Meeting report

Journal of Neuromuscular Diseases
Vol. 12(1): 1–14

DOI: 10.1177/22143602241304993
journals.sagepub.com/home/jnd

Report on the 6th Ottawa International Conference on Neuromuscular Disease & Biology – September 7–9, 2023, Ottawa, Canada

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Abstract

The 6th Ottawa International Conference in Neuromuscular Disease and Biology was held on September 7–9, 2023 in Ottawa, Canada. The goal of the conference was to assemble international experts in fundamental science, translational medicine and clinical neuromuscular disease research. Speakers provided attendees with updates on a wide range of topics related to neuromuscular disease and biology, including methods to identify novel diseases, recent developments in muscle, motor neuron and stem cell biology, expanded disease pathogenesis of known diseases, and exciting advances in therapy development. A summary of the major topics and results presented by these speakers is provided.

Keywords

neuromuscular, conference, NMD, muscle, stem cell

Received: 10 June 2024; accepted: 14 November 2024

Introduction

The 6th Ottawa International Conference in Neuromuscular Disease and Biology opened on September 7, 2023, which was World Duchenne Awareness Day. As one of the first genes to be identified causing neuromuscular disease (NMD), the dystrophin gene was initially described in 1987 as causing Duchenne Muscular Dystrophy (DMD), following intense gene-hunting efforts.[1,2] Follow-up characterization of the native dystrophin gene and protein structure, and how it is altered in patients with DMD and Becker muscular dystrophy,[3] gave rise to the idea of mini- and micro-dystrophin genes that are small enough to fit within existing viral gene therapy vectors but still retain significant dystrophin protein function.[4–6] Such vectors have now been tested in clinical trials and have shown some efficacy.[7,8] Knowledge of the intron/exon structure of the dystrophin gene also led to the idea of using antisense oligonucleotides to force alternative splicing within the dystrophin pre-mRNA to "skip" an exon containing a deleterious mutation, thus allowing production of at least a partially functional protein.[9] Now, several ASO-based therapeutics have been approved by the US Food and Drug Administration (FDA) for treatment of DMD.[10] The evolution in our understanding of the dystrophin gene, protein, and disease pathogenesis, which all have contributed to the development of the currently available therapies for DMD, serves as a blueprint for researchers for therapy development for all NMD. Enhancing these efforts through exchange of ideas and approaches was a key goal of Ottawa NMD 2023.

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After the initial discovery of the *DMD* gene, now over 600 genetic loci have been linked to NMD[11] (https://www.musclegenetable.fr/index.html). NMD include a wide range of diseases affecting motor neuron, peripheral nerve, neuromuscular junction and skeletal muscle, and can include both genetic and acquired disorders. Although most individual NMD are rare, collectively NMDs have an overall prevalence of ~28–53 patients per 100,000 individuals, although this rate can vary significantly between populations.[12–16] The quality of life of NMD patients is significantly affected given that many NMD cause progressive muscle wasting and weakness, disability, and premature death, and few have effective therapies or treatments.

Clinical research is constantly evolving to apply novel methods to identify disease causing genes, to provide an accurate diagnosis for patients affected by NMD and other disorders.[17] Basic research strives to understand the underlying molecular basis of disease pathogenesis and frequently employs animal models of human disease to test new approaches to treatment. The middle ground between basic and clinical research is broached by translational research – a bridging of bench to bedside and bedside to bench. Each of these pillars of research is crucial to identifying new and effective therapies for patients affected with NMD yet, frequently, these researchers and clinicians work in relative isolation. The Ottawa NMD series of conferences is designed to provide a venue for basic, translational, and clinical researchers to learn about recent developments and cutting-edge findings in NMD research, and exchange ideas and approaches that ultimately may improve NMD patient care.

The 6th Ottawa International Conference on Neuromuscular Disease & Biology was held on September 7–9, 2023 in Ottawa, Canada. As in previous years (please see previous conference summaries[18–21]). The aim of the conference was to bring together international world-leading experts in NMD clinical care, translational research and fundamental science. Ottawa represents a natural destination for such a conference given the existence of the University of Ottawa Eric Poulin Centre for Neuromuscular Disease, which brings together over 60 basic, translational and clinical researchers, and over 200 graduate students, postdoctoral fellows, residents and clinical fellows from the region who are all focused on developing innovative therapies to improve NMD patient outcomes. The Centre also celebrates its 25th anniversary in 2024, a milestone which highlights the long-standing and collective commitment of the University, affiliated Hospitals and Research Institutes to this priority area.

The Conference featured 44 internationally recognized invited speakers (13 from Canada, 20 from the United States, and 11 from Europe), and over 350 attendees. For poster sessions, 141 abstracts were submitted for presentation. The keynote address was provided by Dr Rudnicki, a Senior Scientist and Program Director of the Regenerative Medicine Program at the Ottawa Hospital Research Institute (OHRI), as well as Professor in the Department of Medicine and Department of Cellular and Molecular Medicine at the University of Ottawa. Dr Rudnicki overviewed muscle stem cell (*i.e.,* satellite cell) biology in development and differentiation into mature muscle,[22] and the importance of balanced asymmetric versus symmetric cell division for expansion and maintenance of satellite numbers in the mature muscle. He discussed unpublished work using single-cell RNAseq analysis to further characterize the varied cell populations within muscle. Undoubtedly, further elucidation of the cell types and gene expression profiles of cells within developing and mature muscle, and how these programs go awry in diseased muscle, will lead to the identification of novel potential targets for therapeutic intervention.

