

Meeting Report

Report on the 4th Ottawa International Conference on Neuromuscular Disease and Biology – September 5–7, 2017, Ottawa, Canada¹

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INTRODUCTION

Neuromuscular diseases (NMDs) represent a heterogeneous group of acquired and inherited disorders. Although it is difficult to get an accurate figure for the prevalence of NMD, conservative estimates based on more common forms of these diseases ranges from 1 in every 625 to 3500 individuals [1, 2], or about 2- to 11-million people worldwide. Many of these diseases cause progressive muscle weakness, motor impairment, sensory loss, disability and premature death. Although many NMDs have no cure or effective treatment, recent years have seen exciting progress in diagnostics, understanding disease pathophysiology, and development of potential

new therapies. For example, clinical implementation of revolutionary advances in genetic diagnosis, such as next generation sequencing, have accelerated the discovery of new disease-causing genes [3], and the first therapy to treat spinal muscular atrophy (SMA), called Spinraza, was approved recently by the United States Food and Drug Administration [4]. Such progress clearly highlights that we are entering an era of new, impactful discoveries thereby providing much hope for the future of those impacted by NMDs.

Many of these fundamental and clinical advances have emerged due to strong connections and collaborations between basic science researchers and their clinical counterparts. Basic researchers can delve into the molecular mechanisms of disease pathogenesis in model systems and develop novel approaches to treat the underlying and/or secondary defects. However, insight and therapies can only progress so far in flies and mice, and the clinical environment thus provides the complementary

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and necessary expertise into the human condition to truly move NMD research forward. This bridge between clinicians and basic science researchers must remain strong to ultimately improve patient care and outcomes.

The 4th Ottawa International Conference on Neuromuscular Disease and Biology was held in Ottawa, Canada, September 5–7, 2017. The goal of this conference was to assemble international experts in fundamental science, translational medicine and clinical NMD research. Speakers provided attendees with updates on a wide range of topics related to NMD, 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. Through many socializing/networking opportunities, as well as poster sessions, attendees were provided opportunities to stimulate discussion and develop future research collaborations. Summaries of our previous Ottawa NMD conferences are also available [5, 6].

CONFERENCE STRUCTURE

The Conference held both scientific and clinical sessions emphasizing important research and management discoveries for patients with NMDs. The Conference featured almost 40 internationally recognized session speakers (10 from Canada, 19 from the United States, 8 from Europe, and 1 from Australia), and almost 300 attendees. Speakers were chosen based on important scientific and clinical contributions to their specific NMD field and a recognized ability to engage a broad audience at a high level, regardless of attendee stage of career and background.

The conference was divided into 11 sessions. All sessions were based around a disease type (e.g. Amyotrophic Lateral Sclerosis, Myopathies, etc.). Five of the sessions were attended by all conference delegates, and contained a progression of basic science, translational medicine and clinical research approaches to the disease. In addition, we offered three concurrent breakout sessions to allow more in-depth presentations on recent advances in either basic or clinical NMD research. Almost 100 abstracts were submitted for poster presentation, 7 of which were advanced for a short platform presentation during the main scientific sessions. All posters were available for viewing throughout the entire

conference, with two formal poster presentation sessions. Two networking events provided excellent opportunities for social interactions between attendees.

The Conference was hosted by the University of Ottawa Centre for Neuromuscular Disease (CNMD), which is comprised of over 60 basic scientists and clinicians/clinician researchers and over 150 graduate students, postdoctoral fellows, residents and clinical fellows. CNMD clinicians and researchers hold their primary appointment at either the University of Ottawa (uOttawa), The Ottawa Hospital (TOH), Ottawa Hospital Research Institute (OHRI), Children's Hospital of Eastern Ontario (CHEO), CHEO Research Institute (CHEORI) or the uOttawa Heart Institute (uOHI). All session moderators were members of CNMD.

Unfortunately, just prior to the conference, there was a devastating hurricane across Florida and the southern United States and we had several reserved speakers that were understandably unable to attend the conference (Michael Benatar (University of Miami, USA), Leonard Petrucelli (Mayo Clinic – Florida, USA), Steven Burden (Skirball Institute, USA), Donald Sanders (Duke University, USA), Stephan Zuchner (University of Miami, USA). We were grateful to our speakers that offered to present additional talks on their behalf, Drs. Mary Reilly (University College London, UK) and Gil Wolfe (University of Buffalo, USA).

OPENING DAY AND KEYNOTE SPEAKER

Opening remarks were provided by Drs. Jodi Warman Chardon (TOH, CAN) and Robin Parks (OHRI, CAN), Co-Directors of CNMD and two of the conference organizers, who welcomed the conference speakers and attendees to Ottawa. Dr. Bernard Jasmin, co-organizer of the conference introduced the Keynote Speaker for the Ottawa NMD 2017 meeting, Dr. Eric Hoffman. Dr. Hoffman is a Professor in Pharmaceutical Sciences and Associate Dean for Research at Binghamton University, and is well known for his contributions to the identification of the dystrophin protein (implicated in Duchenne Muscular Dystrophy (DMD)), as well as orphan drug development and therapeutic approaches in NMD. Dr. Hoffman's keynote address, "The DMD gene 30 years on - Where has the journey led us?" reviewed the history of DMD, phenotypic presentations of early muscle

hypertrophy then progressive atrophy and the high mutation rate of the dystrophin gene leading to DMD. Dr. Hoffman discussed the variability of the dystrophin protein quantity in different muscles, which influences disease phenotype and has implications for Becker's muscular dystrophy (BMD) and female carriers of dystrophin mutations. In more recent work, Dr. Hoffman demonstrated that microRNA (miRNA) interact with the expansive dystrophin 3' untranslated region of the mRNA [7]. Expression of these miRNA are influenced by inflammatory signals, such as TNF-alpha, showing a link between inflammation and decreased dystrophin expression, thus further reducing dystrophin expression in patients with DMD. Corticosteroids, which are highly prescribed in patients with DMD, reduce expression of these miRNA [8], suggesting another mechanism for their beneficial effect. These inflammation-induced miRNAs would also reduce the effectiveness of antisense oligonucleotide therapies, since they would tend to downregulate expression of the dystrophin mRNA. Dr. Hoffman's Keynote Address provided an excellent kickoff for the Conference.

SESSION 1: INNOVATIONS IN NEUROMUSCULAR DISEASE AND TRANSFORMATIVE TECHNOLOGIES

Given the recent significant advances in genomics and animal modeling, the opening session was devoted to exciting and upcoming technologies, and was moderated by Dr. Robin Parks (OHRI, CAN) and Dr. Jodi Warman Chardon (TOH, CAN). Dr. Robert Baloh (Cedars-Sinai, USA) opened the session by highlighting issues and opportunities in tissue culture and animal models of human disease, including the use of CRISPR/Cas9 gene editing to develop these models. Dr. Baloh also discussed difficulties with different disease modeling systems used for the C9orf72 ALS/FTD (amyotrophic lateral sclerosis/frontotemporal dementia) disorders, specifically focusing on induced pluripotent stem cells (iPSCs), including clonal variability, inter-individual variability, difficulties in generating true mature cell types, complications due to the use and study of mixed population and general variations in protocols used to convert iPSCs into the desired mature cell types [9, 10]. He also presented on the use of organoids to compensate for lack of cellular maturity [11] and microfluidic organs-on-chips, which can be used

to mimic the blood brain barrier [12]. Dr. Charles Gersbach (Duke University, USA) described challenges in treatment approaches for DMD, including the large size of the full-length gene product, which is difficult to accommodate in conventional gene therapy vectors, and the need to target the resident progenitor cell pool in order to obtain true correction. No effective therapeutic options are currently available for patients with DMD, and there is a dire clinical need for novel approaches to treatment. Dr. Gersbach described his very promising work using the CRISPR/Cas9 system to achieve correction in cell culture and an animal model of DMD [13]. Direct intra-muscular injection of an adeno-associated virus (AAV) encoding CRISPR/Cas9 and appropriate guide RNA in *mdx* mice resulted in 70% of fibers showing dystrophin protein at the sarcolemma, which corresponded to correction in about 2% of the genomic DNA.

