Similar morphometric assays will be performed in parallel on cardiac and muscle cells. Moreover, we generated pilot datasets of transcriptomics and proteomics to investigate both cell type and disease. This baseline characterization is imperative in identifying biomarkers to be used in clinical trials and as targets of rescue for our upcoming drug screens.

ABSTRACT POSTER NUMBER: 31

A novel homozygous variant in AHCY causes a rare muscular dystrophy: a new case

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Muscular dystrophies are rare inherited disorders characterized by progressive weakness and degeneration of skeletal muscle. Despite advances in panels and exome sequencing, many patients remain undiagnosed due to the heterogeneity of these conditions and the highly polymorphic nature of associated genes. Standard panels often miss novel or poorly annotated genes, and interpretation of genetic data remains challenging, often resulting in variants of unknown significance, especially in rare disorders with limited functional data. In such cases, there's a need for a personalized approach integrating patient phenotype, tissue-specific data, and advanced bioinformatics.

We report a girl under the age of 18, born to a healthy consanguineous couple, presenting with muscular dystrophy, mild proximal weakness and necrosing myopathic fibers. First-line genetic testing using standard gene panels yielded no diagnosis, requiring deeper investigation through RNA sequencing of muscle tissue and tailored bioinformatic analysis.

This analysis revealed a novel rare homozygous variant in the S-adenosylhomocysteine hydrolase (*AHCY*) gene (c.131C>T ; p.Pro44Leu). Multiple *in silico* tools predict the variant to be pathogenic. Reports in the literature describe patients with *AHCY* variants showing clinical features similar to ours, reinforcing *AHCY* 's disease relevance.

This case underscores the limitations of traditional diagnostic workflows for rare muscle disorders and highlights the importance of personalized transcriptomic analysis. It provides a molecular diagnosis, ending a long diagnostic odyssey and enabling access to therapeutic interventions. Furthermore, this study contributes to growing evidence implicating *AHCY* in muscle disease, a gene not yet well established in the neuromuscular field despite increasing associated pathogenic variants.

ABSTRACT POSTER NUMBER: 32

TWEAK Administration Improves Muscle Function in D2.mdx mice

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Skeletal muscle regeneration relies on signaling pathways that coordinate myoblast proliferation, differentiation, and fusion. The NF-κB pathway plays a key role in this process, with classical NF-κB signaling promoting proliferation and inhibiting differentiation, while the alternative pathway supports myoblast fusion and oxidative metabolism. Dysregulated NF-κB activity contributes to muscle diseases such as Duchenne muscular dystrophy (DMD). TNF-like weak inducer of apoptosis (TWEAK), a cytokine acting through its receptor Fn14, can activate both NF-κB pathways and is upregulated in several muscle disorders. Although chronic TWEAK signaling has been associated with muscle atrophy, it may also promote regeneration by enhancing myoblast proliferation and fusion. Here, we investigated the effects of recombinant TWEAK administration in D2.mdx mice, a model of DMD. TWEAK treatment improved muscle strength and enhanced regenerative capacity following injury. Histological analysis of the gastrocnemius showed increased infiltration of pro-inflammatory macrophages, yet reduced muscle damage and fibrosis. In the tibialis anterior, TWEAK-treated mice exhibited superior repair following acute injury. Mechanistically, TWEAK reduced levels of cIAP1/2 and suppressed classical NF-κB signaling in muscle. Together, these findings suggest that TWEAK modulates muscle inflammation and NF-κB activity to promote functional regeneration in dystrophic muscle. This highlights its potential as a therapeutic strategy for DMD and related myopathies.

ABSTRACT POSTER NUMBER: 33

EV mediated delivery of Wnt7a as a therapy for muscle wasting diseases

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Skeletal muscle comprises nearly half of body weight in all vertebrates, serving critical functions to support vital physiological processes. Muscle regeneration is supported by a heterogenous population of adult stem cells (MuSCs) residing within grooves of myofibers. Upon activation and existing quiescence, MuSCs begin proliferative expansion to give rise to committed progenitor myoblasts and myocytes. Meanwhile, a balance between self-renewal and commitment through utilizing apico-basal or planar cell polarity, prevents depletion of the stem cell pool. In the context of muscular dystrophies or sarcopenia, a reinforced myogenic regenerative response can improve the pathology prognosis where more regeneration is required against an attenuated stem cell niche. Herein, we hypothesize to reinforce the non-canonical Wnt7a signaling contribution to myogenesis in MuSCs, progenitors and mature tissue. We have shown that nanoscale-sized extracellular vesicles can enhance Wnt7a functionality when used as carriers for *in vivo* systemic delivery. We have also proved Fibronectin to be a synergistic agent to Wnt7a. Consequently, we believe we can enhance targeting efficiency and functional activity of Wnt7a by its integration onto small EVs along with a synergizing Fibronectin domain. Heparin binding domain of Fibronectin most optimally synergizes with Wnt7a to encourage motility in myoblasts. A truncated C-terminal Wnt7a preserves functionality of a full-length ligand protein. We argue that systemic co-delivery of Wnt7a and Fibronectin using small extracellular vesicles can be further investigated as a potential ameliorative therapeutic agent alongside curative strategies in muscle wasting diseases.

DG9-PMO Enhances Cardiac and Skeletal Muscle Delivery for Exon 44 Skipping Therapy in Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is a progressive neuromuscular disorder caused by mutations in the DMD gene that results in the absence of dystrophin protein, leading to muscle degeneration and early mortality. Exon-skipping therapies using phosphorodiamidate morpholino oligomers (PMOs) offer a promising strategy by restoring the dystrophin reading frame; however, their clinical impact remains limited by poor cellular uptake. This limitation is especially concerning for dystrophic patients with advanced cardiac conditions, as cardiomyopathy is the leading cause of DMD-associated mortality, and current PMO therapies lack efficient delivery methods to the heart.

Here, we report the development and preclinical evaluation of a novel cell-penetrating peptide, DG9, that can significantly enhance PMO uptake and nuclear localization across multiple types of myocytes. Using a humanized dystrophic mouse model, which lacks both murine and human dystrophin, we show that systemic delivery of DG9-PMO results in robust exon 44 skipping and dystrophin restoration in both skeletal and cardiac muscles, with a 10-fold improvement in the heart compared to unconjugated PMO. Importantly, DG9-PMO preserved cardiac function under β-isoproterenol-induced stress and normalized disease-associated transcriptional profiles, including genes linked to inflammation, apoptosis, and cardiac conduction. The treatment exhibited no observable toxicity and normalized serum biomarkers.

Our findings demonstrate that DG9-PMO is a safe and efficient therapeutic strategy with strong potential to address the unmet need for a cardiac-effective exon skipping therapy in DMD. This work not only advances exon-skipping therapeutics but also underscores the value of targeted peptide delivery platforms in neuromuscular disorders.

ABSTRACT POSTER NUMBER: 35

A Common Thread? Multi-Omics Uncover Denervation in Diverse Myopathies

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BACKGROUND:
Genetic myopathies arise from mutations in many different proteins, beginning with selective muscle involvement before progressing to most skeletal muscles, and in some cases, cardiac or respiratory muscles. Since the molecular drivers of muscle degeneration are not fully understood, treatments remain limited. This underscores the need for deeper exploration of disease mechanisms. Prior research has linked some myopathies with progressive changes at the neuromuscular junction (NMJ). Our study aimed to uncover a shared biological pathway among adult-onset genetic myopathies.

METHODS:
We analyzed skeletal muscle biopsies from 10 individuals with genetically confirmed myopathies and 4 unaffected controls. Samples underwent single-nucleus RNA sequencing (snRNA-seq) and spatial transcriptomics. Publicly available datasets were also examined to evaluate expression of relevant transcripts in other myopathies.

RESULTS:
snRNA-seq revealed a group of myonuclei enriched in myopathy that co-express markers of mature muscle and NMJ-associated genes. These nuclei also express key indicators of a denervation response, including *RUNX1*, *CHRNG*, *NCAM1*, and *MYOG*. Their abundance correlated with the severity of muscle pathology, independent of age or sex. Spatial transcriptomics localized these nuclei near NMJs, and immunostaining showed disrupted patterns of innervation. Analysis of public datasets confirmed elevated expression of NMJ-related genes in additional myopathies, notably myotonic dystrophy and polymyositis.

CONCLUSIONS:
These findings suggest that adult myopathic muscle activates a denervation response that may play a role in disease progression. The consistent presence of similar transcriptional signatures across multiple myopathies indicates that denervation may be a common feature of these conditions.

ABSTRACT POSTER NUMBER: 36

A novel therapeutic effect of lauric acid: preventing loss of ambulation in severe mouse models of muscular dystrophy

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Background:
Cholesterol dysregulation contributes to loss of ambulation and muscle degeneration in multiple forms of muscular dystrophy (MD), rationalizing statin-induced myopathies. Mild mdx and dysferlin-null mice exhibit severe ambulation deficits when hypercholesterolemic. Dodecanoic (lauric) acid (LA), a medium-chain saturated fatty acid, is known to improve aerobic capacity, muscle growth, and cholesterol metabolism. We hypothesized that LA could mitigate cholesterol-induced ambulation decline and muscle pathology in severe MD mouse models.

Methods:
Severe mdx and dysferlin-null mice were generated via knocking out apolipoprotein E (ApoE) and fed a cholesterol-rich diet (CHOL). The therapeutic potential of LA was assessed by comparing outcomes in mice fed CHOL, a LA-rich diet, a combined CHOL/LA diet, or a control diet for 3 months. Ambulatory function, circulating lipids, muscle histology and intramuscular cholesterol metabolism were evaluated. Primary myotubes were treated with CHOL and/or LA to assess myogenesis.

Results:
CHOL-fed mice developed hypercholesterolemia, marked ambulatory dysfunction, and muscle wasting, with fatty-fibrotic infiltration occupying up to 85% of muscle cross-sectional area. Strikingly, the CHOL/LA diet fully preserved ambulatory function and muscle morphology despite no change in circulating cholesterol. LA's protective effects correlated with reduced intramuscular cholesterol, normalized expression of cholesterol-regulating enzymes, and a shift toward oxidative myofiber composition. In vitro, LA enhanced myogenesis by rescuing CHOL-induced defects in myotube formation and myogenic factor expression.

Conclusions:
Lauric acid counters cholesterol-driven muscle dysfunction and degeneration in severe MD models by restoring muscle metabolic and regenerative pathways. These findings support LA as a promising, simple dietary intervention for MD.

ABSTRACT POSTER NUMBER: 37

Chronic loss of the mitochondrial fusion protein OPA1 is detrimental to muscle stem cell maintenance and activity

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Muscle stem cells (MuSCs) are essential for tissue regeneration, yet the mechanisms governing their dysfunction during aging remain unclear. However, mitochondrial dynamics proteins, key regulators of MuSC activity and fate, decline in expression with age. During aging, loss of mitochondrial fission and fusion modulators is associated with MuSC dysregulation. How mitochondrial dynamics contribute to senescent and aged-like phenotypes in MuSCs has not been fully described. Our lab previously implemented a Pax7CreERT2 inducible system to conditionally knock-out the mitochondrial fusion protein OPA1 in MuSCs (OPA1-KO), finding with chronic loss of OPA1, MuSCs have enhanced activation kinetics, proliferation defects, and evidence of mitochondrial dysfunction. Here, we investigated the role of chronic OPA1 loss in MuSCs in the contexts of aging and senescence. With chronic loss of OPA1, MuSCs accumulate senescence markers, experience cell cycle dysregulation at the transcriptional level, and have a dysregulated metabolome. However, the proliferative defects associated with chronic OPA1 ablation may be partially mitigated via supplementation of exogenous metabolites. At 9-months of OPA1 loss, the phenotype of MuSC dysfunction progresses to MuSC depletion basally. Whole-muscle defects were observed at this time, including the onset of muscle wasting phenotypes (reduction in myofiber size and number of myonuclei per myofiber) and fatigability of the
muscle. Mitochondrial dynamics are therefore a critical regulator of MuSC activity and maintenance in an aging context, and whole muscle health depends on the presence of functional MuSCs during aging. This research offers therapeutic insight that may be leveraged to improve MuSC function with age.

Transition to home infusions with a low risk of infusion-associated reactions and functional stability: real-world outcomes from the UK Early Access to Medicines Scheme registry for cipaglucosidase alfa plus miglustat in late-onset Pompe disease

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Late-onset Pompe disease (LOPD) is a rare, inherited metabolic disorder characterised by progressive loss of muscle and respiratory function. Cipaglucosidase alfa plus miglustat (cipa+mig) was accepted into the UK Early Access to Medicines Scheme (EAMS; 50636/0001) in 2021. A prospective observational registry under the EAMS evaluated the real-world safety and effectiveness of cipa+mig in enzyme replacement therapy-experienced adults with LOPD for the first time. Data from standard-of-care assessments were extracted from medical records; no additional measures were mandated. Of 45 patients dosed under the EAMS between November 2021 and September 2023, 37 consented to registry participation. For patients with available data, median (range) 6-minute walk distance increased from baseline (253.5 [10.0–630.0] metres, n=20) to post-baseline visit 1 (397.0 [63.0–656.0] metres, n=15) and returned to baseline at visit 2 (256.0 [126.0–504.0] metres, n=9). Patients received a median (range) of 25.5 (2.0–47.0) home infusions over a median (range) of 344.0 (15.0–649.0) days. Of 44 patients that transitioned to home infusions after one, two and three on-site infusions, 1/13 (7.7%), 3/24 (12.5%) and 1/7 (14.3%) patients reported infusion-associated reactions (IARs), respectively. A total of six patients discontinued treatment during the EAMS; five had received at least one home infusion, and three discontinued due to IARs. The available data demonstrate that cipa+mig was generally well tolerated, with patients transitioning to home infusions with a low risk of IARs alongside evidence of improvement or stability in functional outcomes. Supported by Amicus Therapeutics, Inc.

ABSTRACT POSTER NUMBER: 39

Senotherapeutics and Exercise-Based Strategies to Restore Defective Muscle Stem Cells in Myotonic Dystrophy Type 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) exhibit 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-week 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 fibers (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.

ABSTRACT POSTER NUMBER: 40

The Impact of DEPP1 Overexpression on Skeletal Muscle Function

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INTRODUCTION:
Skeletal muscles play key roles in movement, posture, thermogenesis, and whole-body metabolism. Maintaining optimal muscle mass and function is therefore essential for general health. Autophagy has emerged in the last decades as an essential catabolic process involved in the regulation of muscle mass, function and integrity. However, the autophagy machinery is complex and still partly understood. Recently, we gathered data indicating that Depp1 (C10ORF10) is a FoxO-dependent gene and its expression is increased in various models of skeletal muscle atrophy. However, to date, the impact of Depp1 overexpression on skeletal muscle integrity remains unclear.

METHODS:
DEPP1 was overexpressed for 4 weeks in the tibialis anterior (TA) and gastrocnemius (GAS) of 10-weeks-old mice using intramuscular injections of adeno-Associated viruses (AAV). The impacts of DEPP1 overexpression on muscle mass and contractility were assessed. Immunoblots were used to quantify markers of mitochondrial content (OXPHOS subunits) and autophagy (P62 and LC3). Immunohistochemical approaches were used to assess myofiber integrity and myofibers size.

RESULTS:
DEPP1 overexpression was confirmed by RT-qPCR. Short-term DEPP1 overexpression led to a modest reduction in muscle mass and myofiber cross-sectional area. Analysis of *in-vivo* contractility, autophagy and mitochondria integrity is ongoing, and these results will also be presented.

CONCLUSION:
Our preliminary data support the important role of DEPP1 in regulating skeletal muscle integrity. Further research is under way to further characterize the molecular and cellular mechanisms by which DEPP1 regulates skeletal muscle fitness in health and diseases conditions.

ABSTRACT POSTER NUMBER: 41

Identification and Characterization of Serglycin as a Potential Biomarker in GNE Myopathy

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GNE myopathy (GNEM) is a rare autosomal recessive disorder characterized by progressive skeletal muscle weakness. Symptoms typically manifest between the 2nd to 4th decade of life, beginning with weakness in the tibialis anterior muscle which often presents as foot drop, gait disturbances and/or frequent tripping and falls. Despite advances in the genetic understanding of the disease, the disease course of GNEM remains poorly defined, with considerable variability in age of onset and disease severity even amongst family members carrying the same mutations. Biomarkers play a critical role in rare disease, offering objective and measurable indicators of disease presence, progression, severity, and response to treatments. Currently there are no well-established biomarkers in GNEM, however by applying serum proteomics we recently identified serglycin, a small proteoglycan involved in secretory granule processes, as being significantly decreased in serum samples of patients with GNEM. This finding has been confirmed by ELISA as an alternative analytical approach highlighting the robustness of serglycin as a novel blood biomarker for GNE myopathy. To further characterize serglycin as a potential biomarker of pathophysiological relevance, we investigated its expression across several GNEM cellular models, including two immortalized *GNE* knockout cell lines and patient-derived iPSC models as well as muscle biopsies. Additionally, we evaluated the effects of two drug candidates – each targeting distinct pathways hypothesized to be involved in GNEM – on serglycin levels. Together, these studies highlight the potential of serglycin as a biomarker for GNEM, with important implications for diagnosis, disease monitoring and therapeutic development.

Functional validation of congenital myasthenic syndrome candidate genes: A focus on ETV5 and ATP8A2

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Congenital myasthenic syndromes (CMS) are a group of rare, inherited neuromuscular diseases caused by neuromuscular junction (NMJ) signalling defects and characterised by fatigable muscle weakness. A sizable portion of clinically diagnosed CMS patients do not acquire a genetic diagnosis, suggesting there are unvalidated genes that cause CMS. A streamlined approach using mouse RNA sequencing data and human genomic cohort analysis enabled identification of *ATP8A2* and *ETV5* as high-priority genes for functional preclinical testing to elucidate their role at the NMJ. *ETV5* encodes a transcription factor known to control subsynaptic gene expression in skeletal muscles, and *ATP8A2* encodes a phospholipid flippase that generates asymmetry of aminophospholipids across membranes. Optogenetic *ETV5* and *ATP8A2* zebrafish lines were generated and employed in this project to explore if the phenotypes were consistent with NMJ dysfunction such as fatigable weakness. The analysis of these models includes measurement of spontaneous chorion activity, voluntary movement in light-dark assays, and forced movement using optogenetic stimulation. Furthermore, this model will be analyzed for morphological deficits characterised through brightfield imaging, immunofluorescence, and birefringence. Preliminary analysis of *ETV5* illustrates significant locomotor and morphological deficits while highlighting the feasibility of this high-throughput model. Leveraging these high-throughput zebrafish models is an efficient and cost-effective approach for validation of novel candidate genes. This workflow can be modified for other neuromuscular disorder candidate genes, broadening its impact across multiple diseases. Future directions of this project involve high-throughput drug testing on these models to analyze targeted candidate therapeutics.

ABSTRACT POSTER NUMBER: 43

Music Intervention for Brain-Heart Disease in Pediatric Myotonic Dystrophy Type 1 (DM1): Interim results on feasibility and tolerability

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INTRODUCTION :

Myotonic dystrophy type 1 (DM1) is a rare autosomal dominant inherited multi-systemic disease, affecting the neuromuscular system, as well as cognitive and cardiac function. Congenital DM1 (CDM) is the most severe form and is associated with learning difficulties, autism-like traits, and cardiac conduction defects. There is no approved pharmacological therapy for CDM.

METHODS :

Our interventional study uses an innovative mixed methods approach to evaluate the impact of Dalcroze music education for CDM. Two groups of patients with CDM receive 10 sessions of 45 minutes music intervention each. The primary outcome is the feasibility and tolerability. Secondary and exploratory outcome measures include cognitive and physical assessments, quality of life, sleep quality, and blood and urine biomarkers. We use various questionnaires and wearables for data acquisition.

RESULTS :

We present the results of the first group of 5 patients with CDM (4 male, 1 female) and a mean age of 14.6 years (range 13-18 years). The attendance rate of the interventions was 78%, and the intervention was well tolerated. More results on tolerability and feasibility as well as parental feedback will be available at the time of the poster presentation.

CONCLUSION :

Our study demonstrates the feasibility and tolerability of music education for pediatric patients with CDM and any impacts on brain and heart function. This is a qualitative study with a small sample size. Future studies will improve the understanding of the disease and might provide additional or alternative treatment options in the absence of disease-modifying treatments.

ABSTRACT POSTER NUMBER: 44

Assessing 2-point DIXON and 6-point PDFF fat fraction using full body muscle MRI analysis in patients with oculopharyngeal muscular dystrophy (OPMD)

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INTRODUCTION:
Oculopharyngeal muscular dystrophy (OPMD) is an inherited disorder which features fat infiltration of proximal limb musculature. Studies have shown that quantitative 2-point fat fraction MRI muscle analysis can be utilized to assess disease progression, but none have explored 6-point proton density fat fraction in OPMD patients. This study aims to compare the muscle fat fractions (FF) obtained using 2-point DIXON (2ptFF) and 6-point PDFF (6ptFF) MRI sequences in OPMD patients.

METHODS:
2ptFF and 6ptFF analyses were conducted on 15 OPMD patients, each imaged with a full body axial muscle MRI scan. 97 muscles were cross-sectionally segmented on the in-phase MRI images using ITK-SNAP. FF maps were reconstructed and FF values were extracted from the overlaid segmentation masks. Analyses were conducted comparing the 2ptFF and 6ptFF values.

RESULTS:
The mean difference between 6ptFF and 2ptFF values was 0.028, with 6ptFF being lower. 2ptFF was significantly higher than 6ptFF for FFs in the mid-ranges, with the peak difference seen when FF was 30% by 6ptFF vs 45% by 2ptFF. These difference between the two FF values may be a result of relaxation times.

CONCLUSION:
Differences between 2ptFF and 6ptFF may be helpful for OPMD assessments at different disease stages. At earlier stages with lower fat infiltration, 2ptFF measures might be more sensitive, aiding in diagnosis; whereas, 6ptFF could provide more detailed data on disease progression and muscle changes. Future work incorporating clinical data in relation to FF and examining longitudinal relationships will improve utility of FF MRI.