Motor neuron disease pathogenesis and treatment

Dr Giorgia Querin, (Institut de Myologie, France) discussed the challenges and importance of identifying reliable and valid motor neuron disease biomarkers, which is critical in the development of precision medicine approaches necessary for success in clinical trials.[23] For example, adult forms of SMA are associated with selective grey matter degeneration in the cervical spinal cord with preserved white matter integrity, that can be used as potential imaging biomarkers in spinal MRI.[24] She also discussed liquid biomarkers such as neurofilament proteins, which begin to increase both in the CSF and serum prior to symptom onset in C9ORF72 amyotrophic lateral sclerosis (ALS) and slowly increase over time. In addition, dipeptide-repeat proteins, such as poly glycine-proline protein can be detected in the CSF of these patients and have potential to be a target engagement marker in clinical trials.[25] Dr Hugh McMillan (CHEO, CA) outlined the critical discoveries of SMA that has led to gene editing, gene knockdown, gene addition, and gene replacement therapies for SMA and he discussed the challenges of clinical implementation of these therapies.[26] He reviewed the clinical impact of therapeutic trials and also the potential risks of immune response with Onasemnogene abeparvovec gene therapy.[27,28] Dr McMillan also outlined the adoption of newborn screening across North America.[29] Dr Suma Babu (Harvard Medical School, US) discussed the existing therapies for ALS, such as riluzole, edaravone, albioza/sodium phenylbutyrate-taurursodiol as well as the exciting new therapy tofersen. Tofersen is specific for patients with ALS due to mutations in *SOD1* and is administered intrathecally. Administration of tofersen in the VALOR trial resulted in stabilization of clinical function on the Revised ALS Functional Rating Score and slow vital capacity at 52 weeks.[30–32] Dr Babu highlighted the perpetual adaptive trial design of the HEALEY ALS Platform Trial.

This trial platform includes a placebo controlled and open labelled regimen (3:1) to test several investigational products in parallel and sequentially in persons with ALS to more effectively identify novel treatments[33] (https://www.massgeneral.org/neurology/als/research/platform-trial). Since the Conference, sodium phenylbutyrate with taurursodiol was removed from the market, as the 48-week, randomized, double-blind, placebo-controlled PHOENIX trial (NCT05021536) did not demonstrate a statistically significant difference from baseline in the Revised Amyotrophic Lateral Sclerosis Functional Rating Scale total score, highlighting the challenges in therapy development and interpretation of biomarkers in early motor neuron disease trials.

Challenges and opportunities in amyotrophic lateral sclerosis

Dr Richard Robitaille (University of Montreal, CA) spoke about the "Jekyll and Hyde" duality of glial cells at the neuromuscular junction (NMJ) – they are important in proper maintenance but also in the breakdown of the NMJ.[34,35] In ALS, glial cells show hyperactivity, and Dr Robitaille showed that darifenacin, an FDA approved drug for use on overactive bladder that dampens activity of the muscarinic acetylcholine receptors (mAChR), led to improved NMJ maintenance, muscle strength and survival in the *SOD1*^{G37R} mouse model of ALS. These promising results in mice have led to a phase I Canadian clinical trial in patients with ALS. Dr Justin Ichida (University of Southern California, US) spoke of his work on using ALS patient-derived induced pluripotent stem cells (iPSC) to elucidate new aspects of disease and potential therapies. Dr Ichida was able to differentiate iPSC derived from patients with pathogenic *C9ORF72* mutations into microglial cells (termed induced microglia, iMG,[36]), and showed that these cells had a significantly different gene expression profile compared to those derived from healthy controls. Co-culturing of certain subsets of iMG with motor neuron cells derived from *C9ORF72*-patients appeared to be protective and pointed to CSF1R as being a potential therapeutic target – ASO-mediated down regulation of CSF1R led to phenotypic improvement in a mouse model of *C9ORF72* disease.

Dr Janice Robertson (University of Toronto, CA) discussed the strong relationship between ALS and frontotemporal dementia (FTD), and how many disease-causing genes may be more associated preferentially with ALS or FTD, thus setting up a spectrum of disease phenotypes. *C9ORF72* appears to fall in the middle of this spectrum,[37] and leads to disease likely due to a mechanism involving both loss of function and gain of function for the mutant protein. C9ORF72 is normally found in both the pre- and post-synaptic compartment, and mice that are knocked-out for C9ORF72 show higher levels of GluA1, leading to increased calcium flux and increased sensitivity to kainic acid-induced seizures. C9ORF72 knockout neurons show reduced arborization in culture and when examining spinal arborization. These results clearly show that C9ORF72 function is important in establishing appropriate AMPA receptor levels in neurons and promotes proper arborization of neurons. Dr Léa Lescouzères from the laboratory of Dr Kessen Patten (Institut National de la Recherche Scientifique, CA) provided a short talk entitled, "Calpastatin compensation restores neuromuscular dysfunction in a genetic model of *C9orf72* ALS".