Dr. Christian Lorson (University of Missouri, USA) reviewed the genetic cause of SMA (homozygous deletion or mutation in the *SMN1* gene), and the unique opportunity for therapy development due the presence of a highly homologous second copy of the gene in the human genome. Dr. Lorson outlined his work in developing an antisense oligonucleotide-based therapy targeting Element 1 (E1) of the *SMN2* locus [14]. This approach was very effective in a mouse model of SMA, and extended survival, improved weight gain and "time to right". Dr. Lorson also discussed combinatorial treatments for SMA, including SMN-dependent [15] and SMN-independent [16] approaches. Dr. Charlotte Sumner (Johns Hopkins University School of Medicine, USA) gave an overview of the genetics of SMA and the very promising therapeutics currently approved (Nusinersen/Spinraza) or in development (AVSX-101, scAAV9-SMN1). Dr. Sumner highlighted the importance of early treatment. Infants treated at presymptomatic stages had a marked improvement in motor milestones in the NURTURE study [17], with a 23% decrease in death compared to patients from natural history studies, and 8% achieved independent sitting as of 13 months into the study. Although these data are encouraging, there was a large percentage of treated patients that did not reach appropriate motor milestones; 1 year after treatment 17% did not sit up, 36% did not stand, and 44% did not walk at expected ages. Furthermore, no improvement was observed in 49% of treated patients, and 16% had died from advancement of disease. In short, additional, more effective therapies are required.

SESSION 2: CHALLENGES AND OPPORTUNITIES IN AMYOTROPHIC LATERAL SCLEROSIS THERAPY DEVELOPMENT

Session 2 covered new disease discoveries in ALS and was moderated by Dr. Derrick Gibbings (uOttawa, CAN). Dr. Ian MacKenzie (University of British Columbia, CAN) discussed his work on mutations in the stress granule protein TIA1 (T-cell restricted intracellular antigen-1) in ALS and FTD, identified through whole exome sequencing of an affected family [18]. TIA1 is an RNA-binding protein, which promotes translational arrest of mRNAs in environmentally stressed cells through the formation of highly dynamic stress granules. TIA1 mutations also occur in Welander distal myopathy [19]. The phenotype of patients with ALS due to TIA1 mutation included focal or bulbar weakness, or aphasia, average onset age of 50 (one patient was in their 20s), and the majority of the patients were female. The brain pathology of these patients included large round hyaline Lewy body-like inclusions that were positive for TAR DNA-binding protein 43 (TDP-43). TIA1 mutations affected stress granule (SG) dynamics and led to the accumulation of the insoluble TDP-43 positive inclusions. Dr. Michael Strong (Western University, CAN) reviewed the history of ALS and the inclusion of FTD criteria and explained that 50–60% of patients with ALS have at least mild cognitive impairment and 15% have FTD. Dr. Strong also reviewed the pathology underlying brain atrophy, including the observation that many ALS patients with cognitive impairment have abnormal inclusions of tau protein that is hyperphosphorylated on Thr175 [20]. Dr. Strong compared the overlap between chronic traumatic encephalopathy (CTE) and ALS/FTD, which share a common features in the form of pathological phosphorylation of tau protein [21].

Dr. Clotilde Lagier-Tourenne (Massachusetts General Hospital/Harvard University) reviewed the identification of hexanucleotide (GGGGCC) expansion mutations of C9orf72 as causative in ALS [22]. Dr. Lagier-Tourenne noted that sense and antisense RNA foci accumulate in cells harboring C9orf72 mutations, which is depended on the dose and repeat size, and is associated with age-dependent cognitive changes [23]. Dr. Lagier-Tourenne described an isoform-specific ASOs that prevents accumulation of foci in cells from C9orf72 patients, and alleviates the development of behavioural deficits in mice *in vivo*

[24]. Ms. Maneka Chitiprolu, from the laboratory of Dr. Derrick Gibbings (uOttawa, CAN) gave a short talk entitled, “Mechanisms eliminating stress granules by autophagy: implications for ALS”.

SESSION 3A: CLINICAL SESSION - MYASTHENIC SYNDROMES AND CHANNELOPATHIES

Dr. Hugh McMillan (CHEO, CAN) moderated this session. We are gratefully to Dr. Gil Wolfe (University of Buffalo, USA), for presenting on immune modulation as a therapy for myasthenia gravis (MG) and, on relatively short notice, providing a more general overview of MG clinical management that was originally scheduled to be presented by Dr. Sanders. Dr. Wolfe began the session with his overview of MG and highlighted the need for an International Consensus Statement for the Management of MG [25, 26]. Dr. Wolfe outlined the UCLA/RAND Appropriateness Method (RAM) to help classify and provide clinical recommendations for management of MG. Dr. Stephen Cannon (University of California Los Angeles, USA) provided an overview of the clinical spectrum of the non-dystrophic myotonias and periodic paralysis [27]. He highlighted that sodium channel myotonia may be severe especially in the neonatal period with life-threatening stridor and respiratory compromise, and that genetic testing has replaced clinical neurophysiology (CMAP exercise test) as the first line testing. Dr. Cannon reviewed the clinical significance of specific sodium channel gene mutations causing congenital myasthenic syndromes, focusing on Nav1.4 mutations [28]. Dr. Wolfe returned to discuss exciting advances in immune modulation for MG therapy [29]. MG patients with anti-MuSK antibodies represent 5–10% of the patients that are refractory to conventional therapies. Dr. Wolfe outline the improvement of MuSK antibodies-positive patients with the use of the monoclonal antibody therapy Rituximab (CD20 antagonist) in small trials, and that the clinical benefit was sustained long after the last Rituximab treatment [30, 31]. The predictors for favourable response to Retuximab included an age less than 45 with moderate disease and MuSK antibody-positive, although antibody levels were not predictive of response. Dr. Wolfe also highlighted the effectiveness of sub-cutaneous intravenous immunoglobulin (SCIG) for MG, with stable outcomes and improvements in Myasthenia Gravis Foundation of America

(MGFA) clinical class scores. Dr. Hanns Lochmueller (formerly of Newcastle University, UK, recently relocated to CHEORI, CAN) thoroughly reviewed recent advances in genotype-phenotype correlation in congenital myasthenia syndromes [32]. Dr. Lochmueller described diagnostic criteria and outlined over 30 different molecular causes, 90% due to recessive mutation, with mutation in *CHRNE*, *DOK7*, *RASPN* being the most common. Dr. Lochmueller described the influence of founder effects in certain disorders and the importance of identifying subtypes to tailor therapy. He also highlighted the importance of registries to improve clinical and scientific discoveries, including RD Connect, which has a Biomarkers Biobank and Clinical Trial and Natural History subsections [33].

SESSION 3B: BASIC RESEARCH SESSION – RECENT DEVELOPMENTS IN NEURON/NMJ FORMATION

The first basic research session was moderated by Dr. Jocelyn Côté (uOttawa). Dr. Richard Robitaille (Université de Montreal, CAN) opened the session with a presentation of his work on the role of Schwann cells in regulating synaptic competition to refine synaptic connections at the NMJ. Early during development, multiple presynaptic nerve terminals compete for the control of the same synaptic site, with the “loser” pruned back through the action of glial cells. Dr. Robitaille showed that perisynaptic Schwann cells are able to detect synaptic strength through the purinergic receptor system, and directly influence glial cell pruning of the weaker connections [34]. This work has obvious implications for synapse repair in ALS. Dr. Alison Ebert (Medical College of Wisconsin, USA) described studies outlining the intricate interaction of various neuronal cell types using cell culture systems derived from converted iPSC. Dr. Ebert showed that SMA iPSC-derived astrocytes had significantly higher levels of microRNA-146a (miR-146a), which appeared to be released from the cells and could induce motor neuron death *in vitro* [35]. Such findings suggest a new mechanism in astrocyte-mediated SMA pathology.

Dr. Laurent Schaeffer (University of Lyon, FR) presented his work on missense mutations in agrin that lead to congenital myasthenic syndrome [36]. Two specific patient-derived mutations were discussed, both of which produced a protein that was largely insoluble and affected the natural secretion of

the protein. Follow-up studies suggested that agrin may have a presynaptic function at the NMJ. Dr. Justin Boyer, from the laboratory of Dr. Jeffery Molkentin (Cincinnati Children’s, USA), provided a short talk entitled “Erk1/2 signaling is essential to maintain the satellite cell pool and for muscle regeneration.”