ABSTRACT POSTER NUMBER: 45

A molecular switch: permeability transition pore dynamics regulates mitochondrial membrane potential and quiescence in muscle stem cells

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Muscle stem cells (MuSCs) maintain tissue homeostasis through precise regulation of quiescence and activation states. Recent studies have identified heterogeneity within quiescent MuSC populations, with deeply quiescent cells displaying reduced mitochondrial membrane potential (MMP). This low MMP has been conventionally attributed to passive metabolic depression, but this overlooks physiological constraints requiring maintained mitochondrial function even in quiescent states. In this study, we investigated molecular underpinnings of MMP heterogeneity in MuSCs, addressing potential confounding artifacts and exploring mitochondrial-intrinsic regulatory mechanisms.

Experiments revealed that MMP differences between MuSC subpopulations are not due to mitochondrial potentiometric probes efflux, as MMP_{low} cells showed reduced efflux pump function. Imaging of FACS sorted MuSCs showed that the MMP_{high} and MMP_{low} populations exhibited equivalent mitochondrial content, suggesting that MMP differences reflect genuine mitochondrial regulatory mechanisms. Importantly, we provide compelling evidence that flickering of the mitochondrial permeability transition pore (mPTP), a multi-conductance channel of the inner membrane formed by conformational changes in the ATPsynthase actively regulates mitochondrial polarization state. Our results show that inhibition of mPTP flickering reduces the proportion of MMP_{low} cells by more than 50% and promotes precocious MuSC activation/commitment in cultured EDL fibers. Proximity ligation assays (PLA) also reveal that pore flickering in MMP_{low} cells is linked to enhanced interaction of the PTP-sensitizing protein cyclophilin D (CyPD) with the mPTP at the expense of TRAP1, the repressor of pore opening. Together these results challenge the passive metabolic depression model and identifies a sophisticated layer of mitochondrial control integral to maintaining stem cell quiescence depth.

Phase 1b study of the safety, tolerability, pharmacokinetics, immunogenicity, and efficacy of ARGX-119 in participants with DOK7 congenital myasthenic syndromes

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INTRODUCTION:
Congenital myasthenic syndromes (CMS) are a rare, heterogeneous group of inherited disorders caused by mutations impairing neuromuscular transmission, with no approved treatments. One form of CMS caused by *DOK7* gene mutations (DOK7-CMS) is characterized by a limb-girdle pattern of muscle weakness. DOK7 is a protein coactivator of muscle-specific kinase (MuSK), an essential component of neuromuscular junction (NMJ) development and optimal neurotransmission. ARGX-119, a humanized, agonistic, monoclonal antibody, specifically targets and activates MuSK, which may stabilize, mature, and improve NMJ function in DOK7-CMS, reducing muscle weakness/fatigability, and improving quality of life (QoL).

OBJECTIVES:
To evaluate safety, tolerability, pharmacokinetics, immunogenicity, efficacy of ARGX-119 in adults with DOK7-CMS.

METHODS:
This Phase 1b, multicenter, double-blinded, placebo-controlled study (NCT06436742) enrolled 16 adults with DOK7-CMS. Following a ≤28-day screening period, participants were randomized 4:1 to intravenous ARGX-119 or placebo for 6 doses over the 12-week treatment period, before entering the ~7-month follow-up period. Primary endpoint: safety assessment. Secondary endpoints: ARGX-119 pharmacokinetics, development of antidrug antibodies against ARGX-119, efficacy measures (change over time in key components of the Quantitative Myasthenia Gravis score, Myasthenia Gravis Activities of Daily Living score, and PROMIS Global Health score). Physical function, mobility, and QoL will also be evaluated.

RESULTS:
Estimated completion date: November 2025; interim results will be presented. This will be the first presentation of these data.

CONCLUSION:
This study assesses safety, tolerability, pharmacokinetics, immunogenicity, and efficacy of ARGX-119 in participants with DOK7-CMS.

ABSTRACT POSTER NUMBER: 47

Lipid profiling uncovers peroxisomal roles in metabolic reprogramming and muscle stem cell fate

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Peroxisomes are emerging as key regulators of cellular metabolism, yet their regulation and functional significance in muscle stem cells (MuSCs) remain poorly defined. In this study, we investigate how peroxisomal content and function are dynamically regulated during MuSC state transitions and assess their impact on muscle regeneration. Through single myofiber analysis and transcriptomic profiling, we demonstrate that peroxisome abundance is low in quiescent MuSCs but rapidly increases during activation, preceding lipid droplet accumulation and occurring in parallel with mitochondrial remodeling. Notably, functional interactions between peroxisomes, mitochondria, and lipid droplets intensify during MuSC activation, underscoring their coordinated role in metabolic reprogramming. To directly examine peroxisomal function, we generated an inducible, MuSC-specific *Pex10* knockout (KO) model. Loss of Pex10 led to partial peroxisomal deficiency, disrupting the import of key matrix enzymes essential for lipid metabolism and redox homeostasis. Following cardiotoxin-induced injury, Pex10-deficient MuSCs exhibited impaired proliferation and reduced numbers of Pax7+ cells during early regeneration. Additionally, isolated Pex10 KO myofibers showed fewer quiescent and more activated MuSCs within 24-hour. In vitro, *Pex10* deficiency impaired MuSC proliferation without affecting differentiation potential. Besides, we applied pharmacological treatments to test whether peroxisomal enhancement could restore proliferation and myotube formation in *Pex10*-deficient MuSCs. Finally, transcriptomic analyses revealed broad downregulation of peroxisomal genes in aged MuSCs, implicating peroxisome dysfunction in age-related regenerative decline. Taken together, these findings highlight peroxisomes as central regulators of MuSC metabolism and identify peroxisomal pathways as promising therapeutic targets to enhance muscle regeneration in early stage, especially in aging.

ABSTRACT POSTER NUMBER: 48

Assessing suitability of different subgroups for interventional trials in Duchenne muscular dystrophy

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BACKGROUND:
Duchenne Muscular Dystrophy (DMD) is an X-linked disease, characterized by progressive muscle degeneration. The rarity, heterogeneous phenotype, and progressive nature of DMD highlight the need for novel approaches in trial designs to better evaluate drug efficacy. Strategies based on sample enrichment and/or subgroup-based primary analyses provide a promising alternative to a standard all-comers primary analysis. However, which subgroup is the most suitable for assessing efficacy at a young age is unclear. We tested this with data on vamorolone, a new steroid approved for DMD in boys over 2 years old. The registrational trial previously showed significant primary and multiple secondary endpoints.

OBJECTIVE:
Define subgroups that could increase statistical power compared to an all-comers analysis.

RESULTS:
A literature review identified commonly used subgroups, typically based on age, outcome, or imaging (e.g., fat fraction). Available subgroups were evaluated using data from VBP15-004. Mixed methods for repeated measures (mmrm) were used to compare subgroup estimates with those from an all-comers analysis. It was found that the ≥5-second time to stand from supine (TTSTAND) and 300m-400m six-minute walk distance (6MWD) subgroups demonstrated the best performance. Sample size and power calculations further supported the use of the ≥5 seconds TTSTAND subgroup demonstrating a smaller sample size required than based on the all-comers analysis.

CONCLUSION:
The ≥5-second TTSTAND and 300m-400m subgroups were found to perform better than an all-comers analysis by increasing effect sizes and statistical power. These findings may be used to inform future interventional trial analysis design and inclusion/exclusion criteria.

ABSTRACT POSTER NUMBER: 49

French-Canadian new founder mutation on DTNA gene associated with mild muscular dystrophy

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BACKGROUND:
DTNA encodes α-dystrobrevin, a dystrophin-glycoprotein complex (DGC) component critical for membrane stability and acetylcholine receptor mobility and turnover. The coiled-coil domain of α-dystrobrevin is intolerant of genetic variation in skeletal muscle and can lead to phenotypes ranging from asymptomatic hyperCKemia to mild muscular dystrophy.

CASE PRESENTATION:
We report a 5-year-old French-Canadian male with asymptomatic hyperCKemia (1653-3081 U/L), initially investigated for elevated liver enzymes later attributed muscular origin. The patient exhibited normal strength, gait, and cardiac function with no neurodevelopmental issues to date. Genetic testing revealed a heterozygous DTNA c.1651_1671del (p. Lys552_Arg558del), which was also found in two additional French-Canadian patients with similar clinical presentations in our RQDM (*Réseau québécois de diagnostic moléculaire*) cohort, a relatively new molecular provincial gene panel.

BROADER COHORT CONTEXT:
This variant aligns with 12 individuals from four families harbouring two pathogenic monoallelic DTNA variants affecting the coiled-coil domain of α-dystrobrevin in a recent international article (Nascimento et al., 2023). Three of these four families (seven patients) harbour the same variant as in our Quebec cohort. Furthermore, at least one of these families have French-Canadian origin. Their phenotypes included persistent hyperCKemia (7/7), myalgia (5/7), exercise intolerance (4/7), and mild lower-limb weakness (3/7). Immunofluorescence confirmed pathogenic disruption through marked α-dystrobrevin reduction at the membrane. Muscle biopsies in four of these affected individuals with the same variant showed mixed myopathic/dystrophic features.

CONCLUSION:
The new recurrent c.1651_1671del variant in French-Canadians may represent a founder mutation supporting DTNA screening in unexplained hyperCKemia within this population.

ABSTRACT POSTER NUMBER: 50

48-week data from the Phase 2 open-label FORWARD-53 study of WVE-N531 in boys with Duchenne muscular dystrophy amenable to exon 53 skipping.

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WVE-N531 is an investigational stereopure splicing oligonucleotide with PN (phosphoryl guanidine) chemistry, currently being evaluated in an open-label Phase 2 clinical trial (FORWARD-53) in boys with DMD amenable to exon 53 skipping. Eleven boys (age 5-11; 10 ambulatory and 1 non-ambulatory) initially received WVE-N531 at 10 mg/kg every other week. All 11 boys have advanced to the monthly dosing extension portion of the study. WVE-N531 was safe and well-tolerated through 48 weeks; all treatment-related AEs were mild to moderate in intensity, with no serious AEs or discontinuations. Muscle content-adjusted dystrophin expression, by western blot, stabilized between 24 and 48 weeks of dosing and averaged 7.8%. Eighty-eight percent of boys (7/8) achieved greater than 5% average dystrophin between 24 and 48 weeks.

WVE-N531 effectively targets both myofibers and myogenic stem cells, which is expected to restore regenerative capacity within dystrophic muscles. Muscle histology showed a transition from regeneration to maturation of myofibers, with a statistically significant reduction in muscle fibrosis (28.6% reduction between week 24 to 48; $p<0.01$), and decreases of the median muscle necrosis and inflammation scores from 2 to 1 (representing minimal damage). A 50% decline ($p<0.001$) in creatine kinase, as well as decreases in IL-6 and MCP-1, were also observed. Time-to-Rise results demonstrated a 3.8-second mean improvement versus natural history (statistically significant; $p<0.05$); this value was above the Minimal Clinically Important Difference of 1.4 seconds. Additional functional benefits were observed on the NSAA versus natural history, and in hand grip strength versus baseline.

ABSTRACT POSTER NUMBER: 51

The Role of Genetic Modifiers in Drug Response Safety in Duchenne Muscular Dystrophy

Ahmadiharchegani F[1], Hoffman EP[2], Sabbatini D[3], Pegoraro E[3], Bello L[3], Guglieri M[4], Clemens P[5], on behalf of VBP15 investigators, Dang UJ[1]

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BACKGROUND:
Duchenne muscular dystrophy (DMD) is a rare, progressive X-linked neuromuscular disorder. Vamorolone is a newly approved dissociative steroid. Traditional corticosteroids like prednisone and deflazacort can result in growth stunting, reduced bone biomarkers, and adrenal suppression while vamorolone has shown an improved safety profile, especially on bone health. Genetic modifiers are known to alter disease severity or response to treatment, but have not been investigated at an early age.

OBJECTIVE:
Study associations of genetic modifiers with drug-related safety (growth, bone biomarkers, adrenal function) in young steroid-naïve boys.

RESULTS:
Samples and data were obtained from VBP15-002/003 (n=48) and VBP15-004 trials (n=121). DNA samples were genotyped using the Illumina GDA-PGx platform. The analysis focused on the placebo, prednisone 0.75 mg/kg/day, and vamorolone 6 mg/kg/day treatment groups. Longitudinal analysis was used with a data mask applied to study the association of pre-specified polymorphisms in Vitamin D Receptor, i.e., *VDR* (FokI, GATA, and CDX2 SNPs) and *PDGFD*(*rs361283*) genes with change in height Z-score, bone biomarkers of formation and turnover (Osteocalcin, P1NP, and CTXI), and cortisol levels. No *PDGFD* x adrenal suppression associations were seen. We found that certain *VDR genotypes* were associated with sensitivity to prednisone-related changes in growth velocity (GATA, CDX2) and bone biomarkers (GATA, FokI).

CONCLUSIONS:
Although based on a small sample size and needing additional studies for confirmation, we provide some initial evidence of the association of genetic modifiers at an early age with common steroid safety concerns. These findings may be important for clinical trials and care.

Proximal Femoral Screw Hemiepiphysiodesis in Spinal Muscular Atrophy: A Case Report in the Gene Therapy Era

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Spinal muscular atrophy (SMA) is a rare inherited, severe neuromuscular disease characterized by progressive muscle weakness, atrophy and hypotonia due to biallelic mutations of the survival motor neuron (*SMN*) 1 gene. With the advent of new disease-modifying therapies, the phenotype of spinal muscular atrophy has changed significantly, with a growing number of individuals achieving ambulation. As a result, this may open the door to new orthopedic management. In this case series, two young children with symptomatic spinal muscular atrophy presented with progressive hip subluxation. Both genetic studies revealed *SMN1* deletion with respectively two and three copies of the *SMN2* gene. The first patient was diagnosed with SMA type Ib, while the second, who was non-ambulatory and able to sit independently, was diagnosed with SMA type II. At respectively three years and six months, and four years and two months, they underwent bilateral proximal femoral screw hemiepiphysiodesis without complications nor refractory periods. For the first patient, endurance in the standing position improved and one-year postoperative radiographs displayed improvement of the subluxation for the left hip. For the second patient, independence in mobility and stability in standing position were noted six months post-operatively. This case report underscores proximal femoral screw hemiepiphysiodesis as a well-tolerated procedure for two young SMA patients. Given the lack of data evidence on this topic, clinicians and surgeons should be aware that minimally invasive management of hip dislocations may be considered in patients with spinal muscular atrophy.

ABSTRACT POSTER NUMBER: 53

Skeletal Muscle Transcriptomic Comparison Between Men and Women Individuals with Myotonic Dystrophy Type 1

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BACKGROUND:
Myotonic dystrophy type 1 (DM1) is one of the most common adult-onset muscular dystrophies, caused by an expansion of CTG repeats in the *DMPK* gene. This leads to the accumulation of toxic RNA that sequesters RNA-binding proteins. Clinically, DM1 is highly heterogeneous, with variability in age of onset, disease progression, and severity. Accumulating evidence indicates that DM1 presents distinct sex-specific clinical manifestations, including differences in symptom severity, comorbidities, and disease trajectory. However, the extent to which transcriptomic and splicing alterations in skeletal muscle are sex-dependent in DM1 remains poorly understood.

AIMS:
This study aimed to investigate sex-specific transcriptomic and splicing dysregulation in the skeletal muscle of individuals with DM1.

METHODS:
We re-analyzed RNA-sequencing data from the GSE86356 dataset, which includes 55 tibialis anterior (TA) muscle samples 44 from individuals with DM1 (28 women, 16 men) and 11 from healthy controls (5 women, 6 men). Additionally, we analyzed RNA-sequencing data from BioProject PRJNA1079722, comprising 95 TA muscle samples from individuals with DM1 (58 women, 16 men) and 24 controls (13 women, 11 men).

RESULTS:
Using a bioinformatic pipeline, we identified differentially expressed genes and splicing events based on sex. Various bioinformatics analysis based on sex-specific transcriptomic and splicing dysregulation will also be presented.

CONCLUSION:
Here we provide data showing the existence of sex-specific transcriptomic alterations in skeletal muscle of DM1 patients. These results underscore the importance of incorporating sex as a biological variable in transcriptomic studies of neuromuscular diseases.

ABSTRACT POSTER NUMBER: 54

Interpreting limb girdle muscular dystrophy R1 mutations in the calpain-3 structure

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Limb girdle muscular dystrophy type recessive 1 (LGMDR1) is a progressive decline of the hip and shoulder musculature that affects one in 42,700 people worldwide. LGMDR1 is caused by mutations to the gene for calpain-3, which encodes a calcium-activated cysteine protease abundant in skeletal muscle. Recently, our lab showed by single-particle cryo-EM and biophysical measures that recombinantly-produced calpain-3 forms a trimer of dimers. The trimeric interfaces of the calpain-3 hexamer introduce a novel set of intermolecular interaction sites that might be disrupted by LGMDR1 mutations. Furthermore, the dimer interface is not the typical 5th EF hand pairing seen in other calpains, but a helical bundle that presents another novel interface that could be the site for some mutations. The calpain-3 hexamer is retained in the presence of 10 mM Ca^{2+}, and its cryo-EM structure shows higher resolution with a large conformational change in the six protease core domains on the periphery relative to the calcium-free structure. Calpain-3 is known to bind the N2A region of the gigantic sarcomeric protein titin. When a 37-kDa titin N2A fragment is co-expressed with calpain-3, the hexamer dissociates into dimers, each of which can bind up to four titin fragments. The cryo-EM structure of this calpain-3-titin N2A complex is in progress and may reveal additional sites where LGMDR1 mutations can affect the physiological function of calpain-3 to cause muscular dystrophy. This work is funded by a CIHR Foundation grant to PLD and a Coalition to Cure Calpain-3 Award for Research into Calpain 3 and Calpainopathy.

ABSTRACT POSTER NUMBER: 55

Investigating a potential muscle-driven mechanism of neuromuscular denervation in ALS.

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by motoneuron degeneration and muscle atrophy. In SOD1^{G93A} mice, neuromuscular junction (NMJ) denervation precedes axon degeneration, motoneuron loss, and symptom onset. Peptidyl Prolyl Isomerase A (PPIA) is a chaperone protein highly expressed in the central nervous system of SOD1^{G93A} mice. Activation of the PPIA/EMMPRIN (Extracellular Matrix Metalloproteinases Inducer) pathway increases levels of the matrix metalloproteinase 9 (MMP-9). Notably, MMP-9 expression increases ALS motoneurons vulnerability while its inhibition rescues them and delays both muscle denervation and functional decline. At the NMJ MMP-9 can cleave collagen type IV, a key component of the extracellular matrix (ECM), whose homeostasis is essential for NMJ maturation and stability.
We hypothesize that PPIA/EMMPRIN pathway activation at the NMJ drives ECM dysregulation potentially contributing to NMJ denervation.
We demonstrated that EMMPRIN is expressed at the NMJ in tibialis muscle, and that its expression is increased in SOD1^{G93A} muscles compared to NTg mice. Tibialis muscles interstitial liquid analysis revealed that PPIA and MMP-9 secretion are increased in SOD1^{G93A}. At the NMJ, we demonstrated that collagen type IV turnover is altered, in a way that may drive ECM dysregulation leading to denervation and hinder reinnervation. SOD1^{G93A} myotubes show increased levels of EMMPRIN and secreted PPIA. Interestingly, SOD1^{G93A} myotubes are responsive to PPIA treatment, suggesting an autocrine activation of the pathway in these cells.
This study reveals a potential new muscle-driven mechanism of NMJ denervation in ALS, that may represent a novel therapeutic target.

Risdiplam clinical trial outcomes in individuals with spinal muscular atrophy (SMA) and four SMN2 copies

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BACKGROUND:
SMA is a genetic, progressive neuromuscular disease characterized by motor neuron loss. Three disease-modifying therapies (DMTs) are available for SMA, including risdiplam, an orally administered SMN2 pre-mRNA splicing modifier. SMN2 copy number does not fully predict the disease phenotype; some individuals with SMA and four SMN2 copies continue to decline with age and have significant comorbidities. Evidence suggests earlier treatment with an SMA DMT leads to better outcomes. This research presents results from risdiplam clinical trials that explore the impact of risdiplam on outcomes in individuals with SMA and four SMN2 copies.

METHODS:
Data from SUNFISH Part 2, JEWELFISH and RAINBOWFISH were analyzed. Motor function outcomes, as measured by 32-item Motor Function Measure, Revised Upper Limb Module and Hammersmith Functional Motor Scale–Expanded from SUNFISH and JEWELFISH, and Bayley Scales of Infant and Toddler Development, third edition, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders and Hammersmith Infant Neurological Examination, Module 2 from RAINBOWFISH, were assessed. Outcomes in individuals with four SMN2 copies (≥4 SMN2 copies for RAINBOWFISH) were compared with their corresponding overall study population.

RESULTS:
Treatment outcomes with risdiplam for individuals with four SMN2 copies were consistent with the overall study populations. Data from SUNFISH, JEWELFISH and RAINBOWFISH also showed a consistent risdiplam safety profile between individuals with four SMN2 copies and overall study populations.

CONCLUSIONS:
Individuals with SMA and four SMN2 copies showed benefit from risdiplam in various clinical endpoints, although sample size for this subgroup is small in the risdiplam clinical trial program.

SUNFISH PARTS 1 AND 2: 5-YEAR EFFICACY AND SAFETY DATA OF RISDIPLAM IN TYPES 2 AND 3 SPINAL MUSCULAR ATROPHY

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BACKGROUND:

Risdiplam (EVRYSDI®) is an oral survival of motor neuron 2 (SMN2) pre-mRNA splicing modifier, approved for spinal muscular atrophy (SMA) in over 100 countries. This study investigates the efficacy and safety of risdiplam in patients with Types 2 and 3 SMA.

