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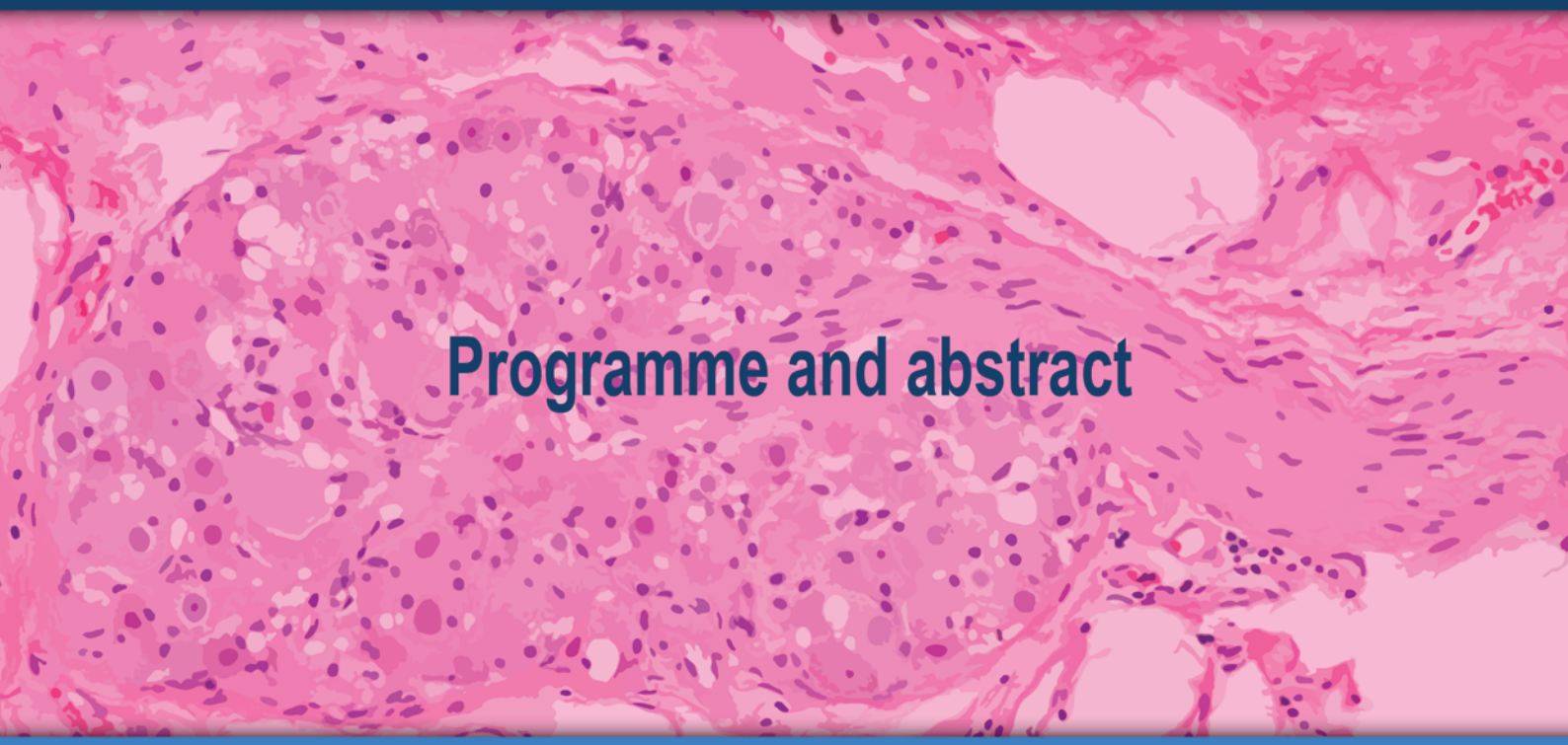
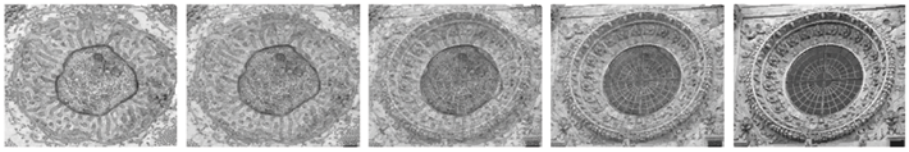
Updates in neuroscience:
Peripheral Nervous System (PNS) in development and disease.



Programme and abstract

Training course on PNS development,
function, damage, regeneration and remyelination.



LECCE, CASTELLO CARLO V, ITALY - JULY 1 - 4, 2014

SCIENTIFIC PROGRAMME

Wednesday – July 2

08:30 Welcome from the scientific organiser
08:45 Student introduction

SESSION I ORGANIZATION OF NERVE FIBERS
Chair: Peter Brophy
09:15 Assembly of Domains of Myelinated Axons – J. Salzer (USA)
09:50 The organization of peripheral myelinated axons – E. Peles
10:25 Coffee break
11:00 The extracellular matrix in myelination – L. Feltri (USA)
11:35 Genetic analysis of Schwann cell development and myelination in zebrafish – W. Talbot (USA)
12.10 LGI proteins in myelination and synapse biology – Dies Meijer (UK)
12:45 Lunch

SESSION II PERIPHERAL NERVE DEVELOPMENT AND MYELINATION
Chair: Ueli Suter
13:45 Mechanisms of early Schwann cell differentiation – P. Brophy (UK)
14:30 Axonal sorting in nerve development: nuclear and cytoplasm control – S. Previtali (Italy)
15:15 Coffee break
15:50 The establishment of cell polarity and initiation of Schwann cell myelination – J. Chan (USA)
16:25 Role of secretases in myelination – C. Taveggia (Italy)
17:10 Epigenetic mechanisms regulating Schwann cell development and myelination – U. Suter (Switzerland)
17:45 Informal student faculty interations

Thursday – July 3

SESSION III AXONAL HEALTH, SUPPORT, DEGENERATION AND REGENERATION
Chair: Giuseppe Lauria
09:00 Adhesion G protein-coupled receptors in PNS development and regeneration – K. Monk (USA)
09:35 Small heat shock proteins in peripheral nerve degeneration – V. Timmermann
10:10 Coffee break
10:30 Painful neuropathies and sodium channels – G. Lauria (Italy)
11:05 The role of Schwann cells in nerve regeneration – K. Jessen (UK)
11:40 Ankyrins and spectrins: all-star protein accumulation machines in axons and Schwann cells – M. Rasband (USA)
12:15 Lunch

SESSION IV: INHERITED NEUROPATHIES, CMTS

Chair: Rhona Mirsky

13:30 Insights from CMT disease into Schwann cell function – D. Sherman (UK)

14:05 Regulation of c-jun expression in Schwann cells - H. Cabedo (Spain)

14:40 Coffee break

15:00 Cellular stress in inherited neuropathies – L. Wrabetz (USA)

15:35 The role of c-Jun in peripheral neuropathies – R. Mirsky (UK)

16:10 Posters

Friday – July 4

SESSION V: INFLAMMATORY, AUTOIMMUNE AND PAINFUL NEUROPATHIES

Chair: Hugh Willison

09:15 Good and bad aspects of inlammation in peripheral nerves: Wallerian degeneration and inherited demyelination – R. Martini (Germany)

09:50 The role of nerve biopsy in the diagnosis of inflammatory neuropathies - A. Quattrini (Italy)

10:25 Coffee break

11:00 Nodo-paranodopathy: beyond the demyelinating and axonal classification in autoimmune neuropathies – Nobuhiro Yuki (Singapore)

11:35 The role of innate and adaptive immunity in mediating human auto-immune nerve disorders – H. Willison (UK)

12:10 Lunch

SESSION VI BENCH TO BEDSIDE AND BACK FOR THERAPY

Chair: Mary Reilly

13:10 Inherited axonal neuropathies – bench to bedside – M. Reilly (UK)

13:45 Inherited demyelinating neuropathies – bedside to bench – M. Shy (USA)

14:20 Coffee break

14:55 New concepts in the therapy of inherited neuropathies – M. Sereda (Germany)

15:20 Challenges of traslating animal work to human nerve regeneration – A. Hoke (USA)

15:55 Innovative biomaterials to promote nerve regeneration – A. Sannino (Italy)

16:30 Informal student faculty interactions

SCIENTIFIC COMMITTEE

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SOCIAL PROGRAMME

Tuesday – July 1

19:30 Patria Hotel: Welcome reception

Thursday – July 3

19:30 Porto Cesareo: Dinner

Friday – July 4

20:00 Convento degli Olivetani: Gala Dinner

Missense mutations in components of the dynein motor complex cause a spectrum of neurodegenerative disorders

Authors: **S. Bervoets1*, K. Peeters1*, J. MacMillan2, B. Castle2, I. Litvinenko3, T. Chamova4, E. De Vriendt1, D. Kancheva1,5, J. Irobi1, V. Timmerman1, P. De Jonghe1, I. Tournev3,6, and A. Jordanova1,5**

* Equally contributing authors
Affiliation: 1Department of Molecular Genetics, VIB & University of Antwerp (UA), Antwerpen (Belgium), 2Genetic Health Queensland, Royal Brisbane and Women's Hospital, Herston (Australia), 3Clinic of Child Neurology, Department of Pediatrics, Medical University-Sofia, Sofia (Bulgaria), 4Department of Neurology, Medical University-Sofia, Sofia (Bulgaria), 5Department of Medical Chemistry and Biochemistry, Molecular Medicine Center, Medical University-Sofia, Sofia (Bulgaria), 6Department of Cognitive Science and Psychology, New Bulgarian University, Sofia (Bulgaria).