SESSION 4A: CLINICAL SESSION: NEUROPATHIES

This session devoted to the clinical presentations and impact of emerging therapies for neuropathies was moderated by Dr. Ari Breiner (TOH, CAN). Dr. Pierre Bourque (TOH, CAN) reviewed the clinical presentations of autoimmune neuropathies, highlighting the clinical classification of the spectrum of Acute Immune Neuropathies/Guillain Barre Syndrome and Chronic Inflammatory Demyelinating Neuropathies (CIDP) as well as their multiple subtypes (i.e. AMAN, acute motor axonal neuropathy; PCB, pharyngeal-cervical-brachial weakness; MMN, multifocal motor neuropathy with conduction blocks; CANOMAD, chronic ataxic neuropathy ophthalmoplegia IgM paraprotein anti disialosyl antibodies; CANDAs, chronic ataxic neuropathy with disialosyl antibodies) and their characteristic antigenic targets [37]. Dr. Bourque succinctly reviewed the evolving nomenclature of neuropathy-related paranodal autoantibodies. He highlighted that treatment recommendations are limited by small sample sizes, however, in the case of CIDP, monthly high doses of oral dexamethasone equivalent to oral prednisone and intravenous immunoglobulin was better than placebo. Dr. Hans Katzenberger (University of Toronto, CAN) presented “Fire and Ice: updates in the assessment and management of acquired neuropathies.” Dr. Katzberg reviewed classic small fibre neuropathy testing, including quantitative sensory thresholds, sympathetic skin responses, laser doppler imaging, intradermal nerve fibre density and cardiac autonomic sensory testing, and discussed the poor performance of proxy tests for small fibre sensory neuropathies [38]. He also highlighted the increased use of nerve ultrasound to assess compressive neuropathies, including for pre- and post-operative evaluations and peripheral nerve trauma. Dr. Katzberg highlighted the importance of lifestyle modifications, in addition to glycemic control, in adults and children to limit diabetic and pre-diabetic neuropathy as well as the appropriate

management (such as Mexiletine) for the common and serious effect of muscle cramps [39].

We thank Dr. Mary Reilly (University College London, UK) for agreeing to expand her talk to include some of the elements that were to be covered by Dr. Stephan Zuchner, who was unable to attend the conference due to the severe weather in Florida. Dr. Reilly outlined the recent increase in gene discovery with the advent of next generation sequencing (NGS) technology. NGS now permits multiple parallel sequencing by either targeted gene panels, protein coding sequences (whole exome sequencing) or the whole genome (whole genome sequencing) [40]. Dr. Reilly reviewed the classification of ‘pure’ neuropathies (i.e. Charcot Marie Tooth Disease, CMT) and discussed that although there are greater than 90 causative CMT genes, more than half of all CMT patients have one of five genetic mutations (*PMP22* duplication 39.5%, *PMP22* point mutation 1.4%, *GJB1* 10.8%, *MFN2* 2.8% and *MPZ* 3.1%) [40]. Dr. Reilly discussed the classification of neuropathies as part of a complex neurological or multisystem disorder, where neuropathy is part of a more complex disease and the diagnosis is more challenging.

SESSION 4B: BASIC RESEARCH SESSION – MUSCLE DEVELOPMENT AND ATROPHY

Dr. David Picketts (OHRI, CAN) chaired the basic research session on muscle development and atrophy. Dr. David Glass (Novartis Institute of Biomedical Research, USA) discussed his work on signaling pathways involved in regulating skeletal muscle mass and function, specifically focusing on the growth differentiation factor (GDF) family of proteins [41]. For example, GDF11 functions similar to myostatin, and acts directly on the muscle to cause a reduction in muscle fibre cross-sectional area, while GDF15 drives a decrease in food intake, inducing essentially anorexia. Dr. Marco Sandri (University of Padova, IT) continued the discussion of cellular pathways that control muscle mass and force generation. Onset of muscle atrophy or cachexia leads to muscle denervation in part due to upregulation of noggin, which directly antagonizes the bone morphogenic protein (BMP) pathway involved in maintaining muscle mass [42]. Dr. Sandri also discussed the importance of optimal mitochondrial function and autophagy in maintaining muscle homeostasis [43].

Dr. Denis Guttridge (Ohio State University, USA) spoke about the role of NF- κ B in DMD pathology. NF- κ B is well known for its role in inflammation, but its expression also inhibits muscle growth [44]. Dr. Guttridge presented his work on the function of NF- κ B in the dystrophic heart, an important area of study since 95% of DMD patients have cardiomyopathy and 25% die from cardiac failure. NF- κ B appears to be involved in recruiting epigenetic regulators (e.g. HDAC1) to the upstream region of many genes, including those involved in calcium handling, thus contributing to the pathological state. Dr. Michael Rudnicki (OHRI, CAN) discussed his work on molecular regulation of muscle stem cell asymmetric division. During cell division, dystrophin-deficient satellite cells fail to form poles properly and undergo mitotic catastrophe, suggesting DMD is not only due to muscle degeneration but also a failure to regenerate properly [45]. Using a high-throughput screen for compounds that affect asymmetric *versus* symmetric satellite cell division, compounds that affect epidermal growth factor receptor (EGFR) signaling were shown to promote symmetric division, and electroporation of an EGF expression plasmid into mouse muscle increased muscle mass. Thus, modulating satellite cell division may be a therapeutic option to increase muscle mass. Dr. Angela Lek from the laboratory of Dr. Lou Kunkel (Boston Children’s Hospital/Harvard University, USA) closed the session with a short talk entitled “Genome-wide CRISPR screen to identify DUX4 modulating pathways.”

AN EVENING AT THE CANADIAN MUSEUM OF HISTORY

A dinner session was convened at the Canadian Museum of History (Gatineau, Canada), with conference co-organizers Drs. Rashmi Kothary (OHRI, CAN) and Bernard Jasmin (uOttawa, CAN) acting as the Masters of Ceremony. Conference delegates were treated to a reception in the Canadian History Hall, a permanent exhibit designed to “explore the journey of a country and its people.” Following the Gala Dinner, which took place in the museum’s Grand Hall, Ms. Danielle Campos provided an entertaining and inspirational talk on her life’s journey (so far) as a person affected by neuromuscular disease. Ms. Campos was a member of the National Canadian Paralympic Swim Team from 1998 to 2006, and won 3 gold, 2 silver, and 2 bronze metals in freestyle and relay

swimming at the 2000 and 2004 Paralympic Games in Australia and Athens, respectively, in addition to many other medals at national and international competitions. She is also a graduate of Fanshawe College (London, ON, CAN) and obtained her Bachelor of Social Work degree from the University of Windsor (Windsor, ON, CAN). Ms. Campos explained that her diagnosis of congenital fibre type disproportion has created some challenges, but her life in sport has provided countless opportunities to travel the world to compete against some of the world's best athletes and meet many exceptional people. A key "take home" message to the basic and clinical NMD researchers in the audience was to keep doing what we do: we provide hope to individuals currently challenged with NMD for a future free of these diseases. It is through our continued efforts and persistence that we will achieve new insight into disease pathogenesis which will ultimately lead to the treatments and cures of tomorrow.

SESSION 5: RECENT ADVANCES IN SPINAL MUSCULAR ATROPHY

The session on SMA was moderated by Dr. Alex MacKenzie (CHEO, CAN). Dr. Melissa Bowerman (University of Oxford, UK) presented her work on circadian rhythm dysregulation in SMA. Dr. Bowerman provided an overview of genes involved in circadian rhythm, including *CLOCK* and *Cry1*, and discussed her studies showing that these genes are dysregulated in a mouse model of SMA at a presymptomatic age [46]. Dr. Bowerman also showed that the *Smn* gene itself displays a diurnal expression pattern, and mouse models of SMA are sensitive to light perturbations. Interestingly, light modulation improves lifespan and weight in a mouse model of SMA. Dr. Rashmi Kothary (OHRI, CAN) discussed his studies suggesting that SMA is more than a disease of motor neurons, and is, in fact, a multi-organ disease [47]. SMN protein is normally expressed at relatively high levels in lymphoid organs, and depletion of SMN protein leads to severe alterations in the thymus and spleen, including a decrease in overall size (for the spleen) and altered histological architecture. Thymocyte development is also altered in the mouse model of SMA, as is cytokine expression. Some of these defects can be corrected when mice are treated by gene therapy (AAV9-SMN).