METHODS:

SUNFISH (NCT02908685) is a multicenter, randomised, placebo-controlled, double-blind study in patients with Types 2 and 3 SMA, aged 2–25 years. Part 1 (*N*=51) assessed safety, tolerability, and pharmacokinetics/pharmacodynamics of risdiplam in patients. Part 2 (*N*=180) evaluated the efficacy and safety of the Part 1-selected dose in Type 2 and non-ambulant Type 3 SMA. Participants were treated with risdiplam or placebo for 12 months, then risdiplam in a blinded manner until Month 24, followed by an open-label extension phase. SUNFISH is now complete.

RESULTS:

The primary endpoint of change from baseline in the 32-item Motor Function Measure (MFM32) total score in patients treated with risdiplam (*n*=120) versus placebo (*n*=60) was met at Month 12. Increases in motor function were sustained over 4 years of risdiplam treatment, measured by MFM32, Hammersmith Functional Motor Scale – Expanded, and Revised Upper Limb Module. After 4 years, no treatment-related safety findings led to withdrawal from SUNFISH Parts 1 or 2.

Here we present final efficacy and safety results from SUNFISH after 5 years (data cut-off 2 October 2023).

CONCLUSION:

SUNFISH provides long-term efficacy and safety data of risdiplam in a broad population of children, teenagers, and adults with Types 2 and 3 SMA.

Muscle and bone growth in the lower legs of typically developing children and children with cerebral palsy: a longitudinal imaging study

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Longitudinal data on skeletal muscle growth in children are scarce, so little is known about developmental trajectories of skeletal muscle size, the relative timing of muscle and skeletal growth, and how cerebral palsy (CP) affects growth. We conducted a mixed longitudinal imaging study of 252 children aged 5-18 years, including 66 children with CP. Using custom-built artificial intelligence methods for accurate, automated segmentation of lower leg muscles and bones from mDixon MRI scans, we measured the volumes of ten individual leg muscles and tibia lengths on 2-3 occasions over, on average, three years. The measurements were used to estimate age- and sex-specific reference curves for growth velocities of muscle volumes and tibia length. At the population level, peak adolescent growth velocity in muscle volume was higher and occurred later in boys (155 cm^3/yr at 14.2 years) than in girls (117 cm^3/yr at 12.1 years) and occurred 1.9 and 1.8 years after peak tibia length velocity in boys and girls, respectively. We show the use of these normative data to identify musculoskeletal growth disorders in ambulant children with CP. In this cohort, median muscle growth velocities in children with CP were ~41% smaller and tibia length velocities were ~11% smaller than in age- and sex-matched typically developing children. This study shows how advances in MRI imaging and analysis methods enable large-scale research on musculoskeletal growth during typical childhood development as well as child- and muscle-specific analyses of the effects of neuromuscular disease on muscle and bone growth.

ABSTRACT POSTER NUMBER: 59

A comprehensive and queryable database with thousands of findings on biomarkers from >20 serum and tissue datasets for DMD

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BACKGROUND:
The promise of fit-for-purpose biomarkers is immense. However, biomarker efforts are often hampered by lack of reproducibility, discordant findings from different technologies, and unclear concordance of signals between studies due to confounders. For Duchenne muscular dystrophy (DMD), biomarkers are urgently needed that inform on pathophysiology, prognosis, pharmacodynamic response, monitoring, etc. Researchers often start from pure discovery and encounter substantial delays in bringing biomarkers into clinical practice as there is no central resource with aggregated overview of published evidence.

OBJECTIVES:
Build a dynamic, searchable, openly available database that compiles evidence on biomarkers for DMD.

METHODS:
Thousands of serum and muscle biomarker proteins from over 20 datasets were compiled, focusing on minimally invasive serum markers. Findings were obtained from supplemental material or standardized processing pipelines when raw data were available. Evidence was aggregated around association with DMD, treatment, age, clinical outcomes, and other biomarkers.

RESULTS:
The website and interactive Shiny application prioritize an intuitive user experience (tutorial and FAQ included), and provide exportable summaries and outputs (tables, figures) of findings. The findings can be filtered by annotations like age and treatment. Continuous updates with new/additional findings will make this a living resource.

CONCLUSIONS:
This knowledge database/tool provides summary estimates around individual studies' effect sizes and helps assess cumulative evidence beyond a single study's findings. This will facilitate a) quick comparison of new findings to published findings, b) new knowledge in terms of nuanced meta-analyses for a specific target, and c) reduce preparatory time and aid with future experimental design.

ABSTRACT POSTER NUMBER: 60

Differential Proteomic Profiling of Cerebrum and Myelin in the mdx Mouse Models of Duchenne Muscular Dystrophy.

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BACKGROUND:
Duchenne Muscular Dystrophy (DMD) results from loss of functional dystrophin, causing progressive muscle degeneration. Dystrophin is expressed in brain and skeletal muscles. The role of dystrophin as a membrane-stabilizer is well understood but its role in the brain is not. Biomarkers and pathways in the cerebral and myelin proteomes are an important research gap.

OBJECTIVE:
(1)Perform untargeted proteomic analysis in the cerebrum (in mdx52) and (2) study myelin proteome in mdx52 and mdx23 mice versus wild-type controls.

RESULTS:
Mass spectrometry was used to obtain proteomic profiles at early (8 weeks or 12 weeks) and late (1 yr) stages in both mdx52 vs BL6 and mdx23 vs BL10 with n=3 to 4 mice in each group, normalized, and analyzed using moderated t-tests. In the cerebrum, only α-Actinin-2 (Actn2; Q9JI91) survived FDR correction (7.0-fold up in mdx52). In the myelin, certain proteins, e.g., Claudin-11 and Rab5c were observed with opposite regulation over time in mdx52 (12 weeks vs at 1 year, compared to wild type), whereas in mdx23, Peroxiredoxin-2 was downregulated at both 8 weeks and 1 year.

CONCLUSIONS:
Parallel analyses reveal cerebrum and myelin proteomic alterations in the *mdx* model. Upregulation of Actn2 in dystrophic cerebrum and dysregulation of antioxidant/structural myelin proteins (e.g., Prdx2 and Ermin) suggest potential compensatory and/or pathological mechanisms in the central nervous system. These initial findings suggest the need for targeted validation of top candidates which could clarify CNS involvement in DMD and guide future interventions including therapeutic strategies.

ABSTRACT POSTER NUMBER: 61

Performance of the 2017 EULAR/ACR Classification Criteria in Indian Patients with Idiopathic Inflammatory Myopathies

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OBJECTIVE:
To evaluate the diagnostic performance of the 2017 EULAR/ACR classification criteria across major idiopathic inflammatory myopathy (IIM) subgroups, using established subgroup-specific criteria as the gold standard.

METHODS:
A total of 156 biopsy-confirmed IIM patients were retrospectively classified according to the EULAR/ACR 2017 criteria. Diagnostic accuracy, sensitivity, and specificity were calculated for each IIM subgroup using the following reference standards: Solomon & Connor's criteria (ASS), ENMC 2018/2024 (DM, IMNM, IBM), Bohan & Peter (PM, DM), and phenotype-based clinical criteria (OM). Concordant and discordant cases were analyzed in detail to identify causes of misclassification.

RESULTS:
The EULAR/ACR 2017 criteria demonstrated excellent diagnostic performance for dermatomyositis (accuracy: 0.97; sensitivity: 1.00; specificity: 0.96) and high specificity across all subgroups (range: 0.83–1.00). Notably, anti-synthetase syndrome (ASS) and immune-mediated necrotizing myopathy (IMNM) cases were entirely missed (sensitivity: 0.00) due to the absence of dedicated classification categories. Moderate sensitivity was observed for inclusion body myositis (IBM) (0.56) and polymyositis (PM) (0.78), with most misclassified cases showing overlapping histopathologic or serological features. Overlap myositis (OM) showed low sensitivity (0.28) due to lack of integration of connective tissue disease features.

CONCLUSION:
While the 2017 EULAR/ACR criteria exhibit strong specificity and accurately classify dermatomyositis, they lack sensitivity for ASS, IMNM, and OM due to limitations in subgroup representation. These findings underscore the need for revised classification frameworks that incorporate serological and histopathological features to improve diagnostic accuracy across the IIM spectrum.

ABSTRACT POSTER NUMBER: 62

Contributions of protein-S-glutathionylation to skeletal muscle differentiation

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Skeletal muscle is a highly regenerative tissue maintained by satellite cells, a population of adult muscle stem cells (MuSC) that can differentiate into multi-nucleated myotubes. During the regenerative process, MuSC must activate and commit to differentiation. We have previously identified that ROS and glutathione homeostasis are essential regulators of MuSC activation and commitment. Our lab has also delineated the role of a post-translational modification, protein-S-glutathionylation, catalyzed by the glutathione-S-transferase GSTP1 in the ROS and glutathione dependent activation of MuSCs. Here we have also uncovered that protein-S-glutathionylation is essential for differentiation. We have shown that using a broad-spectrum inhibitor of glutathione-S-transferases to block protein-S-glutathionylation abolished the differentiation capacity of C2C12 myoblasts into myotubes. Furthermore, we have characterized the changes in gene expression of glutathione-S-transferases during differentiation of C2C12 myoblasts and found that several glutathione-S-transferases (GSTP1, GSTMu1, GSTmu3, GSTT2, GSTZ1, and GSTO2-1) are dynamically regulated during differentiation. These data suggest that differential expression of these regulators may be required to target protein-S-glutathionylation of specific proteins during the process of myogenic differentiation. Thus, we aim to validate the contributions of these glutathione-S-transferases to the differentiation process through siRNA knockdowns or over-expression. In addition, we are identifying the target proteins which are glutathionylated by these glutathione-S-transferases through IP enrichment and proteomics analysis. We envision these targets will include important regulators of myogenic differentiation. Understanding the mechanistic outputs of protein-S-glutathionylation would provide better assessment of the therapeutic potential of modulating ROS and glutathione levels, and the ability to derive more targeted therapies for muscle diseases.

ABSTRACT POSTER NUMBER: 63

Epigenetic Role of Six1 in Muscle Stem Cell Activation

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Skeletal muscle regeneration is driven by muscle stem cells. These cells transition from a quiescent state into an activated one, where they begin expressing genes that orchestrate regeneration and self-renewal. Six1 is a transcription factor that is known to regulate myogenic differentiation, but its role during early stages of muscle stem cell activation is not well understood. This project aims to investigate whether Six1 contributes to chromatin remodeling and transcriptional activation during the initial transition out of quiescence.

We are testing whether Six1 functionally and spatially associate with key co-activators involved in chromatin regulation using proximity ligation assay. To further evaluate functional relevance of these co-activators, muscle stem cells are treated with small molecule inhibitors targeting each co-activator, followed by Assay for Transposase Accessible Chromatin sequencing (ATAC-seq) to measure early changes in chromatin accessibility. These experiments will clarify whether Six1 relies on these coactivators to influence chromatin structure during muscle stem cell activation.

ABSTRACT POSTER NUMBER: 64

Hydrogen sulfide rescues a common redox-dependent mechanism of eccentric contraction-induced force loss in Dmd(mdx) and Actb knockout mice.

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Eccentric contraction (ECC)-induced force drop is one of the most robust and reproducible phenotypes of the dystrophin-deficient *mdx* mouse, which is the most widely studied model of Duchenne muscular dystrophy. Because of its use as a quantitative assessment of therapeutic efficacy in preclinical trials, the mechanism of ECC force loss is of significant clinical importance. We have previously shown that perturbations in redox systems contribute to ECC force loss in dystrophic skeletal muscle. Specifically, we have shown that the combination of (1) excess production of reactive oxygen species (ROS), (2) a compromised ROS buffer, and (3) reduced production of the gasotransmitter hydrogen sulfide (H_2S) renders *mdx* muscle susceptible to ECC force loss and that this force loss is completely rescued by exogenous supplementation with the H_2S donor, sodium hydrosulfide (NaHS). Moreover, we have shown that dystrophin directly interacts with non-muscle cytoplasmic actin isoforms in structures called “costameres”. Here, we demonstrate that EDL muscles from muscle-specific, cytoplasmic β-actin knockout (ms-Actb KO) mice also present with significant ECC force loss, but of less magnitude than measured in *mdx* EDL muscles. Further, muscle-specific transgenic expression of β_{cyto}-actin protects ms-Actb KO muscles from ECC force drop. Consistent with our previous results in *mdx*mice, treatment of ms-Actb KO EDL muscles with the antioxidant *N*-acetylcysteine (NAC) or NaHS prevents ECC force loss to a similar extent as transgenic β_{cyto}-actin replacement. These data implicate increased oxidative stress and diminished H_2S as critical factors of ECC force loss in both dystrophin-deficient and cytoplasmic β-actin-deficient forms of myopathy.

ABSTRACT POSTER NUMBER: 65

Multispectral optoacoustic tomography as non-invasive biomarker for diagnosis and monitoring of neuromuscular diseases

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Despite recent diagnostic advances for neuromuscular diseases (NMDs), many patients with NMDs remain undiagnosed on a molecular level or have diagnostic delays preventing or delaying treatment. MRI has been utilized in defining patterns of muscle involvement and monitor progression. However, MRI scanners are costly, space-consuming, and require patients to lie still for long periods, which is challenging for children.

The last decade has demonstrated the usefulness of multispectral optoacoustic tomography (MSOT) as a non-invasive, non-ionizing radiation imaging technology in many medical fields. Recently MSOT has been used in patients with NMDs, correlating markers (e.g., lipids and collagen) with clinical outcome measures.

We hypothesized that MSOT may provide non-invasive, low-risk data to support NMD diagnosis via biomarkers. MSOT scans are performed, imaging at two positions of predetermined muscle regions on the left and right side of the body. Repeat scans will be obtained at intervals of 6-12 months allow the investigation of changes in intraindividual repeated measurements. It is hypothesized that the quantitative proportion of hemo/myoglobin and amount of collagen and lipid signal differ between different NMDs and correlate with functional clinical assessments. We will present preliminary MSOT results for 20 children with inherited, neuromuscular diseases, supporting the feasibility of this novel imaging technology in a pediatric setting.

ABSTRACT POSTER NUMBER: 66

A case of amyloid myopathy as the initial manifestation of immunoglobulin light chain amyloidosis

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Light chain (AL) amyloidosis is caused by an abnormal plasma cell clone that secretes immunoglobulin proteins that deposit as amyloid fibrils in various tissues, interfering with their structure and function. Amyloid deposition in muscle occurs in only 1.5% of cases and is typically accompanied by other end-organ damage.

An 86-year-old woman presented with an 8-month history of rapidly progressive muscle weakness and weight loss. She could previously walk unassisted, though developed difficulty ambulating and on presentation required a walker. She had no myalgias nor any oculobulbar, respiratory, sensory, or autonomic symptoms. On examination, she had severe muscle atrophy and proximal limb weakness, with milder weakness of her face, neck, and distal limbs. Nerve conduction studies demonstrated low-amplitude compound motor action potentials. Needle electromyography revealed widespread fibrillation potentials and myopathic motor unit action potentials.

Additional investigations showed an elevated serum creatine kinase level and an IgG kappa monoclonal protein with markedly elevated free kappa light chains. A bone marrow biopsy identified a plasma cell dyscrasia. MRI scans revealed diffuse muscle edema and atrophy. A left deltoid muscle biopsy showed significant amyloid deposition, in keeping with amyloid myopathy. Her smouldering myeloma was treated with daratumumab, cyclophosphamide, bortezomib, and dexamethasone.

Our case highlights how amyloid myopathy can represent the initial presentation of AL amyloidosis and its potential for rapid progression. Since there are available treatments to address the underlying plasma cell dyscrasia and prevent this myopathy's progression, clinicians should maintain a high index of suspicion to enable early diagnosis and treatment.

ABSTRACT POSTER NUMBER: 67

A preclinical rationale for combining SMAC mimetics with radiation therapy to reprogram cold rhabdomyosarcoma tumours

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Pediatric rhabdomyosarcoma (RMS) is driven by a failure of skeletal myoblasts to complete terminal differentiation, resulting in aggressive tumours with poor clinical outcomes. This pathology is exacerbated by the immunologically "cold" microenvironment of skeletal muscle and reinforced by immune evasion mechanisms. While multidisciplinary treatment combining surgery, radiation, and chemotherapy has pushed survival rates to 70-90% in low-risk patients, high-risk RMS remains refractory, with 5-year survival plateauing at 20–30%. This highlights a clear need for novel therapeutic strategies. Radiation therapy (RT) remains a cornerstone of treatment, capable of inducing localized immunogenicity. However, its efficacy is limited by stromal immunosuppression, upregulation of anti-apoptotic proteins, and reinforcement of the differentiation block. Inhibitor of Apoptosis proteins (IAPs), commonly overexpressed in RMS, contribute directly to immune resistance and survival signalling. A class of IAP antagonists called SMAC mimetics (SMs) are potent immunomodulators capable of reversing immune resistance and sensitizing tumours to immunogenic cell death. We hypothesize that combining RT with SMs will overcome IAP-mediated resistance, remodel the immune microenvironment, and alleviate the differentiation blockade, thereby converting "cold" RMS into a therapy-responsive, immunogenic tumour. Here, we begin by characterizing baseline traits and molecular responses to SMs in murine RMS cell lines (76-9, "cold," and M3-9-M, "hot"), identifying subtype-specific vulnerabilities. Future work will extend to human RMS cell lines and evaluate the therapeutic and immunologic synergy of RT+SMs in murine RMS models to establish a preclinical rationale for RT+SM combinations as a novel therapeutic strategy in high-risk pediatric RMS.

ABSTRACT POSTER NUMBER: 68

Muscle-specific extracellular vesicles : a novel biomarker for spinal muscular atrophy

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The neuromuscular disease spinal muscular atrophy (SMA) is caused by reduced levels of the SMN protein. There are three approved life-changing and life-saving SMN replacement strategies for SMA. Nevertheless, pre-clinical and clinical studies highlight the need for combinatorial approaches that will include SMN-dependent and -independent treatments. In light of this active and changing SMA therapeutic landscape, there is a pressing need for biomarkers that can inform on severity, disease progression and response to treatment.

Skeletal muscle releases extracellular vesicles (MuVs) that contain lipids, nucleic acids and proteins. Importantly, the number and content of MuVs adapt to and reflect the health status of skeletal muscle. While MuVs have shown biomarker potential in other muscle-wasting conditions, they have yet to be explored in SMA. We therefore set out to explore the potential of MuVs as biomarkers for SMA.

Our analyses of human SMA muscle cells reveals that they exhibit different lipid and protein secretion profiles compared to healthy controls. Similarly, we find significant changes in the MuVs characteristics (number, size, lipid content and protein content) in *Smn*$^{2B/-}$ SMA mice during disease progression. Importantly, treating *Smn*$^{2B/-}$ SMA mice with an SMN restoring treatment (scAAV9-*SMN1*) corrects many of these defects. Interestingly, we also observe MuV changes in hypomorphic Smn-depleted mice (*Smn*$^{2B/+}$, *Smn*$^{+/-}$ and *Smn*$^{2B/2B}$), but not to the extent of those observed in the SMA mice themselves.

Overall, our results suggest that MuVs may be a novel and valid biomarkers for SMA disease progression, SMN levels and response to treatment.

ABSTRACT POSTER NUMBER: 69

Engineering PBMC-derived skeletal muscle organoids as a model for Duchenne muscular dystrophy (DMD)

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Duchenne muscular dystrophy (DMD) is the most severe form of muscular dystrophy, causing progressive deterioration of skeletal and cardiac muscle due to mutations in the DMD gene that disrupt dystrophin production. While numerous therapies aim to restore dystrophin, *no cure currently exists*. Most DMD studies rely on mouse models, but their disease progression differs from humans. Therefore, a **human-derived model** of DMD is needed.

Organoids, or "*mini-organs*," offer a promising approach. These structures are created using induced pluripotent stem cells (iPSCs), which are regular cells reprogrammed to develop into many cell types. **Peripheral blood mononuclear cells (PBMCs)**—immune cells found in the bloodstream—have stem cell-like properties. The goal of this study is to generate dystrophic and healthy **skeletal muscle organoids (SkMOs)** using iPSCs derived from patient PBMCs.

This project aims to **(1)** isolate PBMCs from two samples: a DMD-affected patient and a healthy control. We have successfully isolated PBMCs from the DMD patient's blood and will proceed to **(2)** reprogram these cells into iPSCs. Next, we plan to **(3)** differentiate the iPSCs into myogenic progenitor cells and mature myotubes.

In an initial study, established PBMC-derived iPSCs (SCTi003-A) were used to attempt myogenic differentiation. These iPSCs formed small to medium colonies from Days 1 to 4, and larger three-dimensional colonies from Days 5 to 9. Cells failed to proliferate beyond this point, possibly due to a high initial seeding density.

Overall, SkMOs will provide a *more accurate* model for muscular dystrophy and help advance **personalized treatments** and **clinical trials**.

ABSTRACT POSTER NUMBER: 70

Functional characterization of a dystrophin-null mouse model, DMD-Null, for studying Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a debilitating and fatal X-linked disease affecting 1/5000 males worldwide, characterized by progressive muscle weakness and degeneration. At the molecular level, DMD is caused by mutations in the *DMD* gene encoding for dystrophin, a critical structural protein present in muscle. No cure currently exists for DMD, and research towards effective therapies remains ongoing. One of the most common animal models for DMD studies is the mouse muscular dystrophy (MDX) model, which has a *DMD* nonsense mutation. Unfortunately, despite the shared genetic etiology, MDX mice show a relatively mild dystrophic phenotype compared to affected humans, limiting their overall utility as a research model. One hypothesis for this mild phenotype is that MDX mice express shorter dystrophin isoforms that confer a protective effect. To address this, we aimed to characterize an improved disease model, DMD-Null, that has a total dystrophin knockout and is incapable of expressing shorter isoforms. 12-month functional studies found that DMD-Null mice show a more severe skeletal muscle phenotype than MDX mice across all ages, characterized by profound weakness, decreased exercise tolerance, and impaired muscle regeneration. After isolating muscle fibers for *ex vivo* growth, we also found that muscle progenitor cells from DMD Null mice show impaired proliferation and myogenic differentiation. This work provides a foundation for an improved mouse model for studying DMD, and uncovers novel relationships between dystrophin isoforms and muscle regeneration that could help inform clinical prognosis for DMD patients.

