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Plenaries Sessions
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Abstracts

PLENARY SESSION 01

Theme: *BASIC SCIENCES, MUSCLE AND NERVE DEVELOPMENT*

PL1.1 Modelling Duchenne Dystrophy with embryonic stem cells

Olivier POURQUIE, Strasbourg (France)
Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), CNRS (UMR 7104), Inserm U964, Université de Strasbourg, Illkirch. F-67400, France.

Whereas the in vitro differentiation of certain lineages such as cardiomyocytes or neurons from pluripotent cells is now well mastered, the production of other clinically relevant ones such as skeletal muscle remains notoriously difficult. During embryonic development, skeletal muscles arise from somites, which derive from the presomitic mesoderm (PSM). Based on our understanding of PSM development, we established conditions allowing efficient differentiation of monolayer cultures of mouse embryonic stem (ES) cells into PSM-like cells without introduction of exogenous genetic material or cell sorting. To optimize the differentiation of Embryonic Stem (ES) cells toward the muscle lineage, we used a series of reporter ES cell lines, expressing fluorescent proteins under the control of genes specific for key stages of myogenic development. Our optimized conditions were inferred based on the development of the PSM in vivo and from a microarray series of early developmental stages of this tissue. We next established simple conditions to recapitulate primary and secondary/foetal myogenesis in vitro from these PSM-like cells. Our strategy allowed for the production of contractile fibers from pluripotent cells in vitro with an efficiency comparing well with current cardiomyocytes differentiation protocols. The muscle fibers produced are striated and multinucleated and exhibit post-natal characteristics. They also provide a niche allowing the development of Pax7-positive satellite-like cells. We used these conditions to differentiate ES cells derived from dystrophin-deficient mdx mice. We show that these fibers exhibit a strikingly abnormal organization of the myofibrils accompanied by a dramatic increase in the number of branches. While such a branched phenotype has been reported in vivo in mdx animals or in Duchenne patients, it has been attributed to fusion defects consequent to the cycles of regeneration occurring in dystrophic muscles. Our results rather argue that the defect is intrinsic to the fibers thus challenging current views on the origin of the pathology of Duchenne Muscular Dystrophy.

PLENARY SESSION 01

Theme: *BASIC SCIENCES, MUSCLE AND NERVE DEVELOPMENT*

PL1.2 Regulation of Muscle Satellite Cells

Margaret Buckingham, Paris (France)
Department of Developmental Biology and Stem Cells, CNRS URA 2578, Institut Pasteur, 25–28 Rue du Dr Roux, Paris 75015, France.

Adult skeletal muscle homeostasis and regeneration relies on satellite cells. Unlike muscle progenitor cells in the embryo, most adult satellite cells have activated the myogenic determination gene, Myf5, and thus have acquired muscle identity. However, post-transcriptional mechanisms prevent accumulation of myogenic factors so that satellite cells constitute a reserve cell population which can be mobilized in response to muscle damage. Quiescent satellite cells in their niche on the muscle fibre employ protective strategies against toxins and stress. Once activated, satellite cells proliferate and then begin to differentiate into muscle fibres. This process is accompanied by major metabolic changes, as the differentiating cells progress from a glycolytic to an oxidative state with extensive mitochondrial biogenesis. This leads to increased production of reactive oxygen species (ROS). We show that Pitx transcription factors, also present in the quiescent cell, regulate ROS levels by directly activating genes in the antioxidant pathway. In Pitx2;Pitx3 double conditional mutant mice, ROS levels are abnormally high leading to DNA damage, satellite cell senescence and impaired regeneration. In Pitx3 single mutants, on the other hand, premature differentiation occurs. By manipulating ROS inhibitors in activated satellite cells we show that a moderate

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level of ROS, acting through the p38 kinase pathway, is necessary for the correct timing of the onset of differentiation. Thus the physiological enhancement of ROS production and mitochondrial content is an essential regulator of muscle fibre formation, as well as a response to the rising energy demand of regenerating muscle.

PLENARY SESSION 01

Theme: *BASIC SCIENCES, MUSCLE AND NERVE DEVELOPMENT*

PL1.3 Novel signalling pathways that control muscle mass

Marco SANDRI, Padua (Italie)

Abstract not received

PLENARY SESSION 01

Theme: *BASIC SCIENCES, MUSCLE AND NERVE DEVELOPMENT*

PL1.4 Genetic and epigenetic determinants of myogenesis

Stephen TAPSCOTT, Seattle (Etats-Unis)
Fred Hutchinson Cancer Research Center, Seattle, WA USA

MyoD and NeuroD2 are members of the basic-helix-loop-helix transcription factor family and regulate myogenesis and neurogenesis, respectively. ChIP-seq shows that MyoD and NeuroD2 share similar DNA binding motifs. Both MyoD and NeuroD2 bind to a CAGCTG E-box, whereas the CAGATG E-box is preferred by NeuroD2 and the CAGGTG E-box is preferred by MyoD. To some extent, the specific E-box sequence determines the transcriptional program of NeuroD2 and MyoD. The NeuroD2-specific CAGATG E-box binding site mediates is more highly associated with NeuroD2 regulated genes and has stronger enhancer function in reporter constructs in response to NeuroD2. Therefore, the DNA encoded sequence of the E-box is a genetic determinant of whether a gene will be activated by NeuroD2. However, the specific E-boxes bound by each factor is largely determined by the site accessibility in the chromatin, which varies depending on cell lineage. Therefore, the distinct transcriptional programs of MyoD and NeuroD2 are established by a subset of factor-specific binding motifs and the lineage-determined chromatin context of the cell. Chimeras between MyoD and NeuroD identify the specific molecular attributes that determine neurogenesis and myogenesis.

PLENARY SESSION 02

Theme: *GENETICS IN NEUROMUSCULAR DISEASES*

PL 2.1 Genetics of dystroglycanopathies: new advances in dystroglycan post-translational processing

Kevin CAMPBELL, Iowa City (Etats-Unis)
Howard Hughes Medical Institute, Department of Molecular Physiology and Biophysics, Roy J. and Lucille A. Carver College of Medicine, The University of Iowa, USA

Dystroglycanopathies are muscular dystrophies in which the aberrant post-translational modification of dystroglycan results in loss of an essential link between α-dystroglycan and its laminin-G domain-containing extracullular matrix ligands. Recent genetic data has shown that mutations in at least sixteen genes encoding post-translational enzymes lead to a reduced Xyl-GlcA dissacharide repeat on dystroglycan and cause congenital/limb-girdle muscular dystrophies, which can be accompanied by brain and eye abnormalities. Our previous efforts to understand the molecular mechanism underlying dystroglycan's ability to bind the ECM led to identification of a novel phosphorylated O-mannosyl trisaccharide (N-acetylgalactosamine-β3-N-acetylglucosamine-β4-mannose) on α-dystroglycan, and to the demonstration that addition of this phosphate residue is a prerequisite for formation of the ligand-binding motif. However, the biosynthetic pathway that leads to production of the phosphorylated O-mannosyl glycan—the core saccharide that is extended by LARGE—had not been delineated. We now report that three of the newly identified dystroglycanopathy genes (GTDC2, B3GALNT2, and SGK196) are involved in synthesis of the phosphorylated trisaccharide. We found that GTDC2 is localized at the ER and has a protein O-mannose β1,4-N-acetylglucosaminyltransferase activity, which led us to designate it as POMGNT2. We also demonstrated that GTDC2 and B3GALNT2

can synthesize a GalNAc-β3-GlcNAc-β-terminus at the 4-position of protein O-mannose. The newly identified CMD causative protein—SGK196—phosphorylates the 6-position of O-mannose using ATP, based on which we proposed to designate it as a protein O-mannose kinase (POMK). SGK196 exhibits its phosphorylation activity only when the GalNAc-β3-GlcNAc-β-terminus is linked to the 4-position of O-mannose, indicating that this disaccharide serves as the substrate recognition motif of SGK196. This strict specificity of SGK196 explains why mutations in GTDC2 and B3GALNT2 cause congenital muscular dystrophy even though their product does not directly recognize ECM ligands. We have been also exploring the interaction between the dissachardide repeat added by LARGE (the LARGE-glycan) and its ECM ligands. Using an inducible Large knockdown mouse model we determined that the avidity of α-dystroglycan and its ECM ligands correlates with LARGE-glycan length. Furthermore, muscle formation requires proper LARGE-glycan extension to prevent dystrophy.

PLENARY SESSION 02

Theme: *GENETICS IN NEUROMUSCULAR DISEASES*

PL 2.2 Single gene, gene panel and exome sequencing approaches for neuromuscular diseases

Madhuri HEGDE, Atlanta (Etats-Unis)
Department of Human Genetics, Emory University School of Medicine

Neuromuscular diseases (NMDs) are a group of highly heterogeneous inherited genetic disorders that affect the peripheral nervous system and muscle resulting in gross motor disability. They comprise of over 200 Mendelian disorders all of which are rare individually but have an approximate disease prevalence of 1 in 3,000 altogether. Given the clinical and genetic heterogeneity of NMDs, disease diagnosis is complicated and expensive through traditional biochemical and single gene testing. To improve and expedite molecular diagnosis of NMDs we validated and launched several Next generation sequencing (NGS) based comprehensive panel and sub-panel tests. Comparing the clinical diagnostic yields of single gene (NMD associated) tests with that of the various NMD NGS panel and sub-panel tests NMD comprehensive panel testing has a 4-fold greater diagnostic yield (63%) when compared to single gene testing (15–19%). This presentation will compare clinical utility of targeted NGS panel test with that of whole exome sequencing (WES). Sanger fill-in of low coverage exons, copy number variation (CNV) analysis by comparative genomic hybridization arrays (aCGH) and thorough in-house validation of the assay complements panel testing and allows detection of all types of causative mutations, some of which (about 18%) may be missed by WES.

PLENARY SESSION 02

Theme: *GENETICS IN NEUROMUSCULAR DISEASES*

PL 2.3 Congenital Myopathies: The New Genetic Era

Carsten G. BÖNNEMANN, Bethesda (Etats-Unis)

Abstract not received

PLENARY SESSION 02

Theme: *GENETICS IN NEUROMUSCULAR DISEASES*

PL 2.4 New genes in motor neuron diseases

Wim ROBBERECHT, Leuven (Belgique)
University of Leuven, Belgium

Our understanding of the hereditary causes of motor neuron diseases is advancing rapidly. Here, the impressive progress in unraveling the genetics of amyotrophic lateral sclerosis (ALS) will be critically reviewed. Special attention will be devoted to the heterogeneity of the phenotype of ALS and to the continuum between ALS and frontotemporal lobe degeneration. The role of aberrant RNA processing and failure of proteostasis will be linked to the relevant genetic changes in genes involved in these processes, and novel ways to identify disease causing genes will be discussed.

PLENARY SESSION 03

Theme: *IMMUNE-MEDIATED CONDITIONS IN NEUROMUSCULAR DISEASES*

PL 3.1 Targets of immunotherapy in autoimmune neuromuscular disorders and emerging new therapies

Marinos DALAKAS, Athens / Philadephia (Grèce)
Thomas Jefferson University, Philadelphia USA and University of Athens Medical School, Athens Greece

The main autoimmune neuromuscular disorders (NMD) include the acquired demyelinating neuropathies such as GBS, CIDP and MMN; inflammatory myopathies (polymyositis, necrotizing autoimmune myositis, dermatomyositis and Inclusion Body Myositis), and neuromuscular transmission defects like myasthenia gravis and LEMS. These disorders are currently treated with the following non-specific immunosuppressive or immunomodulating drugs and procedures, applied singly or in combination: Steroids; Azathioprine; Mycophenolate Mofetil; Cyclosporin; Methotrexate; Cyclophosphamide; plasmapheresis; and IVIg. The judicious use of these agents, the means by which they exert their action and the pitfalls or limitations associated with their use will be discussed. Although these agents have had a significant impact in the treatment of autoimmune neuropathies and myopathies, they cause unacceptable side effects after long-term use and they are not always effective in inducing remission, necessitating the need for exploring novel biological agents as future therapeutic options. Advances in biotechnology have promoted the development of a new class of products, mostly in the form of monoclonal antibodies or fusion proteins that offer target-specific immunotherapy. Agents, currently on the market or in ongoing trials, appropriate for target-specific treatment of autoimmune neuromuscular disorders, are directed against the following: 1) T cell Intracellular Signaling Pathways associated with T cell activation, such as monoclonal antibodies against CD52, Interleukin 2-receptor (IL2 R), co-stimulatory molecules or compounds inhibiting Janus tyrosine Kinases JAK1, JAK3; 2) B cells, against key B cell-surface molecules or trophic factors of B cell activation; 3) Complement, against C5, that intercepts the Membranolytic attack complex formation; 4) Cytokines and cytokine receptors, including IL-6, IL-17, the p40 subunit of IL12/1L-23; and 5) Lymphocyte migration molecules. The prospects of applying these biological agents will be discussed in the context of targeting the main immune factors involved in the pathogenesis of each disorder. Their excessive cost and safety profile, as well as the need to establish efficacy with controlled studies, will be highlighted.

PLENARY SESSION 03

Theme: *IMMUNE-MEDIATED CONDITIONS IN NEUROMUSCULAR DISEASES*

PL 3.2 Emerging new concepts in pathogenesis of inflammatory myopathies

Olivier BENVENISTE, Paris (France)
Department of Internal Medicine and Clinical Immunology - Reference Center for Neuromuscular Disorders - AP-HP, UMRS 974, UPMC - INSERM - Pitié-Salpêtrière Hospital, Paris, France

Idiopathic inflammatory myopathies include dermatomyositis, polymyositis, immune-mediated necrotizing myopathy and inclusion body myositis. The myositis-specific antibodies lead to a serologic approach complementary to the clinico-pathological classification, because strong associations of myositis-specific antibodies with clinical features and survival have been documented, determining now myositis subclassifications. The role of these autoantibodies remains elusive, particularly, it is not clear if they are cause or consequence of the myositis. Nevertheless, the striking autoantibody profiles characterizing subsets of myositis patients provide valuable clues to putative antigen triggers and clearly reflect an underlying antigen driven process. Supporting this contention, studies have shown that antibodies targeting histidyltRNA synthetase (anti-Jo-1, encountered during the antisynthetase syndrome) undergo affinity maturation, parallel disease activity, and do not coexist with other "myositis-specific" autoantibodies. Previous work examining human T cell responses in Jo-1 antibody positive myositis patients provides additional evidence that this stereotypical antibody response is driven by underlying antigen-specific T cells. Perhaps most convincing, however, is the combination of muscle and lung inflammation resulting from subcutaneous immunization of various congenic mice with emulsified murine histidyltRNA synthetase that partially replicate the anti-synthetase syndrome in humans. Similarly, antibodies directed against the

signal recognition particle (SRP) that guide polypeptides into the endoplasmic reticulum are associated with severe forms of necrotizing myopathies. We have previously shown that serum titers of anti-SRP auto-Abs closely correlate with CK levels and muscle weakness in patients. We have shown the presence of mouse SRP proteins reactive to human anti-SRP Ab by Western-Blot, immune-fluorescence and immunogold electron microscopy on mouse muscle samples. Furthermore, mice injected with sera or purified IgGs from SRP+ patient showed a significant decrease in muscular strength. This effect is dependant of the complement. These results also provide experimental evidence that injection of anti-SRP Abs may affect muscle function and therefore play a direct role in the pathogenesis of necrotizing autoimmune myopathies.

PLENARY SESSION 03

Theme: *IMMUNE-MEDIATED CONDITIONS IN NEUROMUSCULAR DISEASES*

PL 3.3 Emerging new concepts in pathogenesis of peripheral neuropathy

Hugh WILLISON, Glasgow (Royaume Uni)
College of Medical, Veterinary and Life Sciences, University of Glasgow

Inflammatory changes are widely found in peripheral nerve in a range of acquired and heritable conditions when they play important roles in mediating glial and axonal injury, homeostasis and repair. The term autoimmune neuropathy is used to encompass acute and chronic clinical phenotypes with a varying underlying pathophysiological basis. Research in this area continues to progress rapidly, with the expectation that syndrome biomarkers and new therapies will emerge for use in clinical practice, alongside a deeper understanding of pathophysiology. This presentation will focus on human and animal studies that are providing major new insights into peripheral nerve as an immunologically active organ. Particular attention will be paid to new antibody biomarkers, notably glycolipids and protein components of the nodal complex and to downstream effector pathways, including complement activation. The findings will be illustrated through analysis of human biobanks and animal models. Gaps in knowledge, new concepts and directions for future research will be highlighted.

PLENARY SESSION 03

Theme: *IMMUNE-MEDIATED CONDITIONS IN NEUROMUSCULAR DISEASES*

PL 3.4 Emerging new concepts in pathogenesis of autoimmune channelopathies

Angela VINCENT, Oxford (Royaume Uni)
Emeritus Professor of Neuroimmunology, Nuffield Department of Clinical Neurosciences, University of Oxford, 2012–2017

Over the last 40 years the concept of autoantibodies affecting the number and function of both ligand-gated and voltage-gated ion channels has widened considerably. In the 1970s, myasthenia gravis was shown to be caused by antibodies to acetylcholine receptors (AChRs), and these seminal findings have helped lead the way to the recognition and treatment of other antibody-mediated diseases of the peripheral, autonomic and central nervous systems.

In 2001, antibodies to MuSK were found in some of the myasthenia patients negative for AChR antibodies, and more recently antibodies to LRP4 in a small proportion. MuSK and LRP4 are postsynaptic membrane proteins that are involved in AChR localisation and function. These antibodies are now routinely detected in myasthenia and their pathogenicity is widely accepted.MuSK antibodies are not common in many populations but appear to be present in up to 50% of patients without AChR antibodies in myasthenia in warmer countries. The incidence further south is not yet clear. MuSK antibodies have been shown to passively-transfer disease to mice, but it is still not entirely clear how binding of MuSK antibodies to MuSK leads to neuromuscular transmission failure, although a reduction in AChR numbers at the neuromuscular junction can be found. There may also be presynaptic effects of MuSK antibodies which are not found with AChR antibodies. LRP4 antibodies may act in a similar manner since LRP4 is required for activation of MuSK, but less work has been done on these rare antibodies.

Other antibodies can affect the neuromuscular junction. In the 1990s, antibodies to voltage-gated calcium channels were identified in the Lambert Eaton syndrome, and antibodies to shaker type voltage-gated potassium channels (VGKCs) in acquired neuromyotonia, a condition caused by peripheral nerve hyperexcitatibility that leads to muscle fasciculations,

cramps and pain. Somewhat surprisingly, the VGKC antibodies were also identified in central nervous system disorders, particularly limbic encephalitis (memory loss, sleep disorders and seizures) but this was explained by the fact that the antibodies turned out to be directed at proteins that form part of VGKC complexes in situ (2). The two principal proteins help localise (CASPR2) and modify (LGI1) potassium channel function.

These findings helped to direct more attention to the central nervous system. Neuromyelitis optica is caused by antibodies to the water channel, aquaporin-4 (AQP4) and is a chronic disease that leads to optic nerve and spinal cord inflammation. Other antibodies bind directly to CNS ligand-gated receptors. Antibodies to NMDA receptors (NR1 principally) are found mainly in younger patients, often women and small children, who have a severe encephalopathy with movement disorders. And antibodies to glycine receptors are associated with extreme rigidity and brainstem disturbance which can be life threatening (Carvajal et al in press). Importantly, each of these antibodies bind to extracellular epitopes on the target proteins and there is evidence of their pathogenicity. The conditions, although rare, can now be diagnosed regularly by serological tests and the patients treated with immunotherapies which lead to substantial improvement.

Intriguingly, although rather rare, neuromyelitis optica can present in patients with previous or concurrent myasthenia and myasthenia can also overlap with neuromyotonia and Morvan's syndrome. The ability of the immune system to recognise both peripheral and central ion-channels and receptors or related proteins is proving a challenging but exciting area of clinical neurology. Disclosure: Angela Vincent and the University of Oxford hold patents and receive royalties for antibody tests.

PLENARY SESSION 04

Theme: *THERAPY IN NEUROMUSCULAR DISEASES*

PL 4.1 Loco regional gene therapy for Duchenne Muscular Dystrophy patients

Philippe MOULLIER, Nantes (France)

Abstract not received

PLENARY SESSION 04

Theme: *THERAPY IN NEUROMUSCULAR DISEASES*

PL 4.2 Antisense Oligonucleotide therapies for Duchenne Muscular Dystrophy

Francesco MUNTONI, London (Royaume Uni)
Dubowitz Neuromuscular Centre,
UCL Institute of Child Health & Great Ormond Street Hospital for Children
London, UK

Antisense oligonucleotide (AON) induced exon skipping, also known as splice switching AON, are increasingly being used in Duchenne muscular dystrophy (DMD), where several hundreds of children with deletions eligible to skipping of exons 44; or 45; or 51 or 53 have been studied in phase I, II and III studies.

Two main chemistries are in use at the moment, the 2'O Methyl (2'OMe) AON backbone; and the phosphorodiamidate morpholino (PMO) AON backbone.

Both backbones have been quite extensively studied before in relevant animal models such as the mdx mouse and, the PMO, also in the more severe dystrophic dog models with encouraging results.

These preclinical promising results have been paralleled by similarly encouraging results of the phase I and II studies of both chemistries. More recently however the failure of the only phase III study (in which a 2'OMe AON targeting exon 51 was studied) raised concerns regarding both the rationale for exon skipping induced restoration of the reading frame in DMD, and the possibility that current chemistries may achieve sufficient correction to induce a clinical benefit to DMD patients.

In my presentation I will review both the data from the preclinical model; the efficiency of the current AON to induce dystrophin expression; the biological significance of different levels of dystrophin and the available data from the clinical trials available to date. I will discuss why on the whole the positive data from the current approaches are both encouraging and not the result of chance improvement or "placebo" effect in treated boys. I will also discuss possible reasons for the failed phase III study which will be important to treasure for future study design.

Despite this recent set back, and provided the expectations of what can be achieved with the current chemistries are understood, I remain optimistic that

this approach will demonstrate clinical efficacy in the future. While further chemistry development are likely to be required to refine this approach, I consider the data so far encouraging as examples of addressing- for the first time- the fundamental problem of dystrophin deficiency in DMD boys.

Disclosure. The Author is involved in clinical trials on antisense oligonucleotides in DMD with Prosensa (2' O methyl antisense for skipping exon 45 and exon 53); and as the Chief Investigator, in collaboration with Sarepta Therapeutics, in a European Commission funded study on a morpholino antisense to skip exon 53 (EU grant agreement No. 305370)

PLENARY SESSION 04

Theme: *THERAPY IN NEUROMUSCULAR DISEASES*

PL 4.3 RNA targeted therapies for Myotonic Dystrophy

Charles A THORNTON, Rochester, NY (Etats-Unis)
Department of Neurology, University of Rochester

Much of what we know about RNA toxicity as a mechanism for genetic dominance has come from studies of myotonic dystrophy (DM). The underlying disease process is postulated to involve activation of signalling pathways, aberrant translation of repetitive RNAs, or sequestration of the binding proteins that recognize RNA repeats. Once these disease mechanisms were elucidated, it became possible to identify good targets for therapeutic intervention. Targeted therapies have been developed and tested in animal models, and robust effects have been reported using small molecules, gene therapy vectors, and antisense drugs. Among these, it appears that antisense drugs will be the first targeted treatments to reach clinical trials. This presentation will focus on RNA targeted therapies for DM, in terms of how they were developed, how they can be tested in early phase trials, and what steps may be necessary to optimize their impact on clinical practice.

PLENARY SESSION 04

Theme: *THERAPY IN NEUROMUSCULAR DISEASES*

PL 4.4 Therapy for GNE myopathy (DMRV/hIBM)

Ichizo NISHINO, Tokyo (Japan)

Abstract not received

THEMATIC WORKSHOP 1.1

Theme: *DEVELOPMENT OF NEUROMUSCULAR JUNCTION*

TW 1.1.1 Regulation of muscle gene expression by electrical activity: CtBP mediates repression

Laurent SCHAEFFER, Lyon (France)

Abstract not received

THEMATIC WORKSHOP 1.1

Theme: *DEVELOPMENT OF NEUROMUSCULAR JUNCTION*

TW 1.1.2 Making and Breaking Neuromuscular Synapses

Steven BURDEN, New-York (Etats-Unis)
Molecular Neurobiology Program, Skirball Institute of Biomolecular Medicine, NYU Medical School, N.Y., N.Y. 10016

The formation and maintenance of neuromuscular synapses requires a complex exchange of signals between motor neurons and skeletal muscle fibers leading to the formation of a highly specialized postsynaptic membrane and a highly differentiated nerve terminal. As a consequence, acetylcholine receptors (AChRs) become highly concentrated in the postsynaptic membrane and arranged in perfect register with active zones in the presynaptic nerve terminal, insuring for rapid, robust and reliable synaptic transmission. During development, motor axons approach and recognize muscle that is primed, or prepatterned in the prospective synaptic region. Muscle prepatterning is established by MuSK, a receptor tyrosine kinase, and Lrp4, a member of the LDLR family. Lrp4 associates with MuSK and stimulates MuSK kinase activity, increasing Lrp4 and MuSK expression

and causing the clustering of Lrp4 and MuSK. Once clustered, Lrp4 functions as a direct retrograde signal for presynaptic differentiation, causing motor axons to stop growing and develop specializations required for neurotransmitter release. Nascent synapses are stabilized by neuronal Agrin, which is released by motor nerve terminals and binds to Lrp4, stimulating further association between Lrp4 and MuSK and increasing MuSK kinase activity. Lrp4 thus has a central role in coordinating synaptic differentiation, as Lrp4 not only binds Agrin and stimulates postsynaptic differentiation but also acts in turn as a direct retrograde signal for presynaptic differentiation. Mutations in Agrin, Lrp4 and MuSK, as well as additional genes that function in this signaling pathway, cause congenital myasthenia, and auto-antibodies to Lrp4, MuSK, or AChRs are responsible for myasthenia gravis. I will summarize experiments that have contributed to this model of neuromuscular synapse formation, indicate how this knowledge has provided insight into causes for neuromuscular disease, and describe a therapeutic approach for preserving synapses and treating neuromuscular diseases.

THEMATIC WORKSHOP 1.1

Theme: *DEVELOPMENT OF NEUROMUSCULAR JUNCTION*

TW 1.1.3 Signaling perturbations at the NMJ

David GLASS, Boston (Etats-Unis)

Abstract not received

THEMATIC WORKSHOP 1.2

Theme: *VASCULITIC PERIPHERAL NEUROPATHY*

TW 1.2.1 Classification and pathology of the systemic and non systemic vasculitic neuropathies

Michael P COLLINS, Milwaukee (Etats-Unis)
Medical College of Wisconsin, Milwaukee, WI, USA

Vasculitides are disorders defined by inflammatory destruction of vessel walls. Some are caused by direct infection, but most are autoimmune. Autoimmune vasculitides can be primary or secondary to drugs, infections, cancers, connective tissue diseases and other conditions. When vasculitis affects the PNS, vasculitic neuropathy ensues. Vasculitis nomenclature is a set of names and definitions. Classification systems differentiate patients with vasculitis into cohorts for clinical research. Diagnostic criteria are used to diagnose vasculitis in individual patients. The 1994 Chapel Hill Consensus Conference (CHCC) codified nomenclature for 10 vasculitides. The 2012 CHCC updated these names/definitions and added new vasculitides. Large vessel vasculitides are giant cell arteritis (GCA) and Takayasu arteritis (TAK). Medium vessel vasculitides are polyarteritis nodosa (PAN) and Kawasaki disease (KD). Small vessel vasculitides (SVV) are divided by prevalence of immune deposits. Pauci-immune SVV are ANCA-associated: Granulomatosis with polyangiitis (GPA; formerly Wegener granulomatosis), microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA; formerly Churg Strauss syndrome). Immune complex SVV are anti-glomerular basement membrane disease, cryoglobulinemic vasculitis, IgA vasculitis (formerly Henoch Schönlein purpura), and hypocomplementemic urticarial vasculitis. Variable vessel vasculitides are Behçet disease and Cogan syndrome. Secondary vasculitides are those "associated with systemic disease" or "associated with probable etiology." CHCC2012 also defined single organ vasculitides. Isolated PNS vasculitis is termed nonsystemic vasculitic neuropathy (NSVN). Primary systemic vasculitides commonly associated with neuropathy are EGPA, MPA, and MPA. Secondary vasculitides featuring PNS involvement are HBV-PAN, HCV-cryoglobulinemia, and rheumatoid vasculitis. Neuropathies are infrequent in GCA and rare in TAK, KD, Behçet disease, Cogan syndrome and other immune complex SVV. In 1990, the American College of Rheumatology developed classification criteria for PAN, EGPA, GPA, hypersensitivity vasculitis, IgA vasculitis, GCA and TAK. In 2010, a Peripheral Nerve Society task force classified vasculitic neuropathies into primary systemic, secondary systemic and nonsystemic forms. The latter included NSVN and diabetic radiculoplexus neuropathy. There are no validated diagnostic criteria for primary systemic vasculitides. The Diagnostic and Classification Criteria in Vasculitis study is designed to develop/validate diagnostic criteria and improve/validate classification criteria for MPA, GPA, EGPA, PAN, GCA and TAK. The Peripheral Nerve Society task force derived diagnostic criteria for pathologically definite, pathologi-

cally probable, clinically probable and nonsystemic vasculitic neuropathy.

THEMATIC WORKSHOP 1.2

Theme: *VASCULITIC PERIPHERAL NEUROPATHY*

TW 1.2.2 Clinical approach to vasculitic neuropathies

Alexander FJE VRANCKEN, Utrecht (Pays-Bas)
University Medical Centre Utrecht, The Netherlands

Vasculitic neuropathy presents in ~75% in the context of primary systemic vasculitis or vasculitis secondary to connective tissue disease, infectious disease, malignancy. In nonsystemic vasculitic neuropathy, vasculitis appears limited to the peripheral nervous system.

Except for multi-organ involvement, no clinical or pathological features distinguish systemic from nonsystemic vasculitic neuropathy. Women are more often affected than men (ratio 3:2) and the average onset age is 60. Typically there is a painful multifocal neuropathy with (sub)acute onset of predominantly sensory or sensorimotor deficits. Over time there is progression to generalized polyneuropathy, usually asymmetrical. Cranial nerves can be affected. Occasionally vasculitic neuropathy presents as pure sensory neuropathy, polyradiculoplexoneuropathy, or mimicks Guillain-Barré syndrome.

The differential diagnosis includes diabetes, systemic disease (e.g., sarcoidosis, amyloidosis, malignancy), infection (e.g., leprosy, HIV, hepatitis B & C, Borrelia, CMV), AMSAN variant of Guillain-Barré syndrome. Features such as fever, weight loss, autonomic symptoms, skin changes, musculoskeletal symptoms provide important clues. Because an underlying disease can manifest with the neuropathy, ancillary investigations (blood & urine analysis, imaging) are necessary to search for a cause and evaluate the presence of systemic inflammation and multiorgan involvement.

Nerve conduction studies show axonal neuropathy, often multifocal and more extensive than the clinical deficits. In up to 25% of cases transient 'pseudo' conduction blocks are found, which reflect ongoing Wallerian degeneration and axonal damage from focal ischemic nerve injury due to vasculitis. Electromyography demonstrates a neurogenic pattern, but a myopathic pattern can be seen if there is muscle vasculitis or a disease predisposing to (inflammatory) myopathy.

The gold standard for the diagnosis of vasculitic neuropathy is demonstration of vasculitis in a nerve biopsy. Nerve and/or muscle biopsy sensitivity for vasculitis is between 60–70%. Combined nerve/muscle biopsy (usually sural nerve/gastrocnemius muscle, superficial peroneal nerve/peroneus brevis muscle) can be considered for improved (~15%) yield compared to nerve biopsy alone.

References:
- Gwathmey KG, Burns TM, Collins MP, Dyck PJB. Vasculitic neuropathies. Lancet Neurol 2014;13:67–82.
- Vrancken AFJE, Said G. Vasculitic neuropathy. Handb Clin Neurol 2013;115:463–83.

THEMATIC WORKSHOP 1.2

Theme: *VASCULITIC PERIPHERAL NEUROPATHY*

TW 1.2.3 Treatment of vasculitic neuropathie

Robert DM HADDEN, London (Royaume Uni)
King's College Hospital, London, UK

Any non-immune cause (especially hepatitis B or C infection) should be treated first.

In systemic vasculitis, induction of remission is usually with cyclophosphamide for three months, with intravenous pulses considered safer than daily oral dosing, together with high dose corticosteroids. Cyclophosphamide dose is usually six pulses of 15 mg/kg or 0.6 – 0.7 g/m^2. In milder cases methotrexate is a less toxic alternative. Rituximab is at least as efficacious as cyclphosphamide and probably less toxic but more expensive. Plasma exchange may be used in the most fulminant cases. Intravenous immunoglobulin may be useful for short term benefit in the presence of severe infection.

In non-systemic vasculitic neuropathy, there are no randomised trials of treatment so recommendations are based on case series and on extrapolation from systemic vasculitis. Treatment may be stratified by severity. A few cases improve without treatment. Corticosteroids alone may suffice in many. Rapidly progressive or steroid-refractory cases should additionally receive methotrexate (15 – 25 mg/week) or azathioprine (2.0 – 2.5 mg/kg/d) if of moderate severity, or cyclophosphamide if severe. Pulsed intravenous methylprednisolone is optional for more severe cases.

Monitoring of response is insensitive but usually relies on motor and sensory examination, disability scales, and inflammatory markers; pain alone is an unreliable marker of disease activity.

To prevent relapse, corticosteroids, azathioprine, methotrexate or leflunamide are generally continued for 18–24 months. Any relapse may be treated with corticosteroids usually with cyclophosphamide or rituximab.

Patients may also benefit from treatment of neuropathic pain, optimisation of vascular risk factors, and rehabilitation therapies including orthoses.