Translational research in spinal muscular atrophy

Dr Allison Ebert (Medical School of Wisconsin, US) discussed the role of astrocytes and microglia in SMA pathogenesis. Astrocytes differentiated from iPSC-derived from patients with SMA show many differences from those derived from healthy controls, with shorter processes and an activated phenotype with gene and microRNA misregulation. Knockdown of several of the genes misregulated in the astrocytes and microglia, such as GATA6, CCL5 and IL1, improved cell function.[38] Finally, Dr Ebert showed that Teneurin-4, which is expressed on the surface of healthy astrocytes but aberrantly trafficked in astrocytes derived from SMA patient iPSC cells, appears to promote good electrical activity in motor neurons, clearly showing the importance of support cells in proper motor neuron function. Professor Thomas Gillingwater (University of Edinburgh, UK) began by noting that SMA is not only a neurodegenerative disease but is likely also a developmental disorder, and the reason current therapies are not a "cure" is really a matter of timing – the therapies are not administered early enough to correct early developmental deficits.[39,40] Systematic analysis performed by the CAMARADES (Collaborative Approach to Meta Analysis and Review of Animal Experimental Studies) group clearly supports the idea that early treatment intervention in SMA is better than late and, indeed, treatment of pre-symptomatic patients with Zolgensma showed 100% survival. Analysis of SMN protein levels in different tissues during early stages of development (E12.5-E15.5) showed that SMN protein is typically expressed at higher levels early and then declines during development.[41] Of its many functions, SMN protein also appears to associate with a subset of ribosomes and hold the ribosome in the correct position for translation to occur.[42,43] In the absence of SMN protein, the "translatome" is altered leading to protein production deficits that lead to developmental issues that subsequent therapy administration cannot counteract.

Dr Charlotte Sumner (Johns Hopkins University, US) noted the real variation in response to treatment that is observed in the SMA patient population. Even with adoption of newborn screening for SMA in many countries

worldwide, leading to very early therapy intervention, some patients still show a decline. The reason these therapies are not completely corrective is likely due to very early defects in development that cannot be overcome by subsequent therapy.[44] For example, analysis of the peripheral nerve axon from patients with SMA shows cell bundles with very small axon diameters relative to healthy controls, reminiscent of immature, foetal small axons.[45] Thus, reduced levels of SMN protein appears to lead to arresting of axon development and, perhaps, these immature neurons are more vulnerable to degeneration. However, overexpression of NRG1-III in the SMAΔ7 mouse model of SMA improved axon maturation but did not improve vulnerability to degeneration, suggesting there are still additional pathological issues in these cells.[45] A short talk was provided by Dr Christiano Alves (Massachusetts General Hospital, US) entitled, "Base editing as a genetic treatment for spinal muscular atrophy."

Clinical advances in autoimmune and genetic neuropathies

Dr Kerri Schellenberg (University of Saskatchewan, CA) provided a detailed overview of clinical management of Spinal Bulbar Muscular Atrophy (SBMA), also known as Kennedy Disease. Dr Schellenberg noted that SBMA prevalence is one of the highest in the world among persons of Indigenous descent in Saskatchewan, Canada at 14.7 per 100,000 individuals.[46] Dr Schellenberg provided a clinical overview of this rare x-linked disorder, where men present with motor neuron neurodegeneration with limb and bulbar weakness, tremor, muscle cramps, diffuse fasciculations, sensory neuropathy with gynecomastia, and testicular atrophy.[47] Patients also have respiratory dysfunction with nocturnal hypoventilation, laryngospasm, exertional dyspnea, and orthopnea.[48] Unfortunately, no disease-modifying medications are available, and treatments remain simply supportive. Dr Rami Massie (McGill University, CA) provided an overview of the updated chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) guidelines by the European Academy of Neurology/Peripheral Nerve Society.[49] These consensus-based guidelines simplified the clinical definitions of CIDP, replaced the clinical classification of 'atypical CIDP' with 'CIDP variants', reclassified neuropathies with nodal antibodies (*e.g.,* neurofascin) and chronic immune sensory polyradiculopathy (CISP) as separate diseases, and recommended subcutaneous immunoglobulin (SCIg) as maintenance therapy.[50] Dr Massie also eloquently outlined the ethical challenges of completing randomized placebo-control trials instead of head-to-head trials when there are effective therapies available. Dr Vera Fridman (University of Colorado, US) closed this session by outlining the key clinical features of several of the genetic non-CMT neuropathies, including the porphyrias, mitochondrial neuropathies, lysosomal storage disorders, peroxisomal disorders, and spinocerebellar ataxias with neuropathy. Dr Fridman highlighted critical, evolving, therapeutic considerations, including patisiran[51] and vutrisiran[52] for Hereditary Transthyretin Amyloidosis, and givosiran in acute intermittent porphyria.[53] Given the phenotypic heterogeneity of inherited neuropathies, Dr Fridman emphasized the importance of pursuing exome sequencing in patients with atypical features and who remain undiagnosed after conventional genetic testing, and presented a case study of a patient with a rare presentation of adult-onset Krabbe Disease presenting as an isolated neuropathy.[54]