ABSTRACT POSTER NUMBER: 71

Population-based estimates of the direct and indirect costs of living with oculopharyngeal muscular dystrophy (OPMD) in Canada

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BACKGROUND AND OBJECTIVE:

Patients bear substantial costs of disease which are less commonly assessed in economic evaluations. To inform public policy and cost-effectiveness studies, we estimated the direct and indirect costs of living with oculopharyngeal muscular dystrophy (OPMD) in Canada.

METHODS:

As part of a larger cross-sectional survey of Canadians living with neuromuscular disorders, 109 adults with OPMD (57% female, 65±10 years of age) were asked to complete the work productivity and activity impairment questionnaire (indirect costs), and to report healthcare visits and expenditures for medical/assistive equipment (direct costs). Population-based cost estimates were made by approximation that 7,728 Canadians live with OPMD, and that OPMD begins to burden people at age 55. Non-work and work activity were valued at 35% and 100% of Canadian median wage, respectively.

RESULTS:

Canadians with OPMD bear an estimated annual cost of $70.6 million. Direct costs (healthcare visits: $6.0 million, medical/assistive equipment: $7.0 million) were exceeded by indirect costs (non-work activity impairment: $33.6 million, lost earning capacity: $16.7 million, total work impairment: $7.4 million). Employment status and disease impact were used to group individuals with OPMD. By participant category, mean annualized cost estimates were: 1) *Retired, impacted by OPMD*: $17,400 (~1,595 Canadians); 2) *Working, impacted by OPMD*: $27,500 (~596 Canadians); 3) *Not working due to OPMD*: $64,700 (~340 Canadians); 4) *Impacted by OPMD, not working for other reasons*: $11,700 (~383 Canadians); 5) *Not yet impacted by OPMD*: $0 (~4,814 Canadians).

CONCLUSION:

People with OPMD bear substantial costs that vary with symptom burden and employment status.

ABSTRACT POSTER NUMBER: 72

Early loss of intramuscular motor axons limits capacity for recovery following Smn up-regulating therapy in a mouse model of spinal muscular atrophy.

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Although treatments for spinal muscular atrophy (SMA) have dramatically changed the prognosis for patients, clinical response remains variable. Our objective is to identify post-treatment deficits which will inspire the next generation of therapies. Here we have used the cranial muscles from the SmnΔ7 mouse model of SMA to profile motor unit pathology in differentially vulnerable muscles.

We show that distal motor unit pathology is progressive in all muscles analysed and that loss of intramuscular motor axons precedes muscle denervation. Motor axon loss was evident by P1 in the most vulnerable muscles (40% of axons lost) with progressive loss of up to 77% by P12. Electron microscopy of intramuscular axons confirms the loss of motor axons and reveals a trend towards the selective loss of the more immature axons.

We next administered SMN-inducing therapies to mice at P2 and profiled axon number at P12. Following treatment, all muscles appeared fully innervated, but motor axon loss was still evident in the most vulnerable muscles. Importantly, a delay in treatment by 1 day increased the proportion of muscles with persistent loss of motor axons. Administration of dual Smn-upregulating therapy was unable to offer further protection to motor axons. Collectively this data demonstrates that Smn up-regulating therapy can inhibit motor axon loss, but the capacity is limited by the number of intramuscular axons which remain at the time of treatment onset. This work highlights the importance of developing complementary therapies which can promote motor axon regeneration to act in synergy with Smn-upregulating compounds.

ABSTRACT POSTER NUMBER: 73

Autophagy maintains the regenerative capacity of muscle stem cells

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Muscle stem cells, also known as satellite cells, are essential for muscle regeneration and maintaining muscle homeostasis and regenerative capacity throughout life. Satellite cell fate, including quiescence and their ability to activate and undergo differentiation or self-renewal, is subject to regulation by multiple molecular pathways. Emerging evidence demonstrates a role for cellular metabolism in regulating stem cell fate transitions. Autophagy is a metabolic cellular process of recycling and renewal that is responsible for the removal and repurposing of damaged cellular components and has been shown to mediate stem cell maintenance and differentiation in multiple stem cell systems. In muscle, defective autophagy has been implicated in age-related muscle degeneration, neurodegenerative disorders, and myopathies such as Duchenne Muscular Dystrophy (DMD), the most common and severe form of muscular dystrophy. Our work demonstrates that satellite cells in DMD exhibit reduced autophagy and impaired autophagy dynamics during regenerative myogenesis. Moreover, we found that pharmacological induction of autophagy in DMD satellite cells enhanced their differentiation capacity. Using genetic mouse models to delete the essential autophagy gene *Atg7* specifically in satellite cells, we are examining how autophagy regulates satellite cell fate and their ability to contribute to muscle homeostasis and regeneration. Our data indicates that loss of autophagy impacts the ability of satellite cells to maintain quiescence. Understanding how autophagy contributes to satellite cell function provides insight into the impact of defective satellite cell autophagy in muscle disease. Ultimately, we aim to modulate autophagy to enhance satellite cell regenerative capacity and promote muscle regeneration.

ABSTRACT POSTER NUMBER: 74

Influence of age, CTG repeat, phenotype and training on the Splicing Index in DM1 patients

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OBJECTIVE:
To identify the effect of age, CTG repeat, phenotype and training on the Splicing Index (SI) score in DM1 patients.

METHODOLOGY:
17 DM1 (9 men) participants underwent a 12-week lower-limb strength training program with vastus lateralis biopsies obtained before and after training. RNA extracted from the samples was sequenced along with RNA from vastus lateralis biopsies of 13 non-affected/untrained participants (5 men). Reads were aligned to the hg38 genome with Star. Alternative splicing analysis was done with rMATS. A 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 Provenzano *et al* (PMID: 39836447). Regression models were used to identify variables influencing SI.

RESULTS:
At baseline, SI score varied across participants, with 8 DM1 individuals having SI scores like non-affected participants. These individuals were primarily late-onset or had less than 300 CTG repeats. Age also appeared to influence SI scores as DM1 individuals younger than 40 years had lower SI scores than older individuals. Age, CTG repeats and phenotypes were shown to significantly affect SI score at baseline (variance: 47%). The influence of strength training on SI score is still under analysis.

CONCLUSION:
Response of splicing to strength training is variable and requires further analyses, including testing the effects of age, CTG repeat, and phenotype. It will also be interesting to determine if other splicing events beyond the SI panel are more representative of the splicing dysregulation in vastus lateralis.

ABSTRACT POSTER NUMBER: 75

MOXIe clinical trial overview of Omaveloxolone for patients with Friedreich Ataxia

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Presented by: **Leyderman K**

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BACKGROUND:
We summarize the efficacy and safety data for omaveloxolone in patients with Friedreich ataxia from the MOXIe clinical trial (NCT02255435, EudraCT2015-002762-23) and post hoc analyses.

METHODS:
In MOXIe Part 2, patients aged 16-40 were randomized 1:1 to receive omaveloxolone 150 mg or placebo. The primary outcome was change in modified Friedreich Ataxia Rating Scale (mFARS) from baseline to Week 48—patients could roll over into an open-label extension (OLE). A post hoc propensity-matched analysis compared patients treated with omaveloxolone in the OLE with a natural history cohort not receiving omaveloxolone over 3 years.

RESULTS:
Treatment with omaveloxolone significantly improved mFARS relative to placebo at Week 48, with a difference of −2.41 points for the full analysis set (n=82 [excluding severe pes cavus]; p=0.01) and −1.93 points for the all-randomized population (n=103 [including severe pes cavus]; p=0.03). Transient and reversible changes in aminotransferase levels were observed with omaveloxolone without other signs of liver injury. Headache, nausea, and fatigue were among the more common adverse drug reactions in omaveloxolone-treated patients. In a post hoc propensity-matched analysis, omaveloxolone-treated patients in the OLE progressed by 3 points at Year 3 versus 6.6 points in an untreated matched natural history cohort.

CONCLUSIONS:
Patients who received omaveloxolone showed a significantly stabilized neurological function and slowing of FA progression, as measured by mFARS.

The MOXIe Trial of Omaveloxolone in Friedreich Ataxia: Exploring the Transient Nature of Treatment-Emergent Adverse Events

Lynch D[1], Boesch S[2], Delatycki M[3], Giunti P[4], Goldsberry A[5a], Hoyle C[6], Mathews K[7], Khan S[5a], Meyer C[8a], Murai M[5a], Nachbauer W[2], Perlman S[9], Subramony S[10], Zesiewicz T[11]
Presented by: **Leyderman, K.**

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BACKGROUND:
We examined the frequency, severity, onset, and duration of treatment-emergent adverse events (TEAEs) in patients with Friedreich ataxia treated with omaveloxolone (*n*=51) versus placebo (*n*=52) in the multicenter, double-blind, placebo-controlled trial MOXIe Part 2 (NCT02255435).

METHODS:
The frequency and severity of TEAEs during MOXIe Part 2 were recorded during screening and at Weeks 2, 4, 12, 18, 24, 36, and 48, with a follow-up safety visit 4 weeks after the final dose at Week 52. Median time to onset and duration of TEAEs were examined in participants reporting ≥1 TEAE within 52 weeks of follow-up.

RESULTS:
TEAEs occurred in 100% of participants treated in both arms. Most events were mild or moderate in severity. Omaveloxolone-treated participants showed a higher incidence of TEAEs compared to those placebo-treated within the first 12 weeks of treatment. Events less frequently reported in omaveloxolone-treated participants after versus before Week 12 included elevated ALT/AST, headache, nausea, abdominal pain, fatigue, diarrhea, influenza, vomiting, muscle spasms, and decreased appetite. Most TEAEs had a median duration of ≤1 month.

CONCLUSIONS:
The more commonly reported TEAEs in MOXIe Part 2 experienced by participants who received omaveloxolone compared with placebo were generally reported within the first 12 weeks of treatment and decreased thereafter.

ABSTRACT POSTER NUMBER: 77

Managing aminotransferase elevations in patients with friedreich ataxia treated with Omaveloxolone: expert opinion on use considerations

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BACKGROUND:
Omaveloxolone is an Nrf2 activator approved for Friedreich ataxia (FA) in patients aged ≥16 years. In the MOXIe Part 2 trial, elevations in alanine or aspartate aminotransferase (ALT or AST) were among the most common treatment-emergent adverse events (TEAEs). Omaveloxolone prescribing labels (US and EU) provide guidance for monitoring and managing these elevations. We present expert opinions on use considerations for this topic based on real-world clinical practice that may help inform patient management decisions.

METHODS:
Semi-structured interviews were conducted with 4 FA experts from the US and EU and a US hepatologist.

RESULTS:
The panel provided their interpretations of the label and use considerations. Experts suggested that after 3 months on treatment, the frequency of liver function testing (LFT) may be reduced from monthly to every 6 months and then yearly for patients with normal ALT and AST levels. In real-world practice, clinicians may consider a more conservative approach of treatment interruption if ALT or AST levels increase to >3xULN in the absence of liver dysfunction, due to practical considerations as patients are followed up less frequently than in the trial setting. Per experts' experiences, patients who have a dose interruption due to ALT or AST elevations are indicated for LFT after 2 weeks; those with stabilizing or resolving ALT and AST may be reinitiated with stepwise dose titrations and close monitoring every 2 weeks.

CONCLUSIONS:
There are important clinical practice considerations for patient/laboratory monitoring regarding aminotransferase elevations in the real-world setting, which may inform management decisions.

Strength training improves mitochondrial respiration, H2O2 emission and histological muscle integrity in women with Myotonic Dystrophy Type 1

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Myotonic dystrophy type 1 (DM1) is a hereditary disease characterized by progressive muscle weakness, muscle atrophy, and physical limitations. There is currently no cure for DM1. Thus, rehabilitation is a promising strategy ton counteract muscle impairments. Strength training has shown positive effects in individuals with DM1, improving muscle function as well as mitochondrial content (mass and complex I and complex IV protein levels). This study aimed to evaluate the impact of 12 weeks of strength training on mitochondrial respiration, H_2O_2 emission and skeletal muscle integrity in women with DM1, compared to non-affected/untrained individuals. Muscle biopsies from the vastus lateralis were taken before and after the training in 9 women with DM1 and 9 age-matched controls. Mitochondrial respiration and H_2O_2 emission were measured using Oroboros fluororespirometry and normalized to OXPHOS protein content measured via immunoblotting. Quadruple immunofluorescence (QIF) analyzed key respiratory chain subunits I and IV, and markers of mitochondrial mass (VDAC1). The integrity of muscle fibres and the concentration of reactive oxygen species (ROS) were examined using histological staining techniques. Strength training restored mitochondrial respiration to control levels and reduced normalized H_2O_2 emission (pre- vs post-training: $p<0.01$). Additionally, percentages of fibres with damaged laminin and nuclear clumps showed improvement (pre- vs post-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 enhance mitochondrial respiration, H_2O_2 emission and skeletal muscle integrity in the DM1 population, potentially slowing disease progression.

ABSTRACT POSTER NUMBER: 79

Investigating the role of aquaporin 1 and 4 in skeletal muscle function and pathology in Duchenne muscular dystrophy

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BACKGROUND:
Aquaporins (AQPs) are water channels essential for fluid homeostasis. In skeletal muscle, AQP4 is localized to the sarcolemma, while AQP1 is predominantly found in the microvasculature. In Duchenne muscular dystrophy (DMD), AQP4 is downregulated and AQP1 is upregulated; however, the functional significance of these changes remains unclear.

METHODS:
AQP1 mRNA and protein levels were assessed in wild-type (WT), *mdx*, and AQP4 knockout (KO) mice. Novel double-mutant models (AQP1 KO;*mdx* and AQP4 KO;*mdx*) were generated. At two months of age, WT, AQP1 KO, AQP4 KO, *mdx*, and double-mutant mice underwent voluntary wheel running, grip strength, and open field testing. To assess regenerative capacity, muscles were injected with cardiotoxin, and collected at 7, 14, and 26 days post-injury for histological analysis. Collagen deposition was evaluated using picrosirius red (PSR) staining, and fibrotic gene expression was quantified by qPCR.

RESULTS:
Both AQP1 KO;*mdx* and AQP4 KO;*mdx* mice demonstrated significantly reduced grip strength compared to *mdx* mice. All dystrophic and KO groups exhibited decreased running wheel activity compared to WT, while only AQP1 KO groups displayed significantly reduced open field activity. Notably, AQP1 KO;*mdx* mice presented with more severe histopathology, including increased collagen deposition and fibrotic gene expression compared *to mdx* mice.

CONCLUSIONS:
Loss of AQP1 exacerbates the dystrophic phenotype in *mdx* mice, indicating a compensatory and potentially protective role in DMD. In contrast, AQP1 deficiency in WT mice impaired voluntary locomotor activity without major pathological changes, suggesting that AQP1 function becomes critical during muscle stress or degeneration.

E3 Ligase Parkin mediates muscle stem cell lineage progression

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The regenerative capacity of muscle predominantly depends on its tissue-resident muscle stem cells (MuSCs). MuSC activation and lineage progression is tightly regulated, and recent reserach shows that optimal mitochondrial function is essential for correctly balanced fate decisions. Mitophagy, a selective form of autophagy targeting mitochondria, is a key regulator of mitochondrial homeostasis, though its contribution to MuSC fate decisions and skeletal muscle regeneration has not been fully elucidated. Using a MuSC-specific conditional Parkin knockout mouse model, we investigated the effects of Parkin deletion on myogenic lineage progression. Our results demonstrate that Parkin deficiency reduces mitophagy in quiescent MuSCs and alters the MuSC bioengergetic profile to that which indicates activation. In cardiotoxin-induced injury experiments, Parkin-deficient mice exhibited impaired muscle regeneration, as evidenced by reduced regenerative capacity in the tibialis anterior (TA) muscle. Furthermore, analysis of MuSC fate decisions on isolated single fibers revealed that Parkin deficiency promotes early activation and commitment at the expense of self-renewal. This was accompanied by a reduction in the proliferative capacity of Parkin-deficient MuSCs. Additionally, we identified a nuclear pool of Parkin in quiescent MuSCs, indicating a potential mitophagy-independent role in stem cell function. To further explore this mechanism, Parkin localization was tracked during the transition from quiescence to activation and throughout proliferation, where was seen to localize in the euchromatin and partially colocalized with nuclear speckles. This study highlights the critical role of Parkin in MuSC fate decisions and provides new insights into the mechanisms governing muscle regeneration and longevity.

ABSTRACT POSTER NUMBER: 81

Investigating a miniaturized nebulin for nebulin-related nemaline myopathy gene therapy

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Nemaline myopathy (NM) is a rare congenital disease that leads to muscle weakness and hypotonia, with no curative therapies yet. The majority of NM cases are due to mutations in the nebulin gene (*NEB*), which has coding sequence of ~25kb, coding a structural protein in the thin filaments. Due to its large size, it is hard to administer *NEB* to patients using conventional gene therapy. The central region of NEB protein has super repeats (SR), where each contain seven simple repeats. Each simple repeat has an actin binding motif, and each SR has a troponin interaction site. This leads to regularly spaced protein interactions. Here, we are investigating the potential of a functional miniaturized NEB by deleting *full* SRs. We created zebrafish models with partial and full SR deletions testing whether the latter is tolerated. We found that fish with a full SR deletion were similar to wild-type, while the partial deletion led to a severe phenotype. Therefore, there is a promise to build a functional miniaturized NEB that can be delivered to patients. We are currently testing a 3-AAVMYO gene therapy containing three NEB fragments (~40% of NEB). The fragments will come together at the protein level by protein *trans*-splicing, forming a midiNEB. MidiNEB contains NEB's termini and eight SRs. Interestingly, treated NM mice showed improved survival, with no significant effect on their muscle function (grip strength). Next, we will be designing 4- and 5-fragment midiNEBs, coding for 60% and 80% of full-length NEB, respectively.

ABSTRACT POSTER NUMBER: 82

Tracking early oxygen dysregulation in DMD rat models: toward optical monitoring of disease progression

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Duchenne muscular dystrophy (DMD) is a genetic disorder characterized by progressive muscle degeneration due to dystrophin deficiency. While muscle pathology in DMD has been extensively studied, vascular contributions to disease progression are often overlooked. The objective of this work was to compare the peripheral and cerebral microcirculation in DMD and wild-type rats.
This study employed near-infrared spectroscopy (NIRS) to non-invasively measure tissue oxygen saturation (StO2) in the brain and skeletal muscle of DMD rats and age-matched wild-type controls (n=6) at 4 and 5 weeks of age. NIRS captured changes in oxygenated and deoxygenated hemoglobin under physiological conditions. After the final measurement at 5 weeks, the diaphragm, heart, brain, and skeletal muscle were harvested for molecular and histological analyses.

At both time points, DMD rats showed elevated muscle StO_2 (83.1±3.5% and 80.4±2.9%) compared to wild-type rats (74.7±5.2% and 71.8±2.9% at 4 and 5 weeks, respectively). This corresponds to a lower oxygen extraction rate in DMD compared to healthy rats (21.5% vs. 33.2% at 4 weeks and 23.9% vs. 37% at 5 weeks, $p<0.01$). There were no significant differences in oxygen extraction in the brain between the groups.

The findings suggest dysregulated oxygen delivery and utilization in the skeletal muscle of DMD rats during early disease progression. Ongoing work linking histology to optical biomarkers offers promising avenues for non-invasive monitoring of muscle health and disease progression in DMD, with the potential to track treatment response over time.

ABSTRACT POSTER NUMBER: 83

Identification of early symptomatic gene expression changes in motor neurons in a large animal model of motor neuron disease

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Despite advances in spinal muscular atrophy (SMA) therapies, the mechanisms underlying selective motor neuron (MN) loss in SMA remain unclear. Here we used a porcine model of progressive MN degeneration induced by AAV9-mediated knockdown of *SMN* (AAV9.shSMN), which recapitulates the clinically relevant, cell-specific pathology of human SMA. Longitudinal analysis of plasma neurofilament light chain (NfL) and electromyography (EMG) was used to track disease onset and progression. Injected piglets exhibited elevated NfL 2 weeks post-injection (wpi), preceding hindlimb weakness ~3wpi. EMG revealed consistent denervation and reduced MUNE by ~4wpi. These results indicate 1-, 2-, and 4wpi as key stages in disease progression. To examine molecular changes preceding symptom onset, piglets received intrathecal AAV9.shSMN on postnatal day 5 and were sacrificed at 1wpi (presymptomatic) and 2wpi (early symptomatic). Lumbar MNs were isolated by laser capture microdissection for RNA analysis. *SMN* mRNA levels were reduced by ~79% at 1wpi, confirmed by ddPCR and RNA-seq. Despite this, only *ATP2A3* was significantly downregulated at 1wpi. In contrast, at 2wpi broader transcriptomic alterations were detected, including enrichment of genes related to synaptic and axonal components, ion/anion transport, and cell junction pathways, suggesting early disruption of neuronal communication and membrane transport. This inducible SMA pig model enables high-resolution investigation of presymptomatic MN degeneration and validation of translational biomarkers. Our results highlight 2wpi as a critical timepoint of transcriptomic alterations, providing insight into the molecular cascade preceding MN loss. These findings advance our understanding of MN vulnerability, guiding future therapeutic strategies.

ABSTRACT POSTER NUMBER: 84

Presence of symptoms and signs of neuropathy in a cohort of patients with SCA27B

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Spinocerebellar ataxia 27B (SCA27B) is a recently described cause of autosomal dominant ataxia. It is caused by an intronic GAA repeat expansion in FGF14. Alleles with 250-299 GAA repeats are considered pathogenic with incomplete penetrance and alleles above 300 GAA repeats fully penetrant. Scarce reports suggest expansions as low as 200 GAA repeats can be pathogenic in some individuals.