We performed genome-wide linkage analysis, followed by whole-exome sequencing of a family with an autosomal dominant, axonal form of Charcot-Marie-Tooth disease (CMT2). We identified a novel missense mutation (c.1792C>T, p.Arg598Cys) in the cytoplasmic dynein heavy chain gene (DYNC1H1). This is the second DYNC1H1 mutation described so far in the CMT population. Mutations in DYNC1H1 also cause other neuronopathies, such as spinal muscular atrophy (SMA), that are predominantly located in the tail domain of the protein. We therefore screened this domain of DYNC1H1 in a cohort of sporadic, Bulgarian non-5q SMA patients and identified another novel missense mutation(c.791G>T, p.Arg264Leu). Genotype-phenotype correlations in both families suggest that affected individuals with axonal CMT or SMA, early onset and lower extremity predominance should be screened for mutations in DYNC1H1. Our findings contribute to a better understanding of the mutational spectrum of DYNC1H1 and the importance of axonal transport for neuronal function in humans.

Establishing myelinating cocultures of iPSC-derived human sensory neurons

Authors: **Alex J Clark, David LH Bennett**

Affiliation: Nuffield Department of Clinical Neurosciences, University of Oxford

Human induced pluripotent stem cells (iPSCs) have opened up new methods to better understand disease and have created opportunities for therapeutic approaches. Rapid progress has been made in the differentiation of neural crest derivatives from iPSCs, including sensory neurons.

Here we demonstrate sensory neurons can be successfully differentiated from iPSCs using a combination of small molecular inhibitors. These neurons generate extensive axonal arbors and express a wide array of proteins commonly associated with sensory neurons in vivo, including VGSCs and Brn3a.

Myelinating co-cultures generated from rodent DRG cells and Schwann cells have previously been used to study axon-glia interactions in vitro. In a novel step forward, we show that human iPSC-derived neurons can be myelinated with the addition of rat Schwann cells. Myelinating cocultures have been maintained for up to 6 months with no loss of myelin or neuron integrity. Nodes of Ranvier successfully form between myelin internodes, and have been detected by immunocytochemistry for Caspr. A time course demonstrates that MBP is abundantly detected after just 3 weeks of initiating myelination.

We are currently advancing this model further by differentiating Schwann cells from iPSCs. We will apply these Schwann cells to iPSC-derived sensory neurons to create an entirely human myelinating coculture. We are currently reprogramming fibroblasts from CMT1A patients to pluripotency, and will differentiate these into Schwann cells. We hope that this will enable the establishment of an in vitro human model of mutation specific, demyelinating neuropathies. We aim to use this model to study disease pathophysiology and progression.

The role of paranodes and cytoskeleton in node formation and maintenance

Authors: **Veronica Brivio1 and Peter J. Brophy1**

Affiliation: 1Centre for Neuroregeneration, University of Edinburgh, Edinburgh EH16 4SB, UK

Myelination of axons in the central and peripheral nervous system (CNS and PNS) is required for saltatory propagation of nerve impulses. Myelinated axons are organized in functionally distinct membrane domains, namely the node of Ranvier, the paranode, the juxtaparanode and the internode, and this organization promotes fast conduction. The correct formation and maintenance of these domains is fundamental for the correct propagation of the electrical impulse but the underlying mechanisms are just starting to be unravelled.
Here we have studied the contribution of the paranodes and their linkage to the cytoskeleton to both nodal formation and maintenance in the PNS and CNS by using a combination of knockout and transgenic rescue approaches.
In particular, we show the essential role of the paranodes in clustering nodal proteins before nodes are formed. Also, intact paranodes are essential in timing the dynamics of node formation. In particular myelinating processes with paranodal proteins lacking a linkage to the underlying cytoskeleton have a delay in node formation similar to mice missing the entire paranode. Despite the initial delay, this phenotype recovers.
Further, we have shown the importance of the axonal paranodal cytoskeleton in the maintenance of the node of Ranvier: when the link between Caspr and the underlying cytoskeleton is removed, paranodal junctions disassemble and this affects both nodal and juxtaparanodal components of the node, first in the CNS and later in the PNS. Animals lacking paranodes display a comparable phenotype, suggesting an essential role of the paranodal cytoskeleton in the maintenance of the nodal compartment.

TITOLO: EMERGING BIOLOGICAL ROLE OF LIPIDS IN PERIPHERAL MYELIN PATHOLOGY

Autore: Giovanna Capodivento

Università o Scuola di afferenza: DINOGMI - Università di Genova

Active myelination requires the high demand of lipid biosynthesis in Schwann cell processes, and the disruption of lipid biosynthesis may result in abnormal myelin and PNS pathology. We found in a rat model of CMT1A disease a striking reduction of genes primarily related to lipid metabolism and a significant reduction of sphingomyelin and cholesterol content which mirror the dys-demyelinating phenotype observed in this model. Interestingly, the analysis of purified myelin from sciatic nerves of CMT1A rats showed that the decrease of lipids in pathological nerves is not merely due to the lack of myelin but also to an altered arrangement of these lipids in the myelin sheath. This prompted us to perform a lipidomic profiling in both the sciatic nerve and cerebrospinal fluid of 60-day-old CMT1A rats and wild type littermates. Due to the structural complexity of lipids and the low abundance of many lipid mediators, we carried out the analysis by both shotgun and targeted analysis using high resolution mass spectrometry. In targeted analysis, we focused on the sphingomyelin pathway to clarify the critical step causing the significant reduction of this sphingolipid in experimental CMT1A. As the sphingolipid signal transduction pathway induces apoptosis, differentiation, proliferation, and growth arrest which have been shown to be involved in CMT1A pathogenesis, we are confident with this comprehensive study to improve our knowledge on the molecular mechanisms underlying this myelin disorder and to get insights which may contribute to the development of therapeutic approaches aiming at the preservation of myelin.

Neurology phenotype in Hereditary Transthyretin Amyloidosis

Authors: **AS Carr1, J Gilmore2, P Hawkins2, MM Reilly1**

Affiliation: 1. MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, 8-11 Queen Square, NHNN, London.
2. National Amyloidosis Centre, University College London Medical School, Royal Free Campus, Rowland Hill Street, London

Background
Hereditary transthyretin amyloidosis (ATTR) is associated with progressive peripheral neuropathy, cardiac, gastrointestinal and autonomic failure due to dominantly inherited transthyretin mutations causing accelerated amyloid deposition. With the exception of V30M, the neuropathy phenotype is less well described than cardiac manifestations.

Methods
A cross-sectional and limited follow up study of ATTR patients attending the National Hospital Inherited Neuropathy Clinic. Detailed clinical neurological and electrophysiological data were collected on all patients.

Results
Thirty-seven symptomatic cases were assessed at least once; 45.9% T60A, 13.5% V30M, 8.1% G47V, 5.4% V122I, 5.4% E89K and the rest individual mutations. 100% of T60A patients had Irish ancestry; of the others16.2% were of UK ancestry and the rest were a mixture.
A length-dependant, axonal, sensory followed by motor neuropathy was typical; 6.7% had patchy onset and 10% had demyelinating features. 45.9% (17/37) had neuropathic symptoms at presentation; 1 with distal weakness, 11 with positive sensory, 4 with negative sensory. T60A cases had later onset ($p>0.05$), less positive sensory symptoms ($p:0.09$) and more advanced cardiac disease than V30M and other mutations ($p>0.001$).
There was no correlation between disease duration and CMTNS ($p=0.14$). 20 cases had follow-up assessments, mean (S.D.):2.1 (1.1 years). Measurable, statistically significant and appropriate changes in CMTNS, MRC score and pinprick sensation were seen ($p>0.001$, $p=0.008$, $p=0.02$).

Conclusion
The neuropathy TTR-FAP phenotype varies with genotype. Total MRC score may be a useful outcome measurement as part of a composite scale. This small but representative study mirrors difficulties observed in recent treatment trials regarding sensitivity of current outcome measures.

PILOT STUDIES OF FACILITATED PERIPHERAL NERVE REGENERATION VIA FORMATION OF A SERIAL RELAY: INTRANEURAL SURVIVAL, AXON EXTENSION, AND SYNAPTIC MARKERS OF TRANSPLANTED MOUSE AND RAT MOTOR NEURONS IN A RAT

Authors: **Christopher R. Cashman,1,2,3 Ruifa Mi,2 Ahmet Höke2,3**

Affiliation: 1 MSTP/MD-PhD Program, 2Department of Neurology, 3Department of Neuroscience, Johns Hopkins University, Baltimore, MD

While acute regeneration in the peripheral nervous system is quite successful, regeneration in the chronic setting is much more limited, largely due to Schwann cell atrophy. To overcome this limit of regeneration, we propose a supra-physiological solution, whereby neurons injected within a nerve may extend axons to form end organ specializations and serve as postsynaptic targets of endogenously regenerating fibers to form a relay from the central nervous system to the end organ. To this end, one of the tibial nerves of an immunosuppressed Sprague-Dawley rat was transected seven days prior to cell transplant. Derived mouse or primary rat motor neurons were injected into the denervated distal stump of the rat tibial nerve and survival assessed at one and three weeks post transplantation. Survival of mouse motor neurons was determined by immunofluorescent (IF) staining to be 1.5% and 4.6% at one and three weeks, respectively, while rat motor neurons had improved survived at one and three weeks (3.3% and 15.7%, respectively). Axons were also observed to project distally. Three weeks after injection, the soleus muscle was also collected to determine neuromuscular junction (NMJ) formation ability. No NMJs were detected by IF. Additional studies will focus on the use of immunodeficient RNU rats, NMJ formation assay after two months, as well as repair of the host tibial nerve to form the relay after endogenous regeneration. Relay connection will be assayed with IF staining as well as electrophysiological and pharmacological characterization.