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THEMATIC WORKSHOP 1.3

Theme: *SPINAL MUSCULAR ATROPHY*

TW 1.3.1 Development of therapies for SMA preclinical

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Spinal Muscular Atrophy (SMA) is caused by loss of the SMN1 gene and retention of SMN2. The SMN2 gene differs from SMN1 in that it is inefficient at incorporating exon7 due an alteration of a splice modulator this results in less SMN being produced. We have developed mice which model this situation. In SMA mice early induction of SMN results in rescue whereas late induction has a reduced impact. Using Cre drivers and either reducing SMN or restoring high SMN levels, indicates that CNS is the key tissue to target. We have investigated various methods of restoration of SMN. Using scAAV9-SMN delivered systemically or via intracerebral ventricular (ICV) injection remarkable rescue of SMA mice is obtained. EMG recordings the compound muscle action potential (CMAP) and motor unit number estimates with early delivery indicate an almost complete recovery. We have also created a pig model of SMA by using scAAV9-sh RNA to pig SMN. The scAAV9-shRNA is injected intrathecaly into 5 day old piglets which results in efficient transduction of motor neurons. Pigs developed marked weakness and have reduced MUNE and CMAP with clear signs of denervation detected. Introduction of a second scAAV9 expressing human SMN(scAAV9-hSMN) results in rescue of these pigs. If the rescue is performed when the pigs have symptoms then CMAP but not MUNE is increased and the pigs do show an improved phenotype. The scAAV9-SMN has now received IND and phase 1 trials are planned. In addition to AAV we have investigated the use of antisense oligonucleotide of the morpholino chemistry for treatment of SMA. A series of different ASOs have been investigated strong ASOs result in an extension of survival from 14 days to over 100 days with a single ICV injection. The SMN levels and incorporation of SMN exon 7 shows major increases with ASO treatment. The ASO affect shows a slow decay with time. We have examined CMAP and MUNE with both early and late introduction of ASO rescue therapy. In early rescue both CMAP and MUNE showed marked rescue whereas latter only CMAP showed an increase. We have further increased survival by readministration of the ASO into the CNS at 30 days. Thus SMN restoration with gene therapy, drug compounds or antisense oligonucleotides has a major impact when delivered early in SMA. However it becomes important to consider how to improve therapy latter in the course of the disease.

THEMATIC WORKSHOP 1.3

Theme: *SPINAL MUSCULAR ATROPHY*

TW 1.3.2 Dysregulation of RNA processing in spinal muscular atrophy

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At the post-transcriptional level, expression of protein-coding genes is controlled by a series of RNA

regulatory events including nuclear processing of primary transcripts, transport of mature mRNAs to specific cellular compartments, translation and ultimately, turnover. These processes are orchestrated through the dynamic association of mRNAs with RNA binding proteins and ribonucleoprotein (RNP) complexes. Accurate formation of RNPs in vivo is fundamentally important to cellular development and function, and its impairment often leads to human disease. The survival motor neuron (SMN) protein is key to this biological paradigm: SMN is essential for the biogenesis of various RNPs that function in mRNA processing, and genetic mutations leading to ubiquitous SMN deficiency cause the neurodegenerative disease spinal muscular atrophy (SMA). I will discuss the expanding role of SMN in the regulation of gene expression through its multiple functions in RNP assembly and advances in our understanding of how disruption of SMN-dependent RNA processing pathways can cause motor neuron disease.

THEMATIC WORKSHOP 1.3

Theme: *SPINAL MUSCULAR ATROPHY*

TW 1.3.3 Clinical trials and measures in SMA

Eugenio MERCURI, Rome (Italie)

Abstract not received

THEMATIC WORKSHOP 1.4

Theme: *STEM CELLS AND MUSCLE REGENERATION*

TW 1.4.1 Regulation of satellite cell migration manipulating the amoeboid mechanisms for cell movement to promote skeletal muscle regeneration

Ketan PATEL, Reading (Royaume Uni)

Abstract not received

THEMATIC WORKSHOP 1.4

Theme: *STEM CELLS AND MUSCLE REGENERATION*

TW 1.4.2 Eph/ephrin interactions generate fiber type specificity during muscle development, regeneration and reinnervation

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Each of the 200+ muscles found in adult mammals is unique in its size, shape, and location as well as in the functional connections it forms with other organ systems such as nerves and tendons. When the integrity of a muscle is disrupted, skeletal muscle is remarkable not only in its capacity to rapidly and efficiently regenerate lost cell number and mass, but in the extent to which the pre-existing patterns intrinsic to each muscle are recapitulated. One aspect of patterning that is critical for the specific function of any given muscle is its unique proportion and arrangement of fast and slow myofibers to provide the necessary balance of force and endurance. Four distinct muscle fiber types are present in limb muscles, which are generally divided into 'slow' and 'fast' on the basis of which sarcomeric myosin heavy chain (MyHC) isoform they express: Type I/slow (small diameter, slow twitch, fatigue-resistant, oxidative fibers innervated by slow, fatigue-resistant motor neurons) or Type II/fast (large diameter, fast twitch, fatigable, high force generating, glycolytic fibers innervated by fast motor neurons); fast myofibers are further classified as Type IIa, IIx/d, or IIb, in order of progressively 'faster' phenotypes.

Recent data from our group suggests that classical repulsive guidance signaling through specific pairs of Eph receptors and their ephrin ligands could protect slow myofibers from innervation by fast motor neurons during postnatal development or reinnervation after injury, and from fusion with muscle satellite cells originating on or specified to form fast myofibers. These results not only present a mechanistic explanation for the fiber type-specific innervation/reinnervation and myoblast associations that have been noted for ober 50 years, but also have implications for the understanding of the loss of specific mo-

tor units in disease or aging and the design of exogenous satellite cell-based therapies.

THEMATIC WORKSHOP 1.4

Theme: *STEM CELLS AND MUSCLE REGENERATION*

TW 1.4.3 Wnt signaling(s) and skeletal muscle regeneration

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The author does not wish to be published

THEMATIC WORKSHOP 1.5

Theme: *INCLUSION BODY MYOSITIS*

TW 1.5.1 Critical pathogenic mechanisms associated with the muscle-fiber degeneration specific to Sporadic Inclusion-Body Myositis (s-IBM), and their treatment possibilities

Valerie ASKANAS, Los Angeles (Etats-Unis)
USC Neuromuscular Center, Department of Neurology, University of Southern California Keck School of Medicine, Los Angeles, CA, USA

The pathogenesis of s-IBM, the most common and severe muscle disease of older persons, is complex and it involves multidimensional pathways most of which are still unresolved. Although both muscle-fiber degeneration and mononuclear-cell inflammation are characteristic components of the s-IBM pathology, but how each relates to the pathogenesis and which one plays a more important pathogenic role remain unsettled.

In contrast to usually-treatable polymyositis and dermatomyositis, both of which exhibit the same inflammatory-cell pathology as s-IBM, anti-inflammatory treatments do not usually produce benefit in s-IBM. We consider "aging" of the muscle-fiber cellular milieu, and possibly genetic predispositions, the main risk factors leading to a multifactorial pathogenic cascade that causes s-IBM muscle-fiber degeneration, atrophy and eventually death. s-IBM muscle fiber degeneration includes autophagic vacuoles and intra-muscle-fiber accumulations of ubiquitinated multi-protein aggregates, several of which are conformationally-modified and in congophilic β-pleated-sheet configuration (therefore generically called "amyloid"). Conformationally-modified proteins present as congophilic amyloid within the s-IBM multiprotein-aggregates include: amyloid-β (Aβ), mainly composed of the more toxic Aβ42; phosphorylated tau (including conformationally modified tau); and α-synuclein. Aβ42–oligomers, considered the most toxic form of Aβ, are also present in s-IBM muscle fibers. Because accumulation of these intra-muscle fiber proteinaceous aggregates is associated with and presumably caused by protein unfolding/misfolding, s-IBM is considered a "conformational disorder". In s-IBM, abnormal accumulation of the multi-protein aggregates may result as documented by us a) increased transcription of several proteins, b) their abnormal posttrantslational modifications and misfolding, and c) inadequate protein disposal. These phenomena indicate abnormal "myoproteostasis", which, we postulate, is provoked or abetted by an aging intracellular milieu. Very important pathogenically are the impairments of protein disposal due to the inhibition of both the 26S proteasome and autophagy, endoplasmic reticulum stress, and impaired deacetylation. Our most recent studies point to the important abnormalities associated with mitophagy, which might explain previously-demonstrated mitochondrial abnormalities. We have recently demonstrated in one of our in vitro models of s-IBM, based on the inhibition of autophagy, that treatment with sodium phenylbutyrate (NaPB) improved autophagy, eliminated vacuolization, and significantly decreased Aβ42 and its oligomers. Thus NaPB might be considered a potential treatment of s-IBM patients.

THEMATIC WORKSHOP 1.5

Theme: *INCLUSION BODY MYOSITIS*

TW 1.5.2 How the impaired muscle repair and regeneration contribute to the sIBM pathogenesis, and their treatment possibilities

Massimiliano MIRABELLA, Rome (Italie)
Institute of Neurology, Catholic University, Roma, Italy

Treatment of sIBM remains a challenge because of its complex pathogenesis and resistance to immuno-

suppression due to an underlying muscle degenerative cascade. Satellite cells and fibers presenting some regenerative features are often increased in sIBM and show activation of developmental pathways. Despite activation of repairing mechanisms, truly regenerating muscle fibers are scarce and regeneration is insufficient to counterbalance ongoing fibers degeneration.

We have shown age-related abnormalities accounting for decline in muscle repair ability in sIBM myoblasts that have constitutively impaired regenerative capacity and intrinsic property, upon aging in vitro, to accumulate Aβ. We also isolated and functionally characterized pericyte-derived mesoangioblasts (mAbs) from muscle biopsies and showed that mAbs, only in s-IBM muscle, display a myogenic differentiation defect rescued by overexpression of MyoD, envisaging a possible cell-based therapeutic strategy.

Because of a dual component of persisting inflammation and chronic muscle degeneration, in sIBM exists a complex cross-talk between inflammatory cells and skeletal muscle with activation of local progenitors and in situ recruitment of vessel-associated stem cells. Macrophages may either adversely affect stem cell differentiation or promote fiber regeneration by releasing growth factors, neurotrophins and cytokines contributing to recruit local and circulating myogenic precursors. Consequently balance between fiber degeneration and progenitors activation may be critical for the clinical outcome.

TWEAK is a multifunctional cytokine playing an important role in tissue repair. We have shown dysregulation of TWEAK-Fn14 axis in sIBM: this may induce progressive muscle atrophy and reduce differentiation of muscle precursor cells. TWEAK is a representative molecule linking chronic inflammation with defective regeneration and progressive atrophy. Selective targeting of TWEAK might concurrently suppress sIBM degenerative and inflammatory components counteracting muscle atrophy. Actually the aim to restore muscle mass is common to strategies currently investigated in sIBM patients (bimagrumab, follistatin gene transfer, regular exercise programs). Blocking TWEAK activity may improve IBM mAbs myogenic capacity allowing to use autologous cells in regenerative cell therapy and to activate endogenous mAbs in vivo, inducing them to make new regenerating fibers. Identification and modulation of factors selectively dysregulated in sIBM regulating myogenic differentiation of mAbs, is a more handy approach to enhance muscle regeneration compared to transplantation. Further insights into local cues finely regulating muscle regeneration in sIBM will be critical to design strategies specifically targeting them in the disease treatment, alone or combined with stem cells therapy.

THEMATIC WORKSHOP 1.5

Theme: *INCLUSION BODY MYOSITIS*

TW 1.5.3 How do immune-mediated mechanisms contribute to sIBM pathogenesis, and why dysimmune treatments are largely not effective?

Marinos DALAKAS, Athens (Grèce) / Philadephia (USA)

Abstract not received

THEMATIC WORKSHOP 2.1

Theme: *DEVELOPMENT OF MUSCLE LINEAGES (HEART/ SKELETAL)*

TW 2.1.1 Morphogenesis of muscles: a bird'eye view

Christophe MARCELLE, Melbourne (Australie)
EMBL Australia; Australian Regenerative Medicine Institute (ARMI), Monash University

In amniotes, all skeletal muscle is derived from an embryonic structure known as the somite, with the exception of head muscles, that emerge from the paraxial head mesoderm and prechordal mesoderm. Somite differentiation represents a exceptionally varied microcosm. In just a few hours, a wide spectrum of developmental processes such as the mesenchymal to epithelial transformation, and the converse epithelial to mesenchymal transition take place along with cellular differentiation, migration, morphogenesis and fusion. In the chick embryo, recent advances in electroporation of expression vectors, siRNA constructs and tissue specific reporters in defined regions of somites combined with recent advancements in live cell confocal video microscopy, allow these biological phenomena to be readily observed and hypotheses tested extremely rapidly. This unique strength of the avian system has opened the way to increasingly sophisticated experiments that address questions of interest not only to the somite/muscle field, but are also fundamental in biology. Importantly, an ever-growing

body of evidence indicates that morphogenesis and growth of skeletal muscles in birds is identical to that of mammals.

These technologies have allowed to characterize the morphogenic steps that lead to the formation of the primitive muscle in vertebrates and to identify the embryonic origin of adult muscle stem cells, the satellite cells. Moreover, work we have recently performed identified the neural crest as a central player in the early steps of myogenesis, thus challenging textbook belief that this role is played by the neural tube.

THEMATIC WORKSHOP 2.1

Theme: *DEVELOPMENT OF MUSCLE LINEAGES (HEART/ SKELETAL)*

TW 2.1.2 Head muscle origin, development and regeneration

Eldad TZAHOR, Rehovot (Israël)
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The transition between the proliferation and differentiation of progenitor cells is a key step in organogenesis, and alterations in this process can lead to developmental disorders. The fibroblast growth factor (FGF) - extracellular signal-regulated kinase 1/2 (ERK) signaling pathway is one of the most intensively studied signaling mechanisms regulating both proliferation and differentiation. How a single molecule (e.g., ERK) can regulate two opposing cellular outcomes is still a mystery. Using both chick and mouse models we shed light on the mechanism responsible for the switch from proliferation to differentiation implicating ERK subcellular localization. Analysis of Sprouty mutant embryos in the mouse revealed that increased FGF-ERK signaling suppressed head myogenesis. In chick embryos, manipulation of signaling molecules along the FGF-ERK signaling cascade in vitro and in vivo demonstrated that blockage of this pathway accelerated myogenic differentiation, whereas its activation diminished it. We next examined whether the spatial subcellular localization of ERK can act as a switch between proliferation (nuclear ERK) and differentiation (cytoplasmic ERK) of muscle progenitors. A myristoylated peptide that blocks Importin7-mediated ERK nuclear translocation induced robust myogenic differentiation of muscle progenitor/stem cells in both head and trunk. Our findings, corroborated by mathematical modeling, suggest that ERK shuttling between the nucleus and the cytoplasm provides a switch-like transition between proliferation and differentiation of muscle progenitors.

THEMATIC WORKSHOP 2.1

Theme: *DEVELOPMENT OF MUSCLE LINEAGES (HEART/ SKELETAL)*

TW 2.1.3 Regulation of tissue-specific alternative splicing during development of muscle lineage

Thomas BRAUN, Bad Nauheien (Allemagne)

Abstract not received

THEMATIC WORKSHOP 2.2

Theme: *DYSGLOBULINEMIC AND LYMPHOMA-RELATED PERIPHERAL NEUROPATHIES*

TW 2.2.1 Dysglobulinemic neuropathies

Thierry KUNTZER, Lausanne (Suisse)
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Coexistence of neuropathy and paraproteinemia (monoclonal gammopathy) is a common and complex problem seen in clinical practice with patients over 50 years of age and requires the distinction of specific syndromes.

The different neurological groups include the anti-MAG neuropathy, those associated with Castelman's disorder, POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) syndrome, CANOMAD (Chronic ataxic neuropathy with ophthalmoplegia, IgM paraprotein, cold agglutinins, and anti-disialyl antibodies), and the

neuropathies associated with MGUS, multiple myeloma, Waldenström macroglobulinemia and AL amyloidosis with notable differences in the signs and symptoms among the different groups. The clinical courses of these neuropathies are typically chronic and progressive. A precise distinction of the type of haematologic disorder associated (benign or malignant), investigation of other organs manifestations, and assessment of specific markers are mandatory. There are guidelines to clarify when there is a pathogenic link between the monoclonal gammopathy and the neuropathy (Joint Task Force of the EFNS and PNS, 2010; Nobile-Orazio E. Handb Clin Neurol 2013).These steps are important to initiate an appropriate therapy that may include chemotherapy and/or immunosuppressive treatment targeting the neuropathy and the haematological dysfunction.

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THEMATIC WORKSHOP 2.2

Theme: *DYSGLOBULINEMIC AND LYMPHOMA-RELATED PERIPHERAL NEUROPATHIES*

TW 2.2.2 Lymphoma associated neuropathies

Wolfgang GRISOLD, Vienna (Autriche)
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Paraneoplastic: Paraneoplastic neuropathy in lymphoma is rare. Several reports describe uncharacteristic sensorimotor neuropathies, sensory neuronopathies are rare. A subacute motor neuronopathy has been rarely observed.

Demyelinating neuropathies as GBS and CIDP have been described to occur more frequently in non Hodgkin`s lymphoma than in Hodgkin`s disease. Associations with paraproteins have to be excluded. Vasculitic and autonomic neuropathies are also described in individual cases.

Neoplastic: Neoplastic involvement in lymphoma can occur within the CSF space , by involvement of the dura or outside of the CSF space, or both.

The infiltration of either cranial nerves or peripheral nerves has been termed neurolymphomatosis . The distribution may resemble multifocal neuropathies, symmetric neuropathies or affect individual nerves or plexus. Rarely also an intravascular spread of lymphoma affect peripheral nerves.

Toxic: Treatment of patients with lymphoma often involves neurotoxic drugs in particular vinka alkaloids, which are part of several chemotherapies. Other drugs as nelarabine and potentially intrathecal agents as MTX and cytosin arabinoside can be neurotoxic.

Brentuximab bedotin is an antibody drug conjugate which has been reported to cause neuropathies. Other biological antibody based treatments as rituximab do not seem to cause neuropathy.

As the treatment of lymphomas has been very successful in the past years the knowledge of paraneoplastic , neoplastic and neurotoxic effects is important for patient care. In addition also focal radiotherapeutic interventions have to be considered.

References:

Kelly JJ et al. Lymphoma and peripheral neuropathy: A clinical review. Muscle & Nerve, 2005; 31: 301–313.

Giometto B, et al.. Paraneoplastic neurologic syndrome in the PNS Euronetwork database: a European study from 20 centers. Arch Neurol 2010;67:330–335.

Grisariu Set al. Neurolymphomatosis: an International Primary CNS Lymphoma Collaborative Group report. Blood 2010;115:5005–5011.

Briani C, et al. Spectrum of paraneoplastic disease associated with lymphoma. Neurology 2011;76:705–710.

Grisold W, et al. Peripheral neuropathies from chemotherapeutics and targeted agents: diagnosis, treatment, and prevention. Neuro-oncology 2012;14 Suppl 4:iv45-iv54.

Grisold W , et al. Malignant cell infiltration in the peripheral nervous system. Handb Clin Neurol 2013;115:685–712.

THEMATIC WORKSHOP 2.2

Theme: *DYSGLOBULINEMIC AND LYMPHOMA-RELATED PERIPHERAL NEUROPATHIES*

TW 2.2.3 Treatment options in haemopathic-associated neuropathies

Marinos DALAKAS, Athens / Philadephia (Grèce)

Abstract not received

THEMATIC WORKSHOP 2.3

Theme: *PATHOMECHANISMS OF HEREDITARY NEUROPATHIES*

TW 2.3.1 Understanding the pathomechanisms of peripheral nerve amyloid

Mary M REILLY, London (Royaume Uni)
MRC centre for Neuromuscular Diseases, UCL Institute of Neurology, Queen Square, London

The Familial Amyloid Polyneuropathies (FAP) are a heterogeneous group of autosomal dominant disorders originally described by Andrade in Portuguese patients in 1952. FAP is one of a group of conditions called the amyloidoses that are characterised by deposition of misfolded and aggregated fibrillar proteins with abundant beta pleated structure in the extracellular space.

In familial amyloid polyneuropathy (FAP), the commonest fibril protein deposited as amyloid is a variant form of transthyretin (TTR) but FAP can also occur secondary to deposition of apolipoprotein A-1, gelsolin and very rarely beta 2 microglobulin. Understanding the underlying pathomechanisms of FAP is the key to developing effective therapies.

Over 100 mutations have been described in the TTR gene associated with TTR-related FAP. The most common mutation, Val30Met, presents in the second/third decade in Portuguese patients with a high penetrance. Patients typically present with a small-fibre neuropathic syndrome with prominent pain which progresses relatively rapidly. As the disease progresses large fibre involvement becomes apparent with a progressive sensory-motor neuropathy. Autonomic dysfunction, gastrointestinal symptoms and cardiac involvement is common. Other features include vitreous deposits and, less commonly, nephropathy and leptomeningeal involvement. Cachexia is a common late feature of the disease. Mean survival in untreated patients is approximately 10 years from symptom onset.

The neuropathy and other organ involvement in FAP is due to the deposition of TTR as amyloid. Wild type TTR can be deposited as amyloid in senile cardiac amyloidosis and in FAP, the amyloid deposits contain a mixture of both wild type and mutant TTR. The mutations in TTR appear to increase the tendency of mutant TTR to dissociate from the stable tetrameric form into the pro-amyloidogenic monomeric form.

The exact mechanism by which the TTR amyloid deposits cause the neuropathy are not fully understood but the nerve damage probably at least partially arises from the toxicity of the TTR aggregates early in the disease while accumulation of amyloid in the nerves probably contributes to the pathogenesis of the neuropathy in the late disease stages.

The only specific treatment for TTR amyloidosis for many years was orthotopic liver transplantation to remove the source of abnormal TTR production. Patients with the Val30Met mutation and in the early stages of their disease benefit most from liver transplantation. Understanding the pathomechanisms has led to the development recently of other therapies including TTR stabilizers such as diflunisal and Tafamadis (Tafamadis is licensed in some countries), TTR gene silencing strategies such as antisense oligonucleotide therapy and small interfering RNA therapy (both undergoing clinical trials currently) and anti serum amyloid protein (SAP) monoclonal antibodies.

THEMATIC WORKSHOP 2.3

Theme: *PATHOMECHANISMS OF HEREDITARY NEUROPATHIES*

TW 2.3.2 Understanding the pathomechanisms of hereditary sensory and autonomic neuropathies

Ingo KURTH, Jena (Allemagne)
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Axonopathies are neurodegenerative disorders that mainly affect the long processes of neurons [1, 2]. The longest axons can measure up to one meter posing a major challenge for crucial mechanisms such as trafficking, energy utilization, signaling, and cytoskeletal organization. Axonopathies of cortical motoneurons comprise the group of hereditary spastic paraplegias (HSP/SPG), whereas lower motoneurons are typically affected in distal hereditary motor neuropathies (dHMN). Both diseases are characterized by a length-dependent axonal degeneration and are distinct from motor neuron diseases where a combined degeneration of both upper and lower motoneuron cell bodies occurs. Hereditary neuropathies can affect sensory axons and include hereditary motor and sensory neuropathies (CMT/HMSN) and hereditary sensory and autonomic neuropathies (HSAN).

In HSAN sensory loss ranges from local numbness over loss of proprioception to the complete inability to experience pain and affected individuals are highly exposed to injuries and mutilations. Loss of pain perception also causes abnormal mechanical loading in the distal weight-bearing parts of the skeleton. This can finally result in neuropathic arthropathy and spontaneous fractures. Fracture healing can be severely impaired leading to resorption of bones. The disorder is often complicated by osteomyelitis. Autosomal dominant and autosomal recessive forms of the disorder exist and known culprit genes encode proteins crucial for sphingolipid metabolism, neurotrophin action, axonal transport, DNA methylation, and membrane shaping of organelles [3].

Despite remarkable non-allelic genetic heterogeneity of axonopathies, one of the common emerging disease pathways turns out to be an impairment of the structural organization of organelles. The architecture and curvature of organelle membranes depends on different mechanisms including the insertion of integral membrane proteins into lipid bilayers [4–6]. Proteins of the reticulon family are prototypes in bending and stabilization of the membrane curvature of the ER [7]. This class of molecules inserts a wedge-shaped reticulon-domain composed of hydrophobic hairpins into the outer leaflet of the lipid-bilayers to induce membrane bending. Mutations in reticulon-domain proteins have been shown to cause spastic paraplegias, i.e. mutations in RTN2 (reticulon 2) cause SPG12. Moreover, nearly half of the HSP patients carry mutations in SPAST (SPG4), REEP1 (SPG31), and ATL1 (SPG3A), likewise encoding reticulon-domain containing ER-associated proteins [8, 9]. However, defects in membrane-shaping proteins also contribute to HSAN as illustrated by mutations in FAM134B, encoding a reticulon-domain containing protein of the ER and Golgi-compartment, which we found to be causative for autosomal recessive HSAN2 [10]. Recently, we linked mutations in the reticulon-domain containing GTPase ATL3 to sensory neurodegeneration (HSAN1) [11]. Upon GTP binding the cytosolic GTPase domains of ATL-proteins dimerize. Subsequent GTP hydrolysis causes a major conformational change that pulls ATLs in opposing membranes together to facilitate membrane fusion [12–14]. By this mechanism called homotypic fusion ATL-proteins connect tubules of the ER to networks [15–17]. ATL3 is particularly enriched in three-way junctions of the ER where it facilitates the composition of the polygonal ER network. The HSAN-related missense-mutation p.Tyr192Cys in ATL3 affects a residue in the GTPase-domain that is evolutionarily conserved throughout species and among all three human ATL homologs, suggesting that Tyr192 is important for the proper function of the protein. Remarkably, the mutation p.Tyr196Cys in ATL1 has been identified as cause of autosomal-dominant SPG3A with white matter changes and seizures [18]. Tyr196 in ATL1, which is homologous to p.Tyr192 in ATL3, is located at the interface of the GTPase domains and its replacement by cysteine impaired dimerization in vitro. Mutation in Tyr192 of the ATL3 protein results in a disruption of the cellular ER-network as demonstrated by heterologous overexpression of mutant protein.

HSAN disorders show broad phenotypic overlap with the condition of "congenital inability to experience pain (CIP)". Biallelic loss-of-function mutations in SCN9A, encoding sodium ion channel NaV1.7, have been shown to cause an inability to experience pain by impairing the electrical signaling of morphologically intact axons [19]. However, null alleles of SCN9A have been reported in individuals with loss of large sensory fibers, suggesting that neurodegeneration and lack of ion channel activity are not mutually exclusive.

We recently showed that another type of "congenital inability to experience pain" results from a specific missense mutation in SCN11A, which encodes the NaV1.9 voltage-gated sodium channel. This de novo mutation (p.Leu811Pro) was identified in independent individuals with the condition using whole-exome sequencing. SCN11A / NaV1.9 is expressed in nociceptors, specialized sensory nerves that transmit pain signals from the body periphery to the central nervous system. Mice that carry the missense mutation in Scn11a have reduced sensitivity to pain. Knockin-mice showed self-inflicted tissue lesions, likewise recapitulating aspects of the human phenotype. Mutant NaV1.9 channels in sensory neurons of knockin-mice are functional, but display excessive activity at resting voltages and cause sustained depolarization of pain-sensing neurons. This gain-of-function in the basal activity of NaV1.9 leads to progressive inactivation of other sodium and calcium channels, the principal components of the action potential in pain-sensing neurons. A resultant conduction block prevents signal transmission to the brain as supported by aberrant synaptic transmission in the spinal cord of knockin-mice [20]. The findings raise the possibility that manipulating NaV1.9 activity could be a new pathway for treating pain.

Unraveling the genetic architecture of HSAN and associated pain channelopathies will enlighten the mechanistic understanding of sensory function and provide potential insights in translational concepts, which are urgently needed.

[1] Suter, U. & Scherer, S.S. Disease mechanisms in inherited neuropathies. Nat Rev Neurosci 4, 714–26 (2003).
[2] Zuchner, S. & Vance, J.M. Emerging pathways for hereditary axonopathies. J Mol Med (Berl) 83, 935–43 (2005).
[3] Rotthier, A., Baets, J., Timmerman, V. & Janssens, K. Mechanisms of disease in hereditary sensory and autonomic neuropathies. Nature reviews Neurology 8, 73–85 (2012).
[4] Shibata, Y., Hu, J., Kozlov, M. & Rapoport, T. Mechanisms shaping the membranes of cellular organelles. Annual review of cell and developmental biology 25, 329–354 (2009).
[5] Park, S.H. & Blackstone, C. Further assembly required: construction and dynamics of the endoplasmic reticulum network. EMBO Rep 11, 515–21 (2010).
[6] Pendin, D., McNew, J. & Daga, A. Balancing ER dynamics: shaping, bending, severing, and mending membranes. Current opinion in cell biology 23, 435–442 (2011).
[7] Voeltz, G., Prinz, W., Shibata, Y., Rist, J. & Rapoport, T. A class of membrane proteins shaping the tubular endoplasmic reticulum. Cell 124, 573–586 (2006).
[8] Blackstone, C. Cellular pathways of hereditary spastic paraplegia. Annu Rev Neurosci 35, 25–47 (2012).
[9] Blackstone, C., O'Kane, C.J. & Reid, E. Hereditary spastic paraplegias: membrane traffic and the motor pathway. Nat Rev Neurosci 12, 31–42 (2011).
[10] Kurth, I. et al. Mutations in FAM134B, encoding a newly identified Golgi protein, cause severe sensory and autonomic neuropathy. Nature genetics 41, 1179–1181 (2009).
[11] Kornak, U. et al. Sensory neuropathy with bone destruction due to a mutation in the membrane-shaping atlastin GTPase 3. Brain 137, 683–92 (2014).
[12] Bian, X. et al. Structures of the atlastin GTPase provide insight into homotypic fusion of endoplasmic reticulum membranes. Proceedings of the National Academy of Sciences of the United States of America 108, 3976–3981 (2011).
[13] Byrnes, L. & Sondermann, H. Structural basis for the nucleotide-dependent dimerization of the large G protein atlastin-1/SPG3A. Proceedings of the National Academy of Sciences of the United States of America 108, 2216–2221 (2011).
[14] Liu, T. et al. Lipid interaction of the C terminus and association of the transmembrane segments facilitate atlastin-mediated homotypic endoplasmic reticulum fusion. Proceedings of the National Academy of Sciences of the United States of America (2012).
[15] Hu, J. et al. A class of dynamin-like GTPases involved in the generation of the tubular ER network. Cell 138, 549–561 (2009).
[16] Muriel, M.-P. et al. Atlastin-1, the dynamin-like GTPase responsible for spastic paraplegia SPG3A, remodels lipid membranes and may form tubules and vesicles in the endoplasmic reticulum. Journal of neurochemistry 110, 1607–1616 (2009).
[17] Orso, G. et al. Homotypic fusion of ER membranes requires the dynamin-like GTPase atlastin. Nature 460, 978–983 (2009).
[18] McCorquodale, D. et al. Mutation screening of spastin, atlastin, and REEP1 in hereditary spastic paraplegia. Clinical genetics 79, 523–530 (2011).
[19] Cox, J.J. et al. An SCN9A channelopathy causes congenital inability to experience pain. Nature 444, 894–8 (2006).
[20] Leipold, E. et al. A de novo gain-of-function mutation in SCN11A causes loss of pain perception. Nat Genet 45, 1399–404 (2013).

THEMATIC WORKSHOP 2.3

Theme: *PATHOMECHANISMS OF HEREDITARY NEUROPATHIES*

TW 2.3.3 Understanding the pathomechanisms of distal hereditary motor neuropathies

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Distal hereditary motor neuropathies (HMN) are characterized by predominant degeneration of motor neurons and their long axons, resulting in a length-dependent distal muscle weakness and atrophy. Harding & Thomas (1980) have classified distal HMN into seven subtypes based upon age at onset, mode of inheritance and presence of additional clinical features. Genetic studies reported that dominant or recessive

mutations in 17 genes may cause pure distal HMN or more complex forms: PLEKHG5, DCTN1, REEP1, SLC5A7, DNAJB2, FBXO38, GARS, BICD2, SETX, BSCL2, IGHMBP2, TRPV4, VAPB, ATP7A, HSPB1, HSPB3 and HSPB8. These genes code for proteins with various functions ranging from more general to motor neuron specific functions. The most remarkable group of distal HMN genes are those coding for three small heat shock proteins (HSPB1, B3 and B8). Although regulated by stress, they are constitutively expressed and responsible for quality control and protein folding. The HSPBs are not only molecular chaperones but also involved in many essential cellular processes such as apoptosis, autophagy, splicing, cytoskeleton dynamics and neuronal survival. Tubulin came out as the most striking differential interacting protein to mutant HSPB1. This anomalous binding leads to stabilization of the microtubule (MT) network in a MAP-like manner. A transgenic mouse model for mutant HSPB1 confirmed the enhanced interaction of mutant HSPB1 with tubulin and can be treated by HDAC inhibitors, demonstrating the potential for treating distal HMN. The wild type HSPB1 protein seems to regulate the balance between centrosomal and non-centrosomal MTs. Mutations in HSPB1 have also shown to disrupt the neurofilament (NF) network and cause their aggregation. Neurofilaments are abundant structural proteins in neurons and play a key role in neurodegeneration. Transduction of neuronal cells with mutant HSPB1 affect the NFs axonal transport and binding to the anterograde motor protein kinesin. These deficits were also associated with an increased phosphorylation of NFs as well as an increased phosphorylation of the cyclin dependent kinase Cdk5, which mediates the NF-phosphorylation. Inhibition of Cdk5 restored the NF-phosphorylation and binding to kinesin. Altogether, specific mutations in HSPB1 affect the axonal transport via induced hyperphosphorylation of NFs and stabilization of the MT-network, and can be targeted by drugs in experimental models opening possibilities for treatment of distal HMN.

THEMATIC WORKSHOP 2.4

Theme: *CALCIUM FLUXES AND ION CHANNELS IN MUSCLE*

TW 2.4.1 Periodic Paralysis related to calcium channelopathy

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Periodic paralyses are genetic conditions of autosomal inheritance with an onset in the second decade. They manifest as acute and reversible attacks of muscle weakness. Mutations in the gene encoding the muscular voltage-gated calcium channel are a major cause of hypokalemic periodic paralysis, a form of the disease with a concomitant decrease in blood potassium levels during the attacks. If the causative gene has been known for twenty years, patho-physiology has long remained obscured. In contrast with other channelopathies, no major modification of the biophysical properties was observed in the mutated channel. Recent advances have helped to better understand the mechanism of the attacks with the discovery of a leaking current in the channel. If attacks are better understood, there are still uncertainties in the way this leaking current modifies the properties of the muscle membrane and how these modifications can lead to the observed major ion fluxes occurring across the muscle membrane during attacks. The recent construction of functional models may help to unravel these questions as well as new possibilities of observing abnormal ion fluxes by MRI. All these advances will be summarized and put in the context of treatment of these incapacitating muscle disorders.