Updates in myasthenia gravis and neuromuscular junction disorders

Professor Amelia Evoli (Catholic University, Italy) reviewed clinical diagnostic dilemmas with autoimmune myasthenia gravis (MG), including atypical presentations at onset such as isolated dysphagia, and limb girdle or neck extensor weakness.[55] She described unique features of muscle-specific kinase antibody (Anti-MUSK) positive patients, including early onset, severe muscle atrophy, and paraspinal atrophy with severe scoliosis that improves with treatment.[56] For patients with clinical MG but without antibodies detected by radioimmunoprecipitation assays, live cell-based assays can detect additional antibodies to clustered MuSK, acetylcholine receptor (AChR), and low-density lipoprotein receptor-related protein 4 in 72% of patients.[57] Dr Gil Wolfe (University of Buffalo, US) outlined the key highlights from the updated International Consensus Guidance for Management of Myasthenia Gravis.[58] He reviewed important clinical management updates, including the recommendations that methotrexate may be considered as a steroid-sparing agent in patients with generalized MG who have not tolerated or responded to steroid-sparing agents, and that eculizumab should be reserved for severe and refractory AChR antibody positive MG.[58] Dr Hanns Lochmüller (TOH/CHEO, CA) described the diagnostic challenges with congenital myasthenic syndromes (CMS), with classic features including floppy babies, arthrogryposis, ptosis, and ophthalmoplegia, exercise induced weakness and novel genetic syndromes such as newly identified TEFM.[59] He also reviewed the importance of accurate genetic diagnosis and identifying founder mutations for choosing therapeutic options such as acetylcholinesterase (AChE) inhibitors or beta-agonists based on genotype.[60,61]

Muscle stem cells and development

Dr Natasha Chang (McGill University, CA) reviewed muscle stem cell dysfunction in DMD.[62] She outlined that the process of autophagy is dynamically regulated during

maintenance and differentiation of satellite cells, and that this process appears to be disrupted in satellite cells isolated from *mdx* mice, a mouse model of DMD.[63] Using single cell RNAseq, Dr Chang identified a number of genes and pathways aberrantly regulated in satellite cells from the *mdx* mice relative to healthy animals. These studies have uncovered autophagy as a key process that is acutely regulated during normal muscle differentiation and that likely contributes to the overall pathology of DMD. Dr Laurent Schaeffer (University of Lyon, FR) presented his work on the role of histone variant H2A.Z in DNA repair and in preventing premature aging in muscle fibers. H2A.Z is believed to be important in mitosis and cell proliferation, DNA repair and transcription, and is present at many gene transcription start sites, thus suggesting a crucial function. Although, muscle specific knockout of H2A.Z in mice had minimal effects on gene expression,[64] the muscle cells appeared to show ultrastructural changes consistent with an accelerating-aging phenotype. Muscle from these mice showed increased oxidative stress, with an increase in reactive oxygen species (ROS) production, leading to increased DNA damage. H2A.Z is involved early in recruiting the DNA damage repair machinery to sites of DNA damage, and this process is aberrant in the H2A.Z knockout mice, leading to enhanced DNA damage and accelerated aging.

Dr Melissa Spencer (University of California Los Angeles, US) outlined her work using CrispR/Cas9 genome editing technology to treat DMD.[65] Dr Spencer discussed her research in developing nanoparticles and viral vectors to deliver the gene editing machinery to muscle cells, with good efficiency, and also noted the likely importance of editing muscle satellite cells to achieve a durable response. Dr Andreas Roos (University Duisburg-Essen, DE) closed the session with a short talk entitled, "Bi-allelic variants of *FILIP1* cause congenital myopathy, dysmorphism and neurological defects."

Advances in autoimmune and genetic myopathies

Dr Craig Campbell (Western University, CA) discussed the underlying pathophysiology of DMD and the promise of novel DMD therapies, such as new histone deacetylase inhibitors.[66] Dr Campbell highlighted his work where genome-wide DNA methylation analysis identified an "episignature," a diagnostic and potentially prognostic methylation signature in a large cohort of affected individuals with clinical and molecular diagnosis for DMD.[67] The episignatures identified genes that were hypo- or hyper-methylated and could differentiate between affected patients, treated patients and controls, to improve the variant interpretation process in DMD, provide insight into the underlying disease pathophysiology and serve as a potential blood biomarker.[68] Dr Craig Zaidman (Washington University, US) outlined the early experiences and obstacles in gene therapy in DMD. He described the 1-year outcomes of the ENDEAVOUR gene therapy trial, with muscle transduction with delandistrogene moxeparvovec, an AAV-based gene therapy vector encoding a microdystrophin gene. Dr Zaidman provided multiple video examples of boys with DMD in the trial where motor function had stabilized or improved.[69] Dr Zaidman also reviewed rare but serious complications of gene therapy, including myocarditis and severe acute respiratory distress and death.[70] He described practical considerations to mitigate the risk of therapy in a real-world clinical practice setting, including screening for appropriate anti-AAV antibody titers and liver/cardiac function pre and post infusion, as well as administering additional corticosteroids starting one day pre-infusion and maintaining the corticosteroid regimen for at least 60 days post-infusion.[71] Dr Olivier Benveniste (Institute of Myology, France) provided a detailed overview of recent advances in serological diagnostics of inflammatory myopathies[72] and described specific antibody-associated disease such as elevated risk of malignancy with anti-TIF-1γ, NXP2 or HMGCR antibodies.[73] Professor Benveniste also highlighted the importance of imaging biomarkers in trials, demonstrating that quantitative MRI and phosphorus magnetic resonance spectroscopy could identify differences in sirolimus-treated patients with sporadic inclusion body myositis, which also correlated with functional and strength outcome measures.[74] Finally, he described updated guidelines on therapeutic strategies, including for necrotizing myopathies, which include steroids, immunosuppressants and IVIg, with rituximab remaining an effective second-line therapy.[75]