CEREBELLAR ATAXIA, NEUROPATHY, AND VESTIBULAR AREFLEXIA SYNDROME: A SLOWLY PROGRESSIVE DISORDER WITH STEREOTYPICAL PRESENTATION

Authors: **Cazzato D1, Dalla Bella E1, Dacci P1, Mariotti C2, Lauria G1**

Affiliation: 1Neuroalgology and Headache Unit and 2Clinical Pathology and Genetics Unit, IRCCS Foundation, "Carlo Besta" Neurological Institute, Milan, Italy

Cerebellar ataxia, neuropathy and vestibular areflexia syndrome (CANVAS) is a rare condition with adulthood onset, characterized by progressive impairment of cerebellar, vestibular and sensory functions leading to severe balance impairment. A syndrome characterized by late onset axial and limb ataxia due to bilateral vestibular deficit with cerebellar dysfunction was first described with the acronym of CABV (cerebellar ataxia with bilateral vestibulopathy). More recently, sensory or sensory-motor axonal neuropathy have been included to compose CANVAS. We describe four patients showing a similar clinical picture, characterized by slowly progressive ataxia and sensory disturbances in the feet as presenting features, evolving into severe balance dysfunction. Proprioceptive impairment along with cerebellar and vestibular dysfunctions, were associated with gaze evoked horizontal or down-beating nystagmus, saccadic breakdown of smooth pursuit and abnormal oculo-cephalic reflex, also known as "doll's eye" reflex. Otoneurologic examination demonstrated bilateral vestibular areflexia and impaired visual enhanced vestibulo-ocular reflex (VVOR), which are the prototypical changes in CANVAS patients. Brain MRI revealed vermian cerebellar atrophy. Nerve conduction study showed severe sensory neuropathy with non-lengthdependent distribution and almost completely preserved motor nerve conduction. This patter suggested that CANVAS may be associated with a primary sensory neuronopathy. Possible causes of predominantly sensory neuropathy such as inflammatory, autoimmune, vitamin deficiency, neurotoxic, metabolic or paraneoplastic diseases were ruled out. Genetic disorders, such as dominant spinocerebellar ataxia and mitochondrial diseases characterized by cerebellar atrophy, sensory neuropathy and early ataxia, such as POLG1 and C10ORF2 mutations, were ruled out. Treatment with steroids and IVIG did not change the course of the disease in our patients. In conclusion, CANVAS should be suspected in patients with sensory neuropathy symptoms, early mixed ataxia and eye movement abnormalities suggesting cerebellar and vestibular dysfunction.

TITLE: *IGHMBP2* mutations cause recessive axonal neuropathy: Genetic and functional characterisation in seven families.

Authors: E. Cottenie[1,2], A. Kochanski[3], A. Jordanova[4], J. Baets[4], V. Milic Rasic[5], R. Quinlivan[1], M. Lunn[1], M.G. Hanna[1,2], S. Zuchner[6], M. Harms[7], B.Choi[8], M. M. Reilly[1,2], H. Houlden[1,2]

Affiliation: *[1]MRC Centre for Neuromuscular Diseases, [2]Department of Molecular Neurosciences, UCL Institute of Neurology [3]Neuromuscular Unit, Polish Academy of Science, [4]VIB Department of Molecular Genetics, University of Antwerp [5]Clinic for Neurology and Psychiatry for Children and Youth, University of Belgrade [6]John P. Hussman Institute for Human Genomics, University of Miami, [7]Department of Neurology, Washington University and [8]Department of Neurology, Ewha Womans University*

Abstract:

Background: We analysed two siblings in their 40s with recessive CMT2 using a combination of linkage analysis and exome sequencing. Compound heterozygous mutations in the *IGHMBP2* gene, a helicase enzyme, were identified. A further six families were identifed through Sanger sequencing our cases and the exomes in our collaborators. Mutations in this gene normally lead to the much more severe phenotype of SMARD1 where most children die by one year of age due to respiratory failure. Our patients present in childhood with a typical slowly progressive CMT2, no respiratory weakness or diaphragm problems in adulthood.

Aims: The *IGHMBP2* mutations cause two distinct phenotypes. Genetic and functional studies were performed to better understand the pathways in which the gene is involved and how the difference in genotype can establish a difference in phenotype.

Methods: After genetic analysis, IGHMBP2 protein levels in fibroblasts or lymphoblasts were studied in 8 affected individuals and compared to carriers, controls, and SMARD1 patients. Co-immunoprecipitation was used to look for interacting proteins.

Results: IGHMBP2 levels in CMT2 patients were found to be higher than SMARD1 patients, but reduced compared to controls and carriers. TDP43 was found to interact with IGHMBP2.

Conclusion: Recessive mutations in IGHMBP2 also cause recessive CMT2. This milder phenotype is associated with greater amounts of IGHMBP2 protein in fibrboblasts. Further studies are ongoing to uncover the pathways involved in the cause of disease and we are aiming to compare the results in different celtypes, such as lymphoblasts and motor neurons derived from patient fibroblasts.

Clearance of anti-ganglioside antibodies by endocytosis is a major attenuator of their pathological effects

Authors: **Madeleine E. Cunningham, Rhona McGonigal, Claire Paton, Gavin R. Meehan, Jennifer Barrie, Hugh J. Willison**

Anti-ganglioside autoantibodies associated with Guillain-Barré syndrome injure peripheral axons and myelin by binding to their surface ligands and fixing complement products, thereby driving nerve injury. Many factors, including antibody affinity, quantity and availability can affect the extent of nerve injury. One occurrence which we have recently found to be an important factor in anti-ganglioside antibody-mediated injury is the ability of these antibodies to be endocytosed by ganglioside-expressing cells, especially highly endocytically-active motor nerve terminals.
This uptake phenomenon was investigated in different experimental paradigms using wildtype, GalNAcT-/- and GalNAcT-/--Tg(neuronal) mice, the latter expressing complex gangliosides on neurons only. Following passive immunisation with anti-GD1b antibody, serum levels disappear within 24hrs in wildtype mice but remain elevated at 7 days in GalNAcT-/- mice. GalNAcT-/--Tg(neuronal) mice clear antibody at an intermediate rate. Preliminary results show antibodies against other gangliosides undergo similar processes.
Similar data on clearance are observed in mice which are actively immunised with GD1b. In this paradigm, wildtype mice, which have low levels of circulating antibody, are spared from complement-mediated injury.
These results indicate that wildtype mice, in which GD1b is widely distributed in endocytically active membranes throughout the body, rapidly clear anti-GD1b antibodies from their circulation. In contrast, in GalNAcT-/- ¬which entirely lack GD1b, anti-GD1b antibodies persist harmlessly in the circulation over long periods of time. GalNAcT-/--Tg(neuronal) mice have an intermediate antibody clearance phenotype due to the restriction of GD1b to neuronal membranes. These factors may profoundly affect host vulnerability to antibody-mediated disease by affecting circulating levels of pathological antibodies

Gpr56 is required for Cajal band stability and myelin maintenance in Schwann cells

Authors: **Sarah DeGenova1, Amit Mogha1, Renate Lewis2, Xianhua Piao3, and Kelly Monk1,2**

Affiliation:1Department of Developmental Biology, Washington University in St. Louis, St. Louis, MO 2Hope Center, Washington University in St. Louis, St. Louis, MO 3Division of Newborn Medicine, Department of Medicine, Boston Children's Hospital and Harvard Medical School, Boston, MA

In the peripheral nervous system, Schwann cells (SCs) form myelin by iteratively wrapping their plasma membranes around axons. Then, SC cytoplasm is extruded and compartmentalized to form a compact myelin sheath surrounded by distinct channels of cytoplasm called Cajal bands. Cajal bands are generated by the formation of appositions, or regions where the outer SC membrane adheres to the compact myelin sheath. The function of Cajal bands is poorly understood, though they have been shown to promote microtubule based mRNA transport and to restrict radial growth of myelin. Interestingly, loss of Cajal bands can occur by perturbing structural components of appositions such as Periaxin, and Periaxin mutations cause neuropathy in mouse models and Charcot-Marie-Tooth disease in humans.

We previously showed that the adhesion-GPCR (aGPCR) Gpr126 is essential for SC myelination. The dual roles of aGPCRs in facilitating cell-cell interactions and signaling led us to hypothesize that additional aGPCRs may regulate SC development. We have determined that Gpr56 is also highly expressed in SCs, specifically localized to Cajal bands. Here, we use zebrafish and mouse models to show that loss of Gpr56 impairs formation of Cajal bands, causing demyelination and eventual axon degeneration. We also observed hindlimb clasping by P21 in Gpr56 mutant mice and impaired mobility in gpr56 mutant zebrafish by 3 months, indicative of neuropathy. In sum, these data implicate Gpr56 as a new regulator Schwann cell development and myelin maintenance, and our ongoing work to define the mechanisms of Gpr56 function in Schwann cells will be discussed.