THEMATIC WORKSHOP 2.4

Theme: *CALCIUM FLUXES AND ION CHANNELS IN MUSCLE*

TW 2.4.2 Genetic evidence that Ca2+ is the key inducer of myofiber necrosis in muscular dystrophy

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Muscular dystrophy (MD) refers to a clinically and genetically heterogeneous group of degenerative muscle disorders characterized by progressive muscle weakness and degeneration. Although the primary defect likely results from a loss of sarcolemmal integrity, the secondary molecular mechanisms leading to myofiber necrosis is debated. One hypothesis suggests that elevated cytosolic Ca2+ is an initiating event for many downstream sequelae resulting in myofiber necrosis. Direct measurement of resting Ca2+ levels in myofibers from dystrophic animal models or humans has so far produced equivocal results, with some studies reporting elevated Ca2+ while other studies report no change. Here we undertook a genetic approach to manipulate Ca2+ directly in the mouse by transgenesis

and gene-targeting. Our results uniformly support the Ca2+ hypothesis of MD, such that mouse models with artificially elevated Ca2+ show fulminant disease, while mouse models with enhanced Ca2+ clearance are resistant to MD when crossed with mouse models of this disease. Our collective results span transgenic mice that overexpress TRPC3, Stim1 and NCX1 as ways of enhancing baseline Ca2+ levels in myofibers (all induce MD-like disease), as well as mice expressing dnTRPC3, dnTRPC6, dnOrai1, SERCA1 and NCX1 as ways of reducing pathologic Ca2+ influx (all of which protect from MD disease). We have also generated mice lacking the gene for cyclophilin D (Ppif) and NCX1 (Slc8a1), which renders mitochondria less sensitive to Ca2+-induced necrosis and hence, show less dystrophic disease when crossed with mouse models of this disease. Our results suggest that elevation in resting Ca2+ within the myofiber in MD is the proximal event inducing muscle wasting downstream of an unstable sarcolemma (due to loss of dystrophin or other key structural genes that cause MD).

THEMATIC WORKSHOP 2.4

Theme: *CALCIUM FLUXES AND ION CHANNELS IN MUSCLE*

TW 2.4.3 Identification of a Relay Gate in the type I Ryanodine Receptor from functional analyses of a cluster of disease mutations in TM2

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Mutations in the type I ryanodine receptor (RyR1) that result in malignant hyperthermia and central core disease can be found throughout the RyR1 sequence. Interestingly, a large number of the C-terminal disease mutations are found in two clusters: one in the selectivity/pore-lining region and a second cluster within the second conserved transmembrane domain (TM2) of the channel. We previously found that disease mutations in the RyR1 selectivity filter lead to a form of excitation-contraction "uncoupling" (i.e. reduced calcium release in the absence of store depletion), which results from a disruption in calcium permeation. Here, we determined the effect of 11 different disease mutations in TM2 of RyR1 on EC coupling, bi-directional coupling between RyR1 and the dihydropyridine receptor, and the conductance and gating of RyR1 channels incorporated into planar lipid bilayers. Following expression in RyR1-null myotubes, an uncoupling phenotype was observed for a subset of CCD mutations (L4647P, Delta4647–48, H4651P, and H4651R) located in the center and on one face of the TM2 α-helix. RyR1 function was essentially unaltered for CCD mutations located at either the N-terminal end (T4637A/I, G4638D/S/V, and M4640V) or on the opposing face of the TM2 α-helix (L4650P). Immunocytochemistry and whole-cell patch clamp studies found that all four of the uncoupling mutants in TM2 co-localized with dihydropyridine receptor and enhanced L-type calcium currents. Additional mutations (L4644P/R, L4647R, L4649P, A4653P, and A4655P/R) were engineered to precisely define the boundaries and helix-sidedness of the uncoupling module in TM2. Bilayer studies showed that while potassium and calcium permeation were unaltered by the Delta4647–48, L4650P, and H4651P TM6 mutations, each of these mutants exhibited a markedly reduced average channel open probability due to a modest reduction in channel mean open time and larger increase in channel mean closed time. Together, these results demonstrate that one face of TM2 functions as an important RyR1 relay gate that couples dihydropyridine receptor activation and to channel opening during excitation-contraction coupling and that mutations with this domain disrupt this relay gate function in the absence of change in ion permeation.

THEMATIC WORKSHOP 2.5

Theme: *MYOFIBRILLAR AND GNE MYOPATHIES*

TW 2.5.1 Myofibrillar myopathies - clinical characteristics

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Myofibrillar myopathy is a diagnostic concept based on muscle histopathology findings indicating disorganized myofibrils: dark and hyaline cytoplasmic changes on trichrome stain, abnormal sarcomeric protein aggregations, disintegrated myofibrillar structures on ultrastructure and rimmed vacuoles. Because many patients with a pathological diagnosis of myofi-

brillar myopathy clinically show marked distal weakness there is an overlap with the distal myopathies. This was first known for desminopathy, and later shown for myotilinopathy and zaspopathy. Besides these three genetic disorders mutations in αB-cristallin, BAG3 and Filamin-C cause marked myofibrillar myopathy. Myofibrillar myopathy changes can also be observed in other diseases such as mutated FHL-1, SEPN1, DNAJB6 and HMERF titinopathy, although other clinical or pathology features are usually more characteristic for these disorders. In this workshop we will highlight clinical, epidemiological, muscle imaging and pathology features in this group of muscle diseases with the aim of enhancing diagnostic accuracy for the patients which is of importance for the correct management of each of these diseases. Some are associated with cardiomyopathy or respiratory problems and others are not. Moreover, the molecular pathogenesis linked to defect turnover and recycling of sarcomeric proteins as well as alternatives regarding treatment options will be discussed.

THEMATIC WORKSHOP 2.5

Theme: *MYOFIBRILLAR AND GNE MYOPATHIES*

TW 2.5.2 Diagnostic clues from muscle pathology and MRI in Myofibrillar Myopathies

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Myofibrillar myopathies (MFM) are a group of muscle disorders characterized morphologically by focal disintegration of myofibrils and accumulation of degradation products into inclusions containing desmin and other proteins. These highly heterogeneous disorders are caused by mutations in desmin (DES), α(alpha)B-crystallin (CRYAB), myotilin (MYOT), Z-band alternatively spliced PDZ-containing protein (ZASP), filamin C (FLNC), Bcl-2-associated athanogene-3 (BAG3) and FN3 titin (TTN) domain. Further genetic heterogeneity is suspected since the causative gene remains to be discovered in many patients suffering from MFM.

Individual MFM subtypes have distinct clinical, morphological and muscle imaging features depending on the disease-causative gene. A precise diagnosis requires identification of a causative mutation in each patient.

Muscle biopsies in patients carrying DES or CRYAB mutations show patches of desmin-immunoreactive inclusions that correspond to granulofilamentous material at the ultrastructural level. In patients with MYOT and ZASP mutations prominent desmin and myotilin immunoreactive inclusions, large numbers of vacuoles, inclusion bodies and filamentous bundles on EM are typically seen. A combination of the lesions mentioned above are normally seen in patients with mutations in filamin C rod domain. The presence of multiple cytoplasmic bodies in muscle fibers is typically observed in hereditary myopathy with early respiratory failure (HMERF) resulting from mutations in the FN3 titin domain.

Muscle imaging is a powerful diagnostic tool allowing differentiation between gene-dependent subtypes of MFM. Desminopathy and alphaB-crystallinopathy are characterized by early involvement of semitendinosus, sartorius and gracilis at the mid-thigh, and anterior tibialis and peroneal group at mid-leg. A striking similar pattern of muscle involvement is observed in patients suffering from HMERF. By contrast myotilinopathy, ZASPopathy, and filaminopathy are characterized by early involvement of the semimembranosus, hip adductors and biceps femoris at the mid-thigh, and soleus and medial gastrocnemius at the mid-leg level.

THEMATIC WORKSHOP 2.5

Theme: *MYOFIBRILLAR AND GNE MYOPATHIES*

TW 2.5.3 Protein aggregates and Rimmed Vacuoles: Possible therapeutic interventions in MFM

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The accumulation of proteinaceous inclusions underlies the pathogenesis of a growing list of protein aggregate myopathies or myofibrillar myopathies (MFMs). The histopathologic spectrum of MFMs includes, myofibrillar disorganization, protein inclusions and occasionally rimmed vacuoles. Inherited mutations in myofibrillar proteins such as desmin, myotilin, and filamin-c cause these proteins to aggregate, become insoluble and accumulate in myofibers

leading to MFMs. Moreover, mutations in protein chaperones, such as BCL2-associated athanogene 3 (Bag3) and αB-crystallin, necessary for the proper folding of myofibrillar proteins also leads to pathologically similar MFMs. Protein aggregates can be degraded via autophagy suggesting that enhancing autophagy in MFM may be therapeutic. We will discuss potential therapeutic interventions aimed at enhancing autophagy in skeletal muscle. In addition, we will highlight our studies understanding and manipulating autophagy in skeletal muscle using MFM and protein aggregate myopathy mouse models. These studies suggest that the manipulation of autophagic function in skeletal muscle holds promise in treating MFMs.

THEMATIC WORKSHOP 2.6

Theme: *ENMC SESSION: HIGHLIGHTS FROM RECENT ENMC WORKSHOPS*

TW 2.6.1 Care for adults with DMD - outcome of an ENMC workshop

JES RAHBEK, Aarhus (Danemark)

Over the past 30 years, the life expectancy of Duchenne muscular dystrophy (DMD) patients has increased thanks to advances in the care and treatment of their respiratory and cardiac insufficiency, muscle strength deterioration, and scoliosis. In a country like Denmark, the number of people with DMD aged 18 years and older now exceeds the number younger than 18. While the maximum age for survival was previously approximately 20 years in the Western world, it currently extends to the fourth decade of life.

A survey of the mainly steroid-naive Danish DMD population above the age of 20 (n = 74; age 20–46) revealed that from his mid twenties, a man with DMD is dependent on assisted ventilation 24 hours a day. He is able to sit all day in his powered wheelchair with body and neck support. While he cannot move his limbs, he has a little residual muscle strength in one or more fingers that enables him to use a special joystick for his wheelchair and write on a computer. He is not able to feed himself and when fed, he takes a long time to chew and swallow his food. He cannot raise his voice and may have difficulties in articulation, making his speech difficult to understand. He is dependent on around-the-clock practical assistance. Absence of social and cognitive skills, coupled with an insufficient educational level, may mean that he is poorly equipped to administrate his life and live independently with his helpers.

The adult with DMD will also experience medical complications that previously were not observed. In the Danish cohort, hospital admissions were most often attributable to pneumonia or abdominal pain, the latter often caused by extreme constipation with accumulated air. Surgical treatment of complications substantially increases risk to the lives of the patients because it necessitates anaesthesia at a time where they are medically vulnerable. In a Danish cohort followed for 30 years, the main cause of death was cardiac insufficiency; in half of these cases, cardiac arrest was related to surgery procedures which would normally be considered minor.

The extended life expectancy of DMD patients necessitates centralization of knowledge and follow-up in specialized centres in order to acquire experience with the medical complications and their management. The complexity of the medical care required and the transition from in-patient care to home care with specialized home respiratory equipment and personnel is another challenge in many countries. A specially trained interdisciplinary team working in collaboration with the patient is needed to ensure that his everyday life will function well in his home, on his and his relatives' own terms.

Important topics for further study and discussion include:

- Causes of death
- Medical treatment
- Respiratory insufficiency, oral and upper airway problems
- Endocrine aspects and nutrition
- Motor function and activity
- Organizing care for the adult with DMD
- Quality of life

THEMATIC WORKSHOP 2.6

Theme: *ENMC SESSION: HIGHLIGHTS FROM RECENT ENMC WORKSHOPS*

TW 2.6.2 Biomarkers in DMD: from the discovery to the development toward clinical application and translation in other NMDs

Alessandra FERLINI, FERRARA (Italie)
Department of Medical Sciences University of Ferrara (Ferrara, Italy)

Biomarkers have been defined as cellular, biochemical, molecular alterations or biological characteristics that are measurable in biological material as indicator of normal biological or pathogenic processes (Scotton C et al., 2013). Biomarkers may be used in differential and early diagnosis, and in monitoring of disease progression, regression, or therapeutic response. Duchenne Muscular Dystrophy (DMD) is a severe hereditary muscle disorder due to a variety of dystrophin gene mutations and presenting with variable clinical severity.

Recently novel experimental drugs have been developed for DMD and several trials are ongoing, raising the urgent need of having fine tools for measuring the trial outcomes as well as for optimizing the selection of eligible patients (Archevala-Gomez et al., 2012,; Mendell et al., 2012). The use of clinical parameters measuring muscle strength and function is limited due to their dependence on motivation, large intra-individual variability and slow response time. Conversely, molecular biomarkers may show earlier response to treatment and reflect the different pathophysiological aspects of the disease. The use of biomarker panels for the diagnosis, prognosis, and monitoring of DMD (and more in general of rare chronic neuromuscular disorders) as well as to guide the choice of therapeutic regimens may significantly improve the current clinical practice, by facilitating the evaluation of emerging therapies in drug trials and regulatory approval. These biomarkers will also serve for patients' stratification and for selection of appropriate subjects for clinical trials. This will impact on the economical load, patients' care and novel therapies. Biomarker discovery (proceeding by candidate or via omics analysis) is vital in order to identify exploratory markers to be further validated in larger patients' cohorts and international collaborations are mandatory in the validation stage, since rare diseases patients are small in number. Enhancing cooperation will allow to have validated clinical biomarkers translatable in clinical practice.

References

-Arechavala-Gomeza V, et al., Antisense oligonucleotide-mediated exon skipping for Duchenne muscular dystrophy: progress and challenges. Curr Gene Ther. 2012 12:152–60. Review.

-Mendell JR, et al., Gene therapy for muscular dystrophy: lessons learned and path forward. Neurosci Lett. 2012 Oct 11;527(2):90–9.

-Scotton C, Biomarkers in rare neuromuscular diseases. Exp Cell Res. 2013 Dec 31.

Disclosure of interest: AF is PI of Prosensa and GSK antisense-based clinical trials, participates to EU projects (BIO-NMD and Neuromics) and other research projects on rare diseases.

THEMATIC WORKSHOP 2.6

Theme: *ENMC SESSION: HIGHLIGHTS FROM RECENT ENMC WORKSHOPS*

TW 2.6.3 Autophagy in Muscular Dystrophies: Translational approach

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(Macro)autophagy is an evolutionarily conserved intracellular system by which macromolecules and organelles are delivered to lysosomes for degradation and recycling. This catabolic process is particularly relevant for skeletal muscle that represents 40% of whole-body lean mass, thereby providing a tissue source for amino acids that can be used in times of stress or starvation. Recent research has made it clear that normal autophagy is fundamental for human health, aging and life span, and malfunctioning or failure of autophagy is associated with a wide range of human pathologies including muscular dystrophies [1]. In particular alteration of autophagosome biogenesis has been found relevant for a number of muscular dystrophies including Ullrich congenital muscular dystrophy, DMD, Emery-Dreifuss muscular dystrophy and congenital muscular dystrophy 1A. Alteration of autophagosome-lysosome fusion is instead involved in Vici syndrome, Danon disease and Pompe disease. Activation of autophagic flux has been proved beneficial in mice models of Ullrich congenital muscular dystrophy, DMD, Emery-Dreifuss muscular dystrophy, and inhibition of autophagic flux in a mouse model of congenital muscular dystrophy 1A. A normocaloric low protein diet to reactivate autophagy was performed in patients with COL6-related myopathies. When compared to the baseline samples, LC3II/I ratio and beclin1 appeared increased after treatment both in leukocytes samples and in muscle biopsy of patients. As a group, our current recommendations regarding the design of clinical trials modulating autophagy are the following: 1) every effort should be made to sample patient material under sim-

ilar nutrient states, time of day and site of biopsy. 2) as no single autophagic biomarker is reliable, a panel of several biomarkers should be assessed (i.e. LC3, p62, Lamp1/2) and include both morphologic/biochemical markers as performed via electron microscopy, IHC or immunoblotting along with gene expression studies of these same biomarkers. 3) depending upon the proposed mechanism of therapeutic intervention, measures of autophagy signaling pathways should be evaluated (i.e. mTOR, Akt, FOXO3, TFEB, beclin-1). 4) In some cases, quantitating the changes in other autophagic substrates may be useful (e.g. desmin, ubiquitinated proteins or TDP-43). Finally, 5) it is essential that new methods be developed to monitor autophagic flux in vivo in human patients.

References

[1] Merlini L, Nishino I; On behalf of the Autophagy in muscular dystrophy consortium. 201st ENMC International Workshop: Autophagy in Muscular Dystrophies – Translational Approach, 1–3rd November 2013, Bussum, The Netherlands. Neuromuscul Disord. 2014 (in press).

THEMATIC WORKSHOP 3.1

Theme: *STEM CELLS AND MUSCLE REGENERATION*

TW 3.1.1 A cell-autonomous loss of muscle stem cell self renewal in aged muscle

Bradley OLWIN, Boulder (Etats-Unis)

Abstract not received

THEMATIC WORKSHOP 3.1

Theme: *STEM CELLS AND MUSCLE REGENERATION*

TW 3.1.2 The Role of the Muscle Microenvironment in Cancer-Induced Cachexia

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Cachexia is a debilitating condition characterized by extreme skeletal muscle atrophy that contributes significantly to morbidity and mortality. This wasting state of muscle largely derives from aberrant signaling of pathways that maintain a balance between the anabolism and catabolism of muscle protein. In cachexia, this balance is tipped towards a catabolic state resulting from the activation of the ubiquitin proteasome and autophagy systems that regulate protein breakdown, and reduced Akt and mTOR activities that decrease protein synthesis. Whereas these events are firmly established to reside within the myofiber, less regard has been given to potential contributory factors that might act outside the myofiber within the muscle microenvironment. Recently, our laboratory described that muscles derived from tumor bearing mice and pancreatic cancer patients with weight loss undergo a damage response. This damage was associated with an expansion of both satellite and non-satellite progenitor cells that express Pax7 and commit to a differentiation program. However, we found that due to persistent Pax7 expression, myoblasts are unable to complete their differentiation, and thus unable to fuse to damaged myofibers. The ultimate fate of these cells is to undergo apoptosis, an event controlled by circulating microvesicles. These microvesicles contain miR-21 that signal through TLR7 on myoblasts to mediate cell death via JNK signaling. Thus, we propose a model of cachexia whereby Pax7 functions to impair the regenerative capacity of myogenic cells in the muscle microenvironment to drive muscle wasting in cancer, a mechanism that might also be linked to the death of these myogenic cells mediated through an extracellular vesicle signaling pathway involving miR-21 and TLR7.

THEMATIC WORKSHOP 3.1

Theme: *STEM CELLS AND MUSCLE REGENERATION*

TW 3.1.3 The role of the muscle stem cell niche in regulating regenerative myogenesis

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Satellite cells in adult skeletal muscle are a heterogeneous population composed of stem cells and committed progenitors. Wnt7a signalling dramatically

stimulates the symmetric expansion of satellite stem cells and this expansion requires Fzd7 and Vangl2, both components of the planar cell polarity (PCP) signaling pathway. Importantly, Wnt7a over-expression results in a large expansion of the satellite stem cell population, and Wnt7a deficiency in impaired maintenance of the satellite cell compartment. Therefore, Wnt7a signaling through the planar cell polarity pathway controls the homeostatic level of satellite stem cells and hence regulates the regenerative potential of muscle. In differentiated myofibers, Wnt7a binding to Fzd7 directly activates the Akt/mTOR growth pathway thereby inducing myofibre hypertrophy. Notably, the Fzd7 receptor complex is associated with GNAS1 and PI3kinase in differentiated myofibres but not in myoblasts, and are required for Wnt7a to activate the Akt/mTOR growth pathway. Wnt7a/Fzd7 activation of this pathway was completely independent of IGF-receptor activation. We found that Syndecan-4 (Sdc4) and Frizzled-7 (Fzd7) form a co-receptor complex in satellite cells and that binding of the glycoprotein Fibronectin (FN) to Sdc4 stimulates the ability of Wnt7a to induce the symmetric expansion of satellite stem cells. Newly activated satellite cells dynamically remodel their niche by transient high-level expression of FN. Knockdown of FN in prospectively isolated satellite cells severely impairs their ability to repopulate the satellite cell niche following transplantation into regenerating muscle. Conversely, in-vivo over-expression of FN with Wnt7a dramatically stimulates the expansion of satellite stem cells in regenerating muscle. Therefore, activating satellite cells remodel their niche through autologous expression of FN that provides feedback to stimulate Wnt7a signaling through the Fzd7/Sdc4 co-receptor complex. Thus, FN and Wnt7a together regulate the homeostatic levels of satellite stem cells and satellite myogenic cells during regenerative myogenesis. We generated a truncated Wnt7a variant, consisting of the C-terminal 137 amino acids lacking the conserved palmitoylation sites, which retains full biological activity in skeletal muscle. This includes binding to and signaling through its receptor Fzd7 to stimulate symmetric expansion of satellite stem cells by activating the planar cell polarity pathway, and inducing myofibre hypertrophy by signaling through the AKT/mTOR pathway. Furthermore, this truncated Wnt7a shows enhanced secretion and dispersion compared to the full-length protein. Together, these findings open important new avenues for the development of a Wnt7a as a treatment for muscle wasting diseases and have broad implications for the therapeutic use of Wnts as biologics. M.A.R. is a founding scientist in Fate Therapeutics who have licensed the Wnt7a technology.

THEMATIC WORKSHOP 3.2

Theme: *CONGENITAL MYASTHENIC SYNDROMES*

TW 3.2.1 An emerging subgroup of congenital myasthenic syndromes due to mutations in genes associated with N-linked glycosylation

David BEESON, Oxford (Etats-Unis)
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Congenital myasthenic syndromes (CMS) are hereditary disorders of neuromuscular transmission characterised by fatigable muscle weakness. The number of cases recognised, at around 1:100,000 of the UK population, is increasing with improved diagnosis. The underlying genetic defects are diverse involving a series of different genes with a variety of different phenotypes. Mutations within the muscle acetylcholine receptor form the most common CMS subtype. Here, we highlight a new emerging group of CMS, characterised by a limb-girdle pattern of muscle weakness, which are caused by mutations in genes that encode proteins in the initial steps of the N-linked glycosylation pathway. We used whole exome sequencing to identify mutations in five different genes associated with the N-linked glycosylation pathway that cause CMS. Expression studies in cultured myotubes derived from patients with mutations in GFPT1 or DPAGT1 showed a significant reduction of cell-surface AChR expression $p < 0.0001$. Inhibition of GFPT1 or DPAGT1 enzymatic activity or siRNA silencing of GFPT1 or DPAGT1 expression both resulted in reduced AChR cell-surface expression. Western blot and gene silencing experiments indicate impaired assembly/maturation of the AChR pentamer is responsible for reduced expression at the endplate. Mutations affecting N-linked glycosylation form part of the congenital disorders of glycosylation, and often lead to severe multisystem disorders. Surprisingly although N-linked glycosylation is ubiquitous, occurring in all mammalian cells, symptoms in our patients are often largely restricted to neuromuscular transmission. We speculate that the neuromuscular junction,

and in particular the acetylcholine receptor itself, is particularly sensitive to defects in biochemical pathways affecting glycosylation.

THEMATIC WORKSHOP 3.2

Theme: *CONGENITAL MYASTHENIC SYNDROMES*

TW 3.2.2 Diagnosis and therapy of congenital myasthenic syndromes - clinical update

Hanns LOCHMULLER, Newcastle (Royaume Uni)
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Neuromuscular junction disorders, also called Myasthenic syndromes (MS), are a rare heterogeneous group of acquired (Myasthenia Gravis, MG) and inherited (Congenital Myasthenic Syndromes, CMS) neuromuscular disorders associated with distinctive clinical, electrophysiological, laboratory and ultrastructural abnormalities. The genetic defects in CMS either impair neuromuscular transmission directly or result in secondary impairments, which eventually compromise the safety margin of neuromuscular transmission. More recently, we have identified two genes (DOK7, GFPT1) that cause fatigable weakness of muscles in a limb-girdle distribution, but rarely affecting facial or eye muscles. We will cover the significant progress made in understanding the molecular pathogenesis of CMS, which is important for both patients and clinicians in terms of reaching a definite diagnosis and selecting the most appropriate treatment

THEMATIC WORKSHOP 3.2

Theme: *CONGENITAL MYASTHENIC SYNDROMES*

TW 3.2.3 Agrin and MuSK in congenital myasthenia

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Congenital myasthenic syndromes (CMS) represent a group of genetic diseases characterized by defects in the neuromuscular transmission leading to muscle weakness accentuated by exertion, and are heterogeneous for both their mode of inheritance and their pathophysiology. To date, even if the alteration of several genes have been shown to cause CMS, the molecular origin of about half of these pathologies remains still unknown.

Using a candidate gene approach, the French CMS network has identified mutations in the genes encoding MuSK and agrin. In order to demonstrate that these variants were indeed responsible for the CMS phenotype, MuSK vectors or recombinant agrin proteins, either wild-type or bearing a mutation identified in patients, were tested for their ability to modify MuSK phosphorylation or AChR aggregation in cell cultures or to alter the NMJ structures in rodent muscles. Although the alterations of the agrin-MuSK-Dok7-AChR cascade in vitro were not always those expected, the NMJ structural changes in rodent muscle recapitulated those observed in the patient muscle biopsies. The involvement of the presynaptic compartment was impressive as evidenced by the exuberant axonal growth and questions the role of mutant MuSKs and agrins in these processes. Several other mutations in the genes encoding MuSK or agrin have been subsequently identified, however not to the extent of those in DOK7. In the case of the agrin gene, some mutations may be causing a misleading phenotype of distal myopathy.

This work on human mutations may reveal new functions of agrin and MuSK at the NMJ. In return, it may allow a better understanding of the pathogenic mechanisms underlying CMSs and the adaptive processes taking place at the NMJ in these patients.

THEMATIC WORKSHOP 3.2

Theme: *CONGENITAL MYASTHENIC SYNDROMES*

TW 3.2.4 Observations in recently identified congenital myasthenic syndromes (CMS)

ANDREW G ENGEL, ROCHESTER (Etats-Unis)
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To date 20 CMS disease genes have been identified. In some CMS the disease protein resides at pre-or postsynaptic sites or in the synaptic space. More recently genes involved in protein glycosylation or end-

plate (EP) development and maintenance were identified. The first identified gene affecting glycosylation was GFPT1. In our cohort of 11 pts 5 had intercostal biopsies; all had hypoplastic EPs but only 3 had mild EP AChR deficiency and a low miniature EP potential (MEPP) amplitude. One pt with a mutation disrupting the muscle specific exon never moved in utero, remains quadriplegic at age 8 y, and has a severe autophagic myopathy emphasizing importance of this exon in muscle function. The second identified gene affecting protein glycosylation was DPAGT1. We observed 3 pts who are intellectually disabled and have an autophagic myopathy. The observed missense mutations reduce protein expression, or decrease or abolish enzyme activity, or cause many skipped and few nonskipped alleles. Immunoblots of muscle extracts indicate reduced to absent glycosylation of different proteins, including STIM1 which likely explains the tubular aggregates in this disease. EP development is affected by LRP4, a postsynaptic coreceptor of neural agrin for activating MuSK. Acting in concert with Dok7 and other proteins, LRP4 acts on rapsyn to concentrate AChR at the EP, enhance synapse specific gene expression, and promote postsynaptic differentiation. In a recent study with K. Ohno and B. Ohkawara we identified 2 mutations at the edge of the third beta-propeller domain of LRP4 in a 14-year-old girl with limb-girdle CMS. Both mutations decrease binding affinity of LRP4 for both MuSK and agrin. Intercostal muscle synaptic contacts are disarrayed and hypoplastic but other parameters on neuromuscular transmission are unaffected. Finally, in collaboration with L. Regal and J. Creemers, we identified a pt with isolated PREPL deficiency with myasthenic symptoms since birth, a positive edrophonium test, and growth hormone deficiency without cystinuria who responded transiently to pyridostigmine during infancy. EP studies revealed decreased evoked quantal release and small MEPPs despite robust EP AChR expression. Because PREPL is an essential activator of the clathrin associated adaptor protein 1 (AP1), and AP1 is required by the vesicular ACh transporter, we attribute the small MEPPs to a decreased ACh content of the synaptic vesicles.

THEMATIC WORKSHOP 3.3

Theme: *ELECTROPHYSIOLOGICAL APPROACHES IN DYSIMMUNE NEUROMUSCULAR DISEASES*

TW 3.3.1 Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP): electrodiagnostic evidence

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Making the diagnosis of inflammatory demyelinating neuropathy, in particular when the disease course is chronic, is often difficult. Diagnostic criteria are very important but given the lack of a definitive diagnostic marker and the limitations of laboratory studies, it appears impossible to reach a definitive diagnosis in all patients. By definition, electrophysiological criteria for primary demyelination are designed to exclude abnormalities that can be explained by axonal degeneration. Therefore, lesser degrees of demyelination can not be defined with complete certainty electrophysiologically. Optimised electrophysiological criteria are capable, however, to support the diagnosis with different levels of probability (possible, probable, definite). To reach the best possible sensitivity levels, it is best to examine motor nerves bilaterally and to include proximal stimulation sites in the upper limbs. Electrophysiological criteria for CIDP proposed by the EFNS/PNS Task Force are highly sensitive and specific for primary demyelination and lead to the correct diagnosis when clinical criteria are fufilled in the very large majority of cases.

THEMATIC WORKSHOP 3.3

Theme: *ELECTROPHYSIOLOGICAL APPROACHES IN DYSIMMUNE NEUROMUSCULAR DISEASES*

TW 3.3.2 Guillan-Barrè syndrome: electrodiagnostic issues and challenges

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Department of Neuroscience and Imaging, University "G. d'Annunzio", Chieti-Pescara (IT)

Recent clinical and experimental studies indicated that axonal GBS subtypes with anti-ganglioside antibodies show, besides axonal degeneration, promptly

reversible nerve conduction failure (RCF) affecting which can be recognized only by serial recordings. This explain why Recovery in acute motor axonal neuropathy may be either very rapid and complete or prolonged with poor outcome according to the relative amount of RCF and axonal degeneration in each patient. Not recognizing distal RCF may induce, on the basis of a single test, to diagnose axonal degeneration and to formulate a bad prognosis. Moreover the lack of distinction among demyelinating conduction CB and RCF may fallaciously classify axonal GBS as acute demyelinating inflammatory polyradiculoneuropathies. RCF has been recently demonstrated also in sensory fibers and other clinical GBS variants and subtypes as pharingo-cervical brachial weakness, Fisher syndrome, and acute sensory ataxic neuropathy.These findings suggests that GBS subtypes with antibodies anti-gangliosides belong to a continuous clinical spectrum with a common pathophysiological mechanism characterized by dysfunction/disruption of the node of Ranvier and could be more appropriately classified as nodo-paranodopathies.

Selected references

Capasso M, Notturno F, Manzoli C, Yuki N, Uncini A. Reversible conduction failure in pharingeal-cervical-brachial variant of Guillain–Barré syndrome. Muscle Nerve 2010;42:608–6012.

Capasso M, Notturno F, Manzoli C, Uncini A. Involvement of sensory fibers in axonal subtypes of Guillain–Barrè syndrome. J Neurol Neurosurg Psychiatry 2011;82:664–70.

Uncini A, Kuwabara S. Electrodiagnostic criteria for Guillain-Barrè syndrome: A critical revision and the need for an update. Clin Neurophysiol 2012;123:1487–1495.

Uncini A, Manzoli C, Notturno F, Capasso M. Pitfalls in electrodiagnosis of Guillain-Barrè syndrome subtypes. J Neurol Neurosurg Psychiatry 2010;81:1157–1163.

Uncini A, Susuki K, Yuki N. Nodo-paranodopathy: beyond the demyelinating and axonal classification in anti-ganglioside antibody mediated neuropathies. Clin Neurophysiol 2013;124:1928–1934.

Uncini A, Notturno F, Capasso M. Natura non facit saltus in antiganglioside antibody-mediated neuropathies. Muscle Nerve 2013;48:484–487.

THEMATIC WORKSHOP 3.3

Theme: *ELECTROPHYSIOLOGICAL APPROACHES IN DYSIMMUNE NEUROMUSCULAR DISEASES*

TW 3.3.3 Neuromuscular junction disorders: electrodiagnostic methods

J GERT VAN DIJK, Leiden (Pays-Bas)

Abstract not received

THEMATIC WORKSHOP 3.4

Theme: *NEW APPROACHES OF IDENTIFICATION OF GENE: NEXT GENERATION SEQUENCING*

TW 3.4.1 New systems to interprete data from NGS analysis

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For many years, the sequencing of the human genome was out of reach but recent advances in very high throughput technologies have drastically reduced costs leading to applications in clinical testing. Nevertheless, if it is now technically possible to sequence human genomes, we still have a long way to go to identify all genes responsible for human genetic diseases especially in the context of rare diseases. This specific field harbors today more than 8,000 diseases for which only 2,500 disease-causing genes have been identified. To speed-up genes discoveries the International Rare Disease Research Consortium (IRDiRC) (http://www.irdirc.org/) was created. Its goals are to facilitate the identification of most genes involved in rare diseases as well as 200 new therapies by year 2020. To do so, many funders worldwide combine efforts in a spirit of data sharing. Four projects have already been funded at the European level including the RD-CONNECT project. It is a unique global infrastructure project that links up databases, registries, biobanks and clinical bioinformatics data used in rare disease research into a central resource for researchers worldwide. One key element of this project is to provide a central platform allowing data integration and analysis of various -omics data, first and foremost genomics.

In this presentation, we will review various analysis pipelines, which are used to handle both Whole Exome and Whole Genome Sequencing data as no gold standard already exist. We will also describe the RD-CONNECT existing pipeline and tools as well as future developments that will be progressively implemented in this global reference system that is tightly linked to the Neuromics EU-funded project.

We will describe the three layers of data analysis: raw data analysis leading to variant calling, data annotation and variant prioritization in order to identify bottlenecks and key systems that should be implemented in local pipelines to improve efficiency, allow data comparison and sharing.

Finally, we will demonstrate how data sharing will facilitate rare disease research through the example of the Rare Disease Variant Database from the French Rare Disease foundation.

THEMATIC WORKSHOP 3.4

Theme: *NEW APPROACHES OF IDENTIFICATION OF GENE: NEXT GENERATION SEQUENCING*

TW 3.4.2 User-driven dynamic creation of patient registries: a modular approach to bioinformatics analysis for neuromuscular diseases

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Patient registries are essential, particularly for the rare disease community. Their appropriate design, implementation and deployment enables rapid decision making and ongoing data mining, ultimately leading to improved patient diagnosis and treatment. Unfortunately, a major bottleneck encountered is the static nature of these implemented systems. Static in the way software developers work with stakeholders to determine requirements, design the system, implement the required data fields and functionality for each patient registry. Additionally, static in the way they are designed, making it difficult for disease registries to effectively 'communicate' to share or interrogate patient phenotypic information.