Dr. Thomas Gillingwater (University of Edinburgh, UK) presented his work on targeting the

ubiquitin pathway as a therapy for SMA. Dr. Gillingwater showed that the ubiquitin pathway is disrupted in SMA, including the Uba1 protein which was reduced to ~40% of wildtype levels in the motor neurons of a mouse model of SMA [48], and gene therapy-mediated restoration of Uba1 rescued a mouse model of the disease [49]. Interestingly, mutations in *UBA1* cause a rare form of SMA known as X-linked SMA (XL-SMA), a disease that is clinically similar to SMA [50]. More recent studies in the Gillingwater lab have focused on discovering targets of *UBA1*, which may represent new therapeutic targets for treating SMA. Dr. Stephen Kolb (The Ohio State University Wexner Medical Center, USA) provided an overview of biomarkers for SMA with successes and challenges. Dr. Kolb also presented results from the NeuroNEXT SMA Infant Biomarker Study [51]. These longitudinal natural history studies are crucial to provide the baseline necessary to evaluate the effectiveness of new therapeutics. Ms. Viviane Tran from the laboratory of Dr. Jean-Francois Côté (Institut de Recherches Cliniques de Montreal, CAN) provided a short talk entitled, "ELMO1/2 proteins regulate myoblast function during skeletal muscle development and regeneration."

SESSION 6: PHENOTYPIC AND MOLECULAR INSIGHTS INTO MYOPATHIES

Session 6 was moderated by Dr. Nadine Wipbergeron (uOttawa, CAN). Dr. Carsten Bonnemann (National Institutes of Health, USA) discussed the evolving genetic and clinical landscape of early onset titinopathy. Although not the largest gene in the human genome, titin does have the largest number of exons (approximately 363) in any single gene, and codes for the largest protein (up to 3700 kDa). Due to its large size, titin presents a challenge for molecular diagnosis, since the gene may contain many missense variants with indeterminate pathogenicity, which may be contained in different titin splicing isoforms. Mutations in titin are also associated with several different diseases, including LGMD 2J [52], recessive centronuclear myopathy [53] and recessive core myopathy with heart disease [54]. This complexity has necessitated the development of four iterative pillars for diagnostic confidence: i) genotypic (mutations present in the DNA); ii) phenotypic (what the disease looks like); iii) histotypic (what the tissue structure looks like); and iv) imaging (gross analysis

of muscle involvement by ultrasound or MRI) [55]. Such an approach is useful for many neuromuscular disorders that are difficult to diagnose.

Dr. Alan Beggs (Harvard Medical School, USA) provided an overview of the genetically and phenotypically heterogeneous congenital myopathies. These disorders are typically non-progressive, and are characterized by primary hypotonia and weakness, as well as muscle pathology (i.e. myotubular, CFTD, multimimicore, nemaline). Dr. Beggs discussed in greater detail nemaline myopathy, mutations for which typically occur in genes encoding thin filaments (e.g. nebulin, actin, tropomyosin) and, as demonstrated by Dr. Beggs' group, Kelch-like family member 41 gene (KLHL41) [56]. Dr. Beggs also discussed his work on developing a gene therapy strategy, which has advanced to human clinical trials, to treat X-linked myotubular myopathy (MTM1) [57].

Dr. Kevin Flanigan (Research Institute of the Nationwide Children's Hospital, USA) focused his talk on the nature of the dystrophin gene and mutations that cause Duchenne and Becker muscular dystrophy. Dystrophin is the largest gene in the human genome, and has several alternative transcription and translational start sites, which can impact development of new therapeutics. The United Dystrophinopathy Project Consortium was created to understand how dystrophin gene variations can impact clinical expression of several dystrophinopathies, including DMD [58]. Dr. Flanigan discussed his work on identifying a novel internal ribosome entry site (IRES) in exon 5 of the dystrophin gene, which can be induced to express a nearly functional but somewhat truncated dystrophin protein, and which may be a new target for therapeutic intervention [59].

Dr. Elizabeth McNally (Northwestern University/Feinberg School of Medicine, USA) discussed her work on characterizing genetic modifiers that act on muscle injury and repair. Dr. McNally focused on the beneficial effects of the glucocorticoid steroids used to treat DMD (e.g. prednisone, deflazacort, eplerenone). These steroids function in part to increase the expression of annexins, such as annexin A6, which accumulate at the site of sarcolemmal damage and assist in repair [60]. Indeed, intermittent steroid administration was shown to be beneficial in two mouse/cell models of muscular dystrophy [61, 62], a benefit that was achieved without the common side-effects of chronic steroid use. The session ended with a short talk by Dr. Dwi Kemaladewi, from

the laboratory of Dr. Ronald Cohn (The Hospital for Sick Children, CAN), entitled "Exon inclusion for the treatment of splice site mutations in merosin-deficient congenital muscular dystrophy."

SESSION 7A: CLINICAL SESSION - ADVANCES IN CLINICAL MYOPATHIES

The clinical session on myopathies was moderated by Dr. Jocelyn Zwicker (TOH, CA). Dr. Ariel Breiner (TOH, CA) opened the session by discussing the fundamental principles in NMD ultrasound [63]. Dr. Breiner outlined ultrasound settings for optimal tissue resolution, which is impacted by depth of field, area of focus, frequency (i.e. high frequencies provide better resolution for superficial structures) and gain (brightness to compensate for attenuation of signal at greater tissue depth). Dr. Breiner emphasized that ultrasound is rapid and painless, provides anatomic rather than physiological data, and can act as a potential biomarker in inflammatory neuropathies/myopathies. Ultrasound can also be used to localize the pattern of affected muscles in myopathies and help direct percutaneous muscle biopsies [64]. Dr. Doris Leung (Kennedy Krieger Institute, USA) presented an update on MRI biomarkers for muscle disease to help characterize disease progression and response to therapy, particularly with inflammatory myopathies, for both clinical assessment and as a research tool. Dr. Leung also demonstrated characteristic specific muscle involvement for certain muscular dystrophies, such as sartorius involvement in *SEPN1*-related congenital muscular dystrophies or 'central cloud' effect in collagen VI myopathies [65]. Dr. Leung outlined her work in imaging in patients with FSHD, emphasizing specific muscle patterns that emerge only when sufficient numbers of patient MRIs are assessed from multiple institutions (i.e. selective semi-membranous involvement) [66, 67].

Dr. Nathalie Streichenberger (Centre Hospitalier de Lyon, FR) presented the "Concepts of muscle biopsy: Diagnosis to organisation of the Lyon Myobank." Dr. Streichenberger outlined the development of the large Lyon NMD biobank with over 6000 muscle samples and emphasized the importance of collecting comprehensive clinical information including motor deficits, genetic analysis, EMG data, and muscle imaging, paired to detailed muscle biopsy data. Dr. Streichenberger outlined the diagnostic benefits of three types of biopsy, including needle muscle biopsy, clamp biopsy, and open surgical biopsy as

well as the importance of proper muscle biopsy sample preparation. Finally, Dr. Bernard Brais (McGill University, CAN) presented his work on an emerging autosomal dominant syndrome of muscle hypertrophy with impressive strength and myalgias pain, known as Strongman syndrome. These patients can be difficult to identify given that they are rarely associated with hyperCKemia and can have normal muscle biopsies or slightly larger type 2 fibres. Dr. Brais outlined his work identifying causative genes for NMD, including in large French Canadian families with Strongman syndrome due to *DCST2* mutations [68].

SESSION 7B: BASIC RESEARCH SESSION – ADVANCES IN MUSCLE STEMS CELLS AND DEVELOPMENT

The moderator for this session was Dr. Jeffery Dilworth (OHRI, CAN). Dr. Joe Chakkalakal (University of Rochester Medical Center, USA) presented his work on the relationship between age-related changes in muscle satellite cells and NMJ decline [69]. In aging muscles, the number of satellite cells decline, and there is also a reduction in satellite cell-derived myonuclei at the aged NMJ. Consistent with a specific role for satellite cells at the NMJ, diphtheria toxin-induced elimination of satellite cells led to a loss of NMJ structure, and expression of Sprouty 1 specifically in satellite cells can counteract the age-related loss of NMJ integrity. Dr. Benedicte Chazaud (Institut National de la Santé et de la Recherche Médicale, FR) presented her work on the role of macrophage subsets in controlling muscle extracellular matrix remodeling and fibrosis. Macrophage play a crucial role during normal muscle regeneration, and appear to have different transcriptional and functional characteristics during different stages of regeneration [70].