TITLE: UNRAVELLING THE CROSSTALK BETWEEN DEGENERATING NERVE TERMINALS AND PERISYNAPTIC SCHWANN CELLS AT NEUROMUSCULAR JUNCTION

Authors: Elisa Duregotti[1,2], Samuele Negro[1,2], Michele Scorzeto[1], Cesare Montecucco[1,2] and Michela Rigoni[1,2]

Affiliation: [1] Department of Biomedical Sciences, University of Padua and [2]CNR Institute of Neuroscience Via U. Bassi 58/b, 35121 Padova, Italy; Tel: +39 0498276058; Email: elisa.duregotti@studenti.unipd.it

Perisynaptic Schwann cells (PSCs) are non-myelinating Schwann cells intimately associated with the nerve terminal at the neuromuscular junction (NMJ); they are involved in a variety of physiological functions such as embryonic development and maintenance of adult NMJs, as well as modulation of synaptic activity. Moreover, following nerve damage, PSCs de-differentiate and acquire macrophagic-like activities; these "reactive" PSCs actively participate in nerve regeneration by removing nerve debris, recruiting macrophages and extending long processes to guide nerve regrowth (1).

My project aims at unravelling the cross-talk between degenerating nerve terminals and PSCs and at identifying the molecular mediators released by degenerating nerve terminals that might be involved in PSCs activation. In order to follow the degeneration/regeneration process, we have adopted a novel experimental approach based on the use of two classes of animal presynaptic neurotoxins - α-Latrotoxin (α-Ltx) and snake phospholipase-A2 neurotoxins (SPANs) - as tools to induce a localized and reversible nerve injury at the NMJ (2,3).

Intoxication of cultured primary neurons with α-Ltx and SPANs leads to an increased production of ROS, in particular H_2O_2, which can easily diffuse across membranes. H_2O_2 induces ERK phosphorylation in cultured Schwann cells; moreover, increased levels of P-ERK are observed in Schwann cells co-cultured with neurons upon intoxication as well as in PSCs following toxins injection *in-vivo*. ERK signaling pathway plays a central role in controlling Schwann cells plasticity during nerve repair *in-vivo* (4), but so far the molecular mediators responsible for its activation have not been identified: H_2O_2 produced by degenerating neurons is as a good candidate for this role

References:

(1) Robitaille et al., The Neuroscientist 2003
(2) Duchen et al., The journal of physiology 1981
(3) Harris et al., Experimental Neurology 2000
(4) Napoli et al. Neuron 2012

The role of c-Jun in regenerating nerves

Authors: **Shaline Fazal, Cristina Benito-Sastre, José Gomez-Sanchez, Rhona Mirsky and Kristján Jessen**

Affiliation: Cell and Developmental Biology, University College London (UCL)

After nerve injury, up-regulation of Schwann cell c-Jun distal to the injury is crucial for the conversion of myelin- and non-myelin (Remark) cells to functionally effective repair (Büngner) Schwann cells (Arthur-Farraj et al Neuron 2012, Aug 23;75(4):633-47). Here we show that c-Jun is also activated in Schwann cell nuclei proximal to injury, where ~70% of nuclei are c-Jun+ 1hr after nerve transection. Expression is highest 0-2mm from injury and does not extend beyond 7mm. Elevated c-Jun expression lasts at least for 48 hrs. The function of c-Jun expression proximal to injury remains to be established. The regeneration support provided by the distal nerve stump decreased with time after injury. We find that although c-Jun is rapidly activated in Schwann cells of proximal and distal stumps, c-Jun levels continue to rise in distal stumps from 3d to 7d after injury, but, significantly, decline at 6 weeks. Additional correlation between c-Jun and regeneration was found in mice that show accelerated regeneration because of conditional deletion of the Notch signalling protein RBPj in Schwann cells. We show that c-Jun expression in the nuclei of Schwann cells of regenerating nerves is significantly elevated in RBPj mutants compared to WT controls. Together, these experiments open new questions about the role of c-Jun in Schwann cells proximal to nerve injury, suggest that c-Jun is involved in maintaining the repair Schwann cell phenotype and indicate that enhanced activation of c-Jun could promote nerve repair.

TITLE: Neuregulin 1 trafficking and cleavage in the Peripheral Nervous System

Authors: [1,2,4] Maria Grazia Forese, [3] Davide Mazza, [1,4] Carla Taveggia

Affiliation: [1] Division of Neuroscience at San Raffaele Scientific Institute, Milan, Italy; [2] Vita-Salute San Raffaele University, Milan, Italy; [3] Experimental Imaging Center and [4] INSPE at San Raffaele Scientific Institute, Milan, Italy

Myelin is a specialized membrane produced by glial cells and surrounding large calibre axons, which is fundamental for proper transmission of the action potentials along the axons and for providing trophic support to neurons. In the Peripheral nervous System (PNS), Schwann cells are the glial cells responsible for myelin formation. Neuregulin 1 (NRG1) type III is the key factor responsible for PNS myelination, as the amount of myelin formed by Schwann cells correlates with its level of expression on the axonal membrane.

NRG1 type III belongs to a family of growth factors regulating Schwann cells proliferation, differentiation and ultimately myelination. NRG1 activity is regulated by proteolytic cleavage. In particular the extracellular cleavage of NRG1 type III by two different secretases, BACE 1 and TACE, have opposite effects on myelination. BACE1 promotes myelination whereas TACE inhibits it, suggesting that these events are mutually exclusive. However how this proteolytic processing occurs and whether it is regulated in myelination is currently unknown.

To investigate the mechanisms regulating NRG1 type III processing, we are following NRG1 trafficking and cleavage *in vitro* in dissociated sensory neurons. Preliminary experiments suggest that NRG1 type III is highly dynamic and that its trafficking is controlled by several mechanisms.

The results of these studies will clarify how and when NRG1 type III cleavage occurs and are important to better clarify the role of secretases and NRG1 processing in the formation of PNS myelin.

Role of the α-secretase TACE in Central Nervous System myelination

Authors: **Evelien Fredrickx1,2,3, Elisa Colombo1,2 ,3, Giorgia Dina2,3, Angelo Quattrini2,3 and Carla Taveggia2,3**

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The ADAM family of proteins belongs to the zinc family of proteases that are involved in the ectodomain shedding of several growth factors. We previously demonstrated a key role for the α-secretase TACE in Peripheral Nervous System (PNS) myelination by TACE-mediated cleavage and subsequent inhibition of Neuregulin1 (NRG1) type III activity. Unlike the PNS, in which NRG1 type III is an essential instructive signal for myelination, in the Central Nervous System (CNS) oligodendrocyte (OL) development and myelination are likely controlled by several growth factors some of which undergo cleavage by secretases. To assess whether TACE plays a similar role in PNS and CNS myelination, we investigated its role in vitro and in vivo in OL development and myelination. In vitro experiments showed that immunopanned A2B5+ oligodendrocyte precursor cells (OPCs) grown in conditioned medium from TACE-null DRG neurons undergo marked apoptosis. Further, when cocultured, wild type OPCs poorly myelinate TACE-null DRG neurons, suggesting that neuronal TACE is required for in vitro myelination. In agreement, transgenic mice lacking TACE in CNS neurons are hypomyelinated and have an aberrant myelin sheet throughout development. On the contrary, specific ablation of TACE in OLs in vivo did not affect developmental myelination. Our data strongly suggest that the neuronal α-secretase TACE is required for proper CNS myelination. Further, our studies confirm that secretases are important post-translational regulators of myelination although the mechanisms controlling CNS and PNS myelination are distinct.

TITLE:

The involvement of an RNA binding protein in Charcot-Marie-Tooth disease.

Authors:
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Charcot-Marie-Tooth (CMT) disease represents a large group of clinically and genetically heterogeneous disorders affecting the peripheral nerves. Over 900 different pathogenic mutations in 61 disease-associated genes have been identified for CMT and closely related diseases. Among these, 17 missense mutations in the ubiquitously expressed gene *HSP27* coding for small heat shock protein HSPB1 are responsible for axonal CMT neuropathy (CMT2). This set of mutations can be divided in two subsets regarding the domain in which the mutation resides. Mutations inside the conserved α-crystallin domain are shown to enhance the activity of HSPB1. The CMT2F causing mutation HSPB1-P182L is located outside this α-crystallin domain and does not recapitulate this feature. It results in a much severe phenotype of CMT affecting exclusively motor neurons without any sensory involvement. So far no clear pathomechanism is known for this particular HSPB1 mutation.
We have recently identified an RNA binding protein as a novel interactor of HSPB1. This RNA binding protein shows an increased interaction for the mutant HSPB1-P182L. We are currently studying if and how this RNA binding protein might play a role in the development of CMT. By performing RNA-IP sequencing (RIP-Seq) we identified the mRNA repertoire binding to this mRNA binding protein. Next we will investigate if any of the identified mRNAs are dysregulated in the HSPB1 mutant mouse models recapitulating the CMT disease phenotype.