Molecular insight into myopathies

Dr Jeffrey Dilworth (University of Wisconsin-Madison, US) discussed his work on muscle stem cell adaptation and inflammation. Dr Dilworth noted that there is significant inflammatory cell infiltration in dystrophic muscle, and that diphtheria toxin-mediated elimination of M1 macrophage leads to poor regeneration, suggesting the inflammatory cell infiltration is crucial for proper regeneration. However, paradoxically, Dr Dilworth's studies have shown that inflammatory cytokine signaling from these invading cells can impair the ability of quiescent MuSCs to re-enter the cell cycle. The histone H3 lysine 27 (H3K27) demethylase JMJD3 appears necessary for removing repressive trimethylated H3K27 (H3K27me3) marks at the Has2 locus to initiate production of hyaluronic acid, which in turn established an extracellular matrix competent for integrating signals that direct MuSCs to exit quiescence.[76] Thus, hyaluronic acid could have therapeutic applications to help promote muscle regeneration. Professor Gisèle Bonne (Sorbonne University, FR) presented her work on exploring the phenotypic diversity of

laminopathies caused by mutations in the lamin A/C gene. Lamin protein is found on the inner nuclear envelope, and mutations within the lamin A/C gene cause congenital forms of muscular dystrophy, including Emery-Dreifuss muscular dystrophy and Charcot-Marie-Tooth disease type 2B1, and exhibit significant variability. Prof. Bonne discussed her work on characterizing cell-based models of laminopathies, examining the variability in the disease (such as between family members with the same genetic defect or early- versus late-onset disease), and developing potential new therapies.[77] Prof. Bonne also highlighted that processing and expression of miRNA appears aberrant in lamin A-deficient cells,[78] suggesting a potential role for lamin A/C in influencing miRNA processing.

Professor Silvere van der Maarle (Leiden University Medical Center, NL) discussed our current understanding of the genetics of facioscapulohumeral muscular dystrophy type 1 (FSHD1) and the related FSHD2, and the complex biology of DUX4, the gene that appears aberrantly regulated in FSHD.[79] Prof. van der Maarle discussed therapeutic approaches to treating FSHD, including reversing the chromatin changes that lead to activation of expression of DUX4, thus preventing expression and action of DUX4, and treating the downstream pathways induced in the FSHD muscle (e.g., inflammation).[80] He also discussed his work using nanopore long-read sequencing technology to sequence the entire D4Z4 region, which has provided new insight into locus arrangements that lead to pathogenicity, which will also greatly help in predicting pathogenicity in clinical diagnostic laboratories.[81] The session ended with a short talk by Dr Katelyn Daman, from the laboratory of Dr Charles Emerson (University of Massachusetts Chan Medical School) entitled, "An innate immune cell/FSHD muscle xenograft model to investigate the role of complement in FSHD muscle pathology".

Muscle disease pathogenesis and treatment

Dr Matthew Alexander (University of Alabama, US) introduced the audience to the Center for Precision Animal Modeling (C-PAM, https://sites.uab.edu/precisionmedicine/center-for-precision-animal-modeling-c-pam/), a US National Institute of Health funded service dedicated to developing novel animal models for rare (*i.e.,* < 1 in 2000 individuals) and ultra-rare (*i.e.,* < 1 per 50,000 individuals) human disease. Potential disease-causing gene variants, identified through next generation sequencing or alternative methods, can be submitted to C-PAM for consideration for generation of mutation-specific animal models that can aid in establishing disease causation and mechanism, as well as aid in development of potential therapeutics. Dr Alexander presented work on characterization of a patient-derived mutation in UMA21, a disease-causing gene for the ultrarare disease X-linked myopathy with excessive autophagy (*XMEA*). In a zebrafish model of the disease, edaravone, a drug approved for treatment in ALS, was able to extend life and improve animal motility. Dr Doug Millay (Cincinnati Children's Hospital, US) outlined developing myomaker and myofusion/myomerger to potentially enhance therapeutic delivery to muscle cells. Myomaker and myofusion/myomerger are responsible for mediating the fusion of muscle myoblasts into multinucleated mature fibers. Dr Millay showed that lentivirus vectors with myomaker and myofusion in the viral envelope showed enhanced delivery to injured muscle, with specificity imparted due to the requirement that the recipient cell must also express myomaker.[82] This same approach could efficiently deliver lentivirus vector encoding a microdystrophin gene to muscle tissue in a mouse model of DMD.

Dr Pier Lorenzo Puri (Sanford Burnham Prebys, US) presented his work on elucidating the networks that are engaged when a muscle fiber is denervated. scRNA-seq/snATAC-seq of skeletal muscles after sciatic nerve transection showed selective activation of glial cells and Thy1/CD90-expressing mesenchymal cells.[83] Dr Puri demonstrated that the interaction of these two cell types, mediated in part through NGF/NGFR, is important for NMJ repair. Dr Nicolas Dumont (Université de Montréal) provided a short talk entitled, "Clearance of defective muscle stem cells by senolytics reduces the expression of senescence-associated secretory phenotype and restores myogenesis in myotonic dystrophy type 1."