We clinically evaluated and performed 126 individuals from 70 families followed in our clinic carrying a >200 GAA repeats. Comparisons were performed between individuals with 200-249 (n=18), 250-299 (n=17) and above 300 GAA repeats (n=91), and assessed with Chi-square test for categorical variables and Kruskal-Wallis test for continuous variables. Reduced vibration sensation in the ankles was present in 41% of the patients (44/108), reduced light touch in 11.5% (3/26) and reduced joint position in 0% (0/25). Achillean hyporreflexia was found in 21% (25/119). EMG reports were available for 31 patients. It was abnormal in eight, showing mild axonal sensory neuropathy with a mild motor neuropathy component in two patients. No significant differences between repeat expansion size groups (200-249 vs 250-299 vs more than 300) were identified in any of the categories.

In conclusion, although mild reduced vibration sensation or mild ankle hyporreflexia can be found in SCA27B, peripheral neuropathy is generally absent or mild. This is important for the differential diagnosis with other causes of late-onset ataxia like CANVAS syndrome.

Investigating the impact of muscle stem cell-specific mitochondrial dysfunction on inflammation during muscle regeneration

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Aging and many neuromuscular diseases are known to involve the loss of, and impaired activity of pre-existing adult muscle stem cells (MuSCs) in addition to the generation of a pro-inflammatory environment. A contributor to this phenomenon is mitochondrial dysfunction, leading to impaired cellular energy metabolism, heightened oxidative stress and also plays a central role in triggering systemic inflammation. We have shown that short-term loss of the mitochondrial fusion protein OPA1, a major regulator of mitochondrial dynamics, in MuSCs completely abolishes muscle regeneration. Interestingly, we also observed a significantly higher influx of macrophages into the tissue compared to wild type controls. Indeed, our investigation revealed that depletion of the mitochondrial protein OPA1 in MuSCs causes greater inflammation in regenerating muscle. Specifically, at 4 days post CTX-induced muscle injury, loss of OPA1 in MuSCs leads to an overt infiltration of macrophages beyond the normal level observed as a physiological response to the injury. Therefore, we believe that changes in mitochondrial dynamics and OPA1 in MuSCs promotes signaling to immune cells. We aim to examine the link between mitochondrial dysfunction, skeletal muscle inflammation, and muscle stem cell signaling. Our studies will probe the impact that loss of OPA1 has molecular mechanisms involved during an intracellular immune response and its connection to the MuSC-to-immune cell signaling axis. By investigating the molecular mechanisms underlying this process, we hope this can aid in identifying therapeutic targets.

ABSTRACT POSTER NUMBER: 86

Limb-Girdle Muscular Dystrophies in a Single-Center Adult Population: Clinical Spectrum and Genetic Findings

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BACKGROUND:
Limb-girdle muscular dystrophies (LGMDs) are a heterogeneous group of genetically inherited myopathies characterized by progressive weakness of the limb-girdle muscles. Here we describe the clinical, genetic, and pathological features of a cohort of LGMD patients followed at Montreal Neurological Institute-Hospital.

METHODS:
We conducted a retrospective cohort study including 43 patients with LGMD between 2018-2024. Clinical data, muscle pathology, CK levels, cardiac and respiratory involvement, and genetic findings were collected. Functional status was evaluated using Composite MRC Score and Functional Milestones. Disease progression was assessed longitudinally when data were available.

RESULTS:
A total of 43 patients (median age 50 years (range 20-83) ; 67% females (29/43)) were included, representing 8 LGMD subtypes. The most common subtype was LGMD2L (ANO5 related) accounting for 18% (8/43) of cases. Mean age at symptom onset was 24.6 years, and mean duration of disease was 25 years (2-65y). Pathological data from the biopsies was present in 27/43 patients. Median CK (range) for the cohort was 2737 (from 52 to 10.000). Median severity by MRC sum scale was 47 (20-60). 19 patients required a cane in the history of his condition and 13 patients required wheelchair at a median age of 48 years. 7 patients presented cardiac involvement and 17 respiratory restrictive pattern. Genetic yield was 50% (22 confirmed genetically, 5 negative panel, 8 suspected compound heterozygosity, 6 VUS and 2 pending results).

CONCLUSIONS:
This cohort highlights the clinical and genetic diversity of LGMD. Early diagnosis and multidisciplinary care are essential to optimize management and monitor complications.

ABSTRACT POSTER NUMBER: 87

Telemedicine and Remote Monitoring in Neuromuscular Disease Care: Effectiveness and Challenges in Follow-up Care and Multidisciplinary Coordination

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BACKGROUND: Patients with NMDs often face mobility limitations and geographic barriers to accessing expert care. The COVID-19 pandemic accelerated the adoption of telemedicine and remote monitoring technologies, transforming healthcare delivery for this vulnerable population (1).

OBJECTIVE: To analyze the effectiveness and challenges of telemedicine tools and wearable devices for ongoing patient management in NMDs.

METHODS: This review synthesizes current evidence on the implementation of telemedicine in NMDs. We evaluated outcomes related to patient access, care coordination, satisfaction, and the effectiveness of clinical monitoring across various neuromuscular conditions.

RESULTS: Telemedicine significantly improved access to specialist care, with 100% of European NMD healthcare providers using telemedicine during COVID-19 compared to 60% pre-pandemic (2). Patient satisfaction was consistently high, with 74.4% of neurology patients rating their telemedicine experience as 8/10 or higher (1). Wearable devices and sensor-based systems demonstrated strong correlations with clinical assessments for monitoring mobility, vital signs, and disease progression (3)

CHALLENGES: technology access disparities, particularly among elderly patients requiring caregiver assistance (2). Clinical limitations included the inability to perform comprehensive neurological examinations and assess subtle signs, such as mild weakness or sensory deficits (4). Integration challenges involved workflow adaptation, data privacy concerns, and regulatory inconsistencies (5,6).

CONCLUSIONS: Telemedicine and remote monitoring have significantly improved access to care and care coordination for NMD patients, resulting in high satisfaction and reliable clinical monitoring. However, addressing technology access, clinical limitations, and integration barriers is essential to realize their potential in neuromuscular disease management.

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Train(e)d: Training Platform for Building National Clinical Outcome Assessment Capacity in Neuromuscular Disease

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INTRODUCTION:

With Health Canada's approval of disease-modifying treatments for neuromuscular diseases (NMDs), including conditions such as Spinal Muscular Atrophy (SMA) and Friedreich Ataxia (FA), Clinical Outcome Assessments (COA) have become essential not only for monitoring disease progression and treatment efficacy, but also for responding to reimbursement requirements of regulatory agencies.

Given the rarity of these NMDs it is only in this new era of disease treatment that knowledge gaps, shortages of trained personnel, and inconsistencies in COA delivery have become evident.

To sustainably address these disparities, we developed "Train(e)d", an on-demand virtual COA training platform.

METHODS:

Leveraging NMD4C's community of practice (CoP), an expert faculty was established, composed of clinical evaluators specializing in SMA and FA COAs. This faculty collected real-world case examples of individuals with SMA and FA undergoing COA evaluation, and narrated videos describing the COAs and patient's performance. The resulting material was used to develop educational interactive videos on Moodle LMS, which were subsequently validated by experts to ensure content accuracy.

KEY FINDINGS:

Preliminary qualitative data from beta testers indicate strong appreciation for the tool, particularly in terms of accessibility, content quality, and learning experience, and expressed interest in sharing the tool with peers. Collection of website metrics and post-training surveys will be used to evaluate the platform's effectiveness and user experience.

CONCLUSION:

With interactive features and numerous real-world case examples, Train(e)d represents a first-of-its-kind initiative to support consistency in clinical practice through integration within a national CoP for physiotherapists and occupational therapists.

ABSTRACT POSTER NUMBER: 89

Experience as evidence: mapping the patient journey of Friedreich ataxia in Canada

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INTRODUCTION:
Friedreich Ataxia (FA) is a rare, progressive, multisystemic neuromuscular disorder that presents with nonspecific early symptoms, often resulting in delayed diagnosis and fragmented care. With the recent approval of Omaveloxolone (Skyclarys) in Canada, it is critical to understand the diagnostic and care pathways experienced by individuals with FA to inform person-centered care strategies and optimize treatment access.

METHODS:
A mixed-methods study was conducted to map the healthcare journey of individuals with FA in Canada. Participants completed an online survey capturing four domains: symptom onset, diagnostic experiences, clinical trial and treatment access, and daily life with FA. A subset of participants also took part in semi-structured interviews to explore their experiences in greater depth. Quantitative data were analyzed using descriptive statistics, while qualitative data underwent content analysis to extract key themes.

RESULTS:
Eighty-six individuals participated, including 66 individuals with FA and 20 caregivers or family members. On average, participants reported a 6-year delay from symptom onset to diagnosis. A patient journey map was developed to visualize experiences from initial symptoms through to diagnosis, clinical trial engagement, and current life with FA. Nine themes were identified that illustrate the physical, emotional, psychosocial, and financial toll of living with FA, as well as the barriers encountered in accessing appropriate care and support.

CONCLUSION:
Findings reveal persistent diagnostic delays and significant unmet needs in care and support for Canadians living with FA. Improved awareness, earlier diagnosis, and timely access to innovative therapies are essential to improving outcomes and quality of life.

Characterizing spinal cord pathology in the cuprizone autoimmune encephalomyelitis model: evaluating its potential as a comprehensive model for multiple sclerosis research

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Multiple sclerosis (MS) is a chronic neurodegenerative autoimmune disease characterized by progressive inflammatory demyelination within the central nervous system (CNS). Although MS research has advanced considerably, no single mouse model fully encapsulates all aspects of the disease. Classical models lack the immune component or only affect specific areas of the CNS, while MS lesions are observed widely throughout the CNS. A novel mouse model of inflammatory demyelination, called the cuprizone autoimmune encephalomyelitis (CAE) model, possesses potential in addressing these limitations. The objective of this study is to determine whether the CAE model can replicate MS-like pathology in tissues beyond the brain. The CAE model was induced in 7-8-week-old wildtype C57BL/6 mice by feeding animals cuprizone chow for two weeks followed by an immune boost. Mice were euthanized at various time points to examine disease pathology progression at different stages. Lumbar spinal cord tissue was collected and stained via immunohistochemistry to assess demyelination, axonal degeneration and immune cell abundance/activation. Male mice showed early myelin loss and axonal damage at 2-4 weeks cuprizone exposure, while females exhibited delayed responses peaking at 8 weeks. Microglia activation aligned with neurofilament changes in both sexes. Our findings reveal that the CAE model induces substantial MS-like pathology in the spinal cord. Notable alterations in myelin, axonal health markers, and immune cell activation were observed in male mice. Females exhibit similar trends but with a delayed onset. This discovery suggests the CAE model may offer a more comprehensive view of demyelinating diseases like MS.

ABSTRACT POSTER NUMBER: 91

A dosage study for aav9-mediated gene therapy and the development of combinatorial therapy for the treatment of spinal muscular atrophy

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Spinal Muscular Atrophy (SMA) is a devastating neuromuscular disorder caused by biallelic mutation of the *SMN1* gene. SMA affects 1 in every 8-10,000 live births and although considered rare, newborn screening in Ontario has identified SMA as the third most commonly inherited disorder. While current therapies have improved motor function and increased lifespan for many patients with SMA, there remains a significant population for whom treatment is ineffective. Moreover, even for those who respond well, no therapy is curative and challenges such as regular and invasive re-administration, the "wearing off" of therapeutic effect, and the development of liver toxicity among other treatment-related adverse events, emphasizes the need for further exploration into novel and combinatorial therapies. The work in this study will provide a characterization of an SMA mouse model treated with low dose survival motor neuron 1 (*SMN1)* gene replacement therapy, that will be used as a model to test and compare combinatorial therapies. In this context, our laboratory, in collaboration with Dr. Christiano Alves (Harvard University), is currently exploring the potential of a novel CRISPR-based therapy for the treatment of SMA. This CRISPR-based therapy will be tested in combination with the low dose *SMN1* gene replacement therapy and in combination with a low dose antisense oligonucleotide (ASO) therapy, respectively. This study will determine the potential of our novel CRISPR-based approach for the treatment of SMA, as well as describe the effects of this therapy in combination with currently approved treatments.

ABSTRACT POSTER NUMBER: 92

ClC-1 chloride channel inhibitor NMD712 improves motor function in mouse models of congenital myasthenic syndromes

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A rare neuromuscular condition, congenital myasthenic syndromes (CMS), cause fatigable muscle weakness that can be fatal through respiratory complications. While some disease modifying treatments for CMS are available, these have limited efficacy. Chloride channel 1 receptors (ClC-1) are skeletal muscle specific ion channels that regulate muscle fibre excitability. Partial inhibition of ClC-1 with an orally bioavailable small molecule (NMD712) has been reported to improve muscle function and neuromuscular transmission in rat models of myasthenia gravis (MG), a neuromuscular condition with a similar disease mechanism to CMS. Patients with MG had a clinically improved score following treatment with NMD670, a ClC-1 inhibitor similar to NMD712, in a small, randomized, placebo-controlled clinical trial. We tested NMD712, in established mouse models of two CMS genetic subtypes, *Agrn*-CMS and *ColQ*-CMS. In the *Agrn*-CMS study, mice received daily intraperitoneal (IP) injections of NMD712 or control from postnatal day 7 (P7) to P30, while in the *ColQ*-CMS study, mice received daily IP injections of NMD712 or control from P22 to P43. Control-injected wild-type mice were included for comparison in both studies. We assessed motor behaviour through hindlimb suspension, grip strength, and inverted screen tests and evaluated neuromuscular junction (NMJ) transmission using repetitive nerve stimulation. We report that NMD712 treatment improved survival and grip strength in *Agrn*-CMS mice, and improved inverted screen test performance in *ColQ*-CMS mice. Muscle fibre typing and NMJ morphological analysis are ongoing. Our preliminary results show promise for ClC-1 inhibition as a treatment option for specific CMS subtypes.

Regulation of mitophagy by the NIX pathway during MuSC state transition

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Muscle stem cell (MuSC) regulation requires precise mitochondrial quality control, yet the underlying molecular mechanisms remain incompletely understood. Previous work from our laboratory demonstrated that mitophagy is temporally regulated during quiescent-to-activated MuSC transitions. We established that the PINK1/PARKIN pathway serves as a critical regulator of mitophagy in quiescent MuSCs. Loss-of-function studies revealed that PINK1/PARKIN deficiency disrupts MuSC fate determination by impairing self-renewal and promoting premature myogenic commitment.

Transcriptomic analyses have also identified NIX, a mitochondrial autophagy receptor, as a potential additional contributor to mitophagy regulation in MuSCs, but its role has not been established. We generated a MuSC-specific inducible knockout of NIX to investigate its function. Imaging of FACS-purified MuSCs revealed decreased colocalization between mitochondria and autophagosomes in the absence of NIX, indicating impaired mitophagy. Fate decision kinetics in cultured EDL fibers showed that NIX deficiency increased propensity to activate and commit at the expense of self-renewal. In vivo cardiotoxin injury experiments demonstrated reduced numbers of Pax7-positive MuSCs 21 days post-injury, confirming impaired self-renewal capacity. Ongoing studies aim to define the consequences of these alterations on muscle regeneration and elucidate the molecular mechanisms linking altered mitophagy to impaired fate decisions.

Overview of the Canadian Neuromuscular Disease Registry (CNDR): Applying Real-World Evidence to Improve Disease Research and Outcomes in Canada

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The Canadian Neuromuscular Disease Registry (CNDR) is a national, clinic-based registry that collects longitudinal clinical and natural history data from individuals with neuromuscular disorders. Launched in 2010, the CNDR initially focused on indexing Amyotrophic Lateral Sclerosis (ALS), Duchenne Muscular Dystrophy (DMD), Myotonic Dystrophy (DM), Limb-Girdle Muscular Dystrophy (LGMD), and Spinal Muscular Atrophy (SMA). With recent support from Brain Canada and FSHD Canada, the registry is expanding to include Facioscapulohumeral Dystrophy (FSHD) in 2025. To date, over 7,000 individuals across the lifespan have been enrolled. Participating clinical sites submit disease-specific datasets annually, incorporating standardized metrics on diagnostic tests, therapeutic interventions, and functional status. Data elements are defined in collaboration with clinical experts and reflect established standards of care.

In partnership with the Neuromuscular Disease Network for Canada (NMD4C), and Muscular Dystrophy Canada (MDC), the CNDR offers secure, de-identified data to qualified researchers at no cost. The registry provides data to support care pathway improvement, equitable access to therapy, and robust evidence for health system planning. It has facilitated over 125 academic and industry-led research projects and supported 37 clinical trial or data-sharing initiatives.

Patients and families consenting to clinic-based or self-registration may access research opportunities, surveys, and clinical trials—particularly valuable for those in remote or underserved areas. Current registry development priorities include alignment with ICF standards and implementation of validated patient-reported outcome measures (PROMs). Registry data will be presented, including demographic profiles and baseline clinical characteristics of individuals in Canada affected by neuromuscular disorders (NMDs).

Development of an in-silico workflow to characterize the spliceogenicity and protein effect of splice-altering variants

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Splice-altering variants constitute a significant proportion of disease-causing variants in monogenic neuromuscular diseases. However, determining the pathogenicity and functional impact of splice-altering variants, especially beyond canonical AG/GT sites, remains challenging. Various in-silico tools exist for analysing splicing changes, but none are sufficiently accurate to predict the entire spectrum of splicing defects. Here, we developed a workflow which integrates prominent web-based splicing prediction tools to accurately assess pathogenicity and predict downstream transcript-level effects.

Putative and suspected splice-altering variants associated with neuromuscular phenotypes were retrieved from literature and in-house cases. Pathogenicity is determined through a primary analysis using SpliceAI, Pangolin, HSF, and MaxENT. These tools calculate scores for changes to splice sites and splice site regulatory element ratios (ESE/ESS) with the following thresholds: SpliceAI and Pangolin Δ-splice site ≥ 0.1, HSF and MaxENT Δ-splice site ≥ 10%, HSF Δ-ESE/ESS ≥ 8. The predicted transcript impact is determined through the primary analysis coupled with supporting data from SPIP, dbscSNV, ABSplice, and SpliceVault.

To date, 38 variants have been analyzed. 29/38 variants were splice-altering with the following transcript-level effects: exon skipping (22/29), partial intron retention (20/29), whole intron retention (4/29), and partial exon deletion (4/29). Functional data was available for 17 variants, with predictions being concordant in 16/17 variants.

A comprehensive analysis of splice-altering variants can be achieved by combining an in-silico approach that analyses canonical/cryptic splice sites and ESE/ESS, followed by transcript-level interpretation. Generating a larger variant dataset is required to determine the overall efficacy of the workflow.

ABSTRACT POSTER NUMBER: 96

The Glucocorticoid Receptor Regulates Nuclear Positioning During Skeletal Muscle Differentiation

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Proper myonuclear positioning is essential for skeletal muscle development and function, relying on coordinated interactions between the nucleoskeleton, the LINC (Linker of Nucleoskeleton and Cytoskeleton) complex, and the cytoskeletal network. Although glucocorticoids are widely used to treat muscular dystrophies, the precise role of the glucocorticoid receptor (GR) in muscle differentiation remains incompletely understood. In this study, we investigate the role of GR in regulating nuclear positioning during myogenesis. We demonstrate that GR loss in differentiating myotubes leads to impaired myonuclear alignment, accompanied by reduced Lamin A expression, defective SUN1 recruitment to the nuclear envelope, and mislocalized microtubule-organizing centers (MTOCs), resulting in disrupted microtubule architecture. These findings reveal a previously unrecognized role for GR in coordinating the Lamin A–SUN1–MTOC axis, which is critical for microtubule-guided nuclear positioning during skeletal muscle differentiation. Our work provides mechanistic insight into the benefits of GC therapy in dystrophic muscle and supports the development of selective GR modulators that preserve therapeutic efficacy while minimizing muscle atrophy-associated side effects.

Patient MTRFR iPSC-derived neurons and muscle exhibit cell specific phenotypes and mitochondrial dysfunction.

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The nuclear gene *C12orf65* encodes the protein MTRFR which is responsible for removing the peptide chain from stalled mitochondrial ribosomes[1,2]. Mutations to *C12orf65* cause mitochondrial disease with a spectrum of presentations including optic atrophy, peripheral neuropathy, spastic paraparesis, intellectual disability and distal weakness[3–5]. Patients with MTRFR variants have been diagnosed with Leigh syndrome, Charcot-Marie Tooth and Behr's syndrome, underscoring the variability in clinical manifestations[3,5,6]. This broad phenotypic spectrum emphasises the need for patient-specific and clinically relevant models to better understand the disease. In this study, induced pluripotent stem cell (iPSC) lines were generated from two Behr's syndrome patients with MTRFR variants. These were differentiated to motor neurons, cortical neurons, and muscle to characterize molecular phenotypes in clinically relevant cell types. Patient motor and cortical neurons displayed both increased mitochondrial membrane potential and neurite outgrowth. Comparatively, patient iPSC-derived myoblasts have a decreased mitochondrial membrane potential. Interestingly, patient cortical neurons exhibited swollen mitochondria and increased levels of reactive oxygen species (ROS), which was not observed in motor neurons. Patient motor neurons also have an impaired calcium response, with a decreased peak amplitude and an increased time to peak. Increased mitochondrial membrane potential has been shown to increase the uptake rate of calcium into mitochondria, which may explain the altered calcium response[7]. These results provide novel characterization of MTRFR variants in disease relevant cell types and highlight the cell-type specificity of specific molecular phenotypes.

ABSTRACT POSTER NUMBER: 98

Recessive titinopathies: Clinical and molecular insights from seven patients with biallelic TTN variants

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INTRODUCTION:
Biallelic variants in the titin (*TTN*) gene are associated with a spectrum of recessive skeletal myopathies and muscular dystrophies, with variable age of onset and severity, with or without cardiac involvement. In this study, we report eight patients from seven unrelated families with biallelic *TTN* variants to further characterize the clinical and molecular features of recessive titinopathies.