The influence of PMP22 overexpression on PI3K signaling in a rat model of Charcot Marie Tooth disease 1A

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Charcot-Marie-Tooth disease (CMT) is the most common inherited neuropathy of the peripheral nervous system (PNS) which is characterized by sensory and motor deficits. There is no therapeutic treatment available. The most frequent subform of the neuropathy, CMT1A, is caused by a duplication on chromosome 17p11.2-12 encoding the peripheral myelin protein 22kDa (PMP22). To investigate the still poorly understood disease mechanism, we employ a Pmp22 transgenic rat model (CMT rat) which resembles key pathological hallmarks of the human CMT1A disease, such as peripheral dysmyelination, onion bulb formation and axonal loss, particular well.
In a previous study we could show that Pmp22 overexpressing Schwann cells in CMT rats display an impaired differentiation during development which is caused by a reduced activity of the PI3K/AKT signalling pathway. Importantly, Pmp22 haploinsufficient mice, a murine model for the human hereditary neuropathy with liability to pressure palsies (HNPP), display an increased PI3K/AKT activity in Schwann cells.
We aim to decipher how PMP22-overexpression in CMT rats interferes with the PI3K signalling cascade to obtain a better understanding of the disease mechanism in CMT1. First experiments confirmed a higher PMP22 protein expression from postnatal day (P) 1 to P6 in sciatic nerves of CMT rats. By co-immunoprecipitation experiments during these early postnatal time points we plan to investigate a potentially direct influence of PMP22 on downstream components of the PI3K/AKT signalling pathway.

Autophagy deficit as common pathomechanism leading to CMT

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Charcot-Marie-Tooth neuropathies (CMT) are the most common inherited neuromuscular disorders. Among the causative genes associated with CMT, mutations have been identified in two small heat shock proteins (HSP), HSPB1 and HSPB8, responsible for axonal Charcot-Marie-Tooth neuropathy (CMT2F) and distal hereditary motor neuropathy (CMT2L) respectively. HSPB1 and HSPB8 are widely expressed genes coding for chaperone proteins with essential cellular functions. The pathomechanisms underlying HSPB1 and HSPB8 mutations are unknown. We believe that dysregulation of autophagy is a common pathomechanism by which mutations in HSPB1 and HSPB8 induce neurodegeneration in CMT. Autophagy is a cellular housekeeping process which targets, degrades and recycles aberrant protein aggregates and damaged organelles. There is strong evidence for an essential role for autophagy in maintenance of neuronal and axonal homeostasis. Polynuclear cells of CMT patients demonstrate autophagy deficit in vitro. Furthermore, the recent involvement of HSPB1 in microtubule stability, and the involvement of microtubules in autophagosome formation and transport strongly suggest a role of HSPB1 in autophagy. However, neither the underlying mechanisms nor the pathogenicity of these deficits have been unveiled. Therefore my project aims at revealing the role of HSPB1 and HSPB8 in macroautophagy, by studying the impairment of the autophagic process by CMT-causing mutations, and understanding the role of autophagy in the pathomechanisms of CMT neuropathies. In order to achieve these goals, we are using cell lines and transgenic mice expressing the wild type or selected mutant forms of the small HSPs. Our preliminary data suggest that CMT-causing mutations in HSPB1 impair the autophagy flux.

Whole-exome sequencing in patients with peripheral neuropathy 'plus' syndromes

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Background: Identification of pathogenic mutations causing inherited motor and/or sensory neuropathies may be challenging, since more than 78 disease-related genes have been described. More than 85% of the mutations associated with known Mendelian diseases are located in protein-coding exons. Targeted capture and massive parallel sequencing of these genomic regions (whole-exome sequencing) has been demonstrated to be an efficient method for the identification of novel mutations and genes in patients with inherited neuropathies.

Objective: To report our experience with whole-exome sequencing in patients with suspected inherited neuropathy and additional neurological or systemic features.

Methods: After excluding mutations in inherited neuropathy-related genes according to the phenotype, whole-exome sequencing was performed in 25 individuals from 18 unrelated pedigrees. Enrichment of coding exons and flanking intronic regions was performed with Illumina TruSeq and Agilent SureSelect capture products. Sequencing was performed on HiSeq 1000 and 2000 platforms.

Results: From a total of 18 probands (10 familial cases; 8 sporadic cases), 11 (61%) had sensory neuropathy and ataxia, 4 (22%) had a motor and sensory or pure sensory neuropathy with ophthalmic features (i.e. optic atrophy, retinopathy, ptosis, ophthalmoparesis), 1 (6%) had a sensory neuropathy with upper motor neuron signs, 1 (6%) had sensory neuropathy and deafness, and 1 (6%) had a motor and sensory neuropathy and proximal myopathy.

Conclusion: A probable pathogenic variant was identified in 4 different genes in 4 unrelated probands (1 familial case and 3 sporadic cases). Analyses of novel candidate genes in the remaining cases are ongoing.

Endogenous antibodies contribute to demyelination in a mouse model for CMT1B

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Charcot-Marie-Tooth (CMT) type 1 neuropathies are non-treatable inherited disorders of the peripheral nervous system. Our group could previously identify the substantial pathogenic impact of nerve macrophages in CMT1 mouse models. These reactions are partially comparable to Wallerian degeneration (WD), although in the CMT models occurring in the presence of mechanically non-injured axons. Similar to WD, the myelin sheaths of the mutant nerves are decorated with endogenous antibodies which may play a role in the macrophage-related demyelination. In order to identify the role of these antibodies, we crossbred P0+/- mutants with mice, specifically lacking B-lymphocytes (JHD-/-). As expected, this resulted in a lack of decorating antibodies, and, most interestingly, in a transient amelioration of demyelination in peripheral nerves of young mice. Furthermore, the lack of endogenous antibodies was accompanied by a reduction of endoneurial macrophages. Unexpectedly, in older P0+/- mutants, the absence of endogenous antibodies resulted in disease aggravation accompanied by an increase in immune cell numbers. Therefore, our study suggests that in a mouse model for CMT1B, endogenous antibodies contribute to early macrophage-related demyelination and disease progression, whereas at older ages, B-cells might be necessary to dampen pathogenic immune reactions. Thus, both the innate and adaptive immune system are mutually interconnected in a genetic model for demyelination.

Hereditary Sensory Neuropathy Type 1 (HSN1) secondary to SPTLC1/2 mutations: Investigating the role of deoxysphingolipids in the pathogenesis.

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Background: HSN1 is a progressive sensory motor neuropathy leading to profound loss of sensation with variable motor involvement. Mutations in the genes SPTLC1 and 2, which encode for subunits of the enzyme serine palmitoyltransferase, alter its substrate specificity leading to the build of deoxysphingolipids (dSLs). These are postulated to be neurotoxic

Aims: Determine the effects of dSLs on different neuronal types (sensory versus motor) in-vitro.

Method: Cell survival and neurite outgrowth were assessed in primary motor neuron cultures (wild-type mice embryos) and dorsal root ganglia cultures (7-8 day post-natal pups) following treatment with dSLs (deoxysphinganine and deoxymethylsphinganine) for 12, 24,36 and 48 hours. Immunocytochemistry directed against Beta III Tubulin was used to image the neurons.

Results: preliminary results show that treatment with deoxysphinganine (DSA) and deoxymethylsphinganine (DMSP) caused a significant reduction in motor neuron survival at 48 hours only (DSP:62% when compared to control lipid of 100%, p=0.029, DMSP: 41%, p=0.029). A reduction in DRG survival of 69% was noted at 24 hours when treated with DSA. Further experiments are underway to evaluate the significance of this and to test longer treatment durations (48 hours).

Conclusions: Deoxysphingolipids reduce survival of primary motor neurons and dorsal root ganglia in cultures. DRG cultures appear more vulnerable than motor neurons, mimicking the clinical picture seen in HSN1. Further studies are now needed to investigating the cellular mechanisms involved in the toxicity and how this causes sequential damage to sensory and motor neurons.

AGING AND EPIDERMAL INNERVATION IN SPRAGUE-DAWLEY RATS

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Skin biopsy allows the evaluation of small nerve fibers which are responsible for the perception of thermal sensitivity and pain. This class of nerve fibers is early affected in most peripheral neuropathies. In human subjects, it has been recently demonstrated a correlation between intraepidermal nerve fiber density (IENFD) and aging, which has never been assessed in rats. Aim of this study was to evaluate the age-related IENFD changes in Sprague-Dawley rats, in order to provide normative data useful for experimental trials of neuroprotection.
Two groups of 10 Sprague-Dawley male rats aged 6 and 12 months were examined. Skin biopsy was performed using a 3-mm punch on the right hindpaw under isoflurane-anesthesia. Animals were maintained alive until they were 17 and 21 months, respectively, when follow-up biopsies were performed on the left hindpaw. Specimens were processed for bright-field immunoistochemistry and stained with anti-protein gene product 9.5 (PGP 9.5) antibody. IENFD was quantified using standardized rules using a software for biological image analysis. IENFD declined with aging in both the groups, showing significantly higher values at 6 months (13±0.4) and 12 months (11.4±0.48) compared to 17 months (8.2±0.46) and 21 months (7.9±1.08). Our results demonstrate an age-dependent decrease of IENFD in Sprague-Dawley rats. This finding could be of substantial aid in selecting animals of appropriate age for specific experimental research.

SKIN AND SURAL NERVE BIOPSY FOR THE EVALUATION OF MYELINATED FIBER IN PERIPHERAL NEUROPATHIES: A COMPARATIVE STUDY

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Management of peripheral neuropathies implies an optimal multimodal diagnostic approach, which firstly exploits clinical tools and non-invasive neurophysiological and laboratory tests. In some cases these tools are insufficient and invasive procedures with histological evaluation are needed. Sural nerve biopsy is considered a gold standard procedure for neuropathological evaluation of peripheral neuropathies, even if invasive and non-repeatable. On the other side, skin biopsy is an interesting tool thanks to its minimal invasiveness and repeatability, but its clinical use is currently limited to the study of unmyelinated fibers. The presence in sub-papillary dermis of large myelinated fibers leaves the door open to the possibility of their histopatological evaluation. In this work, therefore, we evaluated the informativeness of skin and sural nerve biopsy on myelinated nerve fibers through optical and electronic microscopy, with a view to determine whether skin biopsy may represent a significant diagnostic option for clinicians facing peripheral neuropathies. We recruited 23 patients with sensory or motor-sensory polyneuropathy who underwent sural nerve biopsy and two skin punch biopsies: the first one, performed at distal leg, was used for the evaluation of small unmyelinated intraepidermal fibers according to international guidelines; the second biopsy was used for morphological analysis of dermal myelinated nerve fibers. Our results demonstrate that skin biopsy is an useful technique to detect most of neuropathological features described above, only found, up to now, by sural nerve biopsy. Further studies are needed to select the conditions where skin biopsy may effectively support minimally-invasive diagnosis and neuropathological follow-up of peripheral neuropathies.