Muscular dystrophies – treatment and pathogenesis

Dr Gerald Pfeffer, (University of Calgary, CA) demonstrated the utility of transcriptomics using principal component analysis, gene ontology and pathway analyses to assist with molecular diagnosis in an undiagnosed myopathy cohort.[84] Dr Pfeffer presented the identification of transcripts that were dysregulated in myofibrillar myopathy [JPX], dystrophic pathological changes [MEG3], and mitochondrial myopathy [GAS5].[84] Dr Katherine Mathews (University of Iowa, US) outlined challenges in pediatric neuromuscular clinical trials that impact the interpretation of clinical outcome measures, including insufficient natural history data, inhomogeneous progression of many NMD, difficulty in finding patients with rare disease to participate in a trial, as well as incorporating changes in biomarkers that can occur as a result of normal developmental and physical growth. Dr Mathews discussed the importance of incorporating real world data into clinical trials, including "wearable" devices and automated outcome measures such as the 95 percentile stride velocity.[85,86] Dr Rabi Tawil (University of Rochester, US) reviewed classic FSHD1 features, where most individuals with FSHD1 have between 4–7 D4Z4 repeats and tend to have more moderate disease including asymmetric muscular involvement. Patients with contractions with 8–10 repeats have later onset, milder

disease, and a higher frequency of non-penetrance (*i.e.,* do not develop symptoms). Despite the relationship between disease severity and repeat size, intra-familial variability indicates the likely presence of other genetic factors influencing disease severity.[87] Dr Tawil described the importance of natural history studies in FSHD to determine the most relevant and sensitive outcome measures of change in muscle strength and function, including muscle, serum and imaging biomarkers.[88]

Diagnostic developments in NMD

Dr Jodi Warman-Chardon (The Ottawa Hospital/CHEO, CA) opened the session by highlighting increased use of muscle magnetic resonance imaging (MRI) as an important diagnostic tool for newly identified genetic neuromuscular diseases to help validate pathogenic genetic mutations as well as identify inflammatory myopathy mimics that clinically resemble muscular dystrophies.[89–92] She outlined the increasing use of quantitative MRI technologies as therapeutic and prognostic biomarkers in natural history studies and clinical trials to provide a more accurate detection of progression of fatty replacement and muscle edema.[93–96] She also discussed the international collaborative ventures to coordinate and advance NMD clinical research such as MYO-Share, an online tool to collect de-identified images, and MYO-Guide, which incorporates machine learning algorithm to identify diagnostic patterns of affected muscles.[92,97] Dr Hernan Gonorazky (Sick Kids Hospital, Canada) reviewed the advances in automatic segmentation and machine learning in muscle MRI imaging.[98] Dr Gonorazky also presented findings of diagnostic muscle MRI patterns for specific limb girdle muscular dystrophies.[99] His team identified specific muscle MRI patterns for more common LGMDs, particularly those that are inherited in an autosomal recessive manner. Less common LGMD muscle MRI analysis is difficult due to data paucity and phenotype heterogeneity.[99] He outlined common rare disease imaging challenges, including lack of coherence in the MRI protocols, reliance on lower extremity muscle MRI for diagnosis instead of whole-body MRI and emphasized that more natural history studies are required to more clearly define disease specific progression.[99] Dr Conrad (Chris) Weihl (Washington University, US) discussed the barriers to molecular diagnosis in NMD, including availability of genetic testing and NMD subspecialty care, limitations of rare disease awareness, complexities of genetic test interpretation of variants of unknown significance and restrictions of payer regulation.[100,101] To improve interpretation of genetic variants, Dr Weihl described a new variant pathogenicity assessment method, named deep mutational scanning (DMS), to assess sarcoglycan SGC cell surface localization for all 6340 possible amino acid changes to measure the effects of all possible missense variants in the *SGCB* gene.[102] The variant functional scores were bimodally distributed and perfectly predicted pathogenicity of known variants in *SGCB* associated LGMD R4/2E. In addition, variants with less severe functional scores more often appeared in patients with slower disease progression, implying a relationship between variant function and disease severity, which clearly highlighted the utility of DMS use in clinical interpretation of SGCB variants.[102]

Mitochondrial disorders

Dr Mireille Khacho (uOttawa, CA) reviewed the importance of stem cells in not only development but also in adult muscle homeostasis, although the number of stem cells in muscle appears to decline with age. Metabolism and mitochondrial function are crucial for stem cell function, and a dynamic balance between mitochondrial fission and fusion correlates with the degree of stem cell activity.[103] In muscle stem cells, the elongated structure of mitochondria in quiescent cells becomes fragmented during activation but returns to the elongated state in the committed myoblast. Loss of mitochondrial fusion protein Optic atrophy 1 (OPA1) specifically in satellite cells prevents mitochondrial fragmentation and activates a glutathione (GSH)-redox signaling pathway and G-alert quiescent state.[104] OPA1-deficient satellite cells also show defects in cell cycle progression, myogenic gene expression, and commitment, highlighting the importance of OPA1 in proper muscle stem cell function. Dr Marco Sandri (University of Padova, IT) noted that in skeletal muscle, mitochondrial fission proteins are reduced in pathological conditions such as ageing sarcopenia, cancer cachexia and chemotherapy-induced muscle wasting. For example, muscle-specific loss of the pro-fission dynamin-related protein (DRP) 1 induces muscle wasting and weakness.[105] Similarly, muscle-specific loss of OPA1 ultimately leads to induction of a catabolic program of muscle loss and systemic inflammation and aging.[106,107] Mechanistically, loss of OPA1 leads to alterations in mitochondrial function that ultimately lead to detection of mitochondrial DNA by TLR9 and an induction of inflammatory gene expression, including IL-6.[106] This inflammatory response triggers expression of the bone morphogenetic protein (BMP) inhibitor Noggin in muscle, which blocks the actions of BMPs on muscle fibers and motor nerves, subsequently causing disruption of the NMJ, denervation, and muscle wasting.[108] At least in a mouse model of cancer cachexia, several genes involved in iron metabolism were altered, and iron supplementation alleviated mitochondrial dysfunction and atrophy, suggesting iron deficiency may be a key precipitating event in muscle wasting disorders.[109]