METHODS:
Genotype-phenotype correlations were evaluated in eight individuals with myopathy or muscular dystrophy, in whom recessive *TTN* loss-of-function (LoF) variants were identified through next generation sequencing.

RESULTS:
Five patients carried biallelic truncations (four compound heterozygous, one homozygous). One patient had a truncating variant in trans with a missense variant predicted to disrupt splicing. Two siblings carried a truncating variant in trans with a canonical splice-site mutation, in whom RNA sequencing confirmed abnormal splicing, and proteomic analysis showed significantly reduced TTN expression ($p = 0.008$). Phenotype correlation showed congenital myopathy phenotype (onset 0-1 yrs) with variable progression characterized by joint contractures and respiratory involvement in four patients with variants affecting the I and/or A-bands. One patient had cardiac involvement. In contrast, four patients with at least one variant in the distal M-band exons presented with later-onset, milder phenotype and no cardiac involvement.

CONCLUSION:
The interpretation of *TTN* variants remains challenging due to the gene's large size and complex splicing pattern. This study expands the phenotypic and molecular landscape of recessive titinopathies. Our findings emphasize the importance of variant location within TTN in influencing disease severity and onset.

What are the Costs of Living with FacioScapuloHumeral Dystrophy? The Socioeconomic Burden of FSHD

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BACKGROUND: People with facioscapulohumeral muscular dystrophy (FSHD) face financial challenges, including direct costs (medical visits, equipment, caregiving) and indirect costs (lost productivity, reduced earning capacity).

OBJECTIVES: To estimate the direct and indirect socio-economic burden of FSHD in Canada.

METHODS: This analysis used a subset of data from the Burden of Inherited and Inflammatory Neuromuscular Disease study, a cross-sectional Canadian survey of 1,456 individuals living with neuromuscular disorders. Participants completed the Health Utilities Index Mark 3 and Work Productivity and Activity Impairment tools.

RESULTS: In the 106 adults with FSHD (52% women) included, 44% of working-age individuals did not work as a consequence of FSHD, despite preserved education levels, with annual cost of $58,827/person in lost earning capacity. Among those employed, productivity loss and absenteeism cost $24,871/year/person. Household incomes were ~$15,000 below the national average. Forty percent reported using personal savings for care. Physical/occupational therapy, massage therapy, and psychological support drove visit burden. Canadians with FSHD (~1,200 individuals) bore an estimated annual cost of $65M/year ($53,000/person), including $51M in indirect costs, $9M for visits, and $5M for equipment. Health-related quality of life (0.27 [0.21-0.33]) was worse than in other chronic diseases such as cancer, diabetes, and dementia. Lower health-related quality of life was associated with being a female ($p=0.032$), younger ($p<0.0001$), and being without paid employment ($p=0.020$).

CONCLUSION: The study reveals significant direct and indirect costs of FSHD and highlights the need to examine health policies through an intersectional lens, considering relationships between demographic, socioeconomic, and health-related factors and longitudinal outcomes.

ABSTRACT POSTER NUMBER: 100

Dystrophic satellite cells undergo mitotic catastrophe and senescence

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Duchenne muscular dystrophy (DMD) is a progressive degenerative muscle disease, caused by the loss of the dystrophin protein. In addition to maintaining muscle structural integrity, dystrophin is also highly expressed in muscle stem cells (satellite cells) and is critical for regulating cellular polarity. The absence of dystrophin leads to the downregulation of the serine-threonine kinase Mark2, loss of cell polarity, and a reduction in asymmetric division, thereby impairing progenitor cell production. Our previous work demonstrated that dystrophic satellite cells exhibit abnormal division patterns and disrupted mitotic spindle orientation. In the current study, we further investigated the mitotic apparatus in dystrophic satellite cells using the *mdx* mouse model. Through *ex vivo* myofiber culture, we found that a significant proportion of dystrophic satellite cells displayed centrosomal abnormalities, which became more pronounced following activation. These abnormalities were associated with disrupted centrosome integrity, nuclear amplification, and nuclear damage. Additionally, dystrophic satellite cells showed an increased proportion of senescent cells, reaching approximately 50% at 72h in culture. siRNA-mediated knockdown of either dystrophin or Mark2 in wild type myofibers showed more senescent cells at 72h, compared to controls. Finally, through flow cytometry, we successfully isolated senescent cells and confirmed that dystrophic muscles contain more senescent satellite cells *in vivo*. Taken together, our findings suggest that impairment of the mitotic machinery in dystrophic satellite cells contributes to mitotic catastrophe, leading to cellular senescence. Understanding these intrinsic defects in dystrophin-deficient satellite cells is crucial for developing targeted therapeutic strategies aimed at mitigating the cellular dysfunction underlying DMD.

ABSTRACT POSTER NUMBER: 101

C9orf72 loss of function leads to cerebellar degeneration in the zebrafish ALS model

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A hexanucleotide (GGGGCC) repeat expansion in *C9orf72* gene represents the most frequent genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), resulting in reduced *C9orf72* mRNA and protein expression. Loss of motor coordination and fine balance are the clinical hallmarks of individuals with ALS/FTD, but the role of the brain region-cerebellum, which controls these functions, remains elusive. Moreover, *C9orf72* is highly expressed in the cerebellum. To investigate the cerebellar pathology in ALS/FTD, we used a *c9orf72* knockdown zebrafish ALS model that replicates the partial loss of C9orf72 observed in patients. We observed both in larval and adult stages a general reduction in the volume of the head, as well as atrophy of the brain and cerebellum in C9-knockdown (KD) zebrafish. Moreover, we observed a general reduction of the GABAergic interneurons and loss of Granule cells in the C9-KD zebrafish cerebellum. Purkinje cells (PCs) have a significant role in encoding and controlling zebrafish swimming, a key motor behaviour. We have identified the loss of Purkinje cells (PCs) in the C9-KD fish, which precedes any observed motor defects, underlying the importance of early cerebellar involvement in the pathogenesis of ALS. Additionally, we performed single-cell RNA sequencing on the C9-KD zebrafish brain, which revealed the functional enrichment of novel genes and biological processes that are dysregulated in the cerebellum. Our study aims to elucidate the specific alterations occurring in the cerebellum, ultimately enhancing our comprehension of the underlying pathogenesis of ALS and paving the way for novel therapeutic target discovery.

ABSTRACT POSTER NUMBER: 102

Characterizing the role of the RNA-binding protein HuR in regulating the structure of neuromuscular junctions in Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a progressive, X-linked disorder caused by mutations in the dystrophin gene, which leads to severe muscle fibre degeneration. Neuromuscular junctions (NMJ) in DMD skeletal muscle display structural and functional abnormalities, including fragmentation of the postsynaptic apparatus. The Human Antigen R (HuR) protein is an RNA-binding protein that stabilizes several target mRNAs during myogenic differentiation, including those involved in synaptic function. Previously, we showed that HuR is upregulated in denervated muscle, where it enhances the stability of transcripts encoding acetylcholine receptor-β (AChR-β) subunits through p38 MAPK signalling (Joassard et al., 2015). However, its specific role in maintaining the structure of NMJs remains unclear. Here, we investigated how HuR affects NMJs in *mdx* (a widely used DMD model) versus wild-type (WT) mice. First, we observed that *mdx* NMJs show pronounced disruptions of the postsynaptic apparatus. Next, we examined HuR expression and localization in multiple muscles at various ages (4 to 24+ weeks) and found a 2- to 4-fold increase in all *mdx* muscles at 4-8 weeks compared to WT, but not at 24 weeks. Given these findings, we also knocked down HuR expression in vivo by injecting an AAV-shHuR in the TA muscles of WT and *mdx* mice. Analysis of NMJs in injected muscles is ongoing. Together, these studies will provide insight into the role of HuR at dystrophic NMJ and reveal its therapeutic potential to restore NMJ integrity in DMD through HuR modulation.

ABSTRACT POSTER NUMBER: 103

SAT-3247: An oral small molecule inhibitor targeting AAK1, a critical effector of skeletal muscle regeneration.

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Satellos is developing the world's first drug specifically designed to target the innate process of muscle regeneration, through regulation of muscle stem cell polarity. Dystrophin protein is expressed in activated muscle stem cells where it is required for establishing apical-basal polarity. In the absence of dystrophin, muscle stem cells undergo reduced numbers of asymmetric divisions and increased numbers of symmetric divisions resulting in a stem cell hyperplasia and reduced numbers of progenitor cells to match the ongoing muscle damage found in Duchenne Muscular Dystrophy (DMD). This deficiency is a significant factor contributing to the progressive muscle loss experienced by people living with DMD. Through the use of an in-situ muscle stem cell screening platform, a highly druggable protein kinase target called adapter associated kinase 1 (AAK1) was identified. The inhibition of AAK1 promotes functional rescue of asymmetric stem cell divisions, resulting in the robust production of progenitors *in vitro* and *in vivo*. Satellos recently announced the nomination of a lead drug candidate SAT-3247, which is a potent, orally available, muscle penetrant, small molecule inhibitor of AAK1. SAT-3247 is efficacious in both mouse and dog pre-clinical models of DMD and has recently completed a phase 1a/b clinical trial in healthy human volunteers and adult Duchenne patients. SAT-3247 was found to be safe, well tolerated and demonstrated potential early signs of impact on improving muscle strength.

ABSTRACT POSTER NUMBER: 104

Identifying cancer cachexia humoral factors affecting muscle stem cell regeneration capacity

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Cancer cachexia is responsible for 20-30% of cancer-associated deaths through extreme muscle wasting. Previous research has suggested that muscle stem cell (MuSC) hyperplasia contributes to muscle mass loss in cancer cachexia. We found that cancer-cell conditioned media markedly impairs MuSC asymmetric division on isolated EDL myofibres resulting in reduced differentiation and increased numbers of undifferentiated cells. To further understand cachectic MuSC dysfunction, we set out to uncover the responsible humoral factors. We first tested the canonical cachectins TNFa, IFNg, and IL-6 and found that they had no effect on MuSC proliferation or differentiation. Then, a targeted screen of cancer-conditioned media for elevated inflammatory cytokines was performed, but the identified cytokines were also insufficient to impair myogenesis. Subsequently, an untargeted screen of available cachectic cancer transcriptomes was performed to match ligand-receptor pairs between the cancers and MuSCs, identifying Epiregulin (EREG) as a potential humoral effector, among others. We made the striking discovery that EREG treatment of MuSC on isolated EDL myofibres resulted in reduced differentiation and increased numbers of undifferentiated cells. Future studies will validate the impact of EREG on MuSC function and investigate intracellular mechanisms of myogenic dysregulation as well as examine the impact of the other identified candidates.

ABSTRACT POSTER NUMBER: 105

The comparative efficacy and safety of risdiplam versus high-dose nusinersen in children with Type 1 SMA

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BACKGROUND:
Risdiplam and nusinersen are therapies approved for treating spinal muscular atrophy (SMA). Type 1 SMA is severe, and untreated infants typically do not survive beyond 2 years. Risdiplam and nusinersen have shown efficacy in separate trials for Type 1 SMA. Without head-to-head trials, indirect comparisons can inform treatment decisions. This study compares the efficacy and safety of the approved risdiplam dose (0.2 mg/kg daily for ages 2 months to <2 years; 0.25 mg/kg daily for ≥2 years and <20 kg) with investigational higher-dose (HD) nusinersen (50/28 mg) in children with Type 1 SMA.

METHODS:
Patient-level risdiplam data were obtained from 58 children in the FIREFISH trial (NCT02913482), and aggregate nusinersen data from 50 children in the DEVOTE trial (NCT04089566). Unanchored matching-adjusted indirect comparisons were used, adjusting for age at first dose, disease duration, and baseline CHOP-INTEND score. Cox proportional-hazards models compared overall and event-free survival.

RESULTS:
After matching, baseline characteristics were similar across groups. The effective sample size for risdiplam was 33.2, a reduction of ~43%. After 15 months of risdiplam treatment, compared with HD nusinersen, the risdiplam group had a 75% reduction in death rate (95% CI 11–93%) and a 76% reduction in the rate of death or permanent ventilation (95%vCI 34–92%).

CONCLUSION:
Risdiplam was associated with a lower risk of death or permanent ventilation compared to HD nusinersen in children with Type 1 SMA with up to 15 months follow-up. Further research from additional sources is recommended to expand these findings.

ABSTRACT POSTER NUMBER: 106

Deciphering the mechanisms involved in the AMPK-induced therapeutic benefits in Myotonic Dystrophy type 1 skeletal muscle

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Myotonic Dystrophy type 1 (DM1) is a multisystemic disease affecting skeletal muscles and other organs, for which no effective treatment currently exists. DM1 is caused by an abnormal expansion of CUG repeats in the 3'UTR of DMPK mRNAs, leading to a toxic gain-of-function of CUG-expanded transcripts. These transcripts accumulate in ribonuclear foci, disrupting splicing regulators, including MBNL1, CUGBP1 and Staufen1, causing a spliceopathy. Multiple signaling pathways are also impaired in DM1 muscle, contributing to the disease mechanism and offering potential therapeutic targets. Our lab previously demonstrated that AMPK signaling is repressed in DM1 skeletal muscles and that its activation through AICAR and exercise improves pathological features (Ravel-Chapuis et al., 2018; Misquitta et al., 2023). However, conventional AMPK activators such as AICAR and metformin present some limitations and have AMPK-independent effects. To investigate whether these therapeutic effects are AMPK-mediated, we generated a DM1 AMPK-deficient mouse model by crossing DM1 mice with AMPK α-floxed mice and delivering AAV-Cre intramuscularly. Preliminary characterization of the injected muscles revealed an approximately 90% reduction in AMPK levels and decreased phosphorylation of its downstream target, ACC, compared to saline-injected muscles. Notably, DM1 AMPK-deficient mice exhibit a greater misregulation of MBNL1 and Staufen1, along with an exacerbation of aberrant splicing patterns. Ongoing experiments aim to further characterize muscle histology and the localization of disrupted RNA-binding proteins (MBNL1 and CUGBP1). Finally, this novel mouse model will prove essential in determining the direct implication of AMPK in the therapeutic benefits following physiological (exercise) and pharmacological (AICAR and others) interventions.

Characterization of Bitter Melon Natural Compounds that Activate AMPK Signaling As Novel Therapeutics For Myotonic Dystrophy Type 1 (DM1)

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Myotonic Dystrophy Type 1 (DM1) is a multisystemic neuromuscular disease that is characterized by severe skeletal muscle wasting and dysfunction. Our lab was first to report that AMPK, a key mediator of skeletal muscle plasticity, is repressed in DM1 and that pharmacological and/or physiological activation (exercise) of AMPK corrects molecular and histopathological features of DM1 (Ravel-Chapuis et al., 2018). Current AMPK activators are either not FDA approved or show mild improvements in DM1 patients. Of relevance, bitter melon natural compounds (BMC) were shown to activate AMPK in L6 myotubes (Tan et al., 2008). Here, we hypothesized that BMC would activate AMPK signaling in DM1 muscle and ameliorate characteristic DM1 pathological features. Therefore, we characterized 26 BMC for their ability to activate AMPK in cultured muscle cells. Compared to the vehicle, BMC 25, 26 and 27 resulted in a ~ 2-fold increase in AMPK activation. MTT assays showed that these compounds maintained over 92% cell viability. Next, female DM1 mice were treated with BMC-25 for 6 weeks; BMC-25 resulted in a sustained activation of AMPK 6 weeks after treatment in hindlimb muscles. This treatment regime with BMC-25 also corrected key DM1-associated mis-splicing events, including rescue of *Serca1*, *Bin1*, *Clcn1* and *RyR1* transcripts. Additionally, BMC-25 increased expression of OXPHOS complexes in DM1 muscle compared to vehicle. Thus, the characterization of natural compounds such as BMC for their ability to activate AMPK in DM1 muscle could result in the rapid identification of novel therapeutics for DM1.

ABSTRACT POSTER NUMBER: 108

Characterization of a novel muscle stem cell subpopulation with low proliferative and metabolic profiles

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Skeletal muscle regeneration depends on satellite cells; however, their regenerative capacity is not uniform as discrete subpopulations differ in quiescence and self-renewal potential. Previous work from our lab showed that ~10% of SCs have never expressed Myf5. This population, termed muscle stem cells (MuSCs; $Pax7^+/Myf5^-$), exhibits enhanced self-renewal and can give rise to committed myogenic progenitors while displaying high stemness, quiescence, engraftment, and asymmetric division. Guided by single-cell RNA sequencing of murine muscle, we identified a previously unrecognized $Pax7^+/Myf5^-$ subpopulation with high expression of Regulator of G-protein Signaling 5 (RGS5). Fluorescence-activated cell sorting enabled isolation of $RGS5^+$ MuSCs, whose identity was confirmed by immunofluorescence and flow cytometry. Compared to bulk $Pax7^+$ MuSCs, $RGS5^+$ cells (i) showed markedly reduced EdU incorporation, delayed MyoD induction, and diminished colony formation, indicating deep quiescence and slow-cycling behavior; (ii) downregulated mitochondrial genes, displayed significantly lower mitochondrial mass and membrane potential, and exhibited signs of mitophagy, consistent with its low proliferation; and (iii) generated significantly less ATP than their $RGS5^-$ counterparts. Notably, $RGS5^+$ MuSCs persisted in dystrophin-deficient mdx mice and retained their low-mitochondrial profile despite the dystrophic niche. Ongoing transplantation experiments (reported separately) show that $RGS5^+$ cells regenerate muscle more efficiently and display higher asymmetric division, similar to previously reported $Myf5^-$ MuSCs. Altogether, these findings pose RGS5 as a promising surface marker for isolating a metabolically low, high-stemness MuSC subset, highlighting the link between mitochondrial state and stem cell quiescence.

ABSTRACT POSTER NUMBER: 109

Therapeutic modulation of the calpastatin/calpain system restores neuromuscular integrity and function in C9orf72 ALS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder characterized by the selective degeneration of motor neurons, leading to muscle atrophy and paralysis. A hexanucleotide repeat expansion (GGGGCC) in the first intron of the *C9orf72* gene represents the most common genetic cause of ALS. Although early dysfunction at the neuromuscular junction (NMJ) is a hallmark of ALS pathogenesis, current therapies offer only modest symptomatic relief, highlighting the urgent need for novel, mechanism-based therapeutic strategies.

In this study, we employed a clinically relevant *C9orf72*-ALS zebrafish model (C9-miRNA) alongside motor neurons derived from patient-induced pluripotent stem cells (iPSCs) to investigate disease mechanisms. We identified a marked downregulation of calpastatin, the endogenous inhibitor of calpains, a family of calcium-dependent proteases previously implicated in neurodegeneration. Targeted restoration of calpastatin activity, via the calpain inhibitor calpeptin and a calpastatin-derived peptide, significantly rescued both locomotor deficits and NMJ structural abnormalities in the C9-miRNA zebrafish model. Furthermore, these interventions improved synaptic vesicle turnover and quantal neurotransmitter release at the NMJ. In iPSC-derived motor neurons, calpastatin-based treatment not only enhanced cell survival but also restored NMJ integrity and functional synaptic transmission.

Our findings identify the calpastatin/calpain axis as a key modulator of NMJ stability and function in *C9orf72*-associated ALS and suggest this pathway as a promising therapeutic target for treating ALS.

ABSTRACT POSTER NUMBER: 110

Longitudinally tracking disease progression and correlation in oculopharyngeal muscular dystrophy patients using whole-body quantitative MRI

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OBJECTIVE:
Oculopharyngeal muscular dystrophy (OPMD) a slowly progressive disorder with ptosis, dysphagia, and proximal weakness lacking reliable biomarkers for disease monitoring. But quantitative MRI (qMRI) offers a non-invasive approach to track progression. This study evaluated 97 muscles at two time points to quantify fat fraction (FF) changes and assess whether diffusion related MRI markers correlate with, or precede, fat infiltration.

METHODS:
Twenty OPMD patients and ten controls were included. Charts were reviewed for demographics (sex, age of onset) and symptoms. All subjects underwent whole-body 3T qMRI using 2-point Dixon and PDFF for FF quantification. Seven patients also underwent diffusion sequences (ADC, IVIM) and T2 mapping at mid-thigh, with an average scan interval of 22.7 ± 0.9 months. In total, 97 muscles were segmented on Dixon, PDFF, and ADC, 24 thigh muscles on IVIM and T2 using ITK-SNAP. Statistical analysis with PRISM included paired Wilcoxon tests and Spearman correlations ($p < 0.05$).

RESULTS:
In initial analysis of seven patients, 4 of 24 thigh muscles showed significant negative correlation between FF and ADC at time point 1 ($R = -0.86$ to -0.93), increasing to nine muscles at time point 2 ($R = -0.79$ to -1.00). Wilcoxon testing revealed significant increases in FF (4 thigh muscles) and ADC (4 muscles in thigh and calf).

CONCLUSION:
Preliminary analysis shows a negative ADC–FF correlation in thigh muscles, suggesting that lower diffusivity may precede fat infiltration and reflect early OPMD involvement. Ongoing work will relate FF changes to ADC, IVIM, T2, and clinical outcomes.