Here we provide an overview of a registry framework that allows scientists and registry curators with standard computing skills to dynamically construct a complete patient registry from scratch, and customise it for their specific needs, with little or no need to engage a software developer at any stage. Our second generation open source rare disease registry framework (RDRF) empowers registry curators to construct one or more patient registries without software developer effort [1, 2, 3, 4]. New data elements for a diverse range of phenotypic and genotypic features can be defined at any time and can then be utilized and shared in any of the created registries. Fine grained, multi-level user and workgroup access can be applied to each data element to ensure appropriate access and data privacy. Results from diverse bioinformatics analyses can then be incorporated into patient registries in a scalable fashion [5, 6].

References

[1] Bellgard, M., et al., A modular approach to disease registry design, Hum Mutat, 2012. 33(10): p. E2356–66.
[2] Bellgard, M., et al., Dispelling myths about rare disease registry system development SCFBM 2013. 8(1): p. 21.
[3] Rodrigues M, Hammond-Tooke G, Kidd A, Love D, Patel R, Dawkins H, Bellgard M, Roxburgh R. The New Zealand Neuromuscular Disease Registry. J Clin Neurosci. 2012 Sep 18.
[4] Bellgard, M et al., Second Generation Registry Framework, under revision, SCFBM, January 2014.
[5] Bellgard M, et al., Rare Disease Roadmap: Navigating the bioinformatics and translational challenges for improved patient health outcomes, submitted, HPT, Nov 2013.
[6] Hunter, A., et al., Yabi: An online research environment for Grid, High Performance, SCFBM, 2012, 7(1).

THEMATIC WORKSHOP 3.4

Theme: *NEW APPROACHES OF IDENTIFICATION OF GENE: NEXT GENERATION SEQUENCING*

TW 3.4.3 FORGE Canada Consortium: outcomes of a 2-year National Rare Disease Gene Discovey Project

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[11]*Centre Hospitalier Universitaire de Québec*

Inherited monogenic disease has an enormous impact on the well-being of children and their families. Over half of the children living with one of these conditions are without a molecular diagnosis due to the rarity of the disease, the marked clinical heterogeneity and the reality that there are thousands of rare disease genes that are yet to be identified. It is in this context that, in 2010, a Canadian consortium was formed to rapidly identify a wide spectrum of pediatric-onset rare disease genes using whole-exome sequencing. FORGE (Finding Of Rare disease GEnes in Canada) brought together clinicians and scientists from 21 Genetics Centres and three Science and Technology Innovation Centres from across Canada. From nation-wide requests for proposals, 264 disorders were selected for study from the 371 submitted; at least one causative gene was identified for 146 disorders (this includes 67 novel disease genes of which 41 have been genetically or functionally validated and 26 are currently under study), yielding a success rate of 55% over a two-year period. Forty-five of these disorders had a neuromuscular component:14 had known genes identified, 7 had potential novel genes, and 24 remain unsolved. We will present our experience with four strategies employed for gene discovery and discuss FORGE's impact in a number of realms; from clinical diagnostics to the broadening of the phenotypic spectrum of many diseases to the biological insight gained into both disease states and normal human development. Lastly, based on this experience, we will discuss the way forward for rare disease gene discovery both in Canada and internationally.

THEMATIC WORKSHOP 3.5

Theme: *THERAPEUTIC APPROACHES IN FACIOSCAPULOHUMERAL DYSTROPHY*

TW 3.5.1 Molecular mechanisms in FSHD: identification of druggable targets

SYLVERE VAN DER MAAREL, Leiden (Pays-Bas)
Leiden University Medical Center, Department of Human Genetics, The Netherlands

Facioscapulohumeral dystrophy (FSHD) is associated with a decondensation of the chromatin structure of the D4Z4 repeat array on chromosome 4 and incomplete suppression of the D4Z4-encoded DUX4 retrogene in skeletal muscle. DUX4 is a germline transcription factor that is normally suppressed in somatic tissue. Its expression in FSHD skeletal muscle activates germline and early developmental programs as well as specific classes of retrotransposons. It also suppresses the innate immune response to viral infection.

In the common form FSHD1, DUX4 expression in skeletal muscle is caused by contraction of the D4Z4 array to a size of 1–10 units on chromosomes 4 that contain a polymorphic DUX4 polyadenylation signal. In the rare form FSHD2, D4Z4 chromatin decondensation and DUX4 expression from chromosome 4 are a consequence of mutations in the SMCHD1 gene on chromosome 18. SMCHD1 is a chromatin modifier that binds to the D4Z4 repeat to maintain a repressive chromatin structure in somatic cells and in FSHD2 patients there is reduced SMCHD1 activity at D4Z4. FSHD2 is a heterogeneous condition since only 85% of cases can be explained by SMCHD1 mutations. SMCHD1 can also act as a modifier of disease severity in FSHD1 families as mutations in SMCHD1 aggravate disease severity in FSHD1 families with borderline D4Z4 repeat sizes.

Collectively these findings suggest specific mechanisms of FSHD pathology which will facilitate the identification of candidate biomarkers for disease diagnosis and progression, as well as the development of therapeutic strategies for FSHD.

THEMATIC WORKSHOP 3.5

Theme: *THERAPEUTIC APPROACHES IN FACIOSCAPULOHUMERAL DYSTROPHY*

TW 3.5.2 Aerobic exercise and cognitive behavior therapy reduce fatigue and slow progression of muscle MR fatty infiltration in FSHD

Baziel VAN ENGELEN, Nijmegen (Pays-Bas)

Objective: The aim of this study was to investigate the effect of aerobic exercise training (AET) and cognitive behavior therapy (CBT) on chronic fatigue in patients with facioscapulohumeral muscular dystrophy (FSHD).

Methods: We performed a multi-center, assessor-blinded, randomized clinical trial (RCT). Fifty-seven patients with FSHD type 1 with severe chronic fatigue were randomly allocated to AET, CBT, or usual care (UC). Outcomes (including a 3 Tesla quantitative MR imaging of the upper leg muscles) were assessed at baseline, immediately following 16 weeks of intervention, and after a 12-week follow-up. A linear mixed model for repeated measurements was used to study the estimated group differences.

Results: Following treatment, both the AET and CBT intervention groups had significantly less fatigue relative to the UC group, with a difference of -9.1 for AET (95%CI: -12.4 to -5.8) and -13.3 for CBT (95%CI: -16.5 to -10.2). These beneficial effects lasted through the follow-up period, with a difference of -8.2 for AET (95%CI: -12.4 to -5.8) and -10.2 for CBT (95%CI -16.5 to -10.1). The patients who received CBT had an increase in registered and experienced physical activity, sleep quality, and social participation. The patients who received AET had an increase in registered physical activity only. The increase in registered physical activity in both groups and the improvement in social participation following CBT were still present at follow-up. MRI determined fat fractions showed decelerated progression of fatty infiltration, most prominently in the adductor magnus muscle. In this muscle fat percentage increased with 1.56% monthly (UC) with a difference of -1.21 for AET (95%CI: -1.99 to -0.34) and -1.73 for CBT (95%CI: - 2.56 to -0.87).

Interpretation: This is the first RCT showing that chronic fatigue can be ameliorated in patients with a muscular dystrophy. AET and/or CBT reduce fatigue and slow progression of fatty infiltration in patients with FSHD.

THEMATIC WORKSHOP 3.5

Theme: *THERAPEUTIC APPROACHES IN FACIOSCAPULO-HUMERAL DYSTROPHY*

TW 3.5.3 Biomarkers and trial readiness in FSHD1 and FSHD2; are we ready for the future?

Sabrina SACCONI, NICE (France)
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Under the genetic and epigenetic point of view, we can distinguish two forms of FSHD, identical in term of clinical phenotype. Autosomal dominant FSHD1 is associated to a pathogenic contraction of D4Z4 4Q permissive allele, while FSHD2 is due to a mutation in SMCHD1 gene in presence of 4QA non-contracted permissive allele.

The recent identification of additive effect of FSHD1 and FSHD2 genetic abnormalities in some families confirmed the central role of toxic retrogene DUX4 in both these diseases. Hence, several therapeutic strategies are being developed, aiming to avoid DUX4 expression or its pathological consequences. More recently, neuroimaging studies bring back the attention to the role of inflammation in FSHD clinical progression and open the way for new therapeutic strategies development.

The future is full of promises for FSHD patients and we need, more than ever, sensitive and specific biomarker(s) able to characterize patients, quantify clinical progression and response to treatment. Some biomarkers are available and some are being developed.

Biomarkers of interest in FSHD can be classified as clinical, epigenetic, genetic, immune and neuroimaging biomarkers. Clinical biomarkers include manual muscular testing, quantified muscular testing, age corrected-Clinical Severity Score, Fatigue Severity Score, pain assessment. Level of hypomethylation correlates with the severity of clinical expression in FSHD2 patients and can be eventually used as a biomarkers for therapy targeting SMCHD1 gene. If DUX4 itself can hardly be used as genetic biomarker because of its limited expression, several genes associated with germline and early stem cell development have been recently identified as targets of the DUX4 transcription factor and are being tested as biomarkers for disease diagnosis and progression. Promising neuroimaging biomarkers (SPECT, MRI), as well as immune biomarkers are also being tested.

In conclusion, this presentation will describe the merits and limitations of known and recently discovered biomarkers for FSHD and the efforts that need to be done by scientific community to be ready in view of future clinical trials.

THEMATIC WORKSHOP 4.1

Theme: *NEW INSIGHT INTO THE MECHANISM OF MUSCLE ATROPHY*

TW 4.1.1 New insight into the molecular mechanism of muscle atrophy

Alfred GOLDBERG, Boston (Etats-Unis)
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Dramatic progress has been made recently in understanding the cellular mechanisms for the atrophy of skeletal muscle that occurs with disuse, nerve injury, fasting, glucocorticoid treatment, and many systemic diseases, especially cancer. In these rapidly atrophying muscles, there is a common program of transcriptional changes, in which a set of atrophy-related genes ("atrogenes") are induced or repressed coordinately. Among the most induced proteins are components of the ubiquitin-proteasome pathway (UPS), especially the muscle-specific ubiquitin ligases, atrogin-1 and MuRF1, which are induced by the FoxO family of transcription factors. Overproduction of FoxO3 causes dramatic atrophy. MuRF1 is critical in the ordered disassembly and degradation of the myofibrillar apparatus, specifically components of the thick filaments. However, a distinct E3, Trim32, catalyzes loss of thin (actin) filaments and the associated Z-band proteins and the cytoskeletal component, desmin. Desmin is phosphorylated in atrophy and degraded in a Trim32-dependent manner. Its loss is critical for the accelerated degradation of thin filaments. The p97/VCP ATPase appears essential for this process, and during atrophy, p97 appears to extract ubiquitinated components from the myofibril, since p97DN mutants also block atrophy, and become tightly associated with myofibrils. In addition, FoxO3 stimulates the expression of genes for the autophagic/lysosomal pathway. Thus, during atrophy, the cell's two main proteolytic systems are activated coordinately to cause breakdown of different cellular components; the loss of contractile proteins via the UPS and mitochondria via autophagy. This program for muscle wasting in disease states is also activated by myostatin and its homolog, Activin A. Antagonists of the myostatin/activin pathway inhibit FoxO-induced proteolysis and are an attractive therapeutic approach. These agents not only dramatically block muscle wasting and cachexia, but also can prolong the viability of tumor-bearing animals. The induction of atrophy upon denervation of even fasting also requires the transcription factor NFκB and its activation by acetylation of p65 subunits. Overexpression of the SIRT1 deacetylase prevents this activation and activation of FoxO and blocks atrophy upon fasting or denervation. Control of protein acetylation and degradation thus are attractive new drug targets.

THEMATIC WORKSHOP 4.1

Theme: *NEW INSIGHT INTO THE MECHANISM OF MUSCLE ATROPHY*

TW 4.1.2 FoxO, autophagia and mitochondria: the crossroad for the control of muscle

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Idiopathic inflammatory myopathies include dermatomyositis, polymyositis, immune-mediated necrotizing myopathy and inclusion body myositis. Clinical phenotype and histopathology examination of muscle biopsies are determinant to distinguish these myopathies from the others but detection of autoantibody is now also crucial. The myositis-specific antibodies and myositis-associated antibodies lead to a serologic approach complementary to the clinicopathological classification, because strong associations of myositis-specific antibodies with clinical features and survival have been documented, determining now myositis subclassifications. The presence of anti-synthetase antibodies is associated with an original histopathologic pattern between polymyositis and dermatomyositis, and defines a syndrome where interstitial lung disease drives the prognosis. Among the antisynthetase antibodies, some of them (anti-PL7 and PL12) have a worst prognosis than others (e.g. anti-Jo1) because of the severity of the interstitial lung disease. On the other hand, myositis may be more common, more severe, and more resistant to treatment for patients with anti-Jo1 than for those with anti-PL12 and anti-PL7. Anti-MDA-5 antibody is specifically associated with frequently amyopathic dermatomyositis, and defines a skin-lung syndrome with sometimes a catastrophic disease course. Anti-TIF1-γ is also associated with dermatomyositis but its presence in adults is highly predictive of cancer association, which then drives the prognosis. On the other hand, anti-Mi2 antibody is associated with a classical

form of dermatomyositis, with a better prognosis since not particularly associated with interstitial lung disease, cancer, nor corticoresistance. Two other specific antibodies, anti-SRP and anti-HMGCR, are observed in patients with immune-mediated necrotizing myopathies and may be very useful to make the difference with muscle dystrophies in case of slowly progressive onset and to allow the initiation of effective therapy (i.e. immunosuppressants). Interestingly, 40 to 60% of the patients presenting an immune-mediated necrotizing myopathies with anti-HMGCR received statins, outlining one of the roles of these drugs in their muscle side effects.

THEMATIC WORKSHOP 4.1

Theme: *NEW INSIGHT INTO THE MECHANISM OF MUSCLE ATROPHY*

TW 4.1.3 Signalling mechanisms mediating muscle homeostasis

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Abstract not received

THEMATIC WORKSHOP 4.2

Theme: *INFLAMMATORY MYOPATHIES*

TW 4.2.1 Specific auto-antibodies in inflammatory myopathies: identification of new clinico pathological syndromes?

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Idiopathic inflammatory myopathies include dermatomyositis, polymyositis, immune-mediated necrotizing myopathy and inclusion body myositis. Clinical phenotype and histopathology examination of muscle biopsies are determinant to distinguish these myopathies from the others but detection of autoantibody is now also crucial. The myositis-specific antibodies and myositis-associated antibodies lead to a serologic approach complementary to the clinicopathological classification, because strong associations of myositis-specific antibodies with clinical features and survival have been documented, determining now myositis subclassifications. The presence of anti-synthetase antibodies is associated with an original histopathologic pattern between polymyositis and dermatomyositis, and defines a syndrome where interstitial lung disease drives the prognosis. Among the antisynthetase antibodies, some of them (anti-PL7 and PL12) have a worst prognosis than others (e.g. anti-Jo1) because of the severity of the interstitial lung disease. On the other hand, myositis may be more common, more severe, and more resistant to treatment for patients with anti-Jo1 than for those with anti-PL12 and anti-PL7. Anti-MDA-5 antibody is specifically associated with frequently amyopathic dermatomyositis, and defines a skin-lung syndrome with sometimes a catastrophic disease course. Anti-TIF1-γ is also associated with dermatomyositis but its presence in adults is highly predictive of cancer association, which then drives the prognosis. On the other hand, anti-Mi2 antibody is associated with a classical form of dermatomyositis, with a better prognosis since not particularly associated with interstitial lung disease, cancer, nor corticoresistance. Two other specific antibodies, anti-SRP and anti-HMGCR, are observed in patients with immune-mediated necrotizing myopathies and may be very useful to make the difference with muscle dystrophies in case of slowly progressive onset and to allow the initiation of effective therapy (i.e. immunosuppressants). Interestingly, 40 to 60% of the patients presenting an immune-mediated necrotizing myopathies with anti-HMGCR received statins, outlining one of the roles of these drugs in their muscle side effects.

THEMATIC WORKSHOP 4.2

Theme: *INFLAMMATORY MYOPATHIES*

TW 4.2.2 Role of innate immunity in dermatomyositis

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Inflammatory myopathies (IM) are a heterogeneous group of myopathies that includes dermatomyositis (DM), polymyositis (PM) and inclusion body myositis (IBM) among others. Although IM share the presence of inflammation, the clinical features and the molecular mechanisms resulting in muscle injury probably differ in each of them [1]. MHC-I overexpression in muscle fibers is a hallmark of IM. Previous studies suggest that both immune and non-immune mechanisms play a role in muscle injury although the relative contributions of each one in each entity are not fully understood [2].

Type I interferons (IFN-I) play a central role in antiviral responses and IFN-I-inducible genes have been largely found in microarray studies in IM [3]. The IFN induction is mainly mediated by the activation of innate immune receptors that recognize pathogens and endogenous danger signals. Several families of innate immune receptors are known, among them toll-like receptors (TLR) and Rig-like receptors (RLR). In PM and DM muscle biopsies, several TLR were found to be increased [4]. Although its presence could be secondary to tissue damage, their activation by endogenous damage signals could have an important role in the perpetuation of the immune response within the muscle.

Using microarray technology from laser microdissected MHC-I positive pathological muscle fibers we have recently found a more prominent role of innate immunity in DM compared to PM and IBM. In particular, RIG-I, a member of the RLR innate immune receptors, was overexpressed in the muscle fibers of DM but not in the others. In vitro studies have demonstrated that RIG-I-activated myotubes induce MHC-I overexpression, secretion of IFNβ and a positive feedback of RIG-I. These results indicate that RIG-I innate immunity contributes to initiating or perpetuating the immune response specifically in DM [5]. The perpetuation of the immune response mediated by RIG-I may occur through the recognition of viruses or damaged-cells derived particles containing, among other molecules, RNAs. Similar innate immunity mechanisms have been proposed in a dystrophic mouse model.

According to these results, modulation of innate immune mechanisms with specific drugs would be a promising approach for the treatment of these diseases.

The authors have no disclosures.

[1] Engel AG F-AC. Myology: basic and clinical 2004: 1321–1486.
[2] Rayavarapu S et al. Skelet Muscle 2013; 3(1):13.
[3] Greenberg SA et al. Ann Neurol 2005; 57: 664–678.
[4] Cappelletti C et al. Neurology 2011; 76: 2079–2088.
[5] Suárez-Calvet X et al. J Pathol 2014 Mar 6. In press.

THEMATIC WORKSHOP 4.2

Theme: *INFLAMMATORY MYOPATHIES*

TW 4.2.3 Treatment of inflammatory myopathies

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The inflammatory myopathies include four distinct entities: Polymyositis (PM), Dermatomyositis (DM), Inclusion Body Myositis (IBM), and Necrotizing Autoimmune Myositis (NAM). A T-cell-mediated cytotoxic process in PM and IBM, a complement-mediated microangiopathy in DM, and a macrophage-mediated antibody-fixing process in NAM, are the hallmarks of the underlying autoimmune processes. A common critical factor that affects therapeutic outcome is the distinction of PM from the difficult-to-treat mimics such as s-IBM and inlammatory dystrophies which may have prominent secondary endomysial inflammation.

In uncontrolled studies, PM and DM respond to prednisone, either alone or in combination with one immunosuppressive drug (Azathioprine, Cyclosporine, Mycophenolate, Methotrexate), to some degree and for some period of time; in contrast, IBM is resistant to most of these therapies, most times. Controlled studies have shown that IVIg is effective and safe for the treatment of DM. The clinical benefit, which can be impressive in patients with early disease, is associated with improvement in the muscle cytoarchitecture and resolution of the aberrant immunopathological

parameters, including interception of complement activation and downregulation of ICAM-I, VCAM, TGF-β, MHC-I and various immunoregulatory and structural genes. IVIg seems to be also effective in patients with PM but offers only minimal and transient help to a small number of patients with IBM. Rituximab seems helpful in some patients with PM, DM or NAM, but a poorly designed controlled study did not show a difference in the outcome measures between Rituximab and placebo. New biological agents currently on the market will be discussed as promising new therapeutic options for the treatment of inflammatory myopathies. They include various monoclonal antibodies or fusion proteins against: a) molecules associated with T-cell-signaling pathways; b) B cells antigens; c) Complement C5; and d) adhesion or transmigration molecules.

THEMATIC WORKSHOP 4.3

Theme: *THERAPEUTIC APPROACHES TO HEREDITARY MYOPATHIES*

TW 4.3.1 Advanced exon skipping antisense strategies for Duchenne Muscular Dystrophy

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Oligonucleotides to modulate pre-mRNA splicing have therapeutic potential for a range of inherited neuromuscular disorders. The classical example is Duchenne muscular dystrophy (DMD), where modulation of pre-mRNA splicing of the DMD gene can restore a viable reading frame and the expression of functional protein. This approach is currently being evaluated in clinical trials. A limitation of such methods is the poor ability to deliver oligonucleotides effectively to affected tissues including skeletal muscle, heart and brain. A possible solution to this problem is offered by the development of advanced oligonucleotides utilising cell-targeting and cell-penetrating peptides conjugated to or complexed with the oligonucleotide of choice. Such compounds provide greatly improved delivery and enhanced potency and are being developed for future clinical studies in both DMD and for other neuromuscular disorders, such as spinal muscular atrophy.

THEMATIC WORKSHOP 4.3

Theme: *THERAPEUTIC APPROACHES TO HEREDITARY MYOPATHIES*

TW 4.3.2 Autologous myoblast cell therapy for oculopharyngeal muscular dystrophy (OPMD): a phase I/IIa clinical study

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Oculopharyngeal muscular dystrophy (OPMD) is an autosomal dominant inherited, slow progressing, late onset degenerative muscular disorder where a small group of specific muscles (pharyngeal and eyelid) are primarily affected, leading to dysphagia and ptosis. Its genetic basis is a trinucleotide repeat expansion ranging from (GCG)8 to (GCG)13 in the N-terminus polyalanine domain of the the poly(A) binding protein nuclear 1 (PABPN1) gene. Mutated expanded PABPN1 protein accumulates as insoluble nuclear inclusions in muscles of OPMD patients. While the roles of PABPN1 in nuclear polyadenylation and in the regulation of alternative poly(A) site choice are established, the molecular mechanisms behind OPMD including

the specificity of affected muscles and the role of these inclusions remain undetermined. So far there is no cure for OPMD patients. The dysphagia compromises their life expectancy, and the classical myotomy is often of transient benefit. Since in a preclinical study, we demonstrated good myogenic capacities of myoblasts expanded from clinically spared muscles, we launched a phase I/IIa clinical study (ClinicalTrials.gov NCT00773227) using autologous myoblast transplantation following myotomy in adult OPMD patients. 12 OPMD patients were included in the study, and were injected with a median of 178 million myoblasts following myotomy. Feasibility, safety end points and potential therapeutic benefit of both autologous myoblast transplantation as well as the surgical procedure were assessed by radioimaging, quality of life questionnaire and drink test. Short and long-term (2 years) safety and tolerability were observed in all patients, with no adverse effects. There was an improvement in the quality of life score for all 12 patients, and no functional degradation in swallowing was observed for the majority of patients. A cell dose dependant improvement in swallowing was even observed in this safety study. This trial supports the hypothesis that a local injection of autologous myoblasts in the pharyngeal muscles is a safe and efficient procedure for OPMD patients. Since the current protocol involves the autologous transplantation of unmodified cells from spared muscles - which still carry the genetic mutation - we are now developing a new gene therapy strategy to genetically engineer patients cells prior to transplantation in order to restore a normal PABPN1 allele.

THEMATIC WORKSHOP 4.3

Theme: *THERAPEUTIC APPROACHES TO HEREDITARY MYOPATHIES*

TW 4.3.3 AAV microdystrophin gene therapy for Duchenne Muscular Dystrophy

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Muscular dystrophies refer to a group of inherited disorders characterized by progressive muscle weakness, wasting and degeneration. So far, there are no strongly effective treatments but new gene-based therapies are currently being developed. In the case of DMD, a number of groups are testing gene therapy with adeno-associated virus vectors expressing engineered micro-dystrophins (AAV-MDs). In our hands, highly sequence optimised AAV-MDs are available for use in mouse, dog and ultimately humans, expressed using a strong synthetic promoter specific for skeletal and cardiac muscle cells have been tested in detail in mdx mice, and in the GRMD dog. Here we report the outcome of a detail study of AAV-canine MD delivery by isolated limb perfusion. No immunosuppression was applied. In skeletal muscles of the treated limb we report widespread vector biodistribution, and sustained high level MD expression. In addition the treated limb exhibited very significantly improved parameters of muscle turnover (regenerative fibres), fibrosis (Collagen type I), 1H-MRI and 31P-NMR spectroscopy, and muscle strength. In the context of immune parameters, AAV administration elicited anti-AAV2/8 antibodies and a transient elevation of serum cytokines, but no evidence of cellular immunity to microdystrophin or to the AAV vector was observed. The current optimised microdystrophin is thus highly functional, not only in mice, but also in a large animal model, and that AAV2/8 vector system yields sustained microdystrophin expression without adverse immune responses. Current studies are focussed on systemic delivery of AAV-canine MD vectors body-wide in the GRMD model.

THEMATIC WORKSHOP 4.4

Theme: *CONGENITAL MYOPATHIES*

TW 4.4.1 Nemaline Myopathy and related diseases: genetics, diagnosis and therapy

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It is generally accepted that nemaline myopathy was first described in 1963 after electron microscopy became available and the nemaline bodies, pathognomonic for nemaline myopathy, could be clearly visualized. It then took 32 years, before slow alpha-tropomyosin (TPM3) was the first gene found to be mutated in nemaline myopathy (1995). This finding

refocused nemaline myopathy thinking from the Z-disc, whose components most obviously accumulate in nemaline bodies, to the thin filament and led relatively rapidly to mutations causing nemaline myopathy being identified in genes coding for other thin filament proteins: nebulin (NEB)(1999), skeletal muscle alpha actin (ACTA1)(1999), slow troponin T (TNNT1)(2000), beta-tropomyosin (TPM2)(2002), and muscle-specific cofilin (CFL2)(2007). More recently, several BTB Kelch proteins have been associated with nemaline myopathies: (KBTBD13)(2010) with rod-core disease, then, with the advent of next generation sequencing (NGS) gene discovery, KLHL40 and KLHL41 (2013) with severe early, including foetal, onset nemaline myopathy. More genes remain to be found. Whether the Kelch proteins associated with nemaline myopathy are also related to the thin filament remains to be determined. Diseases related to nemaline myopathy include actin aggregate myopathy, cap disease and zebra-body myopathy and these have been associated with mutations in some of the genes associated with nemaline myopathy (ACTA1, CFL2, TPM2 and TPM3). Molecular diagnosis of nemaline myopathy is best performed by NGS, because of the high levels of genetic heterogeneity and the major role of the giant protein nebulin. The identification of the proteins affected in nemaline myopathy has allowed investigation of the pathobiology of the disease. This has identified defects in excitation contraction coupling and thin filament length in the pathobiology of nemaline myopathy. This included the identification of increased thin filament sensitivity to calcium associated with ACTA1 mutation and a clinical phenotype of episodic hypercontraction and hypertonicity rather than the universally expected hypotonia and weakness of nemaline myopathy. This hypercontraction has altered the clinical description of nemaline myopathy to muscle dysfunction rather than muscle weakness. The different pathobiologies indicate that different routes to therapy will be needed for mutations associated with nemaline myopathy, even different mutations within one protein.

THEMATIC WORKSHOP 4.4

Theme: *CONGENITAL MYOPATHIES*

TW 4.4.2 Gene replacement therapy for myotubular myopathy

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Loss-of-function mutations in the myotubularin gene (MTM1) cause X-linked myotubular myopathy (XLMTM), a fatal pediatric disease of skeletal muscle characterized by small centrally nucleated myofibers containing abnormal mitochondrial accumulations. By previous local studies in Mtm1-mutant mice we demonstrated potential efficacy of gene therapy to treat the disease. We will present results showing that systemic administration of a single dose of a recombinant serotype-8 adeno-associated virus (AAV8) vector expressing the murine myotubularin to Mtm1-deficient knockout mice at the onset or at late stages of the disease led to robust improvement in contractile force, corrected the muscle pathology and prolonged survival throughout a 6-month study. Similarly, single-dose intravascular delivery of a canine AAV8-MTM1 vector in XLMTM dogs rescued almost completely motor and respiratory functions, and prolonged lifespan in the absence of toxicity and immune response. These results demonstrate the therapeutic efficacy of AAV-mediated gene therapy for myotubular myopathy in small and large animal models, and provide proof of concept for future clinical trials in XLMTM patients.

THEMATIC WORKSHOP 4.4

Theme: *CONGENITAL MYOPATHIES*

TW 4.4.3 Understanding - and improving - sarcomeric function in Nemaline Myopathy

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Nemaline myopathy - the most common non-dystrophic congenital myopathy - is caused by mutations in genes encoding proteins of the thin filament, a major constituent of the muscle's sarcomere. A hallmark feature of nemaline myopathy is muscle weakness, the cause of which remains only partly understood. This presentations aims to provide an overview of the current knowledge regarding the pathophysiology of muscle weakness in nemaline myopathy and to highlight recent developments in targeting thin filament function using calcium sensitizers.

THEMATIC WORKSHOP 4.5

Theme: *THERAPEUTIC APPROACH IN MYOTONIC DYSTROPHY*

TW 4.5.1 Systemic delivery of a peptide-PMO of CAG sequence neutralizes mutant RNA toxicity in a mouse model of DM1

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Expansions of CUG trinucleotide sequences in RNA transcripts provide the basis for toxic RNA gain-of-function that leads to detrimental changes in RNA metabolism. A CTG repeat element normally resides in the 3' untranslated region of the dystrophia myotonica-protein kinase (DMPK) gene, but when expanded it is the genetic lesion of myotonic dystrophy type 1 (DM1), a hereditary neuromuscular disease. The pathogenic DMPK transcript containing the CUG expansion is retained in ribonuclear foci as part of a complex with RNA-binding proteins such as muscleblind-like 1 (MBNL1), resulting in aberrant splicing of numerous RNA transcripts and consequent physiological abnormalities including myotonia. Herein, we demonstrate molecular and physiological amelioration of the toxic effects of mutant RNA in the HSA(LR) mouse model of DM1 by systemic administration of peptide-linked morpholino (PPMO) antisense oligonucleotides bearing a CAG repeat sequence. Intravenous administration of PPMO conjugates to HSA(LR) mice led to redistribution of Mbnl1 protein in myonuclei and corrections in abnormal RNA splicing. Additionally, myotonia was completely eliminated in PPMO-treated HSA(LR) mice. These studies provide proof of concept that neutralization of RNA toxicity by systemic delivery of antisense oligonucleotides that target the CUG repeat is an effective therapeutic approach for treating the skeletal muscle aspects of DM1 pathology.

Disclosures: A.J.L., L.M.M., P.A.P., S.H.C., and B.M.W own stock in Sanofi.

THEMATIC WORKSHOP 4.5

Theme: *THERAPEUTIC APPROACH IN MYOTONIC DYSTROPHY*

TW 4.5.2 Systemic delivery of an antisense oligonucleotide (ASO) targeting DMPK RNA improves the phenotype of DMSXL mice

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Antisense oligonucleotides (ASO) provide therapeutic opportunities for a wide variety of human diseases. Improvements in ASO chemistry and design have advanced the technology such that systemic administration of therapeutic ASOs has produced robust, antisense-mediated target inhibition and therapeutic benefit in multiple species, including in humans. ASOs are clearly an effective therapeutic modality for liver-expressed genes, as exemplified by

the recent FDA approval of the ASO drug, Kynamaro. Extending the power of ASO drugs to extra-heptatic tissues represents one of the next key steps for the technology. Here we describe the identification and characterization of ASOs containing both 2'-methoxyethyl (MOE) and the next generation constrained ethyl (cEt) chemistry, that were designed to target the expression of the DMPK gene in skeletal muscle. This target was selected because in myotonic dystrophy type I (DM1), a CUG expansion within the DMPK gene results in RNA transcripts that are aberrantly retained in the nucleus producing a toxic gain-of-function effect in skeletal muscle and other tissues. . Previous study demonstrated that ASOs targeting transcripts with expanded CUG repeats reverse molecular changes induced by the transgene (Wheeler et al., Nature 488:111–115).

A screen of >3,000 candidate ASOs targeting different regions of the human DMPK gene produced numerous potent DMPK lead ASOs. Two of these leads, one with MOE chemistry that was selective for human DMPK and one with cEt modifications which was active against both human and mouse DMPK. These ASOs completely abolished nuclear foci, preferentially decreased the levels of mutant RNAs (mtRNAs) up to 88%, induced MBNL1 redistribution and corrected the splicing of a subset of mis- spliced pre-RNAs, in DM1 cells in vitro. In DMSXL mice, subcutaneous injection of ASOs reduced the levels of mtRNAs between 30–40% (MOE) and 67–75% (cET) in 6 skeletal muscles and 30% in the heart (cEt-ASO). The cET-lead decreased the number of foci up to 70% in the quadriceps. DMSXL mice treated with cEt-ASO also showed a significant increase in body weight from 17,9 ± 1,9 g to 18,7 ± 1,7 g (P = 0,016), whereas no significant effect was observed in mice treated with the MOE-ASO. cEt-ASO also increased forelimb force determined by the grip test (+3,4 ±7 g, P = 0.008), motor performances determined by the coat hanger test and, induced a complete (cEt) or partial (MOE) disappearance of immature muscle fibers at the histological levels. Both ASOs had no toxic effects.

This study demonstrates: 1) An efficient strategy to select ASOs active in skeletal muscle; 2) The in vivo proof of the principle of a therapeutic antisense strategy for the treatment of DM1.

THEMATIC WORKSHOP 4.5

Theme: *THERAPEUTIC APPROACH IN MYOTONIC DYSTROPHY*

TW 4.5.3 Targeting nuclear expanded repeats to correct RNA gain-of-function effects in DM1

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Myotonic Dystrophy type 1 (DM1) is one of the most common form of inherited neuromuscular disorders in adults characterized by myotonia, progressive muscle weakness and wasting, cardiac conduction abnormalities, cognitive as well as other multisystemic defects. This autosomal dominant disease is caused by an expanded trinucleotide (CTG) n > 50 repeats located in the 3' non-coding region of the DMPK gene. The size of the expansion is generally correlated with the clinical severity and the age of onset of the disease. Expression of pathogenic DMPK transcripts containing expanded CUG repeats (CUGexp-RNAs) results in a toxic RNA gain-of-function mechanism. The CUGexp-RNAs are retained into the nuclei as riboprotein aggregates or foci that sequester MBNL RNA splicing factors leading to functional loss of MBNL and alternative splicing misregulations of a subset of pre-mRNAs. Thus, mis-splicing of CLCN1 and BIN1 pre-mRNAs contributes respectively to myotonia and muscle weakness in DM1. Several strategies are currently under development to reverse toxic CUGexp-RNAs dominant effects.