Dr. Stanley Froehner (University of Washington Medical School, USA) discussed his work on developing simvastatin as a treatment for DMD [71]. Simvastatin is best known as a cholesterol-lowering drug, but it is also known to reduce inflammation, oxidative stress, and fibrosis, all hallmarks of dystrophic muscle. Administration of simvastatin to a mouse model of DMD led to reduced circulating levels of creatine kinase, decreased evidence of inflammation and fibrosis in the muscle, improved specific force, and improved myocardial performance. Dr. Fabio Rossi (University of British

Columbia, CAN) addressed the question of whether muscle fibrosis was a result or a cause of failed regeneration. During normal muscle regeneration, fibro-adipogenic progenitor (FAP) cells expand and infiltrate the damaged muscle, and progress through a series of transformations from secreting primarily inflammatory cytokines, followed by myokines and finally they appear involved in deposition of extracellular matrix. FAP undergo apoptosis at the end of the regeneration, and a failure to die (as may be the case in chronically damaged tissue) actually leads to their involvement in the formation of fatty and fibrotic deposits in the muscle [72, 73]. Dr. Natasha Chang, from the laboratory of Dr. Michael Rudnicki (OHRI, CAN) provided a short talk entitled, “p38-gamma MAPK regulation of *Carm1* mediates asymmetric cell fate of muscle stem cells.”

SESSION 8: FUTURE DIRECTIONS IN NEUROMUSCULAR DISEASE RESEARCH

The final session of the conference was moderated by Drs. Jodi Warman Chardon (TOH, CAN) and Robin Parks (OHRI, CAN). Dr. Kym Boycott (CHEO, CAN) provided a talk entitled “Approaches to solve the remaining unsolved neuromuscular disorders.” Dr. Boycott discussed the large increase in the identification of new disease-causing genes that resulted from the wide-spread adoption of whole exome sequencing. Creation of the International Rare Diseases Research Consortium (IRDiRC) further accelerated the rate of gene discovery [74]. However, challenges still exist, such as persistent lack of data sharing, insufficient infrastructure to allow sharing of large data sets, and how to bring together groups of basic scientists and clinicians working on the same gene. Fortunately, there are several international initiatives designed to bring the rare disease community together, including IRDiRC, PhenomeCentral and Orphanet. Dr. Boycott also pointed out that there are new approaches to enhance the discovery rate for patients that fail to be diagnosed through whole exome sequencing, such as transcriptome sequencing which can detect certain genome rearrangements (e.g. duplications, deletions and inversions) better than whole exome sequencing [75].

The final speaker of the conference was Dr. Nigel Laing (University of Western Australia, AU), who discussed issues surrounding future best practices in NMD. Dr. Laing presented a study showing that whole exome sequencing was a cost-effective method

to diagnose paediatric muscle diseases, relative to traditional diagnostic approaches [76]. Dr. Laing also pointed out that treating individuals with rare diseases actually costs disproportionately more health care dollars in Australia (and, presumably, many other countries), where 2% of the population suffering with rare disease consumes over 10% of the inpatient hospital costs [77]. Finally, Dr. Laing discussed the idea of preconception carrier screen, which is currently offered in several centers in Australia for cystic fibrosis [78, 79], and could be expanded to encompass many NMD conditions [80]. In conclusion, Dr. Laing stated that preconception screening may become more widely available if the average cost of therapies (e.g. Spinraza) exceeds the cost of screening.

CONCLUDING REMARKS

Closing remarks were provided by Drs. Jodi Warman Chardon (TOH, CAN) and Robin Parks (OHRI, CAN), who, on behalf of the organizers, thanked the attendees, speakers and sponsors. The Ottawa NMD 2017 conference accomplished its primary objective of engaging the attendees with high level scientific and clinical presentations in NMD. We hope the conference inspired new collaborations and new ideas that will lead to new understanding of NMD disease pathogenesis, ultimately enhancing diagnosis and treatment options for patients affected by NMDs.

Save the Date! Planning is already underway for the 5th Ottawa International Conference on Neuromuscular Disease and Biology (Ottawa NMD 2019), which will take place on October 17–19, 2019. Once again, we look forward to exciting and dynamic updates from leading basic and clinical researchers in NMD here in our National Capital.

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REFERENCES

- [1] Emery AE. Population frequencies of inherited neuromuscular diseases—a world survey. *Neuromuscular Disorders*: NMD. 1991;1(1):19-29.
- [2] Deenen JC, Horlings CG, Verschuuren JJ, Verbeek AL, van Engelen BG. The Epidemiology of Neuromuscular Disorders: A Comprehensive Overview of the Literature. *Journal of Neuromuscular Diseases*. 2015;2(1):73-85.
- [3] Warman Chardon J, Beaulieu C, Hartley T, Boycott KM, Dymont DA. Axons to Exons: The Molecular Diagnosis of Rare Neurological Diseases by Next-Generation Sequencing. *Current Neurology and Neuroscience Reports*. 2015;15(9):64.
- [4] AJMG, First drug to treat spinal muscular atrophy gets FDA approval: Costly injectable shown to stall debilitating muscle-wasting effects of rare genetic disorder. *American Journal of Medical Genetics. Part A*. 2017;173(4):837-8.
- [5] Chardon JW, Jasmin BJ, Kothary R, Parks RJ. Report on the 3rd Ottawa International Conference on Neuromuscular Biology, Disease and Therapy - September 24-26, 2015, Ottawa, Canada. *Journal of Neuromuscular Diseases*. 2016;3(3):431-42.
- [6] Ferrier A, Ryan SD, McNally EM, Kothary R. New Directions in Biology and Disease of Skeletal Muscle, Meeting Report, 5–8 May 2010, Ottawa, Canada. *Neuromuscular Disorders*. 2011;21:157-9.
- [7] Fiorillo AA, Heier CR, Novak JS, Tully CB, Brown KJ, Uaesoontrachoon K, Vila MC, Ngheim PP, Bello L, Kornegay JN, Angelini C, Partridge TA, Nagaraju K, Hoffman EP. TNF-alpha-Induced microRNAs Control Dystrophin Expression in Becker Muscular Dystrophy. *Cell Reports*. 2015;12(10):1678-90.
- [8] Fiorillo AA, Tully CB, Damsker JM, Nagaraju K, Hoffman EP, Heier CR. Muscle miRNAome shows suppression of chronic inflammatory miRNAs with both prednisone and vamorolone. *Physiological Genomics*. 2018.
- [9] Dolmetsch R, Geschwind DH. The human brain in a dish: The promise of iPSC-derived neurons. *Cell*. 2011;145(6):831-4.
- [10] Sareen D, O'Rourke JG, Meera P, Muhammad AK, Grant S, Simpkinson M, Bell S, Carmona S, Ornelas L, Sahabian A, Gendron T, Petrucelli L, Baughn M, Ravits J, Harms