Polarity dysregulation in dystrophin-deficient satellite cells

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Duchenne muscular dystrophy (DMD) is muscle wasting disease caused by a mutation in DMD gene encoding the dystrophin protein. This protein is highly expressed in skeletal muscle and in activated muscle stem cells (MuSCs). This protein is required to establish apicobasal polarity that is necessary to asymmetric division and efficient regeneration. In addition, asymmetric division depends on the formation of the PAR-complex composed of Pard3, Pard6 and aPKC. We have previously demonstrated that the absence of dystrophin leads to reduced Pard3 polarization on the apical cortex, resulting in a reduction of apicobasal polarity and asymmetric division. However, the specific mechanisms involved remains unclear. We first investigated the composition of the PAR complex in MuSC and established that it contains Pard3, PRKCi and Pard6G. We then examined polarity establishment following cardiotoxin injury and the formation of the PAR-complex in both WT and *mdx* mice. We further found that polarity establishment is sequential in time and differs between the basal and apical sides of the cell. Moreover, despite Pard3 expression being increased in dystrophin-deficient MuSCs, the lack of DMD reduces apicobasal polarity and alters the formation of PAR-complex. Finally, transfection of siMark2 on cultured myofibers revealed that Pard3 is upregulated while displaying reduced polarization analogous to the deficits observed in *mdx* MuSCs. This result supports the involvement of Mark2, the kinase recruited to the basal pole by direct binding to dystrophin. Together, these experiments provide important insight into the dysregulation of polarity caused by dystrophin-Mark2 deficiency in MuSC in Duchenne muscular dystrophy.

A muscular dystrophy associated with bi-allelic LEMD2 variants: Expanding the genotype of nuclear envelopathies

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BACKGROUND:
Genetic defects affecting the nuclear envelope are increasingly recognized as causes of diverse neurological and neuromuscular phenotypes. Variants in nuclear envelope-associated genes can produce pleiotropic clinical manifestations, reflecting their critical roles in nuclear architecture, chromatin organization, and gene regulation.

METHODS AND RESULTS:
We report a 13-year-old boy with congenital myopathy, mild intellectual disability, microcephaly, and respiratory insufficiency. Muscle biopsy revealed myopathic changes and ultrastructural abnormalities of the nuclear envelope. Proteomics identified significantly reduced levels of LEMD2, an inner nuclear membrane protein involved in nuclear envelope integrity. Guided by these findings, re-analysis of exome data uncovered compound heterozygous LEMD2 variants: a novel splice-site mutation (c.854-1G>A) and a rare missense substitution (c.1442G>A; p.Arg481His). In silico modeling predicted destabilization of the protein structure due to the missense variant. Immunofluorescence studies revealed aberrant distribution of nuclear envelope proteins, including Lamin A/C, Lamin B1, Emerin, and Matrin-3. Despite severe neuromuscular symptoms, cardiomyopathy was absent in this case.

CONCLUSION:
Our findings expand the phenotypic spectrum associated with LEMD2 mutations to include an Emery-Dreifuss muscular dystrophy (EDMD)-like myopathy. This study underscores LEMD2's role in nuclear architecture and chromatin organization and highlights the diagnostic value of proteogenomic integration in uncovering rare neuromuscular disorders.

ABSTRACT POSTER NUMBER: 113

Factors that influence adherence to home-based rehabilitation in children and youth with neuromuscular disorders : a scoping review

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INTRODUCTION:
Home-based (HB) rehabilitation exercises are often essential to achieve adequate therapeutic dosage for promoting motor function in paediatric patients with neuromuscular disorders (NMDs). However, adherence to such programs remains a common challenge. Little is known about the factors that influence adherence to HB rehabilitation exercises in this population.

OBJECTIVES:
The primary aim of this scoping review is to describe and map the current state of knowledge on HB programs designed to promote pulmonary and motor function in children and youth with NMDs. A secondary aim is to identify factors that promote or hinder adherence to these programs.

METHODS:
Following JBI guidelines and PRISMA-ScR recommendations, we conducted a comprehensive search of MEDLINE, CINAHL, EMBASE, PsychINFO and Web-Of-Science for studies in English or French from 2010 to 2025. A total of 340 articles were screened, with 68 selected for full text eligibility. Forty studies met inclusion criteria, which included children (4-18 y-o) with NMD and a HB intervention targeting pulmonary or motor function. Data extraction is underway by two reviewers using a standardized charting framework on Covidence.

RESULTS:
Preliminary findings suggest variability in adherence-related outcomes and reporting across studies. Results will be presented, including a summary of study characteristics and a thematic analysis of reported adherence facilitators and barriers.

CONCLUSIONS:
Full scoping review's results will be presented, including trends in study design, intervention types, and key themes related to adherence. Findings aim to inform future research and clinical practice in designing and implementing viable HB rehabilitation programs for children with NMDs.

ABSTRACT POSTER NUMBER: 114

Diagnostic Delay Leading to Inpatient ALS Diagnoses: A Case Series

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BACKGROUND:
Diagnostic delay is common for patients with ALS. Given the increasing number of inpatient ALS diagnoses at our medical center, we investigated the causes of diagnostic delay in this cohort.

METHODS:
A retrospective case series was created of inpatients diagnosed with ALS by the neuromuscular division while admitted to our medical center from 7/1/2024 to 5/1/2024.

RESULTS:
Ten inpatient ALS diagnoses were delivered at our medical center from 7/1/2024 to 5/1/2025. Patients were aged 48-77, with eight presenting with spinal-onset and two with bulbar-onset. The average delay in ALS diagnosis from symptom onset was 15.5 months. Half of the patients were never referred to an outpatient neurologist prior to their ALS-diagnostic admission, and, in the other half evaluated by an outpatient neurologist, ALS was considered as a possible diagnosis only in two. Six patients were transferred from outside hospitals with respiratory failure of undetermined cause. Serum plasma neurofilament levels were elevated in the inpatient cohort compared to a cohort of recent outpatient ALS diagnoses at our center (208 pg/nL inpatient [n=10] v. 60.2 pg/nL outpatient [n= 56], p-value 0.045). By the end of the observation window, four patients died, two were enrolled in hospice, and the remainder required varying levels of assistive care.

CONCLUSION:
A root cause analysis of ALS diagnostic delay uncovered opportunities where a diagnosis could be expedited. Diagnostic delay led to hospitalizations often due to progressive respiratory failure, with patients receiving an ALS diagnosis at terminal stages of disease.

Mitochondrial redox signaling regulates muscle stem cell function through glutathione-s-transferase pi1

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Maintaining muscle stem cell (MuSC) activity is essential for preserving the regenerative capacity of skeletal muscle and preventing dysfunction during in degenerative diseases. We have previously shown that mitochondria play a key role in directing MuSC homeostasis. Specifically, during MuSC activation, mitochondria undergo fragmentation, increasing the production of mitochondrial reactive oxygen species (mtROS) and the synthesis of glutathione. This redox remodeling facilitates the transition of MuSCs from quiescence to activation, promotes cell cycle entry, and influences progenitor cell fate decisions. However, the mechanisms by which these mitochondrial redox changes exert downstream effects remain unclear. Here, we identify protein S-glutathionylation (PSSG), a reversible post-translational modification, as a key redox-dependent signaling mechanism controlling MuSC activity. Notably, inhibition of global glutathione S-transferases with Ethacrynic Acid (EA) significantly impairs MuSC activation, proliferation and progenitor fate decisions. Next, using both genetic and pharmacologic approaches, we show that during MuSC activation, mitochondrial fragmentation and associated increase in mtROS and GSH induce the expression of glutathione S-transferase pi 1 (GSTP1), which mediates PSSG. Importantly, GSTP1 inhibition with TLK117 or siRNA-mediated knockdown significantly impairs MuSC function through impairing cell-cycle kinetics and proliferation. Furthermore, aged MuSCs fail to induce GSTP1 and PSSG effectively, while MuSCs from the Mdx model of Duchenne muscular dystrophy show elevated GSTP1 activity and PSSG, contributing to their dysfunction. These findings uncover a previously unrecognized mechanism by which mitochondrial fragmentation drives redox signaling through GSTP1-mediated PSSG, which is essential for MuSC function, and highlight this axis as a potential therapeutic target in muscle-wasting conditions.

ABSTRACT POSTER NUMBER: 116

Dystrophic muscle phenotypes can be horizontally transferred via fecal microbiome transplantations

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Duchenne Muscular Dystrophy (DMD) is a devastating genetic disorder characterized by progressive muscle degeneration with limited therapeutic options. In addition to skeletal muscle deterioration, patients often suffer from gastrointestinal and cardiac complications, which may be exacerbated by disruptions within gut microbial communities. However, the direct contribution of the gut microbiota to muscle function and disease progression in DMD remains poorly understood.

To investigate this relationship, we performed fecal microbiota transplantations (FMT) over 9 weeks in *mdx* (C57BL/10ScSn-*Dmd*^{mdx}/J) mice, a well established DMD mouse model, and wildtype (C57BL/10; WT) controls. Mice received either homologous (WT:WT; *mdx*:*mdx*) or heterologous (WT:*mdx*; *mdx*:WT) genotype:donor FMTs. As anticipated, *mdx*:*mdx* mice exhibited a progressive decline in grip strength, while WT:WT mice showed improved performance over time. Importantly, *mdx*:WT mice maintained stable grip strength, failing to show the typical decline observed in *mdx* controls. Conversely, WT:*mdx* mice exhibited a significant reduction in grip strength by week 9, suggesting transmissibility of the dystrophic phenotype via the microbiota. Profiling the microbiotas of FMT treated mice revealed dynamic changes over time that appeared to be driven by both the host mouse genotype and the identity of their FMT donor.

These findings provide compelling evidence that gut microbes can modulate muscle function and potentially contribute to the progression or attenuation of muscular dystrophy phenotypes. Our study uncovers a previously unappreciated role for the gut microbiota in DMD pathophysiology and highlights microbial communities as unexploited therapeutic targets for improving overall muscle health outcomes in DMD and other muscular dystrophies.

Retrospective assessment of feeding and nutrition after 2 years of risdiplam treatment in younger children with SMA using the CEDAS

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Children with spinal muscular atrophy (SMA) have severe motor neuron degeneration including bulbar dysfunction which can lead to swallowing and feeding difficulties, poor respiratory health and faltering growth. Routine assessment of bulbar function is essential for children with SMA; however, it is not assessed consistently across studies.

The 6-point graded Children's Eating and Drinking Activity Scale (CEDAS) was developed to improve the definition of feeding-level scoring to reflect both sensory and motor needs, ensuring usability for all children with paediatric feeding disorders. In this analysis, two independent experts used the CEDAS to retrospectively score data collected from children in the risdiplam clinical trials.
In a previous analysis, we reported CEDAS scores in 58 children with Type 1 SMA who received the pivotal dose of risdiplam in the FIREFISH study (NCT02913482). CEDAS scoring confirmed feeding and swallowing findings reported in FIREFISH: 48 (83%) children were able to feed orally at Year 2 (feeding exclusively orally [n=41] and mixed oral and tube feeding [n=7]). The majority of children maintained (48%) or improved (16%) their CEDAS scores over 2 years of treatment. These data showed a maintenance of swallowing function in patients with Type 1 SMA, which differs greatly from outcomes in untreated children in the same age range.

This updated analysis will extend the data set to include CEDAS scoring in 26 children with genetically diagnosed, presymptomatic SMA treated with risdiplam for 2 years in the RAINBOWFISH study (NCT03779334).

ABSTRACT POSTER NUMBER: 118

Drug repurposing of olodaterol and forskolin for congenital myasthenic syndromes

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Congenital myasthenic syndromes (CMS) are a group of rare, genetically and clinically heterogeneous disorders caused by defects at the neuromuscular junction (NMJ). Most CMS subtypes respond positively to the β2 adrenergic agonist salbutamol, including *COLQ*-CMS, which is characterized by abnormal NMJ morphology and fatigable muscle weakness. Salbutamol is thought to stimulate the cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway and increase acetylcholine receptor (AChR) clustering. However, given the cardiac side effects caused by salbutamol treatment and the lengthy process required to obtain approval for use of new drugs in clinic, drug repurposing may help to efficiently uncover novel therapeutic strategies for CMS treatment. The FDA-approved β2 agonist olodaterol has a longer half-life and tighter receptor binding than salbutamol and may therefore require less frequent dosing. Forskolin, a direct cAMP activator may be more effective as it works downstream of salbutamol. This study aims to test olodaterol and forskolin compared to salbutamol in the C2C12 mouse muscle cell line, followed by drug testing in a *colq* knockdown zebrafish model. Initial studies indicate increased AChR clustering in C2C12 following co-treatment of olodaterol with agrin. Forskolin, salbutamol, and olodaterol treatment all increased intracellular cAMP levels compared to control-treated C2C12. *colq* knockdown zebrafish demonstrates altered embryonic neurodevelopment and AChR clustering, as well as fatigable muscle weakness. With this representative model of *COLQ*-CMS, we will determine the potential of forskolin and olodaterol for the treatment of this rare and detrimental condition.

ABSTRACT POSTER NUMBER: 119

AAV-mediated gene editing strategies to treat Collagen VI-related Myopathies

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Collagen VI-related myopathies comprise a phenotypic range of inherited muscle diseases, from mild Bethlem myopathy to severe Ulrich congenital muscular dystrophy. These disorders affect 1.3 – 7.7 per 1,000,000 individuals worldwide and are caused by the reduction or absence of collagen VI function. Patients with impaired collagen VI function experience contraction-induced muscle damage, progressive muscle weakening, and stiffening of connective tissues. Severe forms of inherited collagen VI myopathies appear at birth with decreased muscle tone and weakness. With proactive management, patients can achieve a normal life expectancy, but they face functional impairments that will only be alleviated with the development of a disease-modifying therapy.

In this study, we generated a human-relevant mouse model of Collagen VI deficiency, which carries a bi-allelic recessive loss-of-function COL6A2 1402 A>T nonsense mutation (a mutation found in patients), and used them to test the efficacy of gene therapies for collagen VI-related myopathies. Col6A2R468*/R468* homozygous mice show reduced muscle mass, evidence of muscle damage, significant reduction of overall body mass, and marked diminution of grip strength. We developed a single AAV vector adenine base editor approach capable of converting the nonsense mutation to a silent mutation. Using muscle-directed MyoAAV vectors to deliver the genome editor in 3-day-old Col6A2 R468*/ R468* mice, we demonstrated effective on-target genome modification in the heart and in skeletal muscles throughout the body, with rescue of phenotypic impairments in limb function. Taken together, these data demonstrate the promise of in vivo genome medicine approaches for the treatment of inherited muscle disease.

ABSTRACT POSTER NUMBER: 120

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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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. 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, which is a central cause of DM1 pathology.

OBJECTIVES:
The PGN-EDODM1-101 (FREEDOM-DM1) study is designed to evaluate safety, tolerability, pharmacokinetics, pharmacodynamics (correction of mis-splicing), and potential clinical benefit.

METHODS:
FREEDOM-DM1 is an ongoing Phase 1 randomized, double-blind, placebo-controlled single ascending dose (SAD) study in people with DM1 (NCT06204809). FREEDOM-DM1 consists of dose-ascending cohorts of participants (N=8), each randomized 3:1 PGN-EDODM1 to placebo. Muscle needle biopsies are performed at Baseline, Week 4, and Week 16 to measure tissue drug concentrations and splicing of selected transcripts.

RESULTS:
Initial Phase 1 clinical data showed increasing levels of PGN-EDODM1 in muscle with increasing doses and high levels of mean splicing correction of 12.3% and 29.1% using the 22-gene panel at 5 mg/kg and at 10 mg/kg, respectively, 28 days after a single dose. The emerging safety profile for PGN-EDODM1 is favorable. The 15 mg/kg dose cohort is ongoing.

CONCLUSIONS:
FREEDOM-DM1 data provide initial support for the continued clinical development of PGN-EDODM1 for the treatment of DM1. FREEDOM2-DM1, a multiple ascending dose study, has been initiated (NCT06667453).

ABSTRACT POSTER NUMBER: 121

Characterization of FDA-approved drugs that target the RNA-binding protein Staufen1 and mitigate the atrophy of skeletal muscle fibers following denervation

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Skeletal muscle atrophy is a debilitating condition marked by progressive muscle loss and dysfunction, often linked to aging, inactivity, nerve damage, cachexia, and chronic illness. Our lab previously showed that the transgenic overexpression of the RNA-binding protein Staufen1 (STAU1) induces atrophy in mouse muscle, suggesting that STAU1 may be a novel atrogene (Crawford Parks et al. 2020). More recently, we have observed consistently an early and transient increase in STAU1 levels across different atrophy models including serum-starved myotubes, denervated and suspended hindlimb mouse muscles, as well as in humans subjected to bed rest and dry immersion (Ravel-Chapuis et al. unpublished). Moreover, we observed that the knockdown of STAU1 during atrophy preserved myofiber diameters. Accordingly, we set out to identify FDA-approved drugs that could reduce the expression of STAU1 during atrophy. To this end, a high-throughput ELISA screen of 770 FDA-approved drugs using myoblasts was performed to assess their ability to decrease STAU1 levels. Using our in-vitro atrophy model, three drugs (11, 12 and 15) reduced STAU1 mRNA and protein expression and prevented atrophy by ~90%. Drug 11 was then tested for its efficacy *in-vivo* in male and female mice following sciatic nerve denervation and daily drug administration. Treatment with drug 11 prevented the early increase in STAU1 expression in denervated muscle and significantly mitigated muscle atrophy, as evidenced by the preservation of muscle mass and myofiber cross-sectional areas. Collectively, these data highlight the universal role of STAU1 as a novel atrogene and a relevant target for drug-based therapeutic interventions.

The involvement of PARylation in muscle satellite cell fate and regeneration in a mouse model of muscular dystrophy

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Repair of muscle following injury relies on the function of muscle satellite cells (MuSCs). Upon injury, the MuSC cell cycle is activated, and part of these cells commit to the myogenic lineage by expressing myogenic factors (e.g. Myf5, MyoD and MyoG) and reducing myogenic repressors (e.g. PAX7). Lastly, some MuSCs undergo self-renewal to replenish the quiescent population. Balancing commitment and self-renewal is essential for responding to injuries, which are dysregulated in muscular dystrophy (MD) and aging. Cell fate is shaped by intrinsic factors such as post-translational modifications (PTMs). We propose that enzymes regulating poly-ADP-ribosylation (PARylation), a PTM, regulate MuSC fate and function. PARylation is driven by Poly-ADP-ribose polymerases (PARPs), which attach nicotinamide adenine dinucleotide (NAD+)-derived ADP-ribose groups to proteins. We previously showed that NAD+ availability regulates MuSC function during aging and MD. Here we examined PARylation during regeneration using cultured extensor digitorum longus (EDL) muscle fibers from young wild type (WT) and mdx mice, a model of MD. Fibers were cultured ex-vivo and analyzed at 0 to 72 hours. In WT mice nuclear PARylation increased in tandem with MyoD expression at 48h, indicating commitment. In contrast, mdx MuSCs showed overactive PARylation along with commitment at 0h, possibly reflecting their continuous state of muscle regeneration. At 48h mdx MuSCs exhibited higher PARylation and commitment than WT. These findings suggest that PARylation is dysregulated in MD and may play a role in MuSC fate decisions. Ongoing work aims to test the specific roles of PARPs in MuSC fate regulation.

ABSTRACT POSTER NUMBER: 123

Parylation peril: how poly-ADP-ribosylation affects satellite cell fate and regeneration

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PARylation (Poly-ADP-Ribosylation) is a post-translational modification in which Poly-ADP-Ribose groups are added or removed to regulate cellular processes such as DNA repair, protein recruitment, and pathways governing cell death and fate decisions. This modification is mediated by two classes of enzymes: those that add ADP-Ribose (the PARP family) and those that remove it (PARG). While PARylation is well studied in the context of genome maintenance, its role in tissue regeneration and cell fate decisions is less defined. In skeletal muscle, satellite cells, the precursors to mature muscle fibers, are essential for regeneration following injury. Here, we show that the balance between PARylation and dePARylation is critical for regulating satellite cell fate and muscle repair.

In a cardiotoxin-induced injury model, *Parg*-inducible muscle satellite cell-specific knockout (iMSKO) mice showed a 49% reduction of TA muscle mass at 21 days post-injury (DPI), and a 49% reduction in myofiber diameter at 7 DPI. In ex-vivo cultures, single EDL fibers from *Parg*-iMSKO mice exhibited 40% and 59% reductions in satellite cell activation (PAX7+/MyoD+) and commitment (Myogenin+), respectively, after 48-72 hours, driven by a 32% reduction in cell cycle activation (PAX7+/Ki67+) at 24 hours. These effects on EDL fibers were recapitulated by pharmacological PARG inhibition.

These findings identify PARG as a key regulator of satellite cell dynamics and highlight the importance of PARylation balance in muscle regeneration.

High-Throughput 3-D Myobundle Platform Combining Cyclic Stretch and Electrical Pacing for Eccentric-Contraction Drug Screening in Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) drug discovery is limited by time-consuming and costly in vivo experiments or conventional two-dimensional cell culture assays that neglect the essential mechanical and electrical cues that govern muscle physiology. We are building a high-throughput three-dimensional (3-D) myobundle platform that couples controlled mechanical stretch (uniaxial cyclic strain) with electrical pacing to replicate the eccentric contractions which are considered the gold standard for assessing the susceptibility of DMD muscle to damage. Myoblasts taken from *mdx* mice, a mouse model of DMD, and wild-type littermate controls are isolated via fluorescence-activated cell sorting, suspended in fibrin-based hydrogels, and cast as 150 µL tissues anchored by foam supports on flexible silicone membranes. Thrombin-mediated fibrin polymerization yields stable, anchored myobundles that provide a permissive matrix for myoblast differentiation. A programmable "eccentric contraction" protocol, currently undergoing empirical optimization, applies synchronous cyclic stretch and electrical stimulation as a model of eccentric exercise. Conditioned medium collected after contraction sessions is analyzed for lactate dehydrogenase and creatine kinase as indicators of membrane integrity. Endpoint assessments include whole-mount immunostaining for dystrophin, myosin heavy chain, and sarcomeric α-actinin. Wild-type and *mdx* tissues exposed to the eccentric contraction protocol side-by-side within each six-well plate will facilitate direct comparisons of exon-skipping oligonucleotides, utrophin modulators, and antioxidant compounds under identical biomechanical stimuli. By integrating physiologically relevant strain, electrical pacing, and quantitative biomarker assessments, this model bridges the gap between conventional culture systems and intact muscle. Ultimately, our platform accelerates the preclinical screening and validation of novel therapeutic candidates for DMD.