In this study, we propose to restore MBNL function and correct alternative splicing misregulations in DM1 cells by interfering with abnormal CUGexp-RNA:MBNL sequestration in order to release endogenous MBNL factors. To this purpose, we have engineered a modified MBNLΔ that keeps its RNA binding property but shows reduced splicing activities. To evaluate its ability to inhibit CUGexp-RNA toxicity, we first expressed a GFP-MBNLΔ construct in DM1 muscle cells by using lentiviral vectors. GFP-MBNLΔ colocalized with the CUGexp-RNA foci and splicing misregulations as well as differentiation defects were corrected in DM1-GFP-MBNLΔ muscle

cells. To further assess this approach in vivo, intramuscular injections of AAV-GFP-MBNLΔ vectors were performed in HSA-LR mice expressing 220CTG in skeletal muscles. As observed in DM1 cells in cultures, colocalization of GFP-MBNLΔ with nuclear CUGexp-RNA foci in myofibers indicates that MBNLΔ will compete and release endogenous MBNL from these aggregates. Splicing alterations of several transcripts were normalized or nearly corrected in HSA-LR injected muscles, and the myotonia was abolished. In conclusion, we propose that a modified MBNLΔ gene therapy approach could represent an alternate or complementary therapeutic approach for DM1.

THEMATIC WORKSHOP 5.1

Theme: *PNS SESSION 1: PATHOGENESIS OF IMMUNE MEDIATED NEUROPATHIES*

TW 5.1.1 Immunology and the nerve

Hans-Peter HARTUNG, Dusseldorf (Allemagne)

Abstract not received

THEMATIC WORKSHOP 5.1

Theme: *PNS SESSION 1: PATHOGENESIS OF IMMUNE MEDIATED NEUROPATHIES*

TW 5.1.2 Nodo-paranodopathy: beyond the classification of myelinopathy and axonopathy

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In some anti-ganglioside antibody-mediated neuropathies, human and experimental data suggest a common pathogenic mechanism of dysfunction/disruption at the node of Ranvier resulting in a pathophysiologic continuum from transitory nerve conduction failure to axonal degeneration. The traditional classification of polyneuropathies into demyelinating or axonal may generate some confusion in the electrophysiological diagnosis of Guillain-Barré syndrome subtypes associated with anti-ganglioside antibodies. The axonal forms show, besides axonal degeneration, promptly reversible nerve conduction failure. This may be interpreted, by a single electrophysiological study, as demyelinating conduction block or distal axonal degeneration leading to errors in classification and in establishing prognosis. Moreover the term axonal may be misleading as it is commonly associated to axonal degeneration and not to a transitory, promptly reversible, dysfunction of the excitable axolemma. To focus on the site of nerve injury and overcome the classification difficulties, we propose the new category of nodo-paranodopathy which seems appropriate to various acute and chronic neuropathies associated with anti-ganglioside antibodies and we think better systematizes the neuropathies characterized by an autoimmune attack targeting the nodal region.

THEMATIC WORKSHOP 5.1

Theme: *PNS SESSION 1: PATHOGENESIS OF IMMUNE MEDIATED NEUROPATHIES*

TW 5.1.3 Conduction block: Schwann cell or Axon pathology?

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Conduction block can be produced by either demyelination or axonal dysfunction, if the safety factor for impulse transmission is critically impaired. Guillain-Barré syndrome (GBS) is classified into the two major categories; acute inflammatory demyelinating polyneuropathy (AIDP) and acute motor axonal neuropathy (AMAN), presenting demyelinating and axonal conduction block respectively. Over the past 20 years, major advances have been made in understanding the immunopathogenesis and pathophysiology of AMAN. AMAN is characterized by pure motor involvement, antecedent Campylobacter jejuni enteritis, and serum anti-ganglioside antibodies. Compared with AIDP, AMAN has more rapid progression and earlier nadir, and two patterns of recovery (rapid improvement by resolution of conduction blocks, and slow recovery by axonal degeneration). Electrophysiological studies frequently reveal, as well as axonal degeneration, rapidly reversible nerve conduction block/slowing (reversible conduction failure); the time-course suggests functional or microstructural changes at the nodes and paranodes. It is now established that AMAN is caused by molecular mimicry of human gangliosides by the

bacterial lipo-oligosaccharide. An animal model of AMAN immunized by GM1 was developed, and its histological studies show disruption of nodal sodium channel clusters, and paranodal myelin detachment; these changes are likely to account for conduction block in human AMAN. Conventional electrodiagnostic criteria for AMAN were based on assumption of simple axonal loss, and therefore should be reconsidered. The mechanisms for axonal conduction block due to microstructural nodal/paranodal pathology are discussed.

Kuwabara S, Yuki N. Axonal Guillain-Barré syndrome: concepts and controversies. Lancet Neurol 2013;12:1180–8.

Hiraga A, et al. Patterns and serial changes in electrodiagnostic abnormalities of axonal Guillain-Barré syndrome. Neurology 2005;64:856–60.

THEMATIC WORKSHOP 5.1

Theme: *PNS SESSION 1: PATHOGENESIS OF IMMUNE MEDIATED NEUROPATHIES*

TW 5.1.4 Nerve pathology: still helpful in the diagnosis of immune neuropathies?

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In this presentation, we will speak mainly about Chronic Inflammatory Demyelinating Polyneuropathies (CIDP) and peripheral neuropathies associated to a monoclonal dysglobulinemia which are treatable conditions. In such patients, the diagnosis is made with sufficient certainty in many cases based on clinical, electrophysiological and CSF findings. Nevertheless, CIDP could and should be more often diagnosed by nerve biopsy (NB), especially in atypical cases (Vallat et al., 2003).

Otherwise, a polyneuropathy (PN) can be observed in any type of benign or malignant monoclonal gammopathy and is a frequent complication of plasma-cell dyscrasias. Sometimes aggressive treatments may be indicated if the nerve biopsy demonstrates lesions that are responsible for the polyneuropathy (PN).

CHRONIC INFLAMMATORY DEMYELINATING PERIPHERAL POLYNEUROPATHIES:

When dealing with a chronic isolated polyradiculoneuropathy with electrophysiological pattern of axonal neuropathy associated with subtle signs of demyelination, CIDP should be envisaged. If the clinical and electrophysiological European Federation of Neurological Societies / Peripheral Nerve Society (Joint task force)) criteria are not present, we usually opt for a NB to confirm the diagnosis of CIDP, a treatable disorder. The diagnostic strategy of CIDP, including the decision to perform a NB has been discussed recently by a group of French neurologists (The French study CIDP group).

Sometimes a few perivascular T cells may be seen on paraffin-embedded or frozen sections, while a few macrophages are diffusely scattered in the endoneurium. According to the histopathological diagnostic criteria for CIDP of the AAN (ref), which are still useful, more than five demyelinated fibers must be detected. On semi-thin sections at low magnification, the heterogeneous distribution of these de- and remyelinating lesions between different fascicles and inside some fascicles may be readily visualized in some cases. By EM, various extents of onion bulb proliferations composed of concentric Schwann cell processes may be seen around more or less completely demyelinated axons. Such axons may be devoid of a myelin sheath or wrapped only by a few myelin lamellae indicating a remyelinating process; some macrophages may destroy the myelin sheaths or myelin debris may be visible inside a macrophage well demarcated from the Schwann cell cytoplasm. A loss of nerve axons is a consistent finding; these secondary axonal lesions develop at a variable rate. In almost all cases, there are clusters of regeneration, which generally reflect abortive attempts at repair.

As mentioned below, these morphological lesions are common to CIDP associated with a monoclonal dysglobulinemia and also to some other general disorders. A NB has to be discussed on a case-by-case basis and so will be reserved to clinically and/or electrophysiologically atypical cases including patients with unexcitable nerves (Vallat et al 2003). Without this evidence, a significant number of unrecognized CIDP patients may be classified as having chronic idiopathic axonal neuropathy and will not be treated appropriately.

POLYNEUROPATHIES ASSOCAITED TO A MONOCLONAL DYSGLOBULINEMIA:

The prevalence of neuropathies in patients presenting a monoclonal gammopathy (MG) has not been evaluated with precision in large prospective studies; nevertheless it has been shown that such PN can be observed in any type of benign or malignant MG and are a frequent complication of plasma-cell dyscrasias

such as "monoclonal gammopathy of undetermined significance" (MGUS), multiple myeloma, Waldenström's disease, POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) syndrome, Castleman's disease, and light-chain amyloidosis. These cases of clinically diverse peripheral neuropathies either occur before the discovery of the gammopathy or as complications of it.

In any case, a coincidental association has to be discussed.

A NB may be of particular importance in patients under potentially neurotoxic chemotherapy. Actually, in such cases, the clinical and electrophysiological findings only, cannot discriminate between a PN induced by a therapeutic agent and other mechanisms. Aggressive treatments such as a specific chemotherapy and/or a bone marrow transplant may be indicated if the nerve biopsy demonstrates that the lesions that we will discuss, are responsible for the PN.

. Endoneural immunoglobulin deposits:
- Intramyelin infiltration:
. IgM:

At the present time if anti-MAG antibodies are significantly elevated in the serum of a patient, NB is usually not done. Nevertheless, in a few reported cases, this procedure has revealed other lesions associated to the characteristic widenings of the myelin lamellae such as malignant cells and amyloidosis deposits.These widenings consist of a 23-nm spacing between the separated leaflets of the intermediate line, which contains an electrolucent material. The ultrastructural features of the dense lines remain unchanged. These widened lamellae are associated with a dilatation of the outer mesaxon and are mainly located on the outer part of the sheath. In some fibers, the widening is restricted to the outermost lamellae, and careful examination is required to notice it. Such features are indicative of a dysglobulinemia, and in a patient with PN of unknown origin, should prompt the search for an M component using appropriate immunologic investigations. Widenings of myelin lamellae are observed in Waldenström's macroglobulinemia and in monoclonal gammopathy of undetermined significance (MGUS). Such lesions have also been described in a few cases in the absence of a dysglobulinemic neuropathy. They should not be confused with other types of uncompacted myelin lamellae which may be encountered in other neuropathies such as P0 mutations or in POEMS syndrome.

. IgG ; IgA :

In these patients the antigens are unknown, there is no anti-MAG activity. In a few cases, immunofluorescence of frozen nerve sections with specific antibodies demonstrate annular bindings to numerous myelinated sheaths which correspond to widenings of the myelin lamellae comparable to those described in IgM-anti MAG neuropathies. Sometimes these lesions may be associated to deposits of immunoglobulins in the endoneural interstitial tissue (see next paragraph). Such findings argue in favor of the MG causing the neuropathy. (Vallat et al 2011).

- Deposits in the interstitial tissue:
. Immunoglobulins

Immunoglobulin deposits of any kind: IgG, IgA or IgM or light chains can only be evidenced by nerve biopsy. They must not be confused with amyloidosis on routine stainings and are better identified by immunocytochemistry on frozen sections and by EM. Actually, according to their size, they can be detected either by immunofluorescence on frozen sections or only by EM. Ultrastructurally, they may have a quite characteristic aspect as digitiform, fibrillar or tubular structures etc…(Moorhouse et al 1992); if not, immunoEM using anti-immunoglobulin antibodies has to be done to confirm their specificity of such. In any case, a cryoprotein should be looked for carefully; if present, we recommend to fix it to compare its ultrastructure with the ultrastructure of the endoneural deposits; their ultrastructural similarity would confirm the presence of the abnormal immunoglobulin inside the nerve.

. Amyloid deposits:

Amyloid deposits are also multifocal, so that they are not seen on all the sections of the NB and can be missed. A negative examination does not exclude an amyloid neuropathy. The sensibility of nerve biopsy for the diagnosis of systemic amyloidosis is said to be around 85%.

On paraffin-embedded sections, amyloid deposits appear as round deposits, scattered in the endoneurium and sometimes joined to thickened capillary walls. AL amyloidosis results from the transformation of an immunoglobulin light chain, which may be identified on frozen specimens with a specific antibody. These deposits are stained by Congo red and thioflavine. By EM examination, amyloid deposits are extracellular

and composed of characteristic bundles of unbranched, 7–10 nm-wide fibrils in an irregular matted form; they are sometimes seen in intimate contact with the basement membranes of endothelial cells and of Schwann cells. The severity of the lesions of the myelinated and unmyelinated fibers is common in amyloidosis, whether of genetic or dysimmune origin.

Even in an association of a monoclonal IgG, which may be coincidental, it is recommended to look systematically for a transthyretin (TTR) mutation. Immuno-EM is of value for differentiating amyloidosis due to light chain from TTR amyloidosis which may induce important genetic consequences.

. POEMS syndrome (Crow Fukase syndrome):

Usually, the diagnosis is clinical and is confirmed by increased levels of vascular endothelial growth factor in the serum. As the clinical signs are often incomplete, the diagnosis may be missed; in a few cases VEGF may be normal. So, in such cases a NB is still realized.

Specific immunocytochemistry studies using immunoglobulin antibodies are negative. EM may disclose uncompacted myelin lamellae (UML) which are characteristic features of this condition and may be visible in 1–7% of the myelinated fibers (Vital et al, 2003). Such a myelin modification corresponds to portions of the mesaxon that are neither flattened nor compacted and that are visible at least around one semi-circumference of the axon, on three or more consecutive lamellae; so, there is abnormally widened space between major dense lines. Fragments of cytoplasm are often seen between these lamellae. These unusual lesions are commonly observed in inner and middle layers of the myelin sheaths, but may also concern the outer layer and must be differentiated from widened myelin lamellae, which are observed in patients with an IgM-antiMAG positive activity. So, the presence of numerous UML on a nerve biopsy may serve to distinguish POEMS from nerve CIDP and other demyelinating peripheral nerve disorders, mainly in the early phase of the disorder. The significance of UML with regard to the pathogenesis of POEMS is still unknown.

Otherwise, the nerve fiber lesions are usually characterized by a mixture of segmental demyelination and axonal degeneration. However, in some cases pure axonal lesions are present . Inflammatory infiltration of the endoneurium and occasionally of the epineurium, though sparse, may be seen.

. Malignant cells :

The presence of lymphoma B or T cells in the peripheral nerve parenchyma, usually in the epineurium, again can only be shown by NB; these cells are detected on routine paraffin embedded sections and their specific types are confirmed by immunopathological examination of deep-frozen NB. Cellular infiltrates may disappear after multiple sections, which are required for the immunopathological studies.

In some cases, the NB may also help to find out whether the neuropathy is due to an associated monoclonal gammopathy or to the presence of the lymphomatous cells or a combination of both (Kelly and Karcher 2005; Vallat et al 1995). In such a context, detection of a clonal immunoglobulin or T-cell receptor gene recombination may be very useful to confirm the lymphomatous infiltration. This is commonly performed by using standardized PCR techniques designed to assess the diversity of the junctional regions in immunoglobulin or T-cell receptor genes. PCR techniques require low amounts of DNA and have a good sensitivity (1–5%) to detect clonality (Langerak et al 2007).

In rare cases of angiotropic lymphoma, there are lymphomatous cells visible in the lumen of arterioles in nerve and muscle specimens (Vital et al, 1989).

. CIDP :

If electrophysiological studies are in favor of a demyelinating polyneuropathy which appears to have no direct link with the MG, CIDP is probable and may result from a common immunological disorder whose primary target antigen remains unknown. To diagnose this type of PN is important as CIDP is a treatable disorder (Vallat et al 2010). The most common association is with IgG. As for the "primitive" CIDP cases, nerve biopsy is not done in typical cases, but may be quite useful to diagnose atypical ones (Vallat et al 2003); the pathological lesions have been described in the chapter about CIDP.

So, in these hematologic disorders it is important to discuss a NB because the demonstration of such specific lesions, as early as possible in the course of the PN, will determine treatment options. It seems that usually, this microscopic study is done not early enough, so that a treatment is instaured too late and the evolution unsurprisingly poor. The frequency of these lesions is surely under-estimated. NB may also reveal several processes in the same case; some authors have shown that that finding serum anti-MAG antibodies does not exclude the diagnosis of amyloidosis.

If therapy of the blood disorder is in progress, its possible role needs also to be evaluated so that other strategies or drugs can be instaured.

No disclosure.

References:

AAN (1991) Research criteria for diagnosis of chronic inflammatory demyelinating polyneuropathy (cidp). Report from an ad hoc subcommittee of the american academy of neurology aids task force. Neurology 41:617–618.

Joint Task Force of the EFNS and the PNS. (2010) European Federation of Neurological Societies/Peripheral Nerve Society. Guideline on management of chronic inflammatory demyelinating polyradiculoneuropathy: report of a joint task force of the European Federation of Neurological Societies and the Peripheral Nerve Society--First Revision. J Peripher Nerv Syst 2010; 15:1–9.

Kawagashira Y, Koike H, Tomita M et al. (2010) Morphological progression of myelin abnormalities in IgM-monoclonal gammopathy of undetermined significance anti-myelin-associated glycoprotein neuropathy. J Neuropathol Exp Neurol 69, 1143–1157.

Kelly JJ, Karcher DS (2005) Lymphoma and peripheral neuropathy: A clinical review. Muscle Nerve 31:301–313.

Langerak AW, Molina TJ, Lavender FL, et al. (2007) Polymerase chain reaction-based clonality testing in tissue samples with reactive lymphoproliferations: Usefulness and pitfalls. A report of the biomed-2 concerted action bmh4-ct98–3936. Leukemia 21:222–229.

Moorhouse DF, Fox RI, Powel HC. (1992). Immunotactoid-like endoneurial deposits in a patient with monoclonal gammopathy of undetermined significance and neuropathy. Acta Neuropathol;84:484–94.

The French CIDP Study Group (2008). Recommendations on diagnostic strategies for chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). J Neurol Neurosurg Psychiatry 2008;79:115–8.

Vallat, J.M., Tabaraud, F., Magy, L., Torny, F., Bernet-Bernady, P., Macian, F., and Couratier, P. (2003). Diagnostic value of nerve biopsy for atypical chronic inflammatory demyelinating polyneuropathy: evaluation of eight cases. Muscle Nerve 27, 478–485.

Vallat JM, Sommer C, Magy L (2010) Chronic inflammatory demyelinating polyradiculoneuropathy: Diagnostic and therapeutic challenges for a treatable condition. Lancet Neurol 9:402–412.

Vallat, J.M., Tabaraud, F., Magy, L., Torny, F., Bernet-Bernady, P., Macian, F., and Couratier, P. (2003). Diagnostic value of nerve biopsy for atypical chronic inflammatory demyelinating polyneuropathy: evaluation of eight cases. Muscle Nerve 27, 478–485.

Vallat, J.M., Funalot, B., and Magy, L. (2011). Nerve biopsy: requirements for diagnosis and clinical value. Acta Neuropathol 121, 313–326.

Vallat, J.M., Tabaraud, F., Magy, L., Torny, F., Bernet-Bernady, P., Macian, F., and Couratier, P. (2003). Diagnostic value of nerve biopsy for atypical chronic inflammatory demyelinating polyneuropathy: evaluation of eight cases. Muscle Nerve 27, 478–485.

Vital C, Héraud A, Vital A.(1989) Acute mononeuropathy with angiotropic lymphoma. Acta Neuropathol 78:105–7.

Vital C, Vital A, Bouillot S (2003). Uncompacted myelin lamellae in peripheral nerve biopsy. Ultrastruct Pathol 27:1–5.

Vital C, Héraud A, Vital A. (1989) Acute mononeuropathy with angiotropic lymphoma. Acta Neuropathol 78:105–7.

THEMATIC WORKSHOP 5.2

Theme: *RECENT ADVANCES IN UNDERSTANDING ANTIGENIC TARGETS IN MYASTHENIA GRAVIS*

TW 5.2.1 Defects of immune regulation in Myasthenia Gravis patients with anti-acetylcholine receptor antibodies

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Myasthenia Gravis (MG) is an autoimmune disease in which the thymus frequently presents signs of inflammation and T cells display defects in suppressive regulation. These immune defects can be due to either the defective function of Treg cells or the resistance of Tconv cells to suppression.

In order to determine which cells were responsible for this defect and to address the mechanisms involved, we performed cross-experiment studies using purified thymic Treg cells and Tconv cells from controls and MG patients. We first confirmed that MG Treg cells were defective in suppressing control Tconv proliferation, and we demonstrated for the first time that MG Tconv cells were resistant to Treg cell suppression. The activation of MG Tconv cells triggered a lower upregulation of FoxP3 and a higher upregulation of CD4 and CD25 than control cells. To investigate the factors that could explain these differences, we compared the transcriptomes of purified thymic Treg and Tconv cells from MG patients and controls.

Many of the pathways revealed by this analysis are involved in other autoimmune diseases, and T cells from MG patients exhibit a Th1/Th17/Tfollicular signature. An increase in IL-17-related genes was only observed in Treg cells, while increases in the pro-inflammatory cytokines, IFN-γ, and TNF-α were observed in both Treg and Tconv cells. In addition, the role of TNF-α in the defect in Tconv cells from MG patients was confirmed by functional studies (Gradolatto et al, J. Autoim, 2014). Finally, we showed that thymic epithelial cells from MG patients contribute to the regulation defects of T cells, and normal thymic epithelial cells can partially restore the immunosuppressive features of MG T cells.

Altogether, our results indicate that the immunoregulatory defects observed in MG patients are caused by both Treg cell and Tconv cell impairment and involve thymic epithelial cells, as well as several proinflammatory cytokines, with TNF-α playing a key role in this process. The chronic inflammation present in the thymus of MG patients could provide an explanation for the escape of thymic T cells from regulation in the MG thymus.

THEMATIC WORKSHOP 5.2

Theme: *RECENT ADVANCES IN UNDERSTANDING ANTIGENIC TARGETS IN MYASTHENIA GRAVIS*

TW 5.2.2 Effector mechanisms of autoantibodies in Myasthenia Gravis

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Myasthenia gravis is a prototype autoimmune disease with circulating antibodies against the nicotinic acetylcholine receptor (AChR) or muscle specific kinase (MUSK) or Low density lipoprotein receptor-related protein 4 (LRP4). Although the autoimmune process is initiated in the thymus the bulk of the autoantibodies are produced in the bone marrow by plasma cells.

In AChR MG antibodies against extracellular domains of the AChR mainly a main immunogenic region (MIR) increase the degradation rate of AChRs at the postsynaptic membrane in the presence of complement. The postsynaptic folding is decreased leaving less space for the reduced numbers of AChRs and sodium channels. These phenomena are called antigenic modulation and complement mediated focal lysis, result in a neuromuscular transmission defect leading to muscle weakness. Antigenic modulation is a temperature dependent mechanism requiring clatrhin and microtubules.

In MUSK MG are mainly of the IgG4 subclass. They interfere with the interaction of LRP4 with MUSK. This process does not require the presence of complement nor crosslinking of MUSK at the cell surface. Anti MUSK antibodies bind to the first Ig-Like domain of MUSK preventing the binding of MUSK to LRP4 (I. Koneczny PLOS one 2013 and M.Huijbers PNAS 2013). The disruption of the MUSK-LRP4 complex inhibits the MUSK phosphorylation by neural agrin. Similarly to AChR MG, MUSK MG endplates are severely damaged resulting in severe clinical symptoms.

In contrast LRP4 MG patients have less severe clinical symptoms.(Zisimopoulou J Autoimmunity 2013) Lrp4 is a member of the low-density lipoprotein related receptor family and the receptor for neuronal agrin and activates MuSK Antibodies against LRP 4 are of the IgG1 subclass and may therefore fix complement when and possibly also disrupt the interaction between LRP4 and MUSK.

In all types of autoimmune MG it is crucial to lower the antibody titers rapidly which is best achieved by plasmapheresis in addition it is to immunosuppressive therapy. Targeting plasma cells with proteasome inhibitors opens new perspectives for rapid improvement of MG.

THEMATIC WORKSHOP 5.2

Theme: *RECENT ADVANCES IN UNDERSTANDING ANTIGENIC TARGETS IN MYASTHENIA GRAVIS*

TW 5.2.3 New autoantigens in MG

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Double-seronegative myasthenia gravis (dSN-MG, without detectable AChR and MuSK antibodies, measured with the classical assays), occurring in about 20% of MG patient, presents a serious gap in MG diagnosis and understanding. Recently, autoantibodies against LRP4 have been identified in several dSN-MG sera. Our aim was the development of sensitive assays to be used routinely in the diagnosis of MG for the reliable and efficient detection of antibodies in dSN-MG. We have applied cell based assays (CBA) based on LRP4, MuSK and AChR clusters expressed in HEK293 cells. With these CBAs we have found 18.7% (119/635), 14.3% (100/700) and 3.5% (7/200) in the sera of dSN-MG patients from 12 countries being positive for LRP4, MuSK and AChR clusters, respectively. Interestingly, we found that double positive sera, namely LRP4+/AChR+(7.5%) or LRP4+/MuSK+ (18.2%) are more frequent, compared to AChR+/MuSK+ double positives as described in the literature. Moreover, we found that ocular MG was frequent among the LRP4+ patients. The clinical data of the LRP4-MG patients showed that at disease onset symptoms were much milder than in AChR and MuSK MG. However, although LRP4 antibodies were not detected in any of 90 tested sera from healthy controls and were rare in other neuroimmune diseases, they were detected in 24/104 (23.4%) patients with amyotrophic lateral sclerosis (ALS), suggesting a broader involvement of the LRP4 antibodies. Overall the three CBAs detected autoantibodies in the 36.5% of the dSN-MG patients.

THEMATIC WORKSHOP 5.3

Theme: *NEUROMUSCULAR DISORDERS OF MITOCHONDRIAL FUSION AND FISSION*

TW 5.3.1 Novel phenotypes associated with mitochondrial dynamics deficiency

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Mitochondrial fusion and fission are fundamental processes that govern mitochondrial function from yeast to humans. In mammalian cells, thre large GTPases are important for mitochondrial fusion which requires the coordinated fusion of the outer and inner membranes. The mitofusins Mfn1 and Mfn2 are located on the mitochondrial outer membrane and are involved in early steps of membrane fusion. The dynamin-related protein OPA1 is associated with the inner membrane and is essential for membrane fusion. Mitochondrial fission is mediated by a single dynamin-related protein, DRP1. Loss of either fusion or fission activity results in dysfunctional mitochondria. In addition to its well recognized function of controlling mitochondrial morphology, mitochondrial fusion clearly protects mitochondrial function. The need for exchange of matrix content between mitochondria, including mtDNA copies, explains probably in part the importance of this process.

Mutations in MFN2 and OPA1 genes have been associated with neurodegenerative disorders. MFN2 mutations are a major cause of primary axonal Charcot-Marie-Tooth disease type 2A (CMT2A), an autosomal dominant neuropathy that impairs motor and sensory neurons. OPA1 mutations are responsible for Dominant Optic Atrophy (DOA), that is characterized by retinal ganglion cell degeneration leading to optic neuropathy. Recently, it has been shown that both MFN2 and OPA1 mutations can be responsible for syndromic forms of DOA, including mitochondrial myopathy with COX-negative and ragged red fibres, called DOA "plus" phenotypes. These phenotypes are related to mtDNA instability with multiple mtDNA deletions, suggesting that mitochondrial fusion controls mtDNA integrity. These novel clinical presentations and the link with mtDNA maintenance will be discussed.

THEMATIC WORKSHOP 5.3

Theme: *NEUROMUSCULAR DISORDERS OF MITOCHONDRIAL FUSION AND FISSION*

TW 5.3.2 Neuromuscular complications in patients with autosomal dominant optic atrophy

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Autosomal dominant optic atrophy (DOA) is the most common inherited optic nerve disorder in the general population. It affects at least 1 in 25,000 individuals and OPA1 is the major causative gene in about 60% of families. Visual loss starts in early childhood secondary to the highly tissue-specific loss of one cell type – the retinal ganglion cell (RGC). Although progressive visual failure remains the defining feature of DOA, we recently described the expanding phenotypic spectrum associated with OPA1 disease. Up to 20% of mutational carriers developed a more severe "DOA+" phenotype characterised by prominent neuromuscular features such as myopathy, peripheral neuropathy, ataxia, and chronic progressive external ophthalmoplegia. Histochemical and molecular characterisation of skeletal muscle biopsies revealed the presence of cytochrome c oxidase (COX) deficient fibres and multiple mitochondrial DNA (mtDNA) deletions in the majority of patients harbouring pathogenic OPA1 mutations.

By investigating these tissue samples further, we have uncovered some key pathological consequences of OPA1 mutations, namely:

1. A three-fold increased risk of developing the more severe DOA+ phenotype with missense OPA1 mutations involving the GTPase domain compared with other mutational subgroups. These patients also had significantly worse visual acuities with more pronounced thinning of the RGC layer. The greater deleterious consequences of this specific group of OPA1 mutations on RGC survival is therefore clearly linked to the development of multisystem organ involvement in DOA+, suggesting a dominant-negative effect.

2. The induction of a significant in vivo mitochondrial oxidative phosphorylative defect, with the frequency of COX-deficient muscle fibres being over four times higher in the DOA+ group compared with the pure DOA group.

3. A marked mitochondrial proliferative response and the accumulation of clonally-expanded, somatic mtDNA deletions in these COX-negative muscle fibres.

Our experimental findings have firmly established OPA1 as a novel disorder of mtDNA maintenance. Dissecting the disease mechanisms leading to optic atrophy and neuromuscular involvement in DOA will have important therapeutic implications.

References

1. Yu-Wai-Man P et al. Multi-system neurological disease is common in patients with OPA1 mutations. Brain. 2010;133:771–86.

2. Yu-Wai-Man P et al. OPA1 mutations cause cytochrome c oxidase deficiency due to loss of wild-type mtDNA molecules. Human Molecular Genetics. 2010;19:3043–52.

THEMATIC WORKSHOP 5.3

Theme: *NEUROMUSCULAR DISORDERS OF MITOCHONDRIAL FUSION AND FISSION*

TW 5.3.3 Mitochondrial division and mitophagy in the brain and heart

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Mitochondria divide and fuse at different rates in different cell types. While mitochondria are highly dynamic in the brain, mitochondria are very static in the heart. Nonetheless, altered mitochondrial division and fusion in these two organs are associated with many diseases such as neurological disorders and heart failure. In this talk, I will discuss our recent findings on the physiological functions of mitochondrial division in neurons and cardiomyocytes using tissue-specific mouse knockout for the mitochondrial division protein Drp1. When Drp1 was deleted in Purkinje cells in the cerebellum, mitochondrial tubules elongated due to excess fusion, became large spheres due to oxidative damage, and accumulated ubiquitin and mitophagy markers, leading to respiration defects and neurodegeneration. Treatment with antioxidants rescued cell death in Drp1KO Purkinje cells. When Drp1 was lost in cardiomyocytes, mitochondria also became defective in mitophagy and accumulated oxidative damage, resulting in lethal cardiac failure. Our findings suggest that mitochondrial division serves as a universal mechanism to pro-

tect the organelle from oxidative stress and thus ensures tissue maintenance in mammalian organs.

THEMATIC WORKSHOP 5.4

Theme: *METABOLIC NEUROPATHY*

TW 5.4.1 Underlying Metabolic Mechanisms in the Pathogenesis of Diabetic Neuropathy

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382 million people have diabetes in 2013 and by 2035 this will rise to 592 million. Type 2 diabetes accounts for 90% of all cases with the greatest number of affected people between 40 to 59 years of age. Eighty percent of people with diabetes live in low- and middle-income countries. At least half of all people with diabetes develop diabetic neuropathy (DN), a condition with significant morbidity including foot ulcers and amputations. The underlying pathogenesis of DN is a source of active investigation. A decade ago, the "glucocentric" hypothesis stated that DN was due in large part to impaired glucose flux and metabolism. Excess neuronal glycolysis overloads the mitochondrial electron transport chain, leading to the generation of reactive oxygen species (ROS). This process occurs in parallel with increased flux through the polyol and hexosamine pathways as well as the generation of advanced glycation endproducts, with all 3 pathways further promoting ROS accumulation. A vicious feed forward cycle occurs of continuous impaired glucose flux, disruption of downstream pathways, ROS accumulation and cellular injury.

In the last decade, this hypothesis has been expanded, with two ideas. First, nervous system injury in patients and experimental models of DN are not limited to neurons, axons and nerve terminals, but also include glial cells and endothelial cells of the microvasculature. Secondly, DN is secondary to not only glucose-mediated injury but also nervous system injury secondary to the metabolic syndrome (MS). The MS is a cluster of risk factors that include abdominal obesity, dyslipidemia with high triglycerides and low HDL cholesterol, hypertension and hyperglycemia. Dyslipidemia promotes nervous system inflammation, ROS and apoptosis and in human clinical studies of type 2 diabetes and DN, is more closely related to nervous system injury than hyperglycemia. Additionally, the MS is closely associated with insulin resistance in metabolically active tissues like fat and muscle. Recently insulin resistance was shown to be present in the peripheral nervous system, with disruption of intracellular signaling leading to Mt and endoplasmic reticulum stress, ROS accumulation, DNA damage and the development of DN. In summary, the newly evolving concept that all components of the metabolic syndrome, and not just hyperglycemia, underlie the onset and progression of DN, has broad implications on not only the development of new therapies but also the clinical care of patients.

THEMATIC WORKSHOP 5.4

Theme: *METABOLIC NEUROPATHY*

TW 5.4.2 Metabolic neuropathy: Clinical manifestations and current treatment

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Of the metabolic syndrome (MetS) components, diabetes and pre-diabetes have the strongest evidence supporting a pathogenic link with neuropathy, but each of the other components also have evidence supporting their association with neuropathy in diabetic populations. Specifically, obesity has been shown by multiple investigators to be associated with neuropathy in diabetic patients.(1–3) Similarly, in a study of 427 diabetic patients with mild to moderate diabetic neuropathy, elevated triglycerides correlated with loss of sural nerve myelinated fiber density, a direct anatomical measurement of neuropathy.(4) Furthermore, patients with normoglycemia and neuropathy have the same prevalence of MetS components as those with IGT and neuropathy, and an even higher prevalence of MetS components than those with diabetes and no neuropathy.(5) These results indicate that MetS and its components are likely to be important in non-diabetic populations as well. Given the clustering of MetS components, hypertension, hypertriglyceridemia, dyslipidemia, and particularly obesity are prime candidates to be the essential factors underlying the neuropathy present in patients with type 2 diabetes.