An exome-wide gene identification approach to unravel the molecular pathology of hereditary motor axonopathies.

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Hereditary motor axonopathies are clinically and genetically heterogeneous disorders causing progressive length-dependent degeneration of motor neurons. At present, the majority of patients remain without molecular diagnosis and our understanding of the mechanisms leading to axonal degeneration is incomplete.
A broader knowledge of the genetic background of these diseases, will lead to a better understanding of the molecular pathology and ultimately to the identification of potential targets for effective therapies. To further unravel the genetic factors of motor neuron degeneration, we first applied whole-exome sequencing (WES) to 24 samples of unrelated index patients.
We were able to identify the disease causing mutation in a known causal gene in 7 out of 24 patients (~30%). Interestingly, we identified mutations in 2 genes associated with a different neuropathy phenotype, suggesting phenotypic variability.
In the remaining 17 patient samples, no mutations in known disease genes were found. To identify novel causal genes, we performed WES of 18 additional affected family members of the unsolved index patients. Filtering based on inheritance, impact, frequency and quality of the variants resulted in a range of 10-137 variants per family depending on the amount of samples sent and the structure of the family. Candidate genes will be screened in a follow-up cohort of 163 patients with similar phenotypes using a MASTRTM assay for targeted resequencing. The subsequent functional assays will help to determine the effect of the mutant protein and retrieve the underlying pathomechanisms.

Proximal lesions of median nerve

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Aim: To describe the usefulness of a combined approach by nerve conduction study (NCS) and nerve ultrasound in diagnosing and therapeutical approach in proximal median nerve lesions.

Case reports:
A 56-year old women complained of persisting pain in a right lateral forearm after blood withdrawal. Pain worsened during pronation-supination movements of the elbow. The neurological examination revealed only a mild reduction of pinprick sensation in the territory of the median nerve. NCS of median and ulnar nerves and needle electromyography (EMG) of thenar, hypothenar, pronator teres and wrist and finger flexor muscles provided normal findings. Ultrasound examination (15 MHz linear probe) performed using continuous tracing technique on the inner border of the thin hyperechoic epineural rim showed increased cross sectional area of the median nerve in the proximal third of the forearm (12 mm^2 vs 7 mm^2 on healthy side) without signs of compression . The diagnosis of pronator teres syndrome was made.
A 23-year old men complained of right hand weakness during daily activities. The neurological examination revealed weakness of long flexor of the thumb, index and middle finger muscles, and superficial hypoesthesia in the lateral aspect of the forearm, thumb and index finger. NCS showed absent sensory nerve action potential of the median nerve, reduced compound muscle action potential amplitude of the median nerve recorded from the abductor pollicis brevis muscle without conduction block. EMG showed active denervation in the flexor pollicis longus muscle. Magnetic resonance imaging of the brachial plexus was normal. Ultrasound examination revealed increased cross sectional area of the median nerve in the proximal third of the forearm, without signs of compression (22 mm^2 vs 9 mm^2 on healthy side). The diagnosis of proximal median nerve lesion was made.
Conclusion: The combined approach with NCS/EMG and ultrasound examination allowed to demonstrate non-compressive proximal nerve lesions, driving the therapeutic strategy toward conservative treatments in both the patients. NCS/EMG and ultrasound examinations should be considered in the differential diagnosis of proximal lesions of limb nerves, with the aim to identify site and cause of the damage and address the therapeutic strategies, including the choice of surgical approaches.

A functional interdependency between sulphatide and complex gangliosides is required to maintain nervous system integrity

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Glycolipids are enriched in axonal and myelin membranes and have fundamental roles in the formation and maintenance of both central and peripheral nerve myelinated axons. The node of Ranvier disorganization observed both in mice deficient in glycolipids or structural nodal proteins (Caspr, NF155) suggests a trafficking or targeting interdependency between glycolipids and these proteins.

Sulphatide deficiency in mice (achieved through CST gene knockout) results in age dependent neurodegeneration (death ~1 year) with nodal disorganization characterised by absence of axo-glial transverse bands, invasion of the paranode by juxtaparanodal Kv1.1, lengthening of the nodal Nav1.6 clusters, and reduced conduction velocity. Sulphatide also recruits glial NF155 into lipid rafts to form the paranodal junction. Complex ganglioside deficient mice (GalNAcT-/-) exhibit a milder version of this phenotype, achieving a normal lifespan.

To assess functional interactions and redundancies between sulphatides and complex gangliosides, we generated double-null mice (CST-/- x GalNAcT-/-). These mice have a rapidly progressive degenerative phenotype, dying between P20-P25, which coincides with peak myelination. This suggests there may be an important interaction between sulphatide and complex gangliosides that is essential to nerve integrity, myelin formation and nodal architecture. The double-null mouse phenotype can be rescued by neuron-specific expression of GalNAcT, indicating a critical role for neuronal complex gangliosides.

While the underlying mechanism for this lethality is yet to be uncovered, we are examining the neurodegeneration and nodal organization in both the PNS and CNS of these mice by ultrastructural, immunohistological, behavioral and electrophysiological analysis.

TITLE: DEGENERATING NEURONS RELEASE ALARMINS TO PROMOTE PERISYNAPTIC SCHWANN CELLS ACTIVATION

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Perisynaptic Schwann cells (PSCs) that have been considered for a long time merely as passive supporting players at the synapse, are instead essential components of the neuromuscular junction (NMJ). Following denervation, PSCs can sense axonal injury by detecting danger molecules (so-called DAMPs) released by degenerating nerve terminal and de-differentiate to an earlier developmental stage acquiring macrophagic-like activities. These "reactive" PSCs actively participate in the process of nerve regeneration (1).

We have validated a novel experimental set-up based the use of two classes of animal presynaptic neurotoxins that induce a specific and reversible degeneration of nerve terminals. We detected mitochondrial DNA (mtDNA), cytochrome c and peroxiredoxins in the supernatant of cultured primary neurons upon intoxication with these neurotoxins. We propose these *signalling* molecules as putative inducers of PSCs activation. mtDNA and peroxiredoxins may interact with Toll-like receptors (TLRs), that are widely expressed by SCs. We are currently investigating whether these molecules do activate the NF-kB pathway downstream of TLRs in primary SCs cultures, in co-cultures of SCs with primary motoneurons and at the isolated NMJ. Preliminary data speak in favour of such hypothesis. We are also testing whether exosomes might be employed by neurons as delivery system to communicate with nearby SCs.

Perlecan is recruited by dystroglycan at nodes of Ranvier and binds gliomedin to promote sodium channel clustering.

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Neural saltatory conduction requires clustering of voltage-gated sodium channels at nodes of Ranvier in both peripheral and central nervous system. Our lab previously reported that dystroglycan (DG), a glial laminin receptor, is found in microvilli, and that Schwann cell (SC)-specific ablation of DG causes reduced conduction velocity, abnormal clustering of sodium channel and disorganization of microvilli. Similar alterations were found in mice lacking SC laminins, suggesting that specific laminins and DG complexes at peripheral nodes contribute to microvilli and sodium channel cluster formation. If DG is required for the formation or maintenance of sodium channel clusters, and by which mechanism is unknown. We show that nodal DG faces both the basal lamina and the axon. Additionally, DG is recruited to nascent nodes, where it is required for the formation of heminodes and compact sodium channel clusters. We hypothesize that DG could affect nodes formation by interacting with other components of the nodal gap via its mucin-like domain. Indeed, we find that the DG ligand perlecan is a novel proteoglycans found in PNS nodes, and that perlecan localization at nodes is lost in the absence of DG. Perlecan binds the nodal gliomedin, and enhances sodium channel clustering by favoring interaction of gliomedin to axons. This work identifies perlecan as a heparin-sulfate-proteoglycan required for gliomedin function. This finding indicates that nodal gap proteoglycans have specific roles in peripheral nodes, similar to what previously demonstrated already for central nodes.

The role of target glycolipids in the internalisation of anti-ganglioside antibodies

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In Guillain-Barré syndrome, anti-ganglioside autoantibodies (AGAbs) bind surface ganglioside ligands on peripheral nerve glial and axonal plasma membranes where they induce injury through complement activation with pore formation. It has recently been discovered that endocytosis of AGAbs at endosomally active membranes (eg. motor nerve terminals, MNTs) can massively attenuate motor nerve injury. In order to explore the idea of differential membrane injury based on an endosomal turnover hypothesis we compared the uptake properties of AGAbs of different subclasses with different targets, both ex vivo and in vivo.