ABSTRACT POSTER NUMBER: 125

Multi-Omics Analysis Reveals PARylation as a Regulator of Muscle Health in Models of Obesity and Cancer-Cachexia

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PARylation (Poly-ADP-Ribosylation) is a reversible post-translational modification in which PARP1 uses NAD^+ to attach poly (ADP-ribose) chains to proteins, a process reversed by PARG. PARylation is best known for its role in DNA repair and is targeted by FDA-approved PARP1 inhibitors and PARG inhibitors, which are currently in clinical trials. However, it also has emerging functions beyond genome maintenance, including possible roles in metabolic disorders such as obesity and cancer cachexia.

We employed metabolomics, proteomics, and transcriptomics to investigate the role of PARylation in skeletal muscle, both in vitro and in vivo, by generating inducible, muscle-specific knockouts of *Parp1* and *Parg* (*Parp1*- or *Parg*-iMKO). We assessed their baseline function and responses to metabolic stress using models of cancer cachexia and diet-induced obesity.

Our findings show that genetic ablation of *Parp1* or *Parg* alters PARylation dynamics, resulting in differential responses to metabolic stress, despite minimal changes under baseline conditions. In the cachexia model, *Parp1*-iMKO mice exhibited exacerbated muscle mass loss, whereas *Parg*-iMKO mice showed reduced muscle wasting. Multi-omics analyses suggest these phenotypes may involve PARylation-dependent regulation of the ubiquitin–proteasome system. In the other model of stress, high-fat diet-fed Parp1-iMKO mice exhibited increased weight gain and impaired glycemic control, phenotypes often associated with declining muscle health. The effect of a high-fat diet on *Parg*-iMKO mice is still under investigation.

Together, these data reveal that balanced PARylation is essential for skeletal muscle adaptation to metabolic stress and highlight the importance of future work on PARylation signaling in muscle.

ABSTRACT POSTER NUMBER: 126

Whole-body MRI in neuromuscular disease: muscle fat fraction and diffusion as potential quantitative imaging biomarkers

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OBJECTIVE:
Whole-body MRI (WBMRI) is emerging as a promising muscle disease biomarker. We used WBMRI to compare relationships between fat fraction (FF), apparent diffusion coefficient (ADC), and intravoxel incoherent motion (IVIM) metrics to assess their ability to differentiate oculopharyngeal muscular dystrophy (OPMD) from inflammatory myopathies (IM) and uncover disease-specific muscle pathology.

MATERIALS & METHODS:
In this REB-approved single-centre study, 26 patients (13 OPMD, 13 IM) underwent WBMRI using 2-point Dixon-based imaging for FF quantification, diffusion-weighted imaging (DWI) for ADC, and mid-thigh IVIM sequences to measure microvascular diffusivity (D), perfusion (f), and pseudo-diffusion (D*) coefficients. Using the ITK-SNAP software, 97 muscles were manually segmented in Dixon and DWI, with 24 mid-thigh muscles in IVIM for all patients. FF, ADC, and IVIM metrics were evaluated between OPMD and IM.

RESULTS:
OPMD exhibited more significant negative ADC-FF correlations (52/97 muscles) than IM (41/97), particularly in the shoulder, lumbar, and calf levels. The inverse correlation between FF and ADC was stronger in OPMD ($r=-0.55$, $p<0.0001$) than IM ($r=-0.42$, $p<0.0001$), suggesting more pronounced fat infiltration and diffusion restriction in OPMD. IVIM analysis revealed disease-specific microvascular changes in f and D*, with f correlating positively with FF in IM ($r=0.40$, $p<0.0001$) but not in OPMD ($r=0.03$, $p>0.05$). Muscle-specific differences were identified, with 17 muscles differing significantly in FF and 11 in ADC between OPMD and IM ($p<0.05$).

CONCLUSION:
WBMRI-derived metrics such as FF, ADC, and IVIM reveal distinct patterns of muscle degeneration in OPMD and IM, providing valuable biomarkers for disease quantification and differentiation.

ABSTRACT POSTER NUMBER: 127

Identification of blood-based biomarkers for Myotonic Dystrophy Type 1 (DM1)

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Myotonic Dystrophy Type 1 (DM1) is a genetic neuromuscular disorder characterized by progressive muscle weakness and myotonia, along with a broad range of multisystemic symptoms including cardiac conduction abnormalities, insulin resistance, and cognitive impairments. Despite advancements in understanding DM1 pathophysiology, clinically relevant biomarkers are still needed to improve disease monitoring and support therapeutic development. This study aimed to identify novel circulating protein biomarkers for DM1 using the muscle-specific HSA^{LR} transgenic mouse model. Serum samples from male and female HSA^{LR} and wild-type (WT) mice were analyzed using data-independent acquisition (DIA) mass spectrometry. Among ~1,500 proteins identified for each sex, 79 and 371 were significantly differentially expressed in male and female samples, respectively, compared to WT controls. Functional classification using REACTOME, PANTHER, Uniprot, and Entrez gene databases allowed the identification of candidate biomarkers based on their involvement in biological processes relevant to DM1 pathophysiology. These results provide the foundation for the identification of circulating biomarkers to support disease monitoring, and evaluation of therapeutic efficacy. Validation in human samples is expected to confirm translational potential of these candidates and further support the use of the HSA^{LR} model in biomarker discovery.

ABSTRACT POSTER NUMBER: 128

Age-Associated PARylation Modulates Muscle Stem Cell Dynamics During Regeneration

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In response to muscle damage, muscle satellite cells (MuSCs) activate, proliferate, and differentiate to form new muscle tissue, a process known as myogenesis. MuSC function is regulated by molecular mechanisms that become dysregulated with age, contributing to impaired regeneration. This study investigates the role of poly-ADP-ribosylation (PARylation), a post-translational modification, in myogenesis and how it may influence regenerative decline. PARylation relies on Poly-ADP Ribose Polymerases (PARPs) to transfer ADP-Ribose onto target proteins using NAD^+ as a substrate, modulating metabolic signaling.

To examine the role of PARPs in myogenesis, we inhibited PARP1 and PARP2 activity using Olaparib and traced MuSC fate in isolated extensor digitorum longus (EDL) fibers from young and aged wild-type mice. Aged quiescent MuSCs exhibited elevated levels of PARylation compared to those from young mice. During early myogenesis, aged EDLs showed increased numbers of activated ($PAX7^+/MyoD^+$) MuSCs at 24hrs and committed ($PAX7^-/MyoD^+$) MuSCs at 48hrs. Interestingly, PARP inhibition further increased the number of activated MuSCs at 48hrs and committed MuSCs at 72hrs in both young and aged cultured fibers.

These findings suggest that elevated PARylation in aged quiescent MuSCs may act as a compensatory mechanism to restrain activation and commitment. This is supported by the observation that inhibiting PARylation disrupts this restraint, allowing MuSCs, regardless of age, to progress more rapidly through activation and commitment. These results highlight PARylation as a key regulatory axis in MuSC fate decisions and a potential therapeutic target for preserving muscle health during aging.

ABSTRACT POSTER NUMBER: 129

Dysregulation of the RNA-binding protein HuR impairs neuromuscular junction integrity in muscular dystrophies

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The neuromuscular junction (NMJ) is essential for communication between motoneurons and muscle fibers, relying for its development and maintenance on tightly regulated molecular pathways, including the Agrin-Lrp4-MuSK signaling and synaptic gene expression. We previously identified the RNA-binding protein HuR as a regulator of acetylcholine receptor (AChR) β-subunit mRNA stability. Here, we investigate the broader impact of HuR in the post-transcriptional regulation of synaptically-expressed mRNAs and on NMJ integrity in neuromuscular disorders. In *mdx* and HSA^{LR} mice, well-established models of Duchenne muscular dystrophy (DMD) and myotonic dystrophy type 1 (DM1), respectively, we observed abnormal NMJ morphologies accompanied by elevated levels of HuR, suggesting a potential pathogenic role. Our results show that muscle-specific HuR depletion achieved either through knockout mice (muHuR-KO) or AVV-mediated shRNA delivery, disrupted NMJ architecture. Furthermore, AAV-mediated overexpression of HuR in muscle led to fragmented and ectopic postsynaptic structures. Bioinformatic analyses identified canonical AU-rich elements in multiple synaptic transcripts. Importantly, HuR modulation altered *Musk* mRNA levels, and RNA immunoprecipitation confirmed binding of HuR to *Musk* transcripts. Furthermore, live-cell imaging demonstrated that modulation of p38 MAPK activity, an upstream regulator of HuR localization and mRNA-binding activity, impacted pre-existing AChR clusters and influenced cluster dynamics in myotubes. These findings establish HuR as a master post-transcriptional regulator of NMJ architecture, and indicate that HuR dysregulation may contribute to synaptic defects in DMD and DM1 muscles. This work uncovers a novel pathogenic mechanism, and identifies HuR and its regulatory network as promising therapeutic targets to preserve NMJ function in neuromuscular diseases.

ABSTRACT POSTER NUMBER: 130

ATAD2: A Novel Regulator of Muscle Cell Proliferation, Differentiation, and Autophagy

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Here, we focused on the role of cofactor for oncogenic transcription factors, ATPase Family AAA Domain Containing2 (ATAD2) in regulating skeletal muscle autophagy and myogenesis. We performed siRNA-mediated transient knockdown (KD) of Atad2 in C2C12 myoblasts followed by differentiation for 7 days.

ATAD2 mRNA and protein levels decreased in differentiated C2C12 cells compared to undifferentiated myoblasts. Immunofluorescence revealed that ATAD2 is predominantly localized in the nucleus of C2C12 cells and co-localizes with PAX7 in satellite cells. Atad2 KD significantly increased myoblast number, proliferation and cell viability. This was accompanied by a downregulation of the p53–p21–Rb1 axis and a concurrent upregulation of cyclin D1 and D2, supporting enhanced cell cycle progression. Notably, Atad2 KD significantly elevated the expression of myogenic regulators; MyoD, Myogenin, Myomixer, and Myomaker, and increased the number of myosin heavy chain positive cells during early differentiation (days 0–3). However, by day 7 of differentiation, Atad2-deficient myotubes appeared elongated and atrophied, with a significant reduction in myotube diameter and abundance, associated with a significant upregulation of mRNA expression of Atrogin-1 and MuRF-1. Regarding autophagy, Atad2 KD led to a significant accumulation of LC3B-I, LC3B-II, p62, and Beclin1 proteins without corresponding increases in their mRNA levels, suggesting a blockade in autophagic flux due to autophagosome accumulation. Additionally, genes involved in lysosomal function such as Rab7, Rab29, Rab32, Nrbf2, and Cathepsin L were significantly downregulated, indicating lysosomal dysfunction. Consistently, LAMP1 staining revealed enlarged lysosomes in ATAD2-deficient myoblasts, with increased diameter, suggesting impaired autophagosome–lysosome fusion and disrupted autophagy.

Single-AAV-mediated adenine base editing efficiently restores dystrophin expression and muscle function in mdx 4cv mice

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Duchenne muscular dystrophy (DMD) is a severe genetic neuromuscular disease caused by loss-of-function mutations in the gene encoding dystrophin, a protein crucial for maintaining the structure and function of cardiac and skeletal muscle cells. While there are currently no curative treatments for DMD, emerging approaches using CRISPR-based gene editing technologies have shown promise, although they face challenges related to efficient delivery of CRISPR components and risk of off-target modifications, as well as uncertainties regarding optimal treatment timepoints and long-term efficacy.

Here, we present an adeno-associated virus (AAV) vector-delivered CRISPR-targeted adenine base editor approach to convert the nonsense mutation (TAA) in exon 23 within the dystrophin gene of *mdx*^{4cv} DMD-model mice into a missense mutation (TGG) that restores dystrophin expression. Using muscle-targeted MyoAAV vectors for delivery, we assessed editing rates in *mdx*^{4cv} mice treated at 3-days, 1-week, 3-weeks, 12-weeks, or 6-months of age to identify optimal ages and stages of disease progression for introducing therapeutic edits into *Dmd* alleles. We demonstrate efficient restoration of dystrophin expression and consistent detection of on-target therapeutic edits in both cardiac and skeletal muscles at all ages tested, with the greatest efficiency observed at the 3-week-old timepoint. Interestingly, we observed a higher frequency of functional gene edits (TAA>TGG) in muscle satellite cells compared to mature muscle, suggesting that satellite cells may gain a fitness advantage from restoration of dystrophin reading frame. These data underscore the potential of *in vivo* genome modification to treat DMD at various stages of disease progression.

Development of Muscle Stem Cell-specific Mini-Promoters for AAV-Mediated Gene Therapy Approaches

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Tissue- and cell-type-specific promoters that enable targeted expression of cargo genes while conforming to AAV vector packaging limits would be beneficial for effective gene therapeutic outcomes in Duchenne muscular dystrophy (DMD) and other skeletal muscle diseases. Robust expression in muscle stem cells (satellite cells) is desirable as these cells support early muscle growth and sustain myofiber replacement. However, most existing promoters compatible with AAV size limits exhibit relatively broad expression profiles with diminished activity in quiescent satellite cells.

In this study, we aimed to develop new promoter elements capable of driving robust gene expression in muscle satellite cells following systemic delivery using AAV. To this end, we analyzed multiple ATAC-seq data sets to generate genome-wide chromatin accessibility maps in sorted mouse satellite cells and identify candidate cis-regulatory elements <200 bp in length. This effort nominated 14 short putative regulatory elements (mini-promotors) that we tested for activity through plasmid transfection of primary mouse satellite cells *in vitro*. Several of the tested mini-promoters drove EGFP expression and also enabled adenine-base editing (ABE) to correct the *mdx*^{4cv} nonsense mutation in transfected satellite cells. We are currently testing *in vivo* tissue specificity and potency of these mini-promoters following AAV-mediated delivery of EGFP and ABE cargo genes in *mdx*^{4cv} mice. Our data suggest that some mini-promoter candidates demonstrate comparable potency with greater specificity for satellite cell expression than frequently used muscle promoters, including EFS (250 bp) and MHCK7 (771 bp), underscoring their promise for selective gene delivery *in vivo* and treatment of inherited muscle diseases.

Single AAV vector base editing rescues a nonsense variant in a humanized mouse model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is an X-linked recessive pediatric neuromuscular disorder caused by pathogenic *DMD* variants, of which 15% are nonsense mutations resulting in loss-of-function dystrophin—a critical structural protein in muscle. Dystrophinopathies compromise the mechanical integrity of skeletal myofibers and cardiomyocytes, culminating in life-limiting progressive muscle wasting and cardiorespiratory insufficiency. Adenine base editors (ABEs) are CRISPR-derived technologies enabling template-independent, programmable A-to-G transitions via TadA-mediated deoxyadenosine deamination, however clinical translation has been constrained by off-target RNA and bystander editing, and payload restrictions of clinically validated myotropic adeno-associated viral (AAV) vectors. Here, we report the first therapeutic application of an AAV-compatible ABE for precise correction of a nonsense variant and amelioration of dystrophic pathology in a humanized mouse model of DMD. We established a patient-representative nonsense variant (*DMD*$^{9445C>T}$) amenable to ABE correction on our novel fully-humanized D2.*mdx-hDMD*$^{9445C>T}$ mouse model which recapitulated histopathological features including dystrophin-negative myofibers, endomysial fibrosis, and centralized myonuclei throughout the skeletal musculature at 16-weeks-old. We subsequently screened AAV-compatible compact Cas9 orthologs combined with a series of bespoke TadA8e deaminases with mitigated RNA off-target and bystander activity, achieving 49.9±7.4% *DMD*$^{9445C>T}$ correction in lentiviral-transduced patient-derived primary myoblasts *ex vivo*. Notably, intravenous administration of MyoAAV4A-CK8e-ABE (5E13vg/kg) in 4-week-old D2.*mdx-hDMD*$^{9445C>T}$ mice achieved 85% dystrophin rescue in the heart and 20-40% across skeletal muscles. AAV-ABE treatment significantly improved cardiac function, grip strength, and overall muscle histopathology at 12-weeks post-intervention. These findings substantiate ABEs as efficacious platforms for therapeutic gene-editing and overcome safety limitations essential for clinical translation.

ABSTRACT POSTER NUMBER: 134

Early dynamic functional neuromuscular reinnervation and maturation.

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While neuromuscular junction (NMJ) is a stable and robust synapse, it is can also undergo efficient reinnervation owing to reliable repair mechanisms. These repair processes appear deficient in disease such as Amyotrophic lateral sclerosis (ALS) where NMJs exhibit an abnormal dynamism with denervation-reinnervation cycles. However, the rapid response leading to functional repair remains unexplored, limiting our ability to understand the instability of NMJs in ALS. Hence, we developed a novel *in-vivo* approach to study reinnervation, allowing us to lesion a few axons and denervate downstream NMJs. Axonal photodamage was induced ~50 mm upstream from the NMJs. After a recovery phase (3-28 days), muscles and nerves were dissected, and electrophysiological recordings were performed to study synaptic functions of the reinnervated NMJs and of non-injured control neighbouring NMJs. Confocal imaging was used to assess NMJ innervation integrity. We observed that NMJ reinnervation is still ongoing in a majority of NMJs 3 days after injury, while all NMJs were reinnervated at 5 days postinjury. Synaptic properties are consistent with weaker synaptic output. This weakness was maintained, or even enhanced, at 11 days postinjury, indicative of a critical period of NMJ repair. NMJs showed synaptic properties like the ones observed in intact, uninjured NMJs. Hence, functional NMJ reinnervation is a relatively slow process that encompasses a critical period, before reaching functional maturity. Importantly, morphological reinnervation is an inadequate parameter of the quality of reinnervation. We posit that the reinnervation process is implicated in the instability of the NMJs in disease such as ALS.

208-week efficacy and safety of cipaglucosidase alfa plus miglustat in patients with late-onset Pompe disease treated from PROPEL baseline: muscle function and biomarkers

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The open-label extension (OLE) of the 52-week PROPEL study evaluates long-term efficacy and safety of cipaglucosidase alfa plus miglustat (cipa+mig) in adults with late-onset Pompe disease (LOPD) with or without prior enzyme replacement therapy (ERT) experience.

Here we report 208-week muscle function, biomarker, and safety data, shown as change from PROPEL baseline (CFBL; OLE week 156) for patients receiving cipa+mig.

Sixty-two ERT-experienced and 20 ERT-naïve patients with a mean (standard deviation [SD]) age of 48.8 (13.5) years were treated with cipa+mig. Median (interquartile range) duration of prior ERT was 7.5 (4.3–10.2) years. Mean (SD) CFBL in % predicted 6-minute walk distance was +2.3 (9.8)% for ERT-experienced and +5.8 (11.9)% for ERT-naïve patients. Both groups showed stable mean CFBL for manual muscle test lower extremity (ERT experienced +1.6 [5.3]; ERT naïve +0.3 [4.0]); Gait, Stairs, Gowers' maneuver, Chair test (ERT experienced +0.1 [3.1]; ERT naïve -1.6 [2.8]); and PROMIS® Physical Function (ERT experienced +1.2 [11.1]; ERT naïve +5.1 [12.6]). Mean CFBL in creatine kinase (ERT experienced -160.0 [225.2] U/L; ERT naïve -258.9 (282.2) U/L) and hexose tetrasaccharide (Hex4; ERT experienced -1.9 [3.1] mmol/mol creatinine; ERT naïve -4.3 [2.9] mmol/mol creatinine) improved in both groups. Forty-one patients experienced treatment-related treatment-emergent adverse events (TEAEs) and two experienced serious TEAEs, causing four and two cipa+mig discontinuations, respectively. One fatality occurred following a TEAE unrelated to treatment.

These four-year data support the long-term benefits of cipa+mig in patients with LOPD, including those with over seven years of previous alglucosidase alfa ERT.

An RNA-Seq Analysis Investigating Epigenetic Pathways and Acetylation-Related Dysregulation in Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a severe and fatal genetic neuromuscular disorder characterized by progressive muscle degeneration, fibrosis, and premature loss of function. To date, gene therapies have failed to sufficiently cure DMD, leading to the development of small molecule therapeutics. Preclinical studies have found that protein acetylation is dysregulated in DMD and, specifically, that histone deacetylase inhibitors (HDACis) improve DMD pathology. Furthermore, the pan-HDACi givinostat was approved by the FDA in 2024 to treat DMD symptoms. However, the molecular mechanisms through which givinostat and other HDACis treat DMD remain poorly understood. The purpose of this project was therefore to identify the major acetylation-regulated pathways that become dysregulated in DMD and to identify the specific pathological mechanisms of HDACi action.

To identify robust gene expression signatures (GES) of DMD, we obtained and analyzed multiple RNA-seq datasets from muscle of both patients with DMD and mdx mice (NCBI BioProjects). Intersecting upregulated and downregulated differentially expressed genes (DEG) across datasets formed our GES. Using gene set enrichment analysis (GSEA), the downregulated GES was enriched in gene ontology (GO) terms for RNA binding, transcription factor activity, and aminoacyltransferase activity, while the upregulated GES was linked to GO terms for inflammation, metabolism, and epigenetic regulation, including histone deacetylase binding. To evaluate the effect of epigenetic therapeutic interventions, we compared our GES with DEG profiles following treatment with four HDACis: valproic acid (VPA), sodium butyrate, AR-42, and givinostat. There were no statistically significant alterations of DMD-associated upregulated or downregulated GESs with VPA, butyrate, or AR-42 treatment. In contrast, givinostat treatment reversed DMD GESs. Further, GSEA of givinostat-treated cardiomyocytes revealed modulation of pathways related to inflammation, heart development, and fibrosis; however, none reached statistical significance (FDR ≤ 0.05).

Together, this study 1) described conserved pathological molecular signatures in DMD associated with epigenetics and protein acetylation, and 2) revealed a reversal of these molecular gene expression signatures with the HDACi givinostat. This suggests that the mechanisms regulated by givinostat and other epigenetic modifiers should be further explored within DMD to identify new pathological mechanisms and additional potential therapeutic strategies.