The only component of MetS with an established treatment for the prevention of neuropathy is diabetes. Enhanced glucose control has been shown to decrease the incidence of neuropathy in patients with type 1 diabetes, with little effect in those with type 2 diabetes.(6–9) In type 1 diabetes, enhanced glucose control

can be achieved through diet and exercise and insulin. Similar diet and exercise regimens with the addition of metformin, sulfonylureas, and other less common drugs, provides improved glycemic control but little protection against neuropathy in type 2 diabetes. Diet and exercise in those with pre-diabetes and neuropathy has been shown to increase nerve fiber density, but no controlled clinical trial has been performed to confirm this finding.(10) Furthermore, diet, exercise, and metformin reduce the incidence of diabetes in those with pre-diabetes, but the effect on the prevention of neuropathy is unclear.(11) While effective pharmaceutical treatments exist for hypertension, hypertriglyceridemia, and dyslipidemia, no studies have investigated the effect of these interventions on the prevention or improvement of neuropathy. Similarly, while diet and exercise programs and medications can be effective in the treatment of obesity, no current data exist on the effect of these interventions on peripheral neuropathy in this population. Importantly, diet and exercise regimens have the potential to treat MetS as a whole; however, compliance and long term maintenance on these regimens are notoriously difficult.

Conflicts of interest: Dr. Callaghan receives research support from Impeto Medical and performs center certifications for the ALS Association.

1. Straub RH, Elbracht R, Kramer BK, Roth M, Palitzsch KD, Scholmerich J. Influence of digoxin-like immunoreactive factor on late complications in patients with diabetes mellitus. Eur J Clin Invest. 1994 Jul;24(7):482–7. PubMed PMID: 7957506. Epub 1994/07/01. eng.

2. Tesfaye S, Chaturvedi N, Eaton SE, Ward JD, Manes C, Ionescu-Tirgoviste C, et al. Vascular risk factors and diabetic neuropathy. N Engl J Med. 2005 Jan 27;352(4):341–50. PubMed PMID: 15673800. Epub 2005/01/28. eng.

3. Van Acker K, Bouhassira D, De Bacquer D, Weiss S, Matthys K, Raemen H, et al. Prevalence and impact on quality of life of peripheral neuropathy with or without neuropathic pain in type 1 and type 2 diabetic patients attending hospital outpatients clinics. Diabetes Metab. 2009 Jun;35(3):206–13. PubMed PMID: 19297223. Epub 2009/03/20. eng.

4. Wiggin TD, Sullivan KA, Pop-Busui R, Amato A, Sima AA, Feldman EL. Elevated triglycerides correlate with progression of diabetic neuropathy. Diabetes. 2009 Jul;58(7):1634–40. PubMed PMID: 19411614. Pubmed Central PMCID: 2699859. Epub 2009/05/05. eng.

5. Smith AG, Rose K, Singleton JR. Idiopathic neuropathy patients are at high risk for metabolic syndrome. J Neurol Sci. 2008 Oct 15;273(1–2):25–8. PubMed PMID: 18606421. Pubmed Central PMCID: 2576295. Epub 2008/07/09. eng.

6. Effect of intensive diabetes treatment on nerve conduction in the Diabetes Control and Complications Trial. Ann Neurol. 1995 Dec;38(6):869–80. PubMed PMID: 8526459. Epub 1995/12/01. eng.

7. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. Lancet. 1998 Sep 12;352(9131):837–53. PubMed PMID: 9742976. Epub 1998/09/22. eng.

8. Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, et al. Glucose control and vascular complications in veterans with type 2 diabetes. The New England journal of medicine. 2009 Jan 8;360(2):129–39. PubMed PMID: 19092145. Epub 2008/12/19. eng.

9. Ismail-Beigi F, Craven T, Banerji MA, Basile J, Calles J, Cohen RM, et al. Effect of intensive treatment of hyperglycaemia on microvascular outcomes in type 2 diabetes: an analysis of the ACCORD randomised trial. Lancet. 2010 Aug 7;376(9739):419–30. PubMed PMID: 20594588. Epub 2010/07/03. eng.

10. Smith AG, Russell J, Feldman EL, Goldstein J, Peltier A, Smith S, et al. Lifestyle intervention for pre-diabetic neuropathy. Diabetes Care. 2006 Jun;29(6):1294–9. PubMed PMID: 16732011. Epub 2006/05/30. eng.

11. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002 Feb 7;346(6):393–403. PubMed PMID: 11832527. Pubmed Central PMCID: 1370926. Epub 2002/02/08. eng.

THEMATIC WORKSHOP 5.4

Theme: *METABOLIC NEUROPATHY*

TW 5.4.3 Advances in the diagnosis and clinical interventions for metabolic neuropathy

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Current guidelines recommend that diagnosis of distal symmetric polyneuropathy (DSP) rely on the demonstration of a combination of symptoms, signs and confirmatory neurophysiological or pathological testing1. Reliable recognition of clinical features of DSP is challenging, even for experts, resulting in reliance on nerve conduction studies (NCS)2. However, NCS are frequently normal in early DSP, particularly among patients with preferential small fiber involvement. Skin biopsy with measurement of intraepidermal nerve fiber density (IENFD) has demonstrated excellent diagnostic performance for patients with suspected small fiber neuropathy. Several other techniques, such as corneal confocal microscopy (CCM) hold promise as diagnostic tools. While there are no pharmacological interventions shown to alter the natural history of diabetic or idiopathic DSP, data from a growing number of studies suggest lifestyle interventions, particularly mixed aerobic exercise and strength training, may be effective in enhancing peripheral nerve regenerative capacity, resulting in slowed neuropathy progression and improved patient symptoms.3,4 These strategies likely work by addressing the detrimental effects of obesity and its downstream complications of prediabetic insulin resistance, dyslipidemia and other aspect of the "Metabolic Syndrome"5. There are also a number of symptomatic approaches to DSP with demonstrated efficacy. Current approaches to diagnosis of DSP will be reviewed. Current treatment approaches, including a brief review of symptomatic management will be discussed. New models for therapeutic development, including innovative clinical trial designs for DSP will be discussed.

References:

1. Tesfaye S, Boulton AJ, Dyck PJ, et al. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diabetes Care. 2010 ed. 2010 Oct;33(10):2285–2293.

2. Dyck PJ, Overland CJ, Low PA, et al. Signs and symptoms versus nerve conduction studies to diagnose diabetic sensorimotor polyneuropathy: Cl vs. NPhys trial. Muscle Nerve. 2010 ed. 2010 Aug;42(2):157–164.

3. Smith AG, Russell J, Feldman EL, et al. Lifestyle intervention for pre-diabetic neuropathy. Diabetes Care. 2006 Jun;29(6):1294–1299.

4. Kludig P, Pasnoor M, Singh R, et al. The Effect of Exercise on Neuropathic Symptoms, Nerve Function, and Cutaneous Innervation inPeople with Diabetic Peripheral Neuropathy. Journal of Diabetes and Its Complications. 2013 (26):424–429.

5. Smith AG. Impaired glucose tolerance and metabolic syndrome in idiopathic neuropathy. Journal of the Peripheral nervous system : JPNS. 2012 May;17 Suppl 2:15–21.

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THEMATIC WORKSHOP 5.5

Theme: *NUCLEAR ENVELOPE / NUCLEAR MATRIX*

TW 5.5.1 Emerin-LAP1 Interaction and X-linked Emery-Dreifuss Muscular Dystrophy

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X-linked Emery-Dreifuss muscular dystrophy is caused by loss of function of emerin, an integral protein of the inner nuclear membrane. Mice lacking emerin, however, are essentially normal, suggesting the existence of compensating factors. We show that lamina-associated polypeptide1 (LAP1) interacts with emerin and that conditional deletion of LAP1 from striated muscle causes muscular dystrophy. Striated muscle-selective LAP1 knockout mice also display ventricular systolic dysfunction with abnormal induction of genes encoding cardiomyopathy-related proteins. Skeletal muscle pathology in mice with LAP1 deleted from this tissue is worsened in the absence of emerin. LAP1 levels are significantly higher in mouse than human skeletal muscle, potentially explaining why mice lacking emerin are essentially normal whereas humans develop muscular dystrophy. Reducing LAP1 by approximately half also induces skeletal muscle abnormalities in emerin null mice. Conditional deletion of LAP1 from hepatocytes yields mice that exhibit normal liver function and are indistinguishable from littermate controls, demonstrating a selective role for LAP1 in striated muscle. These results

establish that LAP1 interacts physically and functionally with emerin. They are also consistent with emerging case reports of mutations in the gene encoding LAP1 causing muscular dystrophy in human patients.

THEMATIC WORKSHOP 5.5

Theme: *NUCLEAR ENVELOPE / NUCLEAR MATRIX*

TW 5.5.2 Lamin A/C and striated muscle laminopathies

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The nuclear lamina underlies the nuclear face of the inner nuclear membrane of eukaryotic cells. Because of its well-described roles in maintaining nuclear envelope integrity and providing anchoring sites for chromatin domains, the nuclear lamina plays a significant role in both the nuclear architecture and function. The main components of the nuclear lamina are intermediate filament proteins, the nuclear lamins. LMNA encodes the A-type lamins. The discovery that dilated cardiomyopathy can be caused by LMNA mutations has resulted in a re-evaluation of the function of the nuclear lamina. An emerging body of work supports a new view of the nuclear lamina as a node that integrates and transduces a range of signals. Hence, beyond its classical barrier function, studies of the nuclear lamina are increasingly providing insights into basic aspects of cellular organization and function and providing novel insights into the pathogenesis of human disease.

To explore the pathogenesis of LMNA-cardiomyopathy, we carried out a genome-wide RNA expression analysis of hearts from a mouse model of the disease (Lmna H222P knock-in). This analysis revealed significant differences in expression of genes encoding proteins in stress-activated MAP kinases and AKT/mTOR signaling pathways in the mutated mice (Muchir et al. 2007). We confirmed an aberrant increase in both signaling in hearts from Lmna H222P knock-in mouse hearts (Muchir et al. 2007, Choi et al. 2012). This led us to hypothesize a model of how abnormalities of A-type lamins may lead to cardiomyopathy. We proposed a mechanism for the pathogenesis of LMNA-cardiomyopathy, for which abnormal cardiac activation of MAP kinases lead to an increase in AKT/mTOR signaling, which reduces tolerance to energy deficit (Choi et al. 2012).

These results provide proof of principle for small molecule inhibition as a therapeutic option to delay the onset of heart failure in LMNA-cardiomyopathy. Pharmacological blockade of MAP kinase (Wu et al. 2011; Muchir et al., 2011) or AKT/mTOR (Choi et al. 2012) cascade in Lmna H222P knock-in mice blunts left ventricular dilatation and deterioration in cardiac contractility. While it remains unclear how alterations in A-type lamins lead to activation of these signaling in the heart, these studies clearly show that the abnormal activations are involved in the pathophysiology of LMNA-cardiomyopathy.

Muchir et al. Activation of MAPK Pathway Links LMNA Mutations to Cardiomyopathy in Emery-Dreifuss Muscular Dystrophy. J. Clin. Invest. 2007.

Wu et al. Mitogen activated protein kinase inhibitors improve heart function and prevent fibrosis in cardiomyopathy caused by lamin A/C gene mutation. Circulation 2011.

Choi et al. Temsirolimus activates autophagy and ameliorates cardiomyopathy caused by lamin A/C gene mutation. Sci. Transl. Med. 2012.

THEMATIC WORKSHOP 5.5

Theme: *NUCLEAR ENVELOPE / NUCLEAR MATRIX*

TW 5.5.3 Abnormal nuclear mechanics and mechanotransduction in muscular laminopathies

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Unlike the static pictures found in many text books, cells live in a highly dynamic, mechanically active environment. Cells exert physical forces on intracellular structures and their environment to facilitate movement and migration; at the same time, cells and organelles are subjected to forces from their surroundings, resulting in often substantial deformations. Here, I will focus on intracellular biomechanics, particularly the cell nucleus and its connection to the cytoskeleton.

Recent discoveries provide compelling evidence that the physical properties of the nucleus and its structural organization are critical for a multitude of cellular functions. For example, cell migration requires coordinated movement of the nucleus with the cell body, and neutrophils have evolved highly lobulated nuclei that increase their physical plasticity, enabling passage through narrow spaces. Not surprisingly then, disturbed nuclear structure and mechanics, often caused by inherited mutations in nuclear envelope proteins such as lamins, are responsible for many diseases, including muscular dystrophies, dilated cardiomyopathy, familial partial lipodystrophy, and Hutchinson-Gilford progeria syndrome. My laboratory has developed novel experimental techniques to study the effect of mutations on nuclear structure and mechanics and the physical coupling between the nucleus and the cytoskeleton, as well as to examine how changes in these properties can modulate cellular functions. Using these techniques, we have demonstrated that lamin mutations associated with muscular dystrophies can reduce nuclear stability, thereby rendering cells more susceptible to damage in mechanically active tissues such as muscle and providing a potential explanation for the muscle-specific phenotypes. Interestingly, we also found that many of these mutations impair cellular mechanotransduction signaling, i.e., the ability of cells to respond to mechanical stimulation, which could further exacerbate the muscular phenotypes. In addition, mutations in nuclear envelope proteins that cause muscular dystrophies can also disrupt nucleo-cytoskeletal coupling, which is critical for nuclear positioning, intracellular force transmission, cellular polarization, and migration. I will present these and other recent findings from our laboratory and discuss the close relationship between nuclear mechanics and cellular organization and its role in muscular disease caused by mutations in nuclear envelope proteins.

THEMATIC WORKSHOP 6.1

Theme: *PNS SESSION 2: NEW OPTIONS IN THE TREATMENT OF IMMUNE MEDIATED NEUROPATHIES*

TW 6.1.1 Guillain-Barré syndrome: how to assess and to treat individuals with poor prognosis

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Introduction: Randomized controlled trials showed that intravenous immunoglobulin (IVIg) and plasma exchange (PE) are effective in patients with Guillain-Barré syndrome (GBS)1,2,3. Based upon trial results, these treatments need to be started early in the course of disease, within the first two weeks (or within four weeks for PE) after onset of weakness, presumably before partial irreversible nerve damage has been occurred. Despite standard IVIg or PE treatment, about 25% of patients require artificial ventilation and 20% is still unable to walk after half a year. It is clear that treatment needs to be improved, especially in the group of patients being severely involved and having a slow recovery phase.

Predict the course of disease in GBS by using prediction models: Since the course and severity of disease can vary widely, it is a challenge to predict the course of disease in individual patients. I will discuss two simple prognostic tools that easily can be used at the patient's bedside to predict the prognosis. The Erasmus GBS Respiratory Insufficiency Scale (EGRIS) enables to calculate the chance to need artificial ventilation within two weeks after admission4. It can help to decide if a patient may need direct ICU admission or otherwise very frequent neurological and pulmonary controls at a ward. This scale can be used already at hospital admission and only requires three variables: duration between onset of weakness and admission, presence of facial or bulbar weakness, and the MRC sumscore of 6 pairs of limb muscles.

Another helpful scale is the modified GBS Outcome Scale (mEGOS)5. This scale can be used already one week after hospital admission to predict the chance to walk unaided after 6 months. To determine this chance, it requires the MRC sumscore (range 0–60) at 7 days after admission, the age of the patient and the presence of diarrhea before onset of GBS. There is a website available (www.gbstools.org) that enables to use EGRIS and mEGOS and to do the calculations in a very easy way.

Usage of prediction models in clinical trials: mEGOS is being used in the ongoing Second IVIg Dose (SID)-GBS randomized placebo controlled trial that is running in the Netherlands, and in the international prospective (ISID)-GBS study as part of the international GBS outcome study (IGOS). Both studies investigate the effect of a second IVIg course in GBS patients with a poor prognosis.

GBS patients may have a secondary deterioration or turn out to have acute-onset CIDP: Approximately 10% of GBS patients have a secondary deterioration often named 'treatment-related fluctuation' (TRF). Neurologists should take care for these fluctuations,

since it requires re-treatment with IVIg of PE. Additionally, about 5 percent of patients initially diagnosed as GBS turn out to have acute onset CIDP (A-CIDP). These patients should be treated like CIDP, which indicates repeated IVIg infusions, or even a switch to treatment with corticosteroids. It can be difficult to make a distinction between GBS-TRF and A-CIDP. From a prospective study it turned out that patients initially diagnosed as GBS, who have 3 or more deteriorations, of those having deteriorations after 8 weeks from onset of weakness very likely have A-CIDP6.

References:

1. van Doorn PA, Ruts L, Jacobs BC. Clinical features, pathogenesis, and treatment of Guillain-Barre syndrome. Lancet Neurol 2008;7:939–50.
2. Hughes RA, Swan AV, van Doorn PA. Intravenous immunoglobulin for Guillain-Barre syndrome. Cochrane Database Syst Rev 2012;7:CD002063.
3. Raphael JC, Chevret S, Hughes RA, Annane D. Plasma exchange for Guillain-Barre syndrome. Cochrane Database Syst Rev 2012;7:CD001798.
4. Walgaard C, Lingsma HF, Ruts L, Drenthen J, van Koningsveld R, Garssen MJ, van Doorn PA, Steyerberg EW, Jacobs BC. Prediction of respiratory insufficiency in Guillain-Barre syndrome. Ann Neurol 2010;67:781–7.
5. Walgaard C, Lingsma HF, Ruts L, van Doorn PA, Steyerberg EW, Jacobs BC. Early recognition of poor prognosis in Guillain-Barre syndrome. Neurology 2011;76:968–75.
6. Ruts L, Drenthen J, Jacobs BC, van Doorn PA, Dutch GBSSG. Distinguishing acute-onset CIDP from fluctuating Guillain-Barre syndrome: a prospective study. Neurology 2010;74:1680–6.

THEMATIC WORKSHOP 6.1

Theme: *PNS SESSION 2: NEW OPTIONS IN THE TREATMENT OF IMMUNE MEDIATED NEUROPATHIES*

TW 6.1.2 When and how to treat CIDP?

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Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), is a chronic acquired demyelinating neuropathy that in over 80% of the patients improves with immune therapies. A few variants of CIDP have been described including Distal Acquired Demyelinating Symmetric (DADS), multifocal acquired demyelinating sensorimotor neuropathy also named Lewis Sumner syndrome, purely sensory, purely motor and focal demyelinating neuropathy. If their inclusion as atypical CIDP is useful in prescribing expensive therapy such as intravenous immunoglobulins (IVIg) that are effective in CIDP, it does not help to clarify whether these are phenotypic variants of CIDP or separate diseases. This has also therapeutic implications since motor CIDP may worsen with steroids that is often effective in CIDP. Several immune therapies have been reported to be effective in CIDP including steroids, plasma exchange and IVIg. It is therefore difficult for the clinician to decide when to start treatment in CIDP and what therapy to first use. This decision should consider the efficacy, cost and side effects of these therapies. This also explain why patients with minimal symptoms not interfering with their daily life are usually treated with symptomatic therapy and periodic assessment. A few randomized trial in CIDP have shown a comparable short-term efficacy of IVIg and oral corticosteroids and of IVIg and plasma exchange in and more recent trials have shown that both IVIg and steroids have prolonged efficacy in CIDP. A recent randomized controlled trial comparing the six-month efficacy of IVIg and intravenous methylprednisolone showed that IVIg were more frequently effective and tolerated than steroids during the first six month of treatment, although, when effective, steroids were less frequently associated with deterioration than IVIg after therapy discontinuation. There were not significant differences in the proportion of patients experiencing adverse events even if slightly more adverse events were observed after steroids. It remains unclear whether these advantages are sufficient to balance the much higher cost of IVIg compared to steroids. Even if similarly effective to IVIg, plasma exchange is usually considered the third choice since it is more invasive for the patients and has an higher prevalence of side effects. A number of immunosuppressive agents have been also reported to be useful in CIDP even if their efficacy was not confirmed in randomized trials.

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THEMATIC WORKSHOP 6.1

Theme: *PNS SESSION 2: NEW OPTIONS IN THE TREATMENT OF IMMUNE MEDIATED NEUROPATHIES*

TW 6.1.3 Multifocal motor neuropathy: IVIg and ... what else?

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Multifocal Motor Neuropathy (MMN) is a rare, male predominant, young onset syndrome characterized by progressive asymmetric distal weakness with partial motor conduction block on nerve conduction studies, and clinical improvement following IVIg administration.

MMN should enter the differential diagnosis of any patient with a slowly or stepwise progressive asymmetrical limb weakness without objective sensory abnormalities, upper motor neuron or bulbar signs or symptoms.

The Core Criteria are Slowly progressive or stepwise progressive, focal, asymmetrical limb weakness AND Lack of objective sensory abnormalities except for minor vibration sense abnormalities in lower limbs.

Supportive clinical criteria include predominant upper limb involvement, decreased or absent tendon reflexes in the affected limbs, absence of cranial nerve involvement, cramps and fasciculations in the affected limb, and response in terms of disability or muscle strength to immunomodulatory treatment.

Supportive criteria include elevated IgM anti-ganglioside GM1 antibodies, increased CSF protein, magnetic resonance imaging showing increased signal intensity on T2-weighted imaging associated with a diffuse nerve swelling of the brachial plexus, and objective clinical improvement following IVIg treatment.

Exclusion criteria include upper motor neuron signs, marked bulbar involvement, sensory impairment other than minor vibration loss in the lower limbs, and diffuse symmetric weakness during the initial weeks.

The presence of partial motor conduction block (PMCB) in motor nerve fibers is the electrodiagnostic hallmark of the disease. Some patients with otherwise typical MMN have no detectable PMCB, probably because these blocks are activity-dependent or are located in nerve segments which cannot be assessed by routine electrophysiological examination. Some may have "MMN without PMCB."

Randomized controlled trials show that intravenous immunoglobulin has a beneficial effect on strength and ability. (van Schaik et al. Intravenous immunoglobulin for multifocal motor neuropathy. Cochrane Database of Systematic Reviews 2009 DOI: 10.1002/14651858.CD004429.pub2)

Other immunosuppressants have been trialled:

- In a randomized placebo-controlled trial of mycophenolate mofetil, there was no significant benefit.
- CTX has been used successfully in small numbers of patients but the risks likely outweigh the benefits.
- Rituximab has been trialled in open studies without success.
- Recent open trial of eculizumab suggested a full trial.
- Trials of other immunosuppressants should be undertaken. (Umapathi et al. Immunosuppressant and immunomodulatory treatments for multifocal motor neuropathy. Cochrane Database of Systematic Reviews 2012 DOI: 10.1002/14651858.CD003217.pub4).

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THEMATIC WORKSHOP 6.1

Theme: *PNS SESSION 2: NEW OPTIONS IN THE TREATMENT OF IMMUNE MEDIATED NEUROPATHIES*

TW 6.1.4 Rituximab in anti-MAG neuropathies: Yes or No?

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Within the spectrum of chronic immune-mediated neuropathies, demyelinating neuropathy associated with IgM monoclonal gammopathy and antibodies against myelin-associated glycoprotein (MAG) is a distinct entity that typically presents with progressive sensory ataxia and painful paresthesias in the 4 limbs. The disease may progress slowly over many years in some patients, whereas others develop significant disability mostly due to dysesthesia and ataxia, thus there is a need to develop effective treatments. Despite several arguments in favor of a highly probable autoimmune mechanism underlying this disease, there is insufficient evidence from most pilot studies or randomized controlled trials (RCT) on IgM anti-MAG demyelinating neuropathy to recommend any particular immunotherapy (Lunn and Nobile-Orazio, Cochrane Review 2012). Rituximab is a genetically engineered chimeric murine/human monoclonal antibody directed against CD20, a protein present on the surface of normal and malignant pre-B and mature B cells until differentiation into plasma cells. Its efficacy has been supported by several uncontrolled studies, and the results of 2 recent RCT versus placebo are now available. In the first one (Dalakas et al. 2009), after 8 months, by intention-to-treat (ITT), 4 of 13 rituximab-treated patients improved by ≥ 1 leg INCAT score compared with 0 of 13 patients taking placebo (p=0.096). Excluding one rituximab included patient who had normal INCAT score at entry, and thus could not improve, the results were significant (p=0.036). In addition, the Time to 10m walk was significantly reduced in the rituximab group (ITT; p=0.042). Based on clinical assessments and patient questionnaires, among the 13 placebo-treated patients, 6 had worsened, 7 remained unchanged, and none improved at month 8; in contrast among the 13 rituximab-treated patients, 1 worsened, 5 remained unchanged, and 7 improved. In the second one (Léger et al. 2013), ITT analysis, with imputation of missing ISS values by the last observation carried forward method, showed a lack of mean change in ISS at 12 months (p=0.92). However, changes were observed, in per protocol analysis at 12 months, for the number of patients with an improvement of at least 2 points in the INCAT disability scale (p=0.027), the self evaluation scale (p=0.016), and 2 subscores of the Short Form-36 questionnaire. Consequently, rituximab has no evidence-based efficacy in IgM anti-MAG demyelinating neuropathy, but the limitations of these 2 RCT should be considered.

THEMATIC WORKSHOP 6.2

Theme: *NEW THERAPIES IN MYASTHENIA GRAVIS*

TW 6.2.1 New therapies for myastenia gravis: just for refractory disease

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Myasthenia Gravis (MG) comprises several disease subtypes with distinct pathogenic and clinical characteristics (1). Current treatment is based on a combination of symptomatic medication, thymectomy, long-term and short-term immune therapies.

The development of new therapeutic agents for MG has been relatively marginal when compared to the remarkable expansion in other autoimmune diseases. A possible reason for such disparity is that conventional treatment is effective, as it can control symptoms in most patients. However, this outcome often requires prolonged and even life-long immunosuppression. Moreover, a proportion of patients have refractory disease as they complain of disabling weakness or frequent relapses and/or require too high doses of steroids or other immunosuppressive agents with serious side effects (2). Refractoriness to conventional treatment appears to be more common in patients with thymoma and with muscle-specific kinase (MuSK) antibodies (3–4).

The experience with biological agents in MG is still limited and mostly based on small patient series. Tumor necrosis factor-inhibitors should be avoided as they can induce MG worsening (1). Complement inhibition with eculizumab proved effective in a phase II trial in patients with acetylcholine receptor (AChR) antibodies (5). Many uncontrolled studies on B-cell depletion with rituximab have collectively reported a high rate of positive results, with differences between MuSK-MG and AChR-MG. Recent studies have shown multiple effects of rituximab on the immune system (6–7) and suggested the potential advantage of an early use of this agent in association with selective cytokine inhibition (8). However, until randomized trial data are available, lack of reliable biomarkers and safety concerns limit the use of biological therapies in MG other than refractory disease.

1) Meriggioli MN, Sanders DB. Lancet Neurology 2009;8:475–90
2) Drachman DB et al. Ann NY Acad Sci 2008;1132:305–14
3) Suh J et al. Yale J Biol Med 2013;86:255–60
4) Evoli A, Padua L. Autoimmun Rev 2013;12:931–5
5) Howard JF Jr et al. Muscle Nerve 2013; 48:76–84
6) Sun F et al. Muscle Nerve 2014; 49:487–94
7) Huang H et al. Proc Natl Acad Sci U S A 2010;107:4658–63
8) Mahèvas M et al. Front Immunol 2013;4:494

THEMATIC WORKSHOP 6.2

Theme: *NEW THERAPIES IN MYASTHENIA GRAVIS*

TW 6.2.2 New issues in the rational of thymectomy

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Acquired myasthenia gravis (MG) is an autoimmune disorder caused by autoantibodies that target components of the neuromuscular junction leading to muscle weakness and disabling fatigability. Autoantibodies are mostly against the anti-acetylcholine receptor (AChR), and less frequently the muscle specific kinase receptor or the low density lipoprotein receptor-related protein 4.

MG with anti-AChR antibodies is often associated with thymic abnormalities, such as thymoma or thymic follicular hyperplasia. Active neoangiogenic processes, abnormal chemokine expression and ectopic germinal centres are the main features of thymic hyperplasia. Recently, we demonstrated that interferon-β induced by double-stranded RNA signalisation, plays a central role in thymic events leading to MG. Indeed, interferon-β can orchestrates the overexpression of α-AChR probably leading to dendritic cell autosensitization, the abnormal recruitment of peripheral cells and germinal centre formation.

Thymectomy has become part of the standard therapeutic treatment for MG patients with anti-AChR antibodies. In patients with thymoma, the thymus is removed to avoid spreading of the tumour. In non-thymomatous MG, thymectomy is considered to reduce severity of MG, to slowdown the course of the disease and lead in some cases to stable remission. To better understand how thymectomy can improve MG patients, thymic changes occurring MG thymus will be detailed and the rational of new therapeutic issues to reverse thymic abnormalities will be considered.

THEMATIC WORKSHOP 6.2

Theme: *NEW THERAPIES IN MYASTHENIA GRAVIS*

TW 6.2.3. Antigen-specific apheresis in myasthenia gravis

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In Myasthenia Gravis (MG) the pathogenic autoantibodies target mainly the muscle acetylcholine receptor (AChR) and occasionally the MuSK and the LRP4. Our goal is to develop an antigen-specific therapy, based on the selective extracorporeal autoantibody depletion (immunoadsorption), using sepharose-immobilized extracellular domains (ECDs) of the human AChR subunits and MuSK. We have previously described the expression of these ECDs in the P. pastoris system and their use as efficient immunoadsorbents in vitro. The maximal capacity of the matrix for autoantibodies and its ability to be regenerated after each use, by elution of bound autoantibodies has been determined. Furthermore, the immobilized AChR and MuSK ECDs were proven efficient adsorbents in flow-rate conditions similar to those required during a therapeutic session. In parallel, the possibility for whole-blood adsorption has been investigated. The initial screening showed the same adsorption efficiency when whole blood instead of serum was used. We proceeded to ex vivo whole blood immunoadsorption using immunized animals to determine the efficiency and safety of the procedure. Initial results show a reduction of the antibody titer following a single procedure and no subsequent boost effect. We are currently setting up a protocol for repeated immunoadsorption sessions to maximize the observed effect and its duration. Upon completion of these tests we will proceed to clinical trials of therapeutic immunoadsorption in MG patients. The successful development and application of this antigen-specific therapy for MG could be used as a model for other antibody-mediated autoimmune disorders as well.

THEMATIC WORKSHOP 6.3

Theme: *FOCUS ON NEW INSIGHTS IN METABOLIC MYOPATHIES*

TW 6.3.1 New genes for muscle glycogenosis

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Muscle glycogenosis are a group of metabolic myopathies due to deficiencies of various enzymes involved in the synthesis or degradation of glycogen. Patients may present with exercise intolerance and rhabdomyolysis episodes, or progressive muscle weakness, depending on the deficient defect. Other organs, such as liver or brain, may also be affected.

Two new glycogenosis have been recently described, due to mutations in PGM1 and RBCK1 genes. Patients with phosphoglucomutase (PGM1) deficiency often present with exercise intolerance and rhabdomyolysis, hepatopathy with hypoglycemia, and dysmorphic features. In all cases, abnormal pattern of protein glycosylation may be detected by isoelectric focusing, allowing diagnosis, and thus establishing a connection between glycogenosis and congenital disorders of glycosylation (CDG syndromes).

Patients with RBCK1 mutations exhibit a progressive muscle weakness frequently associated with a severe cardiomyopathy which is a major cause of death. Pathological hallmark of this disease is the presence of polyglucosan inclusions in skeletal and cardiac muscles. However, the link between mutations in RBCK1 gene encoding an E3 ubiquitin ligase, and abnormally branched glycogen accumulation has not been yet established. Interestingly, mutations in this gene have also been reported in children who died from sepsis due to auto-inflammatory disease, with polyglucosan inclusions identified in skeletal muscle, heart and liver.

In addition to extending the list and the clinical spectrum of muscle glycogenosis, both of these diseases pave the way to novel and to date unsuspected physiopathological mechanisms.

References:

- Stojkovic T et al. Muscle glycogenosis due to phosphoglucomutase 1 deficiency. N Engl J Med. 2009;361(4):425–7.
- Tegtmeyer LC et al. Multiple phenotypes in phosphoglucomutase 1 deficiency. N Engl J Med. 2014 Feb 6;370(6):533–42.
- Nilsson J et al. Polyglucosan body myopathy caused by defective ubiquitin ligase RBCK1. Ann Neurol. 2013;74(6):914–9.

THEMATIC WORKSHOP 6.3

Theme: *FOCUS ON NEW INSIGHTS IN METABOLIC MYOPATHIES*

TW 6.3.2 Contribution of exercise tests to study treatment and the phenotypes of metabolic myopathies

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Metabolic myopathies are inborn errors of metabolism affecting muscle. Symptoms and morbidity in glycogen storage diseases (GSD) and fatty acid oxidation defects (FAOD) are caused by a limitation to use carbohydrates or fatty acids, which are the fuels that supply the energy for skeletal muscle contraction during aerobic exercise.

The basis behind the use of exercise to examine and test patients with GSD and FAOD is that exercise increases the energy requirements in skeletal muscle many-fold, and thus either provokes symptoms or induces metabolic derangements that may be detected by measuring key substrates, like plasma lactate and glucose. In addition, by combining exercise with indirect calorimetry and stable isotope methodology important information on the catabolism and oxidation of skeletal muscle substrates may be detected in vivo in humans.

Exercise testing has been used to assess the effects of treatment in patients with GSD and FAOD. The efficacy of carbohydrates as treatments of exercise-induced symptoms and as means of improving exercise tolerance has been established with methods applying exercise testing in McArdle disease (myophosphorylase deficiency) and in very long-chain acyl-CoA dehydrogenase (VLCAD) and carnitine palmitoyltransferase (CPT) II deficiencies. More recently, cycle-ergometer exercise testing in combination with stable isotope methodology and indirect calorimetry has been used to assess the effect of bezafibrate as a treatment to increase fatty acid oxidation in CPT II and VLCAD deficiencies.

In addition, exercise testing may be used to explore the phenotype in a controlled setting in the exercise laboratory. In such a setting, we have demonstrated that debranching enzyme deficiency (GSD type IIIa) is associated with exercise induced symptoms of excessive fatigue and muscle pain (dynamic symptoms), which is in contrast to the typical description of this GSD, as mainly being a liver GSD with a static muscle involvement that develops in the 3rd to 4th decade.

Thus, cycle-ergometry exercise in combination with stable isotopes and indirect calorimetry continues to provide new insight into the metabolic derangements causing disease in metabolic myopathies.

THEMATIC WORKSHOP 6.3

Theme: *FOCUS ON NEW INSIGHTS IN METABOLIC MYOPATHIES*

TW 6.3.3 Exercise-induced rhabdomyolysis: diagnostic guidelines and RYR1-related cases

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Rhabdomyolysis is a common and potentially lethal clinical syndrome that results from acute muscle fibre necrosis with leakage of muscle constituents into blood (CK increase ≥ 10x, with a rapid decrease and decline). Underlying myopathy or muscle metabolic defects are responsible for approximately 10% of the adult cases, with frequent recurrence and a low incidence of acute renal failure or multiple etiologies.