Dr Rita Horvath (University of Cambridge, UK) presented the very diverse phenotypes in mitochondrial diseases. For example, mutations in the mitochondrial gene *TEFM*, which encodes a protein that appears to be involved in transcription elongation of mitochondrial RNA, are

associated with neuromuscular transmission defects similar to a congenital myasthenic syndromes (CMS)-like disease.[110] Mutations in *COQ7*, an key protein in the synthesis of coenzyme Q10 (itself an important electron carrier responsible for shuttling electrons from complex I and complex II to complex III in the inner mitochondrial membrane), present as a motor neuropathy.[111,112] Mutations in *NDUFS6* can lead to a Leigh syndrome-like disorder, with both cognitive and movement deficits.[113] Even patients suffering from inclusion body myopathy can present with mitochondrial dysfunction, in part arising from multiple mitochondrial DNA deletions.[114] The session ended with a short talk by Dr Romane Idoux from the laboratory of Dr Bruno Allard (Institut NeuroMyoGène FR) entitled, "Unraveling the pathophysiology of Bethlem Myopathy using a unique zebrafish model of the disease."

Innovations & transformative technologies in NMD

Dr Michael Benatar (University of Miami, US) discussed the ATLAS study, designed to treat asymptomatic patients that have ALS-associated mutations in superoxide dismutase I (*SOD1*) with tofersen, an FDA-approved antisense oligonucleotide designed to downregulate SOD1 protein levels.[115] Tofersen has already shown efficacy in patients with ALS with pathogenic *SOD1* mutations when therapy was initiated after definitive diagnosis,[32] and it is hoped that earlier intervention prior to symptom onset may lead to even greater efficacy. Mild motor impairment appears to precede clinical manifestation of disease in genetic forms of ALS (*e.g., SOD1, FUS, C9orf72*), and this may also be true of sporadic ALS, an observation that could aid in early diagnosis if more fully understood and characterized,[116] possibly allowing for earlier therapeutic intervention. Dr Dwi Kemaladewi (Children's Hospital of Pittsburgh, US) discussed her work in therapy development for laminin-α2 (*LAMA2*) deficiency. One approach involved use of a deactivated Cas9 gene fused to a transcriptional activator to upregulate expression of LAMA1, which can compensate for loss of LAMA2 in mice.[117] Dr Kemaladewi has improved upon this approach by utilizing a smaller Cas9 gene, which allowed for cloning of the guide RNA within the same vector, and use of a stronger transcriptional activator, thus allowing for enhanced LAMA1 expression from the single-vector system.

Dr Stephan Züchner (University of Miami, US) highlighted innovation in genome sequencing, progressing from Sanger sequencing to next generation sequencing, with an accompanying drop in cost from ~$100 million for the first genome to currently ~$500 per genome. Dr Züchner also noted that the original "whole" genome was actually only about 92% of the genome, but recent efforts have led to creation of the telomere-to-telomere (T-to-T) full genome.[118] He described his work on identifying mutations in the sorbitol dehydrogenase gene (*SORD*), as one of the most frequent causes of recessive hereditary axonal neuropathies.[119] Treatment of a fruit fly model of SORD-deficiency or patient-derived fibroblasts with an aldose reductase inhibitor normalized intracellular sorbitol levels and ameliorated motor and eye phenotypes in the flies. For SORD-deficiency, the road from gene identification to therapy was thus quite rapid. Finally, Dr Züchner spoke to the rise of artificial intelligence in science in general and specifically his development of MAVERICK (a Mendelian Approach to Variant Effect pRedICtion built in Keras), designed to aid in classifying and prioritizing protein-altering single nucleotide variants and indels for potential pathogenicity.[120] Dr Carsten Bönnemann (National Institutes of Health, US), presented two clinical examples of motor neuron disease and pure sensory neuropathy. Mutations in *SPTLC1* usually result in sensory neuropathies, but recently a novel *SPTLC1* mutation was found associated with an ALS-like motor neuropathy.[121] The *SPTLC1* mutation may cause a substrate shift of SPT whereby alanine and glycine are preferred over the canonical serine, resulting in the production of neurotoxic biproducts. Clinical trials are currently underway in patients with mutation in *SPTLC1/2* that give rise to Hereditary Sensory Neuropathy Type 1 (HSAN1), which involves supplementing more serine in the diet (NCT06113055). Dr Bönnemann described a novel sensory neuropathy causing delayed walking, ataxia, dysmetria and pseudoathetosis caused by a mutation in *PIEZO2*. PIEZO2 is a 38-pass transmembrane ion channel involved in proprioceptive reception, specifically detection of stretch sensation and sense of body position.[122]