- MB, Rigo F, Bennett CF, Otis TS, Svendsen CN, Baloh RH. Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a C9ORF72 repeat expansion. *Science Translational Medicine*. 2013;5(208):208ra149.
- [11] Espuny-Camacho I, Michelsen KA, Gall D, Linaro D, Hasche A, Bonnefont J, Bali C, Orduz D, Bilheu A, Herpoel A, Lambert N, Gaspard N, Peron S, Schiffmann SN, Giugliano M, Gaillard A, Vanderhaeghen P. Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits in vivo. *Neuron*. 2013;77(3):440-56.
- [12] Ingber DE. Reverse Engineering Human Pathophysiology with Organs-on-Chips. *Cell*. 2016;164(6):1105-9.
- [13] Nelson CE, Hakim CH, Ousterout DG, Thakore PI, Moreb EA, Castellanos Rivera RM, Madhavan S, Pan X, Ran FA, Yan WX, Asokan A, Zhang F, Duan D, Gersbach CA. In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. *Science*. 2016;351(6271):403-7.
- [14] Osman EY, Miller MR, Robbins KL, Lombardi AM, Atkinson AK, Brehm AJ, Lorson CL. Morpholino antisense oligonucleotides targeting intronic repressor Element1 improve phenotype in SMA mouse models. *Human Molecular Genetics*. 2014;23(18):4832-45.
- [15] Osman EY, Washington CW, Simon ME, Megiddo D, Greif H, Lorson CL. Analysis of Azithromycin Monohydrate as a Single or a Combinatorial Therapy in a Mouse Model of Severe Spinal Muscular Atrophy. *Journal of Neuromuscular Diseases*. 2017;4(3):237-49.
- [16] Kaifer KA, Villalon E, Osman EY, Glascock JJ, Arnold LL, Cornelison DD, Lorson CL. Plastin-3 extends survival and reduces severity in mouse models of spinal muscular atrophy. *JCI Insight*. 2017;2(5):e89970.
- [17] ClinicalTrials.gov. A Study of Multiple Doses of Nusinersen (ISIS 396443) Delivered to Infants With Genetically Diagnosed and Presymptomatic Spinal Muscular Atrophy (NURTURE). 2018 June 25, 2018; Available from: <https://clinicaltrials.gov/ct2/show/NCT02386553>.
- [18] Mackenzie IR, Nicholson AM, Sarkar M, Messing J, Purice MD, Pottier C, Annu K, Baker M, Perkerson RB, Kurti A, Matchett BJ, Mittag T, Temirov J, Hsiung GR, Krieger C, Murray ME, Kato M, Fryer JD, Petrucelli L, Zinman L, Weintraub S, Mesulam M, Keith J, Zivkovic SA, Hirsch-Reinshagen V, Roos RP, Zuchner S, Graff-Radford NR, Petersen RC, Caselli RJ, Wszolek ZK, Finger E, Lippa C, Lacomis D, Stewart H, Dickson DW, Kim HJ, Rogaeva E, Bigio E, Boylan KB, Taylor JP, Rademakers R. TIA1 Mutations in Amyotrophic Lateral Sclerosis and Frontotemporal Dementia Promote Phase Separation and Alter Stress Granule Dynamics. *Neuron*. 2017;95(4):808-816 e9.
- [19] Klar J, Sobol M, Melberg A, Mabert K, Ameer A, Johanson AC, Feuk L, Entesarian M, Orlen H, Casar-Borota O, Dahl N. Welander distal myopathy caused by an ancient founder mutation in TIA1 associated with perturbed splicing. *Human Mutation*. 2013;34(4):572-7.
- [20] Yang W, Strong MJ. Widespread neuronal and glial hyperphosphorylated tau deposition in ALS with cognitive impairment. Amyotrophic lateral sclerosis: Official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases. 2012;13(2):178-93.
- [21] Mszczynski AJ, Strong W, Xu K, McKee A, Brown A, Strong MJ. Pathologic Thr(175) tau phosphorylation in CTE and CTE with ALS. *Neurology*. 2018;90(5):e380-e387.
- [22] Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, van Swieten JC, Myllykangas L, Kalimo H, Paetau A, Abramzon Y, Remes AM, Kaganovich A, Scholz SW, Duckworth J, Ding J, Harmer DW, Hernandez DG, Johnson JO, Mok K, Ryten M, Trabzuni D, Guerreiro RJ, Orrell RW, Neal J, Murray A, Pearson J, Jansen IE, Sondervan D, Seelaar H, Blake D, Young K, Halliwell N, Callister JB, Toulson G, Richardson A, Gerhard A, Snowden J, Mann D, Neary D, Nalls MA, Peuralinna T, Jansson L, Isoviita VM, Kaivorinne AL, Holtta-Vuori M, Ikonen E, Sulkava R, Benatar M, Wu J, Chio A, Restagno G, Borghero G, Sabatelli M, Heckerman D, Rogaeva E, Zinman L, Rothstein JD, Sendtner M, Drepper C, Eichler EE, Alkan C, Abdullaev Z, Pack SD, Dutra A, Pak E, Hardy J, Singleton A, Williams NM, Heutink P, Pickering-Brown S, Morris HR, Tienari PJ, Traynor BJ. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTN. *Neuron*. 2011;72(2):257-68.
- [23] Jiang J, Zhu Q, Gendron TF, Saberi S, McAlonis-Downes M, Seelman A, Stauffer JE, Jafar-Nejad P, Drenner K, Schulte D, Chun S, Sun S, Ling SC, Myers B, Engelhardt J, Katz M, Baughn M, Platoshyn O, Marsala M, Watt A, Heyser CJ, Ard MC, De Muynck L, Daugherty LM, Swing DA, Tessarollo L, Jung CJ, Delpoux A, Utzschneider DT, Hedrick SM, de Jong PJ, Edbauer D, Van Damme P, Petrucelli L, Shaw CE, Bennett CF, Da Cruz S, Ravits J, Rigo F, Cleveland DW, Lagier-Tourenne C. Gain of Toxicity from ALS/FTD-Linked Repeat Expansions in C9ORF72 Is Alleviated by Antisense Oligonucleotides Targeting GGGGCC-Containing RNAs. *Neuron*. 2016;90(3):535-50.
- [24] Lagier-Tourenne C, Baughn M, Rigo F, Sun S, Liu P, Li HR, Jiang J, Watt AT, Chun S, Katz M, Qiu J, Sun Y, Ling SC, Zhu Q, Polymenidou M, Drenner K, Artates JW, McAlonis-Downes M, Markmiller S, Hutt KR, Pizzo DP, Cady J, Harms MB, Baloh RH, Vandenberg SR, Yeo GW, Fu XD, Bennett CF, Cleveland DW, Ravits J. Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110(47):E4530-9.
- [25] Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, Kuntz N, Massey JM, Melms A, Murai H, Nicolle M, Palace J, Richman DP, Verschuuren J, Narayanaswami P. International consensus guidance for management of myasthenia gravis: Executive summary. *Neurology*. 2016;87(4):419-25.
- [26] Sanders DB, Wolfe GI, Narayanaswami P. Developing treatment guidelines for myasthenia gravis. *Annals of the New York Academy of Sciences*. 2018;1412(1):95-101.
- [27] Cannon SC. Sodium Channelopathies of Skeletal Muscle. *Handbook of Experimental Pharmacology*. 2018;246:309-30.
- [28] Wu F, Mi W, Fu Y, Struyk A, Cannon SC. Mice with an NaV1.4 sodium channel null allele have latent myasthenia, without susceptibility to periodic paralysis. *Brain: A Journal of Neurology*. 2016;139(Pt 6):1688-99.
- [29] Silvestri NJ, Wolfe GI. Myasthenia gravis. *Seminars in Neurology*. 2012;32(3):215-26.
- [30] Diaz-Manera J, Martinez-Hernandez E, Querol L, Klooster R, Rojas-Garcia R, Suarez-Calvet X, Munoz-Blanco JL, Mazia C, Straasheijm KR, Gallardo E, Juarez C, Verschuuren JJ, Illa I. Long-lasting treatment effect of rituximab in MuSK myasthenia. *Neurology*. 2012;78(3):189-93.
- [31] Tandan R, Hehir MK 2nd, Waheed W, Howard DB. Rituximab treatment of myasthenia gravis: A systematic review. *Muscle & Nerve*. 2017;56(2):185-96.