Exercise-induced rhabdomyolysis is defined as rhabdomyolysis with exercise preceding the episode of rhabdomyolysis. Most exercise results in some skeletal muscle damages, and unaccustomed and eccentric exercise can cause extensive damage. The mechanisms involved in the initiation of the muscle damage is hypothesized to be either mechanical (muscle damage is the result of direct physical stress on muscle fibres, especially when exercise involves eccentric muscle contractions where the muscle is lengthening while trying to contract) or metabolic (the damage is the result of different energetic deficiencies, involving primarily metabolic substrates). Various exogenous factors influence this: the intensity of the exercise, the physical condition and fitness of the individual, including diet and medication, the temperature and humidity, and the level of hydration. In fact, any healthy individual is probably prone to develop rhabdomyolysis in case of sufficient and severe risk factors.

Recurrent episodes of rhabdomyolysis, a positive (family) history for muscle (metabolic) disorders, and absence of sufficiently explaining exogenous factor point to a underlying genetic cause. The following guidelines can be helpful: rhabdomyolysis 1) evoked by prolonged exercise and/or fasting: defects in fatty acid oxidation; 2) triggered by short-lasting exercise: myophosphorylase deficiency (with second wind phenomenon) or various glycolytic disorders (without second wind phenomenon); and 3) with triggers of various duration: various muscular dystrophies (elevated CK) or RYR1-related disorders (CK usually normal).

Mutations in the RYR1 gene are one of the most common causes of inherited neuromuscular disease, associated with the malignant hyperthermia susceptibility trait, and a variety of congenital myopathies. RYR1 mutations have been recently identified in 14 families presenting with rhabdomyolysis episodes mainly evoked by exercise in absence of anesthetic triggers. Rhabdomyolysis was commonly triggered by exercise and heat and, less frequently, viral infections, alcohol and drugs. Most patients were normally strong and had no personal MH history. Muscle biopsies showed mainly subtle changes. Familial RYR1 mutations were confirmed in relatives with similar or no symptoms. These findings suggest that RYR1 mutations may account for a substantial proportion of patients presenting with unexplained rhabdomyolysis and/or exertional myalgia.

THEMATIC WORKSHOP 6.4

Theme: *PATHOMECHANISMS UNDERLYING MOTOR NEURON DEATH AND NEW THERAPEUTIC APPROACHES IN ALS/NMD*

TW 6.4.1 New therapeutic targets for neuroprotection in ALS/NMD

PAMELA SHAW, SHEFFIELD (Royaume Uni)

Abstract not received

THEMATIC WORKSHOP 6.4

Theme: *PATHOMECHANISMS UNDERLYING MOTOR NEURON DEATH AND NEW THERAPEUTIC APPROACHES IN ALS/NMD*

TW 6.4.2 Dysregulation of RNA processing in motor neuron degeneration

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Loss of motor endplates, axonal degeneration and cell death of motor neurons are characteristic pathological features of motor neuron diseases. The identification of gene defects for familial Amyotrophic lateral sclerosis (ALS) and other motor neuron diseases has pointed to distinct pathophysiological mechanisms but it is still unclear how they lead to synaptic loss, axonal degeneration and ultimatively to motor neuron cell death. The survival motor neuron (SMN) protein which is deficient in spinal muscular atrophy (SMA) also interacts with RNA binding proteins such as hnRNP R, TDP-43 and FUS. Smn interacts with the C-terminus of these proteins where mutations have been identified in familial forms of ALS. A prominent phenotype of SMN deficient motor neurons is reduced axon elongation in the absence of altered motor neuron survival pathways. The axonal defects are similar to those observed in motor neurons overexpressing mutant forms of TDP-43, but TDP-43, in particular M337V mutant TDP-43 alters signalling pathways of endoplasmatic reticulum (ER) stress. The subcellular transport of mRNAs for β-actin and other transcripts for axonal growth and presynaptic function is severely affected in Smn deficient motor neurons, and local translation of these mRNAs in axonal terminals is disturbed. Similar observations have been made in motor neurons after depletion of TDP-43. The consequences are disturbed axon elongation, reduced growth cone size and functional deficits in neurotransmission that are caused by disturbed integration and clustering of voltage-gated calcium channels and other proteins for presynaptic function in axon terminals. The deficit in clustering of voltage-gated calcium channels in growth cones of SMN-deficient motor neurons and in motor neurons expressing mutant TDP-43 is accompanied by a significantly reduced frequency of spontaneous Ca2+ transients. In mild models of SMA, this defect can be compensated by enlargement of motor units. PTEN signalling restores defective axon growth and excitability by stimulating local protein synthesis in axons. These findings point to common pathomechanisms in SMA and ALS, and to new strategies which can be used to restore altered RNA metabolism in these different forms of motor neuron disease.

THEMATIC WORKSHOP 6.4

Theme: *PATHOMECHANISMS UNDERLYING MOTOR NEURON DEATH AND NEW THERAPEUTIC APPROACHES IN ALS/NMD*

TW 6.4.3 Cellular autonomy and motor neurone degeneration

SIDDHARTHAN CHANDRAN, Edinburgh (Royaume Uni)

Abstract not received

THEMATIC WORKSHOP 6.5

Theme: *UBIQUITIN-PROTEASOME COMPLEX IN HEART AND SKELETAL MUSCLES*

TW 6.5.1 Regulation of ubiquitin-mediated turnover of sarcomeric proteins

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The dynamic turnover of muscle sarcomeres is regulated by two major pathways, the ubiquitin-proteasomal system (UPS) and autophagy-lysosomal (AL) degradation pathways. Muscle-specific ubiquitin ligases like MAFBx/atrogin-1 and MURFs target muscle proteins for degradation, classically via the UPS, including sarcomeric proteins like myosin as well as signalling proteins, metabolic enzymes and transcription factors. Several UPS and AL-associated proteins associate directly with the sarcomere, including the giant sarcomeric muscle protein titin that plays an essential role as a molecular ruler for determining sarcomere length. Furthermore, titin has multiple signalling domains, including a C-terminal kinase domain (TK) located in the M-band. This kinase shows unusual sequence features, including a mechanosensitive regu-

latory domain, raising the question whether its main function is catalytic, that of a scaffold, or both. Lange et al. [1] reported a signalling complex where TK interacts with the autophagy adaptor protein nbr1, a complex implicated in load-dependent muscle protein turnover regulation. Nbr1 binds a homologous autophagy-associated scaffold protein, p62/SQSTM1, which in turn interacts with at least two ubiquitin E3 ligases, MURF-1 and -2. This complex is developmentally tightly regulated, with MURF isogenes also showing fibre-type specific expression patterns [2, 3]. To understand the interplay between sarcomeric kinase scaffolding and load-dependent muscle protein turnover via autophagy, we generated genetically altered mouse lines carrying truncated versions of Nbr1, lacking either the C-terminal region following its PB1 domain (tr), or lacking the PB1 domain alone (ΔPB1). These animals can be bred to homozygosity and can reach over 1 year in age, but show distinct muscle phenotypes. Unloading and loading experiments in skeletal and cardiac muscle show that load-dependent muscle protein turnover is dependent on full-length Nbr1, whereas complete knockout of p62 is compensated. Proteomic analysis reveals altered turnover of metabolic enzymes, sarcomeric proteins, and signalling proteins as well as components of the endosomal pathway. Surprisingly, sarcomeric components whose turnover depends on Nbr1 include M-band titin and myomesin, whereas previously identified cytoskeletal targets for autophagosomal turnover were restricted to the peri-myofibrillar Z-disk protein filamin-C [4]. In conclusion, the sarcomere interacts as a mechanosensitive scaffold with autophagy adaptors that are critical and non-redundant for cardiac and skeletal muscle maintenance, as well as for atrophy and repair responses. Our studies may have wider implications for muscle and cardiac responses to cellular stress, aggregate clearance and protein homeostasis. Targeting signalling pathways like TK-nbr1-p62 that modulate muscle protein turnover and remodelling may provide new approaches to alter the biology of hereditary muscle diseases, muscle wasting, and heart failure.

References

1. Lange, S., et al., The Kinase Domain of Titin Controls Muscle Gene Expression and Protein Turnover. Science, 2005. 308: p. 1599–1603, Epub ahead of print March 31.

2. Perera, S., et al., Developmental regulation of MURF ubiquitin ligases and autophagy proteins nbr1, p62/SQSTM1 and LC3 during cardiac myofibril assembly and turnover. Dev. Biol., 2011. 351(1): p. 46–61.

3. Perera, S., B.S. Mankoo, and M. Gautel, Developmental regulation of MURF E3 ubiquitin ligases in skeletal muscle. J. Muscle Res. Cell Motil., 2012. 33(2): p. 107–122.

4. Ulbricht, A., et al., Cellular Mechanotransduction Relies on Tension-Induced and Chaperone-Assisted Autophagy. Curr Biol, 2013. 23(5): p. 430–5.

THEMATIC WORKSHOP 6.5

Theme: *UBIQUITIN-PROTEASOME COMPLEX IN HEART AND SKELETAL MUSCLES*

TW 6.5.2 UPS dysfunction in hereditary cardiomyopathy (MYBPC3 / LMNA)

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Adequate protein turnover is essential for maintaining cardiac homeostasis. Several protein quality controls are involved in protein homeostasis, including molecular chaperones and co-chaperones, the autophagy-lysosomal pathway, and the ubiquitin-proteasome system (UPS). In the last decade, a body of evidence has underlined a major role of the UPS in cardiac physiology and pathology. Particularly, recent studies have shown that dysfunctional proteasomal function leads to cardiac disorders, including hereditary cardiomyopathies. Hypertrophic and dilated cardiomyopathies are the two most prevalent inherited cardiomyopathies. Both are primarily transmitted as an autosomal-dominant trait and mainly caused by mutations in genes encoding components of the cardiac sarcomere, including a relevant striated muscle-specific E3 ubiquitin ligase. A growing body of evidence indicates UPS impairment in inherited cardiomyopathies as determined by measurement of the level of ubiquitinated proteins, the activities of the proteasome and/or the use of fluorescent UPS reporter substrates. While the paradigm for UPS impairment in HCM is MYBPC3, encoding cardiac myosin-binding protein C (cMyBP-C), this is CRYAB, encoding αB-crystallin for dilated cardiomyopathy (DCM). Other genes associated with DCM are also tightly regulated by the UPS. This is for example the case for LMNA encoding lamins A/C. Mutations in LMNA

caused Emery-Dreyfuss Muscular Dystrophy, DCM with conduction and/or rhythm defects. I will present typical examples of UPS impairment in mouse models of inherited cardiomyopathies, suggest potential mechanisms leading to and consequences of UPS impairment. Finally, I will underline available therapeutic options to restore proteasome activity and therefore cardiac homeostasis and function.

References
Vignier*, Schlossarek* et al., Circ Res 2009.
Schlossarek et al., Basic Res Cardiol 2012.
Schlossarek et al., J Muscle Res Cell Motil 2012.
Cattin et al., Hum Mol Genet 2013.
Schlossarek, Frey & Carrier, J Mol Cell Cardiol 2014.

THEMATIC WORKSHOP 6.5

Theme: *UBIQUITIN-PROTEASOME COMPLEX IN HEART AND SKELETAL MUSCLES*

TW 6.5.3 Ubiquitin Proteasome system and skeletal myopathies

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The ubiquitin-proteasomal system (UPS) is pivotal for the selective degradation or recycling of cytoplasmic and nuclear intracellular proteins and plays a major role in the regulation of protein homeostasis. One major function of the ubiquitin–proteasome system (UPS) is to prevent accumulation of non-functional, potentially toxic proteins. Degradation via the UPS system requires the attachment of ubiquitin to target proteins by a peptide linkage, allowing its transport to the 26S proteasome complex. Substrate recognition for ubiquitination is highly specific and provided by the E3 ubiquitin ligases.

Proteasomal dysfunction is considered to play a role in the pathogenesis of protein aggregate myopathies (PAM), a wide group of muscular conditions characterized by accumulation of proteins within muscle fibers. PAM includes myofibrillar myopathies (MFM), myosin storage myopathy (MSM) and actinopathy, among many others. The vast majority of PAM is caused by mutations in genes coding for cytoskeletal proteins, including components of the myofibrillar apparatus. We have recently identified a new protein aggregate myopathy, which is associated with gene defects in striated muscle-specific E3 ubiquitin ligases, MuRF1 and MURF3 that mediate the degradation of myosin heavy chains via the UPS.

This new entity is myopathologically characterized by subsarcolemmal accumulation of myosin and myosin-associated proteins and at the ultrastructural level by fragmented sarcomeres with preserved A-bands and M-bands but absent Z-discs. The selective accumulation of thick filaments, that are neither ubiquitinated, nor labeled with antibodies against the 20S proteasome subunit, indicates a specific impairment of their degradation due to gene defects in the striated muscle-specific E3 ubiquitin ligases.

THEMATIC WORKSHOP 7.1

Theme: *CELL THERAPY IN NEUROMUSCULAR DISEASES*

TW 7.1.1 Mouse satellite cells

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The classical skeletal muscle stem cell is the satellite cell. Satellite cells contribute to both muscle growth and regeneration and are capable of self-renewal (1).

We have used an in vivo transplantation model system to investigate the effect of different environmental factors on the ability of mouse satellite cells to contribute to muscle regeneration and functionally reconstitute the satellite cell pool. Satellite cells from normal donor mice contribute robustly to muscle regeneration only when the host satellite cell niche has been modulated prior to engraftment (2, 3). Although muscle maintenance and regeneration is impaired with increasing age and as a consequence of muscular dystrophies, satellite cells from both aged and young,

male and female normal mice (4) and dystrophic donor mice contribute effectively to muscle regeneration when grafted into a permissive host environment. We are currently investigating the factors that control donor satellite cell-mediated muscle regeneration.

References

1. Collins CA, Olsen I, Zammit PS, Heslop L, Petrie A, Partridge TA, et al. Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell. 2005;122(2):289–301.

2. Boldrin L, Zammit PS, Muntoni F, Morgan JE. Mature adult dystrophic mouse muscle environment does not impede efficient engrafted satellite cell regeneration and self-renewal. Stem Cells. 2009;27(10):2478–87.

3. Boldrin L, Neal A, Zammit PS, Muntoni F, Morgan JE. Donor Satellite Cell Engraftment is Significantly Augmented When the Host Niche is Preserved and Endogenous Satellite Cells are Incapacitated. Stem Cells. 2012; 30(9):1971–84.

4. Neal A, Boldrin L, Morgan JE. The satellite cell in male and female, developing and adult mouse muscle: distinct stem cells for growth and regeneration. PLoS One. 2012;7(5):e37950.

THEMATIC WORKSHOP 7.1

Theme: *CELL THERAPY IN NEUROMUSCULAR DISEASES*

TW 7.1.2 Cell therapy for muscular dystrophies: update on the autologous myoblast cell therapy trial for oculopharyngeal muscular dystrophy

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Cell therapy was first proposed in the 80's as a potential treatment for muscular dystrophies, based upon early results obtained in mdx mice: dystrophin expression was restored in this model by intramuscular injections of normal myoblasts. These results were quickly followed by clinical trials for patients suffering from Duchenne Muscular Dystrophy (DMD) in the early 90's, based mainly upon intramuscular injections of allogenic myoblasts. The clinical benefits obtained from these trials were minimal, if any, and research programs concentrated then on the various pitfalls that hampered these clinical trials. New therapeutic venues were then explored, such as the use of stem cells with myogenic potential, which have been described in various populations, including bone marrow, circulating blood or muscle itself. These stem cells presented the main advantage to be available and not exhausted by the numerous cycles of degeneration/regeneration, which characterize muscular dystrophies. However, the different stem candidates have also shown their limits in terms of efficiency to participate to the regeneration of the host. A state of the art summary of the various ongoing clinical and preclinical trials and of the various cell types that are currently available will be presented. In particular, the results of the phase I/IIa clinical trial (ClinicalTrials.gov NCT00773227) of autologous myoblast transplantation following myotomy in pharyngeal muscle of OPMD patients will be described. This study included 12 patients with clinical diagnosis of OPMD and indication for cricopharyngeal myotomy. The feasibility and safety end points of both autologous myoblast transplantation as well as the surgical procedure were assessed by videoendoscopy in addition to physical examinations. Potential therapeutic benefit was also assessed through videoendoscopy and videofluoroscopy of swallowing, quality of life score, dysphagia grade, and a drink test. Patients were injected with a median of 178 million myoblasts following myotomy. Short and long-term safety and tolerability were observed in all patients, with no ad-

verse effects. There was an improvement in the quality of life score for all 12 patients, and no functional degradation in swallowing was observed for the majority of patients. A cell dose dependant improvement in swallowing was even observed in this safety study. This trial supports the hypothesis that a local injection of autologous myoblasts in the pharyngeal muscles is a safe and efficient procedure for OPMD patients.

THEMATIC WORKSHOP 7.1

Theme: *CELL THERAPY IN NEUROMUSCULAR DISEASES*

TW 7.1.3 Human artificial chromosomes and iPS cells for ex vivo gene therapy of muscular dystrophies and beyond

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Mutations in the dystrophin gene affect skeletal muscle and cause Duchenne muscular dystrophy (DMD), ultimately resulting in premature death. Gene and cell therapy for DMD is difficult1, since skeletal muscle is the most abundant human tissue and dystrophin is the largest gene in the human genome. A few years ago we described the amelioration of dystrophic mdx mice by combining Human Artificial Chromosome (HAC)-mediated dystrophin gene-replacement with mesoangioblast myogenic stem/progenitor cells, using a HAC containing the entire dystrophin locus (DYS-HAC) and mouse pericyte-derived mesoangioblast transplantation as a model system2. Nevertheless, in order to extend this strategy to human mesoangioblasts their proliferative capacity needs to be extended to ensure survival after HAC transfer.

Here we describe the reversible immortalization of human mesoangioblasts using lentiviral vectors encoding human telomerase and Bmi-1 to bypass replicative senescence. Cells have been characterised for proliferation and transgene expression, remaining non-transformed, not tumorigenic and myogenic. Notably, this strategy allowed the transfer of a novel DYS-HAC into reversibly immortalized DMD mesoangioblasts, which had a normal karyotype after expansion in culture. Preliminary results also showed that DYS-HAC-corrected reversibly immortalized DMD mesoangioblasts were integrated into regenerating myofibers upon transplantation into immunodeficient mdx mice.

However, degeneration-regeneration cycles of dystrophic muscles can exhaust the pool of adult progenitors for ex vivo expansion, as we have shown for limb-girdle muscular dystrophy 2D (LGMD2D, characterized by α-sarcoglycan deficit)3. Therefore, LGMD2D fibroblasts or myoblasts were reprogrammed to induced pluripotent stem (iPS) cells and then differentiated to mesoangioblast-like cells3. These cells were expanded, genetically corrected and then transplanted into ad hoc generated α-sarcoglycan-null immunodeficient mice. Hybrid human myofibres expressing α-sarcoglycan were identified in the xenotransplants and upon murine intra-specific transplantation these cells caused functional amelioration of the dystrophic phenotype3,4. Importantly, this approach was also extended to DMD using the DYS-HAC for genetic correction. Finally, preliminary evidence of use of this platform for disease modelling and tissue engineering will be discussed. This strategy offers proof-of-principle of safety and efficacy of HACs and iPS cells for cell therapies of muscular dystrophies, providing also encouraging data for their potential use in drug development and tissue engineering for muscle disorders.

1. Benedetti S et al., FEBS Journal, 2013.
2. Tedesco FS et al., Science Translational Medicine, 2011.
3. Tedesco FS et al., Science Translational Medicine, 2012.
4. Gerli MFM et al., Journal of Visualized Experiments, 2014.

THEMATIC WORKSHOP 7.2

Theme: *NEW PATHOMECHANISMS OF AMYOTROPHIC LATERAL SCLEROSIS*

TW 7.2.1 Genetics of Amyotrophic Lateral Sclerosis

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ALS is usually described as a progressive disorder of upper- and lower motor neurons leading to muscle weakness and death due to respiratory failure on average 3 years after the onset of symptoms. In clinical practice, however, we experience a large variability in the clinical expression of the disease. An important gap in our knowledge of ALS pathophysiology is whether motor neurons in ALS die from a complex interaction between multiple factors or from the manifestation of a unique cause. In other words, is ALS one disease or just a phenotype of a large number of diseases with many causes? Filling this gap in knowledge may have important consequences for molecular diagnostic and therapeutic strategies in ALS and possibly other complex/neurodegenerative diseases. ALS could be considered either as a collection of single, unique rare diseases or a diagnostic continuum in which ALS can be sub-classified according to the relative contribution of genetic, environmental/lifestyle and phenotypic factors. Genetics in ALS may help reclassify ALS into separate disease entities. A family history of ALS is obtained in about 5 % but the distinction between familial and apparently sporadic ALS may be artificial and genetic factors play a role in all types. For several years, only one gene was known to have a role in ALS pathogenesis, SOD1. In the last few years there has been a rapid advance in our genetic knowledge of the causes of ALS. Relatively common mutations in the TDP-43, FUS and C9orf72 genes, and rare mutations in many more genes have been identified. In this presentation, I will discuss the genetic architecture of ALS, highlight some of the genes implicated in pathogenesis, and describe their phenotypic range and overlap with other diseases.

THEMATIC WORKSHOP 7.2

Theme: *NEW PATHOMECHANISMS OF AMYOTROPHIC LATERAL SCLEROSIS*

TW 7.2.2 Overlap between Amyotrophic Lateral Sclerosis and Frontotemporal Dementia

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Amyotrophic Lateral Sclerosis (ALS) has traditionally been considered a neurodegenerative disease exclusively involving the motor system. However, recent clinical, imaging neuropathological and genetic evidence point to considerable overlap with frontotemporal dementia (FTD). In recognition of this, consensus criteria have been proposed to categorize the various forms of cognitive and behavioural impairment associated with ALS, although recent data suggest these criteria will require adjustment to include deficits in language, theory of mind and social cognition.

A prospective population based study of ALS has shown that 13% of prevalent patients have full blown FTD, characterized by personality change, irritability, poor insight and pervasive deficits on frontal executive tests. Up to 25% of incident patients have evidence of executive impairment, characterized by deficits in verbal fluency, judgement and impulse control . Executive impairment tends to progress, and is a strong negative prognostic indicator. Up to 40% of ALS patients also develop behavioural impairment, and a proportion of patients also exhibit deficits in social cognition and theory of mind. Behavioural impairment in the absence of cognitive impairment can also occur, and both cognitive and behavioural dysfunction can precede or follow the onset of motor symptoms. Up to 40% of patients with ALS remain cognitively intact when tested longitudinally, indicating that ALS is a clinically heterogeneous condition.

Structural and functional imaging of groups of patients with ALS also demonstrate extensive extra-motor involvement, including changes in the deep grey matter. These findings are supported by neuropathological studies, although there is also evolving evidence to suggest substantial heterogeneity, indicating that ALS and ALS-FTD represent a syndrome rather than a single disease entity.

The recently described hexanucleotide repeat expansion on chromosome 9p21, which occurs in up to 11% of those with ALS , is associated with a distinct clinical , imaging and pathological phenotype with higher rates of cognitive and behavioural impairment and shorter survival. Those carrying the C9orf72 repeat expansion are also more likely to have family members with neuropsychiatric conditions including psychosis and a history of suicide. Other Mendelian inherited genes are also associated with an ALS-FTD phenotype, although it remains unclear as to whether distinct phenotypic characteristics can be discriminated.

Future studies will aim to further characterize the neuropsychological, imaging and pathological aspects of the extra motor features of ALS, and will help to provide a robust classification system that is both clinically and biologically relevant.

THEMATIC WORKSHOP 7.2

Theme: *NEW PATHOMECHANISMS OF AMYOTROPHIC LATERAL SCLEROSIS*

TW 7.2.3 Molecular mechanisms in Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS), the commonest form of degenerative motor neuron disease, is a heterogeneous syndrome with clinical, pathological and genetic overlap with frontotemporal dementia (FTD). Arresting the disease process in ALS and other neurodegenerative conditions is currently proving to be challenging because i) patients present with end stage disease, ii) we have a rudimentary understanding of the initiating biological events in motor neuron death and iii) our disease models are inadequate. No longer viewed as one disease with a single unified cause, ALS can be viewed as resulting from a complex convergence of genetic susceptibility, age-related loss of cellular homeostasis, and possible environmental influences. The rapid increase in recent years of the number of genes in which mutations have been associated with ALS has led to in vitro and in vivo models that have generated a wealth of data indicating disruption of specific biochemical pathways and sub-cellular compartments, including protein misfolding, mRNA splicing, axonal transport, oxidative stress, proteosome and mitochondrial dysfunction. However, clinical observation of characteristic regional patterns of involvement and progression, together with electrophysiological and brain-imaging studies suggest that ALS is a 'system degeneration'. This dichotomy between cellular and systems neurobiology raises the fundamental questions of what initiates the disease process and how it is propagated. Is the essence of ALS a cell-to-cell transmission of pathology with, for example, a 'prion-like' mechanism, or does the cellular pathology follow degeneration of specific synaptic networks? Elucidating the interaction between cellular degeneration and system level degeneration will aid modeling of the disease in the earliest phases, improve the development of sensitive markers of disease progression and response to therapy, and expand our understanding of the biological basis of clinical and pathological heterogeneity. In particular, the identification of the C9orf72 gene provides a roadmap for developing novel treatments based on specific targeting of the genetic mutation.

THEMATIC WORKSHOP 7.3

Theme: *NEW THERAPIES IN METABOLIC MYOPATHIES*

TW 7.3.1 Therapeutic approaches in lipid storage myopathies

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Lipid storage myopathy (LSM) is characterized, from the pathological point of view, by an abnormally increased lipid storage in muscle fibers, due to alterations of lipid metabolism pathways.

Nowadays, there are three different types of biochemically determined LSMs: 1) Disorders of fatty-acid oxidation (FAO), 2) Defects of trygliceride metabolism, 3) Defects of trygliceride and membrane phospholipid biosynthesis.

In the first group there are: Primary carnitine deficiency (PCD), Multiple acyl-coenzyme A dehydrogenase deficiency (MADD), Carnitil-Palmitoyl Transferase 2 (CPT2) (1) and others, whereas Neutral lipid storage disease with ichthyosis (CGI-58), and Neutral lipid storage disease with myopathy (PNPLA2) are in the second one; finally, in the third group, there is the Recurrent acute rhabdomyolysis in childhood (LPIN1) (2,3).

The diagnosis of these disorders is not easy but necessary because some of them are treatable conditions and, applying specific laboratory tests including morphological, biochemical and molecular genetic analyses, it is possible to reach the correct diagnosis.

From the clinical point of view, the infantile forms are mainly characterized by cardiomyopathy, liver failure, hypoglicemia, metabolic acidosis and delayed motor milestones whereas the most relevant aspects in adults are myalgia, recurrent episodes of massive rhabdomyolysis, exercise intolerance and progressive myopathy.

A muscle biopsy with a massive lipidosis could suggest to expand the laboratory investigations to better define the different types of lipid disorders (4).

In fact, urinary organic acid profile, measurement of blood carnitine and acylcarnitines and a search for some morphological aspects in the smear as Jordan's anomaly could properly address to the diagnosis.

To confirm the diagnostic suspect, molecular genetic analysis is required looking for mutations in already known genes as CPT2, ETFDH, ETFA, ETFB, ABDH5, SLC22A5 and PNPLA2 (5).

In this complex situation, the therapeutic approaches (3) suggested for lipid storage myopathies are:

a) Individuals with PCD may show striking improvement with high-dose oral L-carnitine supplementation (100–300 mg/Kg/day for chronic therapy).

b) There is also an increasing evidence indicating that several cases of MADD, due to ETFDH mutations, are Riboflavin-responsive (100–400 mg/day) with a dramatic improvement of patients clinical conditions. In case of incomplete rrcovery, patients could benefit of an additional CoQ10 supplementation.

c) It is necessary to raise the attention of patients suggesting avoidance of exacerbating factors as fasting, prolonged physical exercise and excessive alcohol ingestion and to maintain a normal carbohydrate diet in case of infections to reduce lipolysis.

d) Specific dietary recommendations for patients with long-chain, very-long chain fatty acid oxidation deficiency or with CPT2 deficiency.

e) In addition, there are a number of new therapeutic approaches that are ongoing to improve the opportunities to better treat patients with LSMs (6).

In conclusion, Lipid Storage Myopathies are challenging diseases but a considerable number of them can be satisfactorily treated, in some cases saving life of patients.

Bibliography

1) O. Musumeci, M Aguennouz, G P Comi, C R, M Autunno, A Bordoni, S Baratta, F Taroni, G Vita, A Toscano Identification of the infant-type R631C mutation in patients with the benign muscular form of CPT2 deficiency. Neuromuscular Disorders, 2007, 17:960–3.

2) Bruno C, Dimauro S. Lipid Storage Myopathies Curr Opin Neurol. 2008 Oct;21(5):601–6.

3) Laforêt P, Vianey-Saban C. Disorders of muscle lipid metabolism: diagnostic and therapeutic challenges. Neuromuscul Disord. 2010 Nov;20(11):693–700.

4) Liang WC, Nishino I. Lipid storage Myopathy - Curr Neurol Neurosci Rep. 2011 Feb;11(1):97–103.

5) Clinical features and ETFDH mutation spectrum in a cohort of 90 Chinese patients with late-onset multiple acyl-CoA dehydrogenase deficiency. Xi J, Wen B, Lin J, Zhu W, Luo S, Zhao C, Li D, Lin P, Lu J, Yan C. - J Inherit Metab Dis. 2013 Dec 20. [Epub ahead of print].

6) van de Weijer T, Havekes B, Bilet L, Hoeks J, Sparks L, Bosma M, Paglialunga S, Jorgensen J, Janssen MC, Schaart G, Sauerwein H, Smeets JL, Wildberger J, Zechner R, Schrauwen-Hinderling VB, Hesselink MK, Schrauwen P. Effects of bezafibrate treatment in a patient and a carrier with mutations in the PNPLA2 gene, causing neutral lipid storage disease with myopathy. - Circ Res. 2013 Mar 1;112(5):e51–4.

THEMATIC WORKSHOP 7.3

Theme: *NEW THERAPIES IN METABOLIC MYOPATHIES*

TW 7.3.2 Therapies in glycogen storage myopathies

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Glycogen storage diseases encompass now more than sixteen genetically distinct entities. Recent advances in therapy have re-opened the field of muscle glycogen storage diseases. The pathogenesis of the most common muscle glycogen storage diseases, GSD2/Pompe disease, is more complex than originally thought, as the primary lysosomal dysfunction is also altered by autophagy and lipofuscin build-up processes. These findings are presumably related to the partially failure of enzyme replacement therapy in up to 1/3 of patients with exceedingly long-lasting Pompe disease. However, with esteemed more than 2000 worldwide treated Pompe patients and new upcoming modified enzymes, enzyme replacement therapy is still highly encouraging for diminution of the individual disease burden of Pompe patients at any age. Chaperones are still under investigation for enhancement of enzyme replacement therapy, and quite recently, drugs like albuterol were tested to booster the efficacy of enzyme replacement therapy. Latest results of gene therapy in muscle glycogen storage diseases open the avenue for the translation into human protocols. So, Pompe disease is the role model for all types of glycogen storage diseases and beyond. E.g., enzyme replacement therapy might become an option in other glycogen storage diseases. New and

old drugs are under investigation in a variety of animal models, e.g. in a canine model of glycogen storage disease type 3 and primary muscle cells from human glycogen storage disease type 3 patients, rapamycin, a specific inhibitor of mTOR, has proven to be an effective therapy. In polyglucosan body disease/glycogen storage disease type 4, rapamycin and starvation was proven protective against uncontrolled elongation of glycogen chains leading to the formation of an insoluble form of glycogen, the polyglucosan bodies. Thus, the translation for experimental findings are on the run. Finally, exercise therapy and nutritional aspects are under investigation in more detail for their supplementary treatment impact in a variety of muscle glycogen storage diseases.

Disclosures: BS received advisory board and speaker honoraria from Genzyme, a Sanofi company and Biomarin Pharmaceutical Inc.

THEMATIC WORKSHOP 7.3

Theme: *NEW THERAPIES IN METABOLIC MYOPATHIES*

TW 7.3.3 Experimental therapy in mitochondrial disorders

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The genetic and biochemical intricacy of mitochondrial bioenergetics explains in part the extreme heterogeneity of mitochondrial disorders, a formidable challenge for both diagnostic workup and treatment. Although energy failure associated with reduced ATP biosynthesis appears to be critical in individual or combined MRC-complex defects, other mechanisms are also likely to be involved, and are probably predominant in the pathogenesis of specific syndromes, such as alterations of cellular reduction-oxidation status, production of reactive oxygen species (ROS), compromised Ca2+ homeostasis, and altered mitochondrial dynamics, autophagy or apoptosis. We have started a long-term program on the development of experimental therapy in recombinant mouse models with mitochondrial dysfunction and in mutant cybrids. A first strategy is centered on the activation of the mitochondrial biogenetic program by AMPK or SIRT1 agonists. Both AMPK and SIRT1 induce expression of energy genes, partly by targeting the transcriptional coactivator PGC-1α. We found that pharmacological stimulation of AMPK or SIRT1 partly corrects the motor deficit and biochemical abnormalities of mice characterized by impaired assembly of cytochrome c oxidase. Other ongoing strategies are based on increasing the expression of antiapoptotic factors, which leads to remarkable clinical improvement in COX-defective animals; or on the ability of mitochondrial aminoacyl-tRNA synthetases to correct the functional defect in cybrids carrying mt-tRNA mutations. In addition, specific conditions, e.g. Ethylmalonic Encephalopathy and MNGIE, characterized by accumulation of toxic substances derived from mitochondrial dysfunction have been effectively treated in mouse models by AAV-mediated gene targeting to the liver. Taken together, our results suggest that a spectrum of different approaches are effective in experimental mitochondrial dysfunction. Some of these can be rapidly transferred to clinical trials in humans.

THEMATIC WORKSHOP 7.4

Theme: *PARANEOPLASTIC PERIPHERAL NEUROPATHY*

TW 7.4.1 Paraneoplastic sensory neuropathies: clinical and pathophysiological aspects

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Paraneoplastic sensory neuronopathy belongs to a group of inflammatory disorders affecting the dorsal root ganglia. It is one of the classical paraneoplastic neurological syndromes and most patients have anti-Hu antibodies and small cell lung carcinoma. Cases with other intracellular onconeural antibodies including anti-CV2/CRMP5 and anti-amphyphisin antibodies have been reported and seronegative cases or cases with other cancers may occur. Although immunoglobulin deposits containing anti-Hu antibodies can be found around sensory neurons, the disorder is probably mediated by cytotoxic T-cells. SNN is the most frequent paraneoplastic neurological syndrome but it represents only a small proportion of patients with sensory neuronopathy. Identifying these patients early in the course of their disease is important since it may lead to an early diagnosis of the tumor which is probably the best way of stabilizing the neuropathy and improving the tumor prognosis. Other paraneoplastic sensory neuropathies involve antibodies toward pe-

ripheral nerve membrane antigens and include neuropathies with Waldenström's disease and anti-MAG monoclonal IgM and small fiber involvements in patients with anti-CASPR2 antibodies, Morvan's syndrome and thymoma. Contrarily to antibodies directed toward intracellular antigens, antibodies recognizing cell surface antigens are probably pathogenic.