Internalisation at both axonal and perisynaptic Schwann cell membranes was examined using ex vivo triangularis sterni muscle preparations from wildtype, GalNAcT-/-, GalNAcT-/-(neuronal) and GalNAcT-/-(glial) mice. GalNAcT-/- have no complex gangliosides and therefore no target, while the latter two express complex gangliosides on neurons and glia, respectively. Application of anti-GT1b antibody demonstrated extensive tissue binding in all mice expressing complex gangliosides. The degree of internalisation at physiological temperatures correlated with ganglioside expression, being extensive in WT, absent in GalNAcT-/-, and intermediate in GalNAcT-/-(neuronal) and GalNAcT-/-(glial) mice.

In vivo, internalisation was examined by assessing circulating AGAb levels following intraperitoneal injection of AGAb. We observed differential rates of serum clearance and internalisation, dependent on target availability and antibody subtype. Our findings demonstrate that neural membranes are capable of internalising circulating AGAbs of varying specificity, according to levels and site of target ganglioside expression. This is fundamental to our understanding of the differential susceptibility of neural membranes to AGAb injury in different clinical populations.

Proteome analysis of peripheral myelin identifies novel myelin proteins and candidate neuropathy loci

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Many aspects of peripheral myelin biogenesis and pathophysiology are poorly understood. We hypothesized that only a subset of all myelin proteins has been identified. By combining gel-based and gel-free proteomic approaches, we identified 545 proteins in purified mouse sciatic nerve myelin, including 36 previously known myelin constituents. By mass spectrometric quantification, the predominant myelin protein zero (P0, MPZ), periaxin (PRX), and myelin basic protein (MBP) constitute 21, 16, and 8% of the total myelin protein, respectively. This suggests that their abundance was previously misestimated because of technical limitations regarding protein separation and visualization. Finally, the systematic comparison of our compendium with the positions of human disease loci allowed us to identify novel candidate genes for hereditary demyelinating neuropathies. We have also developed a protocol to systematically identify alterations of the peripheral myelin proteome in pathological situations. Such a differential approach was first applied to a mouse model of demyelinating polyneuropathy caused by the loss of prion protein (PrPC). Here, we found a considerably altered abundance in myelin of septin 9 (SEPT9), the protein affected in hereditary neuralgic amyotrophy (HNA). We are now analyzing the biology of septin proteins in peripheral nerves using the tools of biochemistry, immuno-visualization, and mutagenesis in mice. Together, our results demonstrate the capacity of unbiased proteome analysis for a more complete molecular understanding of peripheral nerves in health and disease.

Mouse Schwann Cells Need Both NRG1 and Cyclic AMP to Myelinate.

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Genetically modified mice have been a major source of information about the molecular control of Schwann-cell myelin formation, and the role of -neuregulin 1 (NRG1) in this process in vivo. In vitro, on the other hand, Schwann cells from rats have been used in most analyses of the signaling pathways involved in myelination. To correlate more effectively in vivo and in vitro data, we used purified cultures of mouse Schwann cells in addition to rat Schwann cells to examine two important myelin-related signals, cyclic adenosine monophosphate (cAMP), and NRG1 and to determine whether they interact to control myelin differentiation. We find that in mouse Schwann cells, neither cAMP nor NRG1, when used separately, induced markers of myelin differentiation. When combined, however, they induced strong protein expression of the myelin markers, Krox-20 and P0. Importantly, the level of cAMP signaling was crucial in switching NRG1 from a proliferative signal to a myelin differentiation signal. Also in cultured rat Schwann cells, NRG1 promoted cAMP-induced Krox-20 and P0 expression. Finally, we found that cAMP/NRG1-induced Schwann-cell differentiation required the activity of the cAMP response element binding family of transcription factors in both mouse and rat cells. These observations reconcile observations in vivo and on neuron-Schwann-cell cultures with studies on purified Schwann cells. They
demonstrate unambiguously the promyelin effects of NRG1 in purified cells, and they show that the cAMP pathway determines whether NRG1 drives proliferation or induces myelin differentiation. (235 words)

Identification of genetic and molecular factors that function in the gpr126-mediated peripheral myelination program

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Myelination in the peripheral nervous system requires Gpr126, an orphaned adhesion family G protein-coupled receptor (aGPCR). In zebrafish and mouse gpr126 mutants, Schwann cells associate with, but fail to wrap axons (Monk et al. 2009, Monk et al. 2011, Mogha et al. 2013). As an aGPCR, Gpr126 is purported to have dual roles in cell-cell/cell-matrix interactions, via its large extracellular N-terminal region, and in signal transduction through its 7-pass transmembrane domain. We are characterizing these potential molecular functions of Gpr126 in Schwann cells using a multisystem approach.

To define critical domains of Gpr126 and its endogenous ligands, we are performing structure-function analyses of Gpr126. Our biochemical, in vitro, and in vivo data suggest a model in which Schwann cell-derived Laminin binds this extracellular region to drive radial sorting and development. We are also performing genetic and chemical enhancer/suppressor screens in zebrafish to identify interactors with gpr126. We have identified multiple candidate mutants that enhance or suppress a hypomorphic allele of gpr126, and we are currently identifying the causative lesions via next-generation sequencing. Additionally, our in vivo pharmacological screen has revealed over twenty compounds that rescue myelination in gpr126 mutants. We hypothesize that a subset of these small molecules function as exogenous agonists for Gpr126. Taken together, our work has identified multiple genetic and molecular interactors of Gpr126 in Schwann cell development and myelination.

XBP-1 SPLICING TO DETECT COMPOUNDS THAT REDUCE UPR ACTIVATION IN CMT1B

Background: Charcot-Marie-Tooth (CMT) 1B is the second most common form of autosomal dominant CMT1 and results from mutations in myelin protein zero (MPZ). Recent studies have demonstrated the role of endoplasmic reticulum (ER) accumulation of misfolded MPZ and unfolded protein response (UPR) activation in the pathogenesis of multiple MPZ mutations. UPR activation is mediated by three arms, one of which involves inositol requiring enzyme 1 (IRE1) that splices the mRNA of the transcription factor X-box binding protein-1(XBP-1). Our group has previously demonstrated that treatment with phosphatidylcholine curcumin relieves ER stress by reducing the UPR activation in both cell based assays and murine models.
Objective: Our goal is to investigate a series of designed compounds with structural similarity to curcumin for their ability to alter the XBP-1 splicing arm of the UPR as candidate therapies for some cases of CMT1B.
Method: We cotransfect Cos7 cells with MPZwt or mutant MPZ and a plasmid containing XBP1 fused out of frame with enhanced green fluorescent protein (EGFP). With UPR activation, EGFP is pulled into frame by XBP-1 splicing and results in the emission of nuclear green fluorescence. Cells are either left untreated or pretreated, according to manufacturer's instructions. We have received a series of compounds developed by AndroScience based on the structure of curcumin which we will test in a blinded fashion.
Results: We have confirmed that XBP1 assay is a valid and reproducible method to test UPR activation in vitro and that curcumin treatment reduces XBP1 splicing. Results about the effects of the new compounds are underway and will be completed by the meeting.
Conclusions: We hypothesize that the curcumin derivatives we are testing, may show a better activity than curcumin itself in reducing UPR activation. If this proves correct we will identify the compounds that show the greatest activity and test them in knockin mice with CMT1B.

DEFINING THE PERIPHERAL NERVE ENVIRONMENT IN AMYOTROPHIC LATERAL SCLEROSIS AND MOTOR NEUROPATHIES: MORPHOLOGICAL AND WG EXPRESSION STUDIES IN HUMAN MOTOR NERVE BIOPSIES

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The causes and pathogenesis of Amyotrophic Lateral Sclerosis (ALS) remain poorly understood. To date, only few treatments can prolong survival of ALS patients, as well an identified biomarker is still lacking for diagnosis. Therefore, the characterization of pathways involved in the pathogenesis of this disease could help in the identification of new biomarkers and to provide insight into the development of new therapeutic strategies.
We demonstrated specific alterations in human ALS motor nerves, supporting the utility of the biopsy of the motor branch of the obturator nerve for an early differential diagnosis of selected cases of Lower Motor Neuron Disease. Specific histopathologic diagnostic criteria have been established.
To investigate the molecular changes underlying ALS, we performed whole genome expression (GE) profiling study on motor and sural nerve biopsies. Based on pathologically-validated diagnosis, the following groups of patients have been studied: Group 1, ALS (N:10); Group 2, Motor Neuropathy (N:8).
Total RNA was isolated from nerves. GE study was performed using the Illumina Beadchips. Sample clustering analysis based on the absolute correlation metric parameter was also generated with GenomeStudio software. The dendrograms were able to show a good segregation of our group samples. Differentially expressed genes (DEGs) were identified. We carried out a pathway analysis, in order to assign biological meaning to the group of DEGs. Our preliminary data suggest similar gene expression profiles in the different diagnostic groups and stimulate further analyses in order to unravel the pathogenetic pathways underlying the development of ALS and other PNS disorders.