- [32] McMacken G, Abicht A, Evangelista T, Spendiff S, Lochmuller H. The Increasing Genetic and Phenotypical Diversity of Congenital Myasthenic Syndromes. *Neuropediatrics*. 2017;48(4):294-308.
- [33] Thompson R, Johnston L, Taruscio D, Monaco L, Beroud C, Gut IG, Hansson MG, t Hoen PB, Patrinos GP, Dawkins H, Ensini M, Zatloukal K, Koubi D, Heslop E, Paschall JE, Posada M, Robinson PN, Bushby K, Lochmuller H. RD-Connect: An integrated platform connecting databases, registries, biobanks and clinical bioinformatics for rare disease research. *Journal of General Internal Medicine*. 2014;29(Suppl 3):S780-7.
- [34] Darabid H, Arbour D, Robitaille R. Glial cells decipher synaptic competition at the mammalian neuromuscular junction. *The Journal of neuroscience: The Official Journal of the Society for Neuroscience*. 2013;33(4):1297-313.
- [35] Sison SL, Patitucci TN, Seminary ER, Villalon E, Lorson CL, Ebert AD. Astrocyte-produced miR-146a as a mediator of motor neuron loss in spinal muscular atrophy. *Human Molecular Genetics*. 2017;26(17):3409-20.
- [36] Huze C, Bauche S, Richard P, Chevessier F, Goillot E, Gaudon K, Ben Ammar A, Chaboud A, Grosjean I, Lecuyer HA, Bernard V, Rouche A, Alexandri N, Kuntzer T, Fardeau M, Fournier E, Brancaccio A, Ruegg MA, Koenig J, Eymard B, Schaeffer L, Hantai D. Identification of an agrin mutation that causes congenital myasthenia and affects synapse function. *American Journal of Human Genetics*. 2009;85(2):155-67.
- [37] Bourque PR, Chardon JW, Massie R. Autoimmune peripheral neuropathies. *Clinica Chimica Acta; International Journal of Clinical Chemistry*. 2015;449:37-42.
- [38] Ebadi H, Perkins BA, Katzberg HD, Lovblom LE, Brill V. Evaluation of proxy tests for SFSN: Evidence for mixed small and large fiber dysfunction. *PloS One*. 2012;7(8):e42208.
- [39] Maxwell SK, Kokokyi S, Breiner A, Ebadi H, Brill V, Katzberg HD. Characteristics of muscle cramps in patients with polyneuropathy. *Neuromuscular Disorders: NMD*. 2014;24(8):671-6.
- [40] Rossor AM, Tomaselli PJ, Reilly MM. Recent advances in the genetic neuropathies. *Current Opinion in Neurology*. 2016;29(5):537-48.
- [41] Egerman MA, Glass DJ. Signaling pathways controlling skeletal muscle mass. *Critical Reviews in Biochemistry and Molecular Biology*. 2014;49(1):59-68.
- [42] Sartori R, Sandri M, BMPs and the muscle-bone connection. *Bone*. 2015;80:37-42.
- [43] Romanello V, Sandri M. Mitochondrial Quality Control and Muscle Mass Maintenance. *Frontiers in Physiology*. 2015;6:422.
- [44] Shintaku J, Guttridge DC. Reining in nuclear factor-kappaB in skeletal muscle disorders. *Current Opinion in Clinical Nutrition and Metabolic Care*. 2013;16(3):251-7.
- [45] Dumont NA, Wang YX, von Maltzahn J, Pasut A, Bentzinger CF, Brun CE, Rudnicki MA. Dystrophin expression in muscle stem cells regulates their polarity and asymmetric division. *Nature Medicine*. 2015;21(12):1455-63.
- [46] Walter LM, Deguise MO, Meijboom KE, Betts CA, Ahlskog N, van Westering TLE, Hazell G, McFall E, Kordala A, Hammond SM, Abendroth F, Murray LM, Shorrock HK, Prosdocimo DA, Halder SM, Jain MK, Gillingwater TH, Claus P, Kothary R, Wood MJA, Bowerman M. Interventions Targeting Glucocorticoid-Kruppel-like Factor 15-Branched-Chain Amino Acid Signaling Improve Disease Phenotypes in Spinal Muscular Atrophy Mice. *EBioMedicine*. 2018;31:226-42.
- [47] Nash LA, Burns JK, Chardon JW, Kothary R, Parks RJ. Spinal Muscular Atrophy: More than a Disease of Motor Neurons? *Current Molecular Medicine*. 2016;16(9):779-92.
- [48] Groen EJM, Gillingwater TH. UBA1: At the Crossroads of Ubiquitin Homeostasis and Neurodegeneration. *Trends in Molecular Medicine*. 2015;21(10):622-32.
- [49] Powis RA, Karyka E, Boyd P, Come J, Jones RA, Zheng Y, Szunyogova E, Groen EJ, Hunter G, Thomson D, Wishart TM, Becker CG, Parson SH, Martinat C, Azzouz M, Gillingwater TH. Systemic restoration of UBA1 ameliorates disease in spinal muscular atrophy. *JCI Insight*. 2016;1(11):e87908.
- [50] Wishart TM, Mutsaers CA, Riessland M, Reimer MM, Hunter G, Hannam ML, Eaton SL, Fuller HR, Roche SL, Somers E, Morse R, Young PJ, Lamont DJ, Hammerschmidt M, Joshi A, Hohenstein P, Morris GE, Parson SH, Skehel PA, Becker T, Robinson IM, Becker CG, Wirth B, Gillingwater TH. Dysregulation of ubiquitin homeostasis and beta-catenin signaling promote spinal muscular atrophy. *The Journal of Clinical Investigation*. 2014;124(4):1821-34.
- [51] Kolb SJ, Coffey CS, Yankey JW, Krossschell K, Arnold WD, Rutkove SB, Swoboda KJ, Reyna SP, Sakonju A, Darras BT, Shell R, Kuntz N, Castro D, Parsons J, Connolly AM, Chiriboga CA, McDonald C, Burnette WB, Werner K, Thangarajh M, Shieh PB, Finanger E, Cudkowicz ME, McGovern MM, McNeil DE, Finkel R, Iannaccone ST, Kaye E, Kingsley A, Rensch SR, McGovern VL, Wang X, Zaworski PG, Prior TW, Burghes AHM, Bartlett A, Kissel JT. Natural history of infantile-onset spinal muscular atrophy. *Annals of Neurology*. 2017;82(6):883-91.
- [52] Hackman P, Vihola A, Haravuori H, Marchand S, Sarparanta J, De Seze J, Labeit S, Witt C, Peltonen L, Richard I, Udd B. Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin. *American Journal of Human Genetics*. 2002;71(3):492-500.
- [53] Ceyhan-Birsoy O, Agrawal PB, Hidalgo C, Schmitz-Abe K, DeChene ET, Swanson LC, Soemedi R, Vasli N, Iannaccone ST, Shieh PB, Shur N, Dennison JM, Lawlor MW, Laporte J, Markianos K, Fairbrother WG, Ganzler H, Beggs AH. Recessive truncating titin gene, TTN, mutations presenting as centronuclear myopathy. *Neurology*. 2013;81(14):1205-14.
- [54] Chauveau C, Bonnemann CG, Julien C, Kho AL, Marks H, Talim B, Maury P, Arne-Bes MC, Uro-Coste E, Alexandrovich A, Vihola A, Schafer S, Kaufmann B, Medne L, Hubner N, Foley AR, Santi M, Udd B, Topaloglu H, Moore SA, Gotthardt M, Samuels ME, Gautel M, Ferreira A. Recessive TTN truncating mutations define novel forms of core myopathy with heart disease. *Human Molecular Genetics*. 2014;23(4):980-91.
- [55] Oates EC, Jones KJ, Donkervoort S, Charlton A, Brammah S, Smith JE 3rd, Ware JS, Yau KS, Swanson LC, Whiffin N, Peduto AJ, Bournazos A, Waddell LB, Farrar MA, Sampaio HA, Teoh HL, Lamont PJ, Mowat D, Fitzsimons RB, Corbett AJ, Ryan MM, O'Grady GL, Sandaradura SA, Ghaoui R, Joshi HB, Marshall JL, Nolan MA, Kaur S, Punetha J, Topf A, Harris E, Bakshi M, Genetti CA, Marttila M, Werlauff U, Streichenberger N, Pestronk A, Mazanti I, Pinner JR, Vuillerot C, Grossmann C, Camacho A, Mohassel P, Leach ME, Foley AR, Bharucha-Goebel D,