THEMATIC WORKSHOP 7.4

Theme: *PARANEOPLASTIC PERIPHERAL NEUROPATHY*

TW 7.4.2 Update on Lambert-Eaton myasthenic syndrome

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Autoimmune disorders of the neuromuscular synapse are associated with different well defined antibodies, including the acetylcholine receptor (AChR), muscle-specific kinase (MuSK), low-density lipoprotein receptor-related protein 4 (Lrp4), or voltage-gated calcium channels (VGCC). More than 80% of these patients have AChR antibodies (AChR MG) with a reported incidence varying between 2 and 20 per million inhabitants per year, and a prevalence of 1–2 per 10.000 persons. The prevalence of the Lambert-Eaton myasthenic syndrome (LEMS) and the other forms of myasthenia are about 20 times more rare.

Both LEMS and AChR MG have a tumour and non-tumour associated form, where LEMS is associated with small cell lung cancer (SCLC) and AChR MG with thymoma. The non-tumour related forms of these disorders show a bimodal distribution in their relation to sex and age, with the age of 40–50 years as a cut-off point. Below this age predominantly female patients, with a high prevalence of HLA DR3-B8 are affected, while above this age the majority of the patients is male with no clear HLA association. Both LEMS and AChR MG are caused by IgG1 or IgG3 antibodies. LEMS starts almost exclusively with proximal weakness of the legs, and with disease progression weakness extends in a caudal-cranial direction. AChR-MG classically starts with ptosis and diplopia, followed by generalized, predominantly proximal weakness. A characteristic ptosis with ophthalmoplegia is rare in LEMS, which makes this disorder more difficult to diagnose. LEMS is associated with, most often mild, autonomic dysfunction, which is not seen in AChR MG. In LEMS associated with SCLC the symptoms of the disease develops much faster in a shorter period of time than in the non-tumour variant. The DELTA-P score can help to predict the presence of an underlying SCLC at the first visit of the patient. It is an easy score based purely on clinical findings.

Treatment of non-tumour LEMS consists of symptomatic treatment with 3,4-diaminopyridine or the use of prednisolone and immunosuppressive agents for more severely affected patients, while patients with LEMS and SCLC are treated by chemotherapy. A detailed analysis of the clinical signs and symptoms is very helpful in distinguishing the different forms of myasthenia.

THEMATIC WORKSHOP 7.4

Theme: *PARANEOPLASTIC PERIPHERAL NEUROPATHY*

TW 7.4.3 Paraneoplastic dysautonomia (from the PNS-Euronetwork Database)

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Introduction: In paraneoplastic syndromes of the peripheral nervous system (PNS), sensory ganglia are often targeted by the immunological attack.

Subacute sensory neuronopathy (SSN) is a classical paraneoplastic syndrome. There is a clear predominance of SSN with well-established clinical features and oncological and immunological associations.

Dysautonomia can occur as an isolated paraneoplastic peripheral neuropathy and is also a classical syndrome. The lack of extensive clinical and epidemiologic studies on these rare disorders prompted establishment of the PNS Euronetwork by 20 consortium members from 11 European countries. The partnership collected information on 979 patients, resulting in the largest database on PNS to date (Giometto et al, 2010). In this study we report data related to the patients with paraneoplastic dysautonomia.

Results: 33 patients (31 adults and 2 children) with dysautonomic paraneoplastic neurological syndromes (dPNS) were recruited (6% of the total study population) from 5 centres. All were definite PNS. Most patients had an additional PNS, most frequently a peripheral, usually sensory, neuropathy. Dysautonomia was the sole PNS in only 27% of patients. Chron-

ic pseudo-obstruction was the commonest syndrome (73%) .

All of the 31 adults had serum onconeural antibodies 80% of which were against Hu protein. 76% of the patients had detectable tumours, usually SCLC or NSCLC. Of the 25 Hu antibody-positive adult patients, 19 had tumours (13 SCLC, 3 NSCLC, 1 liver carcinoma, and 1 unknown primary) leaving 7 in whom no tumour was found after follow up of up to 8 years. CSF was analysed from 12 patients. 10/12 had a cell count of 10 or higher and 9/12 had a total protein of > 0.5 g/l. 2/4 CSF had oligoclonal bands.

5 patients were lost to follow up. Of the other 27 patients, 77% died: 55% from their dPNS, 15% from their tumour, 1 from sepsis, and 1 during mediastinoscopy. Average time from onset to death from PNS was 8 months (range 1 – 24 months). dPNS improved in 2/14 patients after tumour treatment. Both of these patients had NSCLC. dPNS did not improve in any of the 13 patients given immunotherapy.

Conclusions: We suspect that dysautonomic PNS is underdiagnosed as only 5/19 of our Centres reported cases. In addition, the majority of cases (73%) developed in the setting of at least one other PNS, in particular Subacute Sensory Neuronopathy, which we speculate may dominate the presentation and mask the autonomic features. Dysautonomic PNS was most strongly associated with lung tumours, especially SCLC, and Hu antibodies. It improved only rarely with tumour treatment and none of our 13 patients improved with immunotherapy. Prognosis was poor. When dysautonomia developed, 77% died from their PNS, all within 2 years of onset.

No disclosure

Reference

Bruno Giometto, Wolfgang Grisold, Roberta Vitaliani, et al. Arch Neurol. 2010;67(3):330–335.

THEMATIC WORKSHOP 7.5

Theme: *INTERNAL MUSCLE CELL STRUCTURE*

TW 7.5.1 Formation and maintenance of triads during development and pathological conditions

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Myology Center of Research, UMRS 974 UPMC - INSERM, Paris, France

Mutations in Amphiphysin-2/BIN1 are associated with autosomal recessive centronuclear myopathy (ARCNM), a muscle disorder characterized by myofibers with centrally positioned myonuclei, defects in T-tubules and abnormal triads. How Amphiphysin-2 orchestrates T-tubule formation and ARCNM-associated mutations lead to muscle morphological defects remains elusive. We developed an in vitro system where myotubes mature into myofibers with peripheral myonuclei, myofibrils, T-tubules and triads. We established the main molecular mechanisms involved in the positioning of myonuclei and triad formation. We found that Amphiphysin-2 interacts with N-WASP in newly formed myofibers as well as in adult muscle and that this interaction is disrupted by Amphiphysin-2 ARCNM mutations. We established that N-WASP and Amphiphysin-2 are required for nuclear positioning, T-tubule biogenesis and triad formation, but not myofibrillogenesis. We also demonstrated that N-WASP and Amphiphysin-2 are involved in the maintenance of triads in adult myofibers. Finally, we showed that N-WASP distribution is disrupted in muscle from ARCNM, ADCNM patients and more slightly in XLCNM and DM1 patients. Our results support a role for N-WASP in Amphiphysin-2-dependent nuclear positioning, T-tubule biogenesis and triad formation in skeletal muscle formation and in CNM pathophysiology.

THEMATIC WORKSHOP 7.5

Theme: *INTERNAL MUSCLE CELL STRUCTURE*

TW 7.5.2 Molecular basis of sarcoplasmic reticulum organization in skeletal muscle fibers

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The sarcoplasmic reticulum (SR) is a specialized form of endoplasmic reticulum that plays a key role in the regulation of muscle contraction by controlling release of Ca2+ following membrane depolarization in a process named excitation-contraction (e-c) coupling. In skeletal muscle, e-c coupling occurs at specialized intracellular junctions, named triads, where two terminal cisternae of the SR are positioned to flank, on opposite sites, a transverse (T)-tubule. The terminal cisternae membrane that faces the T-tubule represents

the junctional SR. This is the SR region where the ryanodine receptors Ca2+ release channels and additional proteins, including triadin, junctin and calsequestrin, assemble into a large multi-protein complex. The domains where this machinery assembles are also referred to as Calcium Release Units (CRUs). The junctional SR is selectively aligned with the junction between the A and the I bands of the sarcomere and is in direct connection with the longitudinal SR, a large network of tubules that are interconnected one with each other in forming a network that surrounds individually each single myofibril and that covers both the A and the I bands of the sarcomere. Regardless of the distinction in junctional and longitudinal domains, tubules and cisternae of the SR share a continuous lumen delimited by a single continuous membrane, thus corresponding to a single, though very large, organelle.

It would then appear that, to efficiently position the Ca2+ store close to the contractile apparatus, the entire SR is organized to surround each individual myofibril and that proteins of the CRUs are precisely targeted to the junctional SR. In the recent years we have started to identify and characterize some of the proteins that contribute to stabilize the SR around the myofibrils and to dissect the complex molecular mechanisms that target and organize some of the proteins of the CRU at the junctional SR. Current knowledge on these issues will be presented.

1. Rossi et al., Biochem J. 2014 458 (part 2), Pages 407–417.
2. Randazzo et al., J Cell Biol. 2013 Feb 18;200(4):523–36.
3. Golini L et al., J Biol Chem. 2011 Dec 23;286(51):43717–25.
4. Cusimano et al., Proc Natl Acad Sci U S A. 2009 Mar 24;106(12):4695–700.
5. Rossi et al., Traffic. 2008 Jul;9(7):1044–9.
6. Bagnato et al., J Cell Biol. 2003 Jan 20;160(2):245–53.

THEMATIC WORKSHOP 7.5

Theme: *INTERNAL MUSCLE CELL STRUCTURE*

TW 7.5.3 The molecular basis of the stereotyped muscle organization

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Spontaneous and depolarization-induccd calcium releases during excitation- contraction coupling of skeletal muscle fibres occur at complex sites called calcium release units (CRUs). The composition, structure and positioning of CRUs in adult muscle are stereotyped, although subject to species and fiber-type dependent variations. The units are composed of a highly complex macromolecular complex involving direct molecular interactions between a membrane component of the transverse tubules (the voltage sensing CaV1.1 channels or dihydropyridine receptors, arranged in tetrads associated with the four subunits of RyR1); the calcium release channels of the sarcoplasmic reticulum, SR (ryanodine receptors, RyRi and RyR3); other SR membrane proteins (triadin and junctin); the luminal protein calsequestrin; and the docking protein junctophilin. CRUs have specific positions relative to the cross striations of the myofibrils. A basic if simplified understanding of these arrangements and how they develop is obtained from ultrastructural observations of differentiating cells in vitro and in vivo and from observations of null mutations. A) Positional clues from differentiation: 1) the initial position of transverse (T) tubules is longitudinal; 2) initial CRU complexes are also longitudinal, but specifically located relative to the myofibrils; 3) the shifts from longitudinal to transverse orientations of T tubules and triads are coordinated events. B) Composition clues from development: 1) CRU differentiate through a hierarchical sequence from peripheral couplings to longitudinal dyads/triads to transverse triads while acquiring their components; 2) early peripheral couplings do not have Cav1.1 or RyR. Hence an independent docking protein (identified as junctophilin by H. Takeshima) is required; 3) calsequestrin, RyR and Cav1.1 are acquired in sequence. C) Clues from null mutations: 1) RyR1 are targeted to CRUs and form arrays in the absence of CaV1.1 (in dysgenic mice); 2) CaV1.1 are targeted to CRUs but do not form tetrads in the absence of RyR1 (in dyspedic mice); 3) triadin is appropriately targeted to CRUs in dyspedic muscle in vitro and in vivo; 4) RyR1 and RyR3 are targeted to CRUs independently of each other when expressed in a dyspedic cell line, but the latter do not associate with triadin, they do not restore skeletal type e-c coupling and do not induce tetrad assembly by CaV1.1; 5) when coexpressed in vivo RyR1 and RyR3 assume different position (junctional versus parajunctional) within the triads. 6) calsequestrin is targeted to and retained in CRUs in the absence of RyRs; 7) a null mutation for triadin ablates the visible CASQ anchors to the jSR membrane.

Supported by NIH grant HL 48093.

Slides: Triads 3 D drawing: the macromolecular complex and some of its constituents: RyR. DHPR tetrads, CASQ, triadin-junctin

The stereotyoed positioning of triads: at the Z line (zfish?) or A-I junction (mammals)

Show feet, tetrads and CASQ network

Figurefrom Barine…SORRENTINO TYPE 1 AND TYPE 3 NULL

Clues from development:

T tubule are initially longoitudinal

Longituinal Triads are at the A-I junction BEFORE T tubules are transverse.

PRESUMABY SR assocaitin owht myofibrils f0drags the T tubules transversely

Z fish at 48 hrs versus mouse or chicken at severa weeks.

A hierarchical sequence from peripheral couplings to longitudinal triads to transverse triads.

THEMATIC WORKSHOP 8.1

Theme: *THERAPEUTIC APPROACH TARGETING THE HEART IN NEUROMUSCULAR DISEASES*

TW 8.1.1 RNA-based or gene-based therapies

Lucie CARRIER, Hamburg (Allemagne)
Department of Experimental Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

An overview of gene therapies for heart failure will be first presented. Then, I will focus on RNA-based therapeutics, such as exon-skipping using antisense oligoribonucleotides (AONs) or RNA trans-splicing that we recently applied for hypertrophic cardiomyopathy (HCM). HCM is an autosomal-dominant disorder and the leading cause of sudden cardiac death in young athletes. It is mainly characterized by left ventricular hypertrophy (LVH), diastolic dysfunction and increased interstitial fibrosis. HCM is often caused by mutations in MYBPC3, encoding cardiac myosin-binding protein C (cMyBP-C). Most MYBPC3 mutations result in truncated proteins. Findings in humans and in cat/mouse models indicate that haploinsufficiency is the most prevalent HCM mechanism. Moreover, marked reduction of cMyBP-C results in LVH and dysfunction in mice and in severe neonatal, lethal forms in humans. RNA-based therapeutics were evaluated in Mybpc3-targeted knock-in (KI) mice that carry a homozygous G>A transition in exon 6, resulting in 3 different aberrant mRNAs and proteins. An alternative variant (Var-4) deleted of exons 5–6 was also identified in wild-type (WT) and KI mice. 5'-trans-splicing was induced between the endogenous mutant Mypbc3 pre-mRNA and an engineered pre-trans-splicing molecule (PTM) carrying a WT-Mybpc3 cDNA sequence. PTMs were packaged into adeno-associated virus (AAV) for specific transduction of cultured cardiac myocytes and the heart in vivo. Full-length repaired Mybpc3 mRNA represented 33% and 0.15% of total Mybpc3 transcripts in cardiac myocytes and in the heart, respectively. Repaired cMyBP-C protein was detected by immunoprecipitation in cells and in vivo. Exon-skipping enhancing expression of Var-4 mRNA was induced by AON-5 and AON-6 that mask exonic splicing enhancer motifs in exons 5 and 6. AONs were inserted into modified U7snRNA and packaged in AAV. Transduction of cardiac myocytes or systemic administration in newborn KI mice markedly increased Var-4 mRNA and protein, reduced aberrant mRNAs, and rescued the cardiac phenotype in mice. We provide the first evidence of successful 5'-trans-splicing in vivo and of cardiac functional rescue by exon skipping. These findings open new horizons for causal therapy of severe forms of genetic diseases with cardiac involvement.

References

Gedicke-Hornung*, Behrens-Gawlik* et al., EMBO Mol Med 2013.

Mearini*, Stimpel* et al., Mol Therapy – Nucl Acids 2013.

Behrens-Gawlik et al., Pflügers Arch – Eur J Physiol 2014.

THEMATIC WORKSHOP 8.1

Theme: *THERAPEUTIC APPROACH TARGETING THE HEART IN NEUROMUSCULAR DISEASES*

TW 8.1.2 Prevention and treatment of heart involvement in muscle diseases

Denis Duboc, Henri Marc Becane, Rabba Ben Yaou, Bruno Eymard, Pascal Laforet, Anthony Béhin , Tania Stojkovic, Karim Wahbi
Cardiology, Hospital Cochin and Myology Institute, Hospital de la Salpétrière, Paris, France

Many muscle diseases are concerned by cardiovascular complications.

The most dramatic are cardiac sudden death and heart failure. But atrial fibrillation with the risk of systemic emboli and the venous thrombo embolism pathology take an important place in the morbidity of these diseases.

In this presentation we will show the specificities of these complications and propose some guidelines to prevent them, and particularly to prevent the risk of sudden death in DM1 and in laminopathies and to limit the progression of left ventricular dysfunction in dystrophinopathies.

THEMATIC WORKSHOP 8.1

Theme: *THERAPEUTIC APPROACH TARGETING THE HEART IN NEUROMUSCULAR DISEASES*

TW 8.1.3 Targeting HDACs (histone deacetylase) in cardiovascular disease

Joseph A HILL, Dallas (Etats-Unis)
University of Texas Southwestern Medical Center

Heart failure, a syndrome culminating the pathogenesis of many forms of heart disease, is highly prevalent and projected to be increasingly so for years to come. Major efforts are directed at identifying means of preventing, slowing, or possibly reversing the unremitting progression of pathological stress leading to myocardial injury and ultimately heart failure. Indeed, despite widespread use of evidence-based therapies, heart failure morbidity and mortality remain high. Recent work has uncovered a fundamental role of reversible protein acetylation in the regulation of many biological processes, including pathological remodeling of the heart. This reversible acetylation is governed by enzymes that attach (histone acetyltransferases, HAT) or remove (histone deacetylases, HDACs) acetyl groups. In the case of the latter, small molecule inhibitors of HDACs are currently being tested for a variety of oncological indications. Now, evidence has revealed that HDAC inhibitors blunt pathological cardiac remodeling in the settings of pressure overload and ischemia/reperfusion, diminishing the emergence of heart failure. Mechanistically, HDAC inhibitors reduce stress-induced cardiomyocyte death, hypertrophy, and ventricular fibrosis. Looking to the future, HDAC inhibitor therapy may emerge as a novel means of arresting the untoward consequences of pathological cardiac stress, conferring clinical benefit to the millions of patients with heart failure.

THEMATIC WORKSHOP 8.2

Theme: *TREAT NMD ALLIANCE: OMICS TECHNOLOGIES FOR TRANSLATIONAL RESEARCH IN NEUROMUSCULAR DISORDERS*

TW 8.2.1 Genomics and gene discovery in hereditary neuropathies

Jan SENDEREK, Munich (Allemagne)
Friedrich-Baur-Institut, Department of Neurology, Ludwig Maximilians University, 80336 Munich, Germany

Peripheral neuropathy is one of the most common referrals to neurologic clinics. Most cases are acquired (due to diabetes mellitus, alcoholism or autoimmune or infectious diseases), however, several genetic forms do exist as well. Mutations in more than 50 genes affecting diverse glial or neuronal functions have been associated with different forms of hereditary neuropathies. This has led to a substantial improvement in diagnostics of the disease and in the understanding of implicated pathophysiological mechanisms. As for other Mendelian conditions, gene discovery in hereditary neuropathies has been largely fostered by the decoding of the complete human genome at the turn of the Millennium. More recently, highly-parallel genetic technologies (e.g., whole exome sequencing and whole genome sequencing) have been adapted for the study of inherited neuropathies. Application of these technologies has enabled researchers and clinicians to identify numerous new neuropathy genes and to recognize previously unpredicted genotype-phenotype relationships. It has also been proposed that hereditary neuropathies may occasionally be inherited in a more complex fashion, as an oligogenic disorder. A number of patients has been described who carried mutations at more than one locus, but at present the extent to which possible oligogenic inheritance accounts for the phenotype is unknown. In addition to providing an overview of genetic and functional data concerning various neuropathy forms, this review focuses on the current and future impact of advances in DNA sequencing on gene discovery and diagnostics of hereditary neuropathies.

THEMATIC WORKSHOP 8.2

Theme: *TREAT NMD ALLIANCE: OMICS TECHNOLOGIES FOR TRANSLATIONAL RESEARCH IN NEUROMUSCULAR DISORDERS*

TW 8.2.2 Proteomics and pathophysiological signatures in myofibrillar myopathies

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Myofibrillar myopathies (MFM) are a group of usually autosomal dominant inherited muscle disorders characterized by focal disintegration of myofibrils and by the formation of intramyoplasmic protein aggregates. Known diseases genes encode proteins that are located at or associated with the Z-disc. We applied a highly sensitive proteomic approach to decipher the composition of pathological protein aggregates in different MFM subtypes. The aim was to identify novel disease-relevant proteins and subtype-specific proteomic profiles.

Skeletal muscle samples from 67 MFM patients were included in this study. Aggregate samples and intraindividual control samples (from normally looking muscle fibers) were collected from 10µm muscle sections by laser microdissection and analyzed by a label-free mass spectrometric approach for identification and relative quantification of proteins. We detected 4470 different proteins in the samples and 287 of these showed a statistically significant accumulation in aggregate samples with a ratio >1.5 compared to controls (mean ratio 4.5). Z-disc and Z-disc-associated proteins, especially desmin, filamin C and their binding partners, constituted the most abundant group of over-represented aggregate proteins followed by proteins involved in protein quality control and protein degradation, extracellular and sarcolemmal proteins, components of signaling pathways and regulators of myofibrillar organization. Subgroup analysis revealed a characteristic basic pattern of aggregate composition but also significant differences regarding the accumulation ratio, order and proportion of individual proteins that enabled the definition of subtype-specific proteomic profiles. In addition, some disease-causing mutations could be detected directly on the protein level.

Our proteomic data provide important new insights into the composition of pathological protein aggregates in MFM and expand our knowledge about proteins and pathways that seem to be involved in pathogenesis. The identification of specific proteomic profiles in different MFM subtypes and the detection of mutations directly on the protein level can be useful in differential diagnosis of protein aggregation myopathies.

THEMATIC WORKSHOP 8.2

Theme: *TREAT NMD ALLIANCE: OMICS TECHNOLOGIES FOR TRANSLATIONAL RESEARCH IN NEUROMUSCULAR DISORDERS*

TW 8.2.3 Biomarker discovery in Duchenne Muscular Dystrophy

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The lack of non-invasive biomarkers to be used as surrogate endpoints in Duchenne muscular dystrophy (DMD) patients is one of the unmet needs in clinical practice. The availability of objective measures able to correlate with or being predictive of clinical benefit could be used as secondary clinical endpoints, thus reducing the time and money needed to map response to treatment while speeding up drug approval.

We have set up a multi-step discovery pipeline to identify proteins and metabolites present in body fluids (serum, plasma and urine) associated with DMD pathology. Candidates are identified with high-throughput proteomic and metabolomics technologies in cross-sectional studies (cases vs controls); the most promising candidates are first validated with an independent assay and then tested in 2 independent patient cohorts. Multivariate analysis is then used to identify biomarkers correlating with clinical scores. As an extra validation layer, candidate biomarkers are studied in 2 large longitudinal cohorts.

Matrix Metalloproteinase-9 (MMP-9) is our most advanced candidate. Levels of MMP-9 were measured in serum of 116 patients followed up in Newcastle (NCL), Leiden (LUMC) and London (UCL). MMP-9 levels were higher in DMD patients com-

pared to age-matched healthy controls. A strong significant correlation was present between serum and plasma MMP-9 levels in DMD patients. Elevated MMP-9 levels were confirmed by gelatin zymography, showing a strong correlation between the results obtained with the ELISA and with gelatin zymography. Longitudinal data from 66 DMD patients followed up in Leiden and Newcastle for over 5 years (168 samples) showed that MMP-9 serum levels significantly increase over time. Hence, MMP-9 is a good candidate biomarker to monitor disease progression in DMD patients. These data are supportive to evaluate the potential of MMP-9 as a biomarker to determine the early response to treatments.

Disclosures: AAR discloses being employed by LUMC which has patent applications on exon skipping. As co-inventor on some of these patients, AAR is entitled to a share of potential royalties.

THEMATIC WORKSHOP 8.3

Theme: *DIAGNOSTIC BOUNDARIES OF LIMB GIRDLE MUSCULAR DYSTROPHIES*

TW 8.3.1 Proximal weakness and atrophy: is this a limb girdle muscular dystrophy?

John VISSING, Copenhagen (Danemark)
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With the advent of progress in unravelling the molecular background of a great variety of muscle diseases, it has become increasingly clear that what was believed to be a single disease with a clear phenotype can have multiple genetic backgrounds. The opposite is often also the case, i.e. that distinct aberrations in single genes may show a wide range of phenotypes. Thus, classical lumping of disease entities according to phenotypic presentation has been challenged by molecular genetics that often split such entities. One could then argue that the current classification system of inherited muscle diseases could be replaced by a classification that relies exclusively on the molecular genetic defect. However, lumping diseases according to phenotype has many advantages, because clinical appearance is what the clinicians look at the first time he/she sees the patient, and therefore is a pivotal entry port for onward diagnostic strategy.

Limb girdle muscular dystrophies, inherited by a recessive trait, encompass a group of muscle diseases that originally were lumped together according to criteria of 1) proximal weakness and atrophy, 2) dystrophic appearance of muscle tissue, and 3) elevated creatine kinase levels. Many patients in this category can have prominent involvement of distal muscle groups as well (for instance dysferlinopathies and anoctamine 5 deficiency), yet still are designated a limb girdle muscular dystrophy. Conversely, patients who are not coined as a limb girdle muscular dystrophy can have severe proximal atrophy and weakness. A number of these have a clear underlying pathology, which is very much unlike a muscular dystrophy (for instance, polymyositis and some muscle glycogenosis and endocrine myopathies). However, several disease entities within the muscular dystrophy domain also can present with pronounced proximal affection of muscles, yet are not classified as limb girdle muscular dystrophy. Examples of that are several types of late-onset forms of congenital muscular dystrophies, myofibrillar myopathies and facioscapulohumeral muscular dystrophy.

The symposium and current presentation will discuss these overlapping phenotypes, and make suggestions for some adjustments as to how we classify muscular dystrophies based on their clinical appearance.

THEMATIC WORKSHOP 8.3

Theme: *DIAGNOSTIC BOUNDARIES OF LIMB GIRDLE MUSCULAR DYSTROPHIES*

TW 8.3.2 Overlap of phenotypes in dominantly inherited limb girdle muscular dystrophies vs. other dystrophies

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The original classification of the dominantly inherited limb girdle muscular dystrophies has been the basis for the identification of a number of different disease genes. Within this group of disorders however it is abundantly clear that phenotypic and genetic heterogeneity is the norm, challenging the basis on which we make these diagnoses and extending the phenotypes seen in association with mutations in specific

genes. This is especially true for the disorders overlapping genetically with the myofibrillar myopathies, where several disease genes now have been reported in association with an "LGMD" presentation as well as the more typical mixed proximal/ distal presentation. This is likely to become even more the case as patients are no longer diagnosed by a targeted gene approach but by next generation sequencing.

The way that this impacts on our classification of these diseases but even more importantly on the information that we can give to patients to help with management and prognosis will be discussed.

THEMATIC WORKSHOP 8.3

Theme: *DIAGNOSTIC BOUNDARIES OF LIMB GIRDLE MUSCULAR DYSTROPHIES*

TW 8.3.3 Phenotypic transitions from congenital to limb girdle muscular dystrophies

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Congenital muscular dystrophies (CMD) are defined as conditions with onset at birth or within the first 6 months of life. The last 20 years have seen a dramatic improvement of the understanding of the molecular basis of these conditions with now more than 20 molecularly defined variant, mostly related to mutations in genes encoding for extracellular matrix proteins or proteins with definitive or suspected glycosyltransferase function; or structural components of the nuclear envelope. Several of these proteins have a clear role in allowing adequate muscle formation / satellite cell function, providing an explanation for the prenatal onset of muscle pathology,

The availability of robust genetic testing has allowed to explore the boundaries of the phenotypic spectrum of these conditions, allowing to identify in several instance the occurrence of milder, late onset phenotypes which are for all purposes limb girdle muscular dystrophy (LGMD) variants.

This is clearly the case both for protein of the extracellular matrix such as laminin alpha2, responsible both for a severe CMD but also a –so far not frequently recognized- LGMD form; mutations in the glycosyltransferases, with a few of the mutant genes (such as FKRP or ISPD, but more recently also GMPPB) which are common causes for adult onset LGMD; and also for LMNA mutations, a nuclear envelope protein more frequently affected in an adult onset muscular dystrophy but also now described in CMD.

In my presentation I will describe our experience so far with the diagnosis of these conditions and provide some insight on when to suspect them.

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THEMATIC WORKSHOP 8.4

Theme: *ARTHROGRYPOSIS*

TW 8.4.1 Arthrogryposis a general overview and syndromes

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Arthrogryposis, or arthrogryposis multiplex congenita, is a descriptive term for the presence of multiple congenital contractures at birth. The etiology is heterogeneous and more than 200 conditions are known in which multiple congenital contractures are a predominant sign. The birth prevalence in western Sweden was found to be 1 in 5100 live births (Darin et al 2002) and has been estimated to be between 1 in 3000 to 1 in 12000 live births in different population-based studies.

All forms of arthrogryposis are associated with decreased movements in utero and can be classified from a clinical point of view into three categories: involvement of the limbs only, limb involvement with other abnormalities, or limb involvement with major central nervous system dysfunction. Arthrogryposis can also be classified from an etiologic point of view into disorders of the developing motor system (brain, spinal cord, neuromuscular system or connective tissue) or to abnormalities in the environment of the fetus.

The most common cause of arthrogryposis is amyoplasia representing 1/3 to 1/4 of all affected individuals (Kroksmark et al 2006, Hall et 2014), followed by dysfunction of the central nervous system and distal arthrogryposes syndromes. Amyoplasia and the distal arthrogryposes syndromes will be dealt with by other speakers in this workshop. The patients with central

nervous system dysfunction and arthrogryposis were more severe compared to other subgroups of patients with arthrogryposis in terms of joint contractures at birth, feeding difficulties during infancy, onset of independent walking and mortality. Mental retardation is common in this subgroup. The aim of this presentation is to give a general overview on arthrogryposis, to discuss the group with major central nervous system involvement and other syndromatic causes, such as fetal akinesia deformation sequence and pre- and perinatal disorders of neuromuscular transmission associated with arthrogryposis.

No disclosure.

References:

Darin et al. Multiple congenital contractures: Birth prevalence, etiology and outcome. J Pediatr 2002;140:61–7.

Hall et al. Amyoplasia revisited. Am J Med Genet Part A 2014;164A:700–30.

Kroksmark AK, et al. Muscle involvement and motor function in amyoplasia. Am J Med Genet Part A 2006;140A:1757–67.

THEMATIC WORKSHOP 8.4

Theme: *ARTHROGRYPOSIS*

TW 8.4.2 Amyoplasia: current concepts

Eva KIMBER, Uppsala (Suède)
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Amyoplasia is the most common cause of arthrogryposis, about 1/4 - 1/3 of all cases. Amyoplasia is a highly specific clinical diagnosis. The term amyoplasia implies no muscle growth/ development, and, typically, some muscles are absent or very thin and replaced by fatty or connective tissue, while other muscles are normal. Amyoplasia is a sporadically occuring condition, and there is no known genetic cause. There is no recurrence risk. As in all arthrogryposis, the cause of joint contractures in amyoplasia is decreased fetal mobility. Case studies have demonstrated that vascular compromise/decreased blood flow to the fetus in early pregnancy, gestational week 9–12, is involved in the pathogenesis. It is thought that anterior horn cells in the spinal cord are damaged, resulting in muscle hypoplasia and joint contractures. Contractures are at their maximum at birth, and are usually not progressive. In ¾ of all cases there are joint contractures symmetrically in all four limbs. Involvement of only upper limbs or only lower limbs occurs, as well as asymmetrical involvement. There may also be involvement of the jaws and spine. Typical joint positions at birth are inward rotation and adduction of the shoulders, extended elbows, flexed wrists and fingers, varying contractures in lower limbs with external rotation and abduction of the hips with flexion in the knees or flexion of the hips with extended or flexed knees and bilateral club feet. The face often has a rounded appearance. There is often a midline facial hemangioma and also dimples over affected joints. In about 10% of all cases abdominal wall defects are also present at birth. Children with amyoplasia usually have no intellectual impairment, and are very adapt at finding solutions and ways to compensate for their motor disabilities. They often need extensive treatment with physiotherapy, orthoses, orthopedic surgery etc, and the treatment involves a team with experience of diagnosis and treatment in arthrogryposis. Stretching and muscle activation are important mainstays in treatment, and especially important in early life.

No disclosures.

Hall et al. Amyoplasia revisited. Am J Med Genet Part A 2014;164A:700–30.

Kroksmark AK, et al. Muscle involvement and motor function in amyoplasia. Am J Med Genet Part A 2006;140A:1757–67.

THEMATIC WORKSHOP 8.4

Theme: *ARTHROGRYPOSIS*

TW 8.4.3 Distal arthrogryposis: a developmental myopathy

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Distal arthrogryposis (DA) belongs to a group of syndromes, arthrogryposis multiplex congenita, with congenital contractures affecting several joints in different body areas. Distal arthrogryposis involves mainly hands and feet but additional features such as facial involvement, scoliosis, ophthalmoplegia and hearing deficits have formed the basis for a clinical classification including at least 10 different forms.

The most common forms are classified as DA1 and DA2B. DA1 is restricted to hands and feet whereas DA2B also shows facial involvement and corresponds to the Sheldon-Hall syndrome. A more severe form known as the Freeman-Sheldon syndrome is classified as DA2A.

Recent progress in genetics have disclosed that several of the DA syndromes are associated with dominant mutations in genes encoding skeletal muscle sarcomeric proteins that are expressed during early development such as embryonic and perinatal myosin (MYH3 and MYH8), beta-tropomyosin (TPM2), troponin I (TNNI2), troponin T (TNNT3) and myosin binding protein C (MYBPC1). These observations have lead to the hypothesis that the pathogenesis of DA syndromes in many cases is caused by a developmental myopathy.

Muscle biopsy performed in patients with DA has supported this concept although the embryonic isoform of myosin heavy chain that is frequently mutated in cases of DA1 and DA2 is not expressed postnatally. However, abnormal expression of perinatal myosin as well as abnormal fiber size variability among type 1 fibers have been observed in patients with DA associated with MYH3 mutations. In cases where the mutated protein is expressed postnatally, such as troponin I (TNNI2), more clear evidence of myopathic changes have been obtained.

In DA1 and DA2 there is no clear correlation between the clinical phenotype and the type of mutated protein, since mutations in different genes can be found in the same clinical syndrome and different syndromes can be associated with mutations in the same gene.

No disclosure