Generation of sensory neurons and Schwann cells from human-iPS cells of CMT patients: a suitable tool to test new developing drugs

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Human induced pluripotent stem cells (iPSC) provide an invaluable resource for regenerative medicine, modeling of human diseases, and to test new developing drugs. Within a collaborative project aimed at identifying new chemical drugs to interfere with peripheral nerve myelination, we decided to generate human dorsal root ganglia (DRG) neurons and Schwan cells to test efficacy and toxicity of promising drugs. We induced iPSC from fibroblasts obtained by two healthy subjects and 2 CMT1B patients (D102Tfsx12 and S78L mutations). iPSC clones were multipotent, as they could generate cells of different germ layers either in vitro or in vivo in nude SCID (immunodeficient) mice. Then, iPSC were grown in embryo bodies and differentiated in neural crest stem cells by bFGF, EGF and NOGGIN, as revealed by positivity for p75NTR and HNK1. FACS sorted neural crest stem cells (p75NTR/ NHK1 positive) were subsequently plated on fibronectin/laminin-coated coverslips in neurobasal medium supplemented with growth factors, ascorbic acid and cAMP, and differentiated in DRG sensory neurons. These cells showed diffuse positivity for neurofilaments, beta-tubulin type III and calcitonin gene related peptide (CGRP), and formed extended axonal network. Conversely, neural crest stem cells plated on matrigel-coated coverslips in Mesenpro medium supplemented with bFGF and neuregulin 1-ECD generated Schwan cells. iPSC-derived Schwann cells showed spindle shape and birefringence appearance in phase contrast, and immunohistochemical positivity for S100, GFAP and Sox10. Both iPSC-derived DRG sensory neurons and Schwan cells treated with experimental drugs were viable and did not show differences in differentiation and survival. Our results confirm that iPSC-derived neurons and Schwann cells may be a useful tool to text efficacy or toxicity of experimental therapies on human peripheral nerves in vitro.

TITLE: Study of the degeneration and regeneration of the neuromuscular junction in models of autoimmune neuropathies.

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The neuromuscular junction (NMJ) is a 'tripartite' synapse, composed of the presynaptic nerve terminal (NT), the muscle and perisynaptic Schwann cells (pSCs). NMJ functionality is essential for the execution of body movements and is compromised in a number of disorders. Our laboratory is currently exploring the cross-talk between NT and pSCs using animal presynaptic neurotoxins (α-latrotoxin from the black widow spider and some snake presynaptic PLA2 neurotoxins named SPANs) as tools to induce an acute and reversible neuronal degeneration (1). We have identified some mediators released by degenerating NT that are "sensed" by pSCs; these molecules trigger pSCs activation both in *vitro* and in *vivo*, thus ensuing axonal regeneration and muscle reinnervation. Our aim is to extend such analysis to the degeneration/regeneration processes taking place at the NMJ following autoantibodies-mediated damage.
In axonal forms of Guillain-Barrè Syndrome (GBS) autoantibodies against specific gangliosides (glicolipids highly enriched in many neural tissues, including the presynaptic NMJ membrane) are produced and in turn activate complement cascade, leading to nerve degeneration (2). Such process is electrophysiologically and morphologically akin to α-latrotoxin-mediated degeneration (3,4). We propose that the intra- and intercellular signaling that takes place at the NMJ following animal neurotoxins poisoning might be common to different forms of motorneurons damage. Therefore we plan to perform both in *vitro* and in *vivo* experiments employing monoclonal antibodies against specific gangliosides plus complement and GBS patients sera to characterize the molecular mechanisms that take place at the damaged NMJ during degeneration and regeneration of nerve terminals.

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A dominant negative mutation in FBXO38 is a cause of distal hereditary motor neuropathy (dHMN)

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Background: The dHMNs are a genetically heterogeneous group of diseases characterised by distal lower motor neuron (MN) weakness. Only 20% of cases have mutations in known genes.

Aims: To identify the causative gene and underlying pathomechanism in a large unresolved dHMN pedigree.

Methods: We employed whole exome sequencing to identify a mutation in FBXO38 in two unrelated families with dHMN. FBXO38 is a co-activator of KLF7 transcriptional activity, a transcription factor with a role in neuronal development and repair. We used luciferase reporter constructs to examine the effect of the FBXO38 mutation on KLF7 transcriptional activity. In addition, we examined neurite outgrowth in primary mouse embryonic MNs infected with lentivirus expressing mutant FBXO38.

Results: We identified a p.Cys206Arg mutation in FBXO38 as the causative mutation in two dHMN pedigrees. Mutant FBXO38 was found to impair KLF7-mediated transactivation of a KLF7-responsive promoter construct in both HEK293T cells and patient-derived fibroblasts. This transcriptional dysregulation was associated with impairment of neurite outgrowth in primary embryonic mouse MNs expressing mutant FBXO38.

Conclusions: The p.Cys206Arg mutation in FBXO38 is causative of dHMN, highlighting the importance of FBXO38 and KLF7 in MNs.

Modulation of Neuregulin-1 signaling in P0S63del-CMT1B Neuropathy.

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Myelin Protein Zero (P0) is a highly abundant transmembrane glycoprotein synthesized by myelinating Schwann cells. More than 120 mutations of the P0 gene (Mpz) are associated with various forms of neuropathy, many of them with dominant inheritance. In particular, the deletion of serine 63 in the extracellular domain of P0 (P0S63del) is responsible for the demyelinating Charcot-Marie-Tooth type 1B and it has been shown to produce a similar neuropathy in transgenic mice. P0S63del is a misfolded protein retained in the endoplasmic reticulum (ER) that fails to be incorporated into myelin. This results in the activation of a pathogenetic unfolded protein response (UPR), via a dose-dependent, toxic gain of function mechanism (Wrabetz et al., 2006; Pennuto et al., 2008). S63del nerves manifest not only demyelination, but also a developmental hypomyelination. To explore the hypothesis that increasing a key pro-myelinating axonal signal, such as NRG1 Type III, could ameliorate the phenotype, we crossed S63del mice with transgenic mice that over express an HA-Nrg1 Type III (HANI) fusion protein (Velanac et al., 2011). In HANI/+// S63del sciatic nerves we found an increase of myelin thickness, more obvious in smaller caliber axons, as compared to S63del nerves. Conversely, axons with larger diameter remained hypomyelinated as compared to WT, with a g-ratio similar to S63del nerves. Surprisingly, myelin protein and gene expression levels were not increased in HANI/+// S63del sciatic nerves despite a significant increase of phospho-AKT, that was not followed by a correspondent Krox20 protein expression. Furthermore, the UPR was still active at a level comparable to that of S63del nerves. However the neurophysiological parameters were significantly ameliorated in HANI/+// S63del. Taken together with previous results in HANI/Q215X/CMT1B mice, these data provide preliminary proof of principle that modulation of Nrg1 Type III activity could be a general potential treatment for MPZ-related neuropathies.

Investigating the causes for impaired nerve regeneration in aging rats

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It is well established that aging has a negative impact on nerve regeneration. Multiple explanations for this have been proposed, including: 1) older neurons may be less able to regenerate, 2) older Schwann cells may be less supportive of regeneration, and 3) there may be a delay in degeneration and clearance of axonal and myelin debris. To test these hypotheses, I performed nerve grafting with 2 month old and 18 month old Brown-Norway male rats. I sutured 1 cm sciatic nerve grafts from young or aged rats into sciatic nerves of young or aged rats. Regeneration was allowed to occur until the tissues were harvested at 3 days, 7 days, 2 weeks, and 6 weeks. Once harvested, the grafts and distal stumps were examined for debris clearance and axonal regeneration. My current data suggest that placement of aged nerve grafts into nerves of young rats impairs regeneration of young neurons, while the placement of young nerve grafts into the nerves of aged rats improves regeneration of aged neurons. Moreover, the clearance of myelin debris in young grafts is slowed when sutured into the nerves of aged rats, and clearance in aged grafts is faster when sutured into young rats. This data is in agreement with previous studies that suggest delayed clearance inhibits regeneration in aged animals. We are currently using this grafting model to explore the causes of slow clearance, whether it be delayed Wallerian degeneration, cytokine secretion, macrophage infiltration, and/or phagocytosis by Schwann cells and macrophages

Phosphorylation of LKB1/Par-4 Establishes Cell Polarity at the Schwann Cell-Axon Interface to Initiate and Control Myelin Extent

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Abstract:
The Schwann cell (SC)-axon interface represents a novel membrane specialization that integrates axonal signals to coordinate cytoskeletal dynamics resulting in the initiation of myelination. However, the manner in which this polarized membrane domain is generated remains unresolved. Here we demonstrate that the tumor suppressor protein LKB1/Par-4 is asymmetrically localized to the SC-axon interface and colocalizes with the polarity protein Par-3. As LKB1 mutations are epistatic to other Par protein functions, we propose that LKB1 may be a central regulator of cellular asymmetry in the SC. We demonstrate that asymmetric localization is dependent on the phosphorylation of LKB1 at serine-431 and correlates with the expression of myelin-specific proteins. SC-specific deletion of LKB1 disrupts the localization of Par-3 and significantly attenuates developmental myelination, delaying the initiation and altering the myelin extent into adulthood. We demonstrate that phosphorylation of LKB1 in SCs by protein kinase A (PKA) is necessary and sufficient to establish the asymmetric localization of LKB1 and Par-3 and rescues the hypomyelination phenotype observed in the SC-specific knockout of LKB1. The establishment of cell polarity in the SC may have widespread implications concerning the coordinated organization of multiple signaling complexes, highlighting novel mechanisms that couple SC-axon contact to the redistribution of specific membrane components that are necessary for myelination.

My name is Natasha Sukhanov and I'm a PhD student at Prof.Ori Peles lab. As I understood, you spoke with Prof. Peles and agreed to consider my participance in the Training course on PNS development, function, damage, regeneration and remyelination.

My current project aims are to find new drugs, pathways and genes affecting myelination during development. In order to find drugs affecting myelination, I'm performing a high throughput screening of FDA approved compound library on myelinated DRG cultures. To find new genes and pathways involved in myelination, I will compare expression profiles of myelinated vs. non myelinated neurons in DRG cultures. For this purpose I've established a system that allows me to profile isolated neuronal cell bodies from their distal axons which will be