Future directions in neuromuscular disease

Dr Elizabeth McNally (Northwestern University Feinberg School of Medicine) described her research identifying modifier genes in a mouse model of sarcoglycan deficiency and translating that knowledge to develop novel therapies for other NMDs. A genome wide screen for modifiers of the Sgcg-null mouse identified latent transforming growth factor β (TGFβ) binding protein 4 (LTBP4), an extracellular matrix protein found in muscle that inhibits a precursor form of TGFβ.[123] Dr McNally developed a monoclonal antibody that blocked a protease-sensitive hinge region of LTBP4, thus stabilizing the protein and enhancing its anti-TGFβ function. Administration of the LTBP4-directed antibody to dystrophic mice led to enhanced muscle stability and function, clearly showing the potential of this new therapeutic. Dr McNally also described her work in the Murphy Roths Large mouse strain, which exhibits enhanced healing properties, and indicates the unique properties of the extracellular matrix as contributing to the enhanced muscle regeneration

in this mouse.[124] Prof. Annemieke Aartsma-Rus (Leidin University Medical Center, NL) discussed the 4 different ASO-based therapeutics available for treatment of DMD, which function through inducing exon skipping to remove the mutated region of the DMD primary transcript, thus producing a shorter but functional protein. However, these ASO only result in recovery of 1–5% of wildtype dystrophin levels, so there is certainly room for improvement.[125] One promising approach to enhance muscle uptake is to conjugate a therapeutic ASO to cell-penetrating or homing peptides. Prof. Aartsma-Rus described her ongoing work on use of phage display to identify novel peptides that could be conjugated to ASO to enhance targeting to healthy or dystrophic skeletal or heart muscle.

Prof. Rafael Yanez-Munoz (Royal Holloway University of London, UK) discussed his efforts to improve AAV safety and efficacy, specifically for SMA. He noted that administration of AAV to human patients has resulted in many severe adverse events and even deaths.[126] Rather than re-engineer the AAV capsid, Prof. Yanez-Munoz has worked on optimizing the vector genome, including using codon-optimized versions of the *SMN1* gene.[127] Prof. Yanez-Munoz also discussed a new *in vitro* model of the blood-brain barrier (BBB) that can be used to test vectors for increased translocation across the BBB, and thus hopefully lead to greater delivery and uptake by the CNS.[128] Finally, he discussed his work on *in utero* gene therapy demonstrating that non-integrating lentivirus vectors are effective at delivering a therapeutic gene to the spinal cord of embryos,[129] which may have utility for very early treatment of patients with SMA.

Finally, two of Canada's leading organizations supporting NMD research and patient care, Muscular Dystrophy Canada (MDC) and Amyotrophic Lateral Sclerosis Society of Canada (ALS Canada), provided compelling, high-level overviews of their impact in advocacy and support for research in NMD. Stacey Lintern, Chief Executive Officer MDC, and Dr Homira Osman, Vice President, Research and Public Policy MDC, discussed MDC's mission to "enhance the lives of those affected by NMDs by continually working to provide ongoing support and resources while relentlessly searching for a cure through well-funded research". Dr David Taylor, Vice President, Research & Strategic Partnerships, ALS Canada, shared the mission of ALS Canada, to "work with the ALS community to improve the lives of people affected by ALS through support, advocacy and investment in research for a future without ALS." In addition to providing tremendous support for those affected by NMD, both MDC and ALS Canada are major supporters of basic, translational, and clinical research in NMD in Canada, as well as providing competitive scholarships and fellowships to help train the next generation of NMD researchers and clinicians. The NMD research and clinical communities in Canada are greatly indebted to these tremendous organizations.

Conclusion

The Ottawa NMD series of conferences continues to be an important forum to review advances in basic, translational, and clinical research and clinical care in NMD, and to celebrate the successes and discuss the challenges in the NMD field. Plans are now well underway for the Ottawa NMD 2025 conference, scheduled for September 11–13, 2025.

Acknowledgements

The conference was co-organized by Drs. Robin Parks (Ottawa Hospital Research Institute (OHRI), CA), Jodi Warman-Chardon (The Ottawa Hospital (TOH)/Children Hospital of Eastern Ontario, CA), Bernard Jasmin (University of Ottawa (uOttawa), CA), and Rashmi Kothary (OHRI, CA). The session moderators included CNMD clinicians and scientists including: Drs. Robin Parks, Jodi Warman-Chardon, Rashmi Kothary, Bernard Jasmin, Ari Breiner, Aymeric Ravel-Chapuis, Hanns Lochmuller, Maxime Rousseaux, Beth Pringle, Hugh McMillan, Jocelyn Zwicker, Mary-Ellen Harper, Pierre Bourque, and Nadine Wiper-Bergeron.

As with the prior five Ottawa NMD conferences, we are extremely indebted to Ms. Heidi Hickey (OHRI) for her attention to detail and tireless efforts to ensure that the conference was efficiently organized. We also thank Michael Hendley (OHRI) and his team for preparing and managing our online website and registration system. Finally, we sincerely thank all our speakers, moderators, and attendees for their enthusiastic participation in Ottawa NMD 2023.

The Conference was kindly supported by unrestricted educational funds provided by the following: Gold Sponsors – Ottawa Hospital Research Institute, University of Ottawa (Faculty of Medicine, Brain and Mind Research Institute, Office of the Vice President - Research and Innovation), Canadian Institutes of Health Research (Institutes of Genetics), Argenex, Amylyx, Medunik Canada, PepGen, Pfizer, Sanofi, Sarepta Therapeutics, Roche, Novartis.

Silver Sponsors – Alnylam Pharmaceuticals, Biogen, Mitsubishi Tanabe Pharma Canada.

Bronze Sponsors and Conference Supporters - Aurora Scientific, Muscular Dystrophy Canada, Amyotrophic Lateral Sclerosis Society of Canada.

Publication of the Ottawa NMD 2023 meeting report is sponsored by the Journal of Neuromuscular Diseases and IOS Press.

Statements and declarations

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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