- Collins J, Connolly AM, Gilbreath HR, Iannaccone ST, Castro D, Cummings BB, Webster RI, Lazaro L, Vissing J, Coppens S, Deconinck N, Luk HM, Thomas NH, Foulds NC, Illingworth MA, Ellard S, McLean CA, Phadke R, Ravenscroft G, Witting N, Hackman P, Richard I, Cooper ST, Kamsteeg EJ, Hoffman EP, Bushby K, Straub V, Udd B, Ferreira A, North KN, Clarke NF, Lek M, Beggs AH, Bonnemann CG, MacArthur DG, Granzier H, Davis MR, Laing NG. Congenital titinopathy: Comprehensive characterisation and pathogenic insights. *Annals of Neurology*. 2018.
- [56] Gupta VA, Ravenscroft G, Shaheen R, Todd EJ, Swanson LC, Shiina M, Ogata K, Hsu C, Clarke NF, Darras BT, Farrar MA, Hashem A, Manton ND, Muntoni F, North KN, Sandaradura SA, Nishino I, Hayashi YK, Sewry CA, Thompson EM, Yau KS, Brownstein CA, Yu TW, Allcock RJ, Davis MR, Wallgren-Pettersson C, Matsumoto N, Alkuraya FS, Laing NG, Beggs AH. Identification of KLHL41 Mutations Implicates BTB-Kelch-Mediated Ubiquitination as an Alternate Pathway to Myofibrillar Disruption in Nemaline Myopathy. *American Journal of Human Genetics*. 2013;93(6):1108-17.
- [57] Childers MK, Joubert R, Poulard K, Moal C, Grange RW, Doering JA, Lawlor MW, Rider BE, Jamet T, Daniele N, Martin S, Riviere C, Soker T, Hammer C, Van Wittenberghe L, Lockard M, Guan X, Goddard M, Mitchell E, Barber J, Williams JK, Mack DL, Furth ME, Vignaud A, Masurier C, Mavilio F, Moullier P, Beggs AH, Buj-Bello A. Gene therapy prolongs survival and restores function in murine and canine models of myotubular myopathy. *Science Translational Medicine*. 2014;6(220):220ra10.
- [58] Flanigan KM, Dunn DM, von Niederhausen A, Soltanzadeh P, Gappmaier E, Howard MT, Sampson JB, Mendell JR, Wall C, King WM, Pestronk A, Florence JM, Connolly AM, Mathews KD, Stephan CM, Laubenthal KS, Wong BL, Morehart PJ, Meyer A, Finkel RS, Bonnemann CG, Medne L, Day JW, Dalton JC, Margolis MK, Hinton VJ, Weiss RB. Mutational spectrum of DMD mutations in dystrophinopathy patients: Application of modern diagnostic techniques to a large cohort. *Human Mutation*. 2009;30(12):1657-66.
- [59] Wein N, Vulin A, Falzarano MS, Szigyarto CA, Maiti B, Findlay A, Heller KN, Uhlen M, Bakthavachalu B, Messina S, Vita G, Passarelli C, Brioscchi S, Bovolenta M, Neri M, Gualandi F, Wilton SD, Rodino-Klapac LR, Yang L, Dunn DM, Schoenberg DR, Weiss RB, Howard MT, Ferlini A, Flanigan KM. Translation from a DMD exon 5 IRES results in a functional dystrophin isoform that attenuates dystrophinopathy in humans and mice. *Nature Medicine*. 2014;20(9):992-1000.
- [60] Demonbreun AR, Quattrocchi M, Barefield DY, Allen MV, Swanson KE, McNally EM. An actin-dependent annexin complex mediates plasma membrane repair in muscle. *The Journal of Cell Biology*. 2016;213(6):705-18.
- [61] Quattrocchi M, Barefield DY, Warner JL, Vo AH, Hadhazy M, Earley JU, Demonbreun AR, McNally EM. Intermittent glucocorticoid steroid dosing enhances muscle repair without eliciting muscle atrophy. *The Journal of Clinical Investigation*. 2017;127(6):2418-32.
- [62] Quattrocchi M, Salamone IM, Page PG, Warner JL, Demonbreun AR, McNally EM. Intermittent Glucocorticoid Dosing Improves Muscle Repair and Function in Mice with Limb-Girdle Muscular Dystrophy. *The American Journal of Pathology*. 2017;187(11):2520-35.
- [63] Katzberg HD, Brill V, Breiner A. Ultrasound in Neuromuscular Disorders. *Journal of Clinical Neurophysiology: Official publication of the American Electroencephalographic Society*. 2016;33(2):80-5.
- [64] Simon NG, Noto YI, Zaidman CM. Skeletal muscle imaging in neuromuscular disease. *Journal of Clinical Neuroscience: Official journal of the Neurosurgical Society of Australasia*. 2016;33:1-10.
- [65] Leung DG. Magnetic resonance imaging patterns of muscle involvement in genetic muscle diseases: A systematic review. *Journal of Neurology*. 2017;264(7):1320-33.
- [66] Leung DG, Carrino JA, Wagner KR, Jacobs MA. Whole-body magnetic resonance imaging evaluation of facioscapulohumeral muscular dystrophy. *Muscle & Nerve*. 2015;52(4):512-20.
- [67] Leung DG, Wang X, Barker PB, Carrino JA, Wagner KR. Multivoxel proton magnetic resonance spectroscopy in facioscapulohumeral muscular dystrophy. *Muscle & Nerve*. 2018;57(6):958-63.
- [68] Laberge AM, Michaud J, Richter A, Lemyre E, Lambert M, Brais B, Mitchell GA. Population history and its impact on medical genetics in Quebec. *Clinical Genetics*. 2005;68(4):287-301.
- [69] Liu W, Klose A, Forman S, Paris ND, Wei-LaPierre L, Cortes-Lopez M, Tan A, Flaherty M, Miura P, Dirksen RT, Chakkalakal JV. Loss of adult skeletal muscle stem cells drives age-related neuromuscular junction degeneration. *eLife*. 2017;6.
- [70] Varga T, Mounier R, Horvath A, Cuvellier S, Dumont F, Poliska S, Ardjoune H, Juban G, Nagy L, Chazaud B. Highly Dynamic Transcriptional Signature of Distinct Macrophage Subsets during Sterile Inflammation, Resolution, and Tissue Repair. *Journal of Immunology*. 2016;196(11):4771-82.
- [71] Whitehead NP, Kim MJ, Bible KL, Adams ME, Froehner SC. A new therapeutic effect of simvastatin revealed by functional improvement in muscular dystrophy. *Proceedings of the National Academy of Sciences of the United States of America*. 2015;112(41):12864-9.
- [72] Joe AW, Yi L, Natarajan A, Le Grand F, So L, Wang J, Rudnicki MA, Rossi FM. Muscle injury activates resident fibro/adipogenic progenitors that facilitate myogenesis. *Nature Cell Biology*. 2010;12(2):153-63.
- [73] Judson RN, Zhang RH, Rossi FM. Tissue-resident mesenchymal stem/progenitor cells in skeletal muscle: Collaborators or saboteurs? *The FEBS Journal*. 2013;280(17):4100-8.
- [74] Boycott KM, Rath A, Chong JX, Hartley T, Alkuraya FS, Baynam G, Brookes AJ, Brudno M, Carracedo A, den Dunnen JT, Dyke SOM, Estivill X, Goldblatt J, Gonthier C, Groft SC, Gut I, Hamosh A, Hieter P, Hohn S, Hurles ME, Kaufmann P, Knoppers BM, Krischer JP, Macek M Jr, Matthijs G, Olry A, Parker S, Paschall J, Philippakis AA, Rehm HL, Robinson PN, Sham PC, Stefanov R, Taruscio D, Unni D, Vanstone MR, Zhang F, Brunner H, Bamshad MJ, Lochmuller H. International Cooperation to Enable the Diagnosis of All Rare Genetic Diseases. *American Journal of Human Genetics*. 2017;100(5):695-705.
- [75] Cummings BB, Marshall JL, Tukiainen T, Lek M, Donkervoort S, Foley AR, Bolduc V, Waddell LB, Sandaradura SA, O'Grady GL, Estrella E, Reddy HM, Zhao F, Weisburd B, Karczewski KJ, O'Donnell-Luria AH, Birnbaum D, Sarkozy A, Hu Y, Gonorazky H, Claeys K, Joshi H, Bournazos A, Oates EC, Ghaoui R, Davis MR, Laing NG, Topf A, Kang PB, Beggs AH, North KN, Straub V, Dowling JJ, Muntoni F, Clarke NF, Cooper ST, Bonnemann CG, MacArthur DG. Improving genetic diagnosis in Mendelian

- disease with transcriptome sequencing. *Science Translational Medicine*. 2017;9(386).
- [76] Schofield D, Alam K, Douglas L, Shrestha R, MacArthur DG, Davis M, Laing NG, Clarke NF, Burns J, Cooper ST, North KN, Sandaradura SA, O'Grady GL. Cost-effectiveness of massively parallel sequencing for diagnosis of paediatric muscle diseases. *NPJ Genomic Medicine*. 2017;2.
- [77] Walker CE, Mahede T, Davis G, Miller LJ, Girschik J, Brameld K, Sun W, Rath A, Ayme S, Zubrick SR, Baynam GS, Molster C, Dawkins HJS, Weeramanthri TS. The collective impact of rare diseases in Western Australia: An estimate using a population-based cohort. *Genetics in medicine: Official Journal of the American College of Medical Genetics*. 2017;19(5):546-52.
- [78] Archibald AD, Massie J, Smith MJ, Dalton DG, du Sart D, Amor DJ. Population-based genetic carrier screening for cystic fibrosis in Victoria. *The Medical Journal of Australia*. 2014;200(4):205-6.
- [79] Massie J, Ioannou L, Delatycki M. Prenatal and preconception population carrier screening for cystic fibrosis in Australia: Where are we up to? *The Australian & New Zealand Journal of Obstetrics & Gynaecology*. 2014;54(6):503-9.
- [80] Molster CM, Lister K, Metternick-Jones S, Baynam G, Clarke AJ, Straub V, Dawkins HJ, Laing N. Outcomes of an International Workshop on Preconception Expanded Carrier Screening: Some Considerations for Governments. *Frontiers in Public Health*. 2017;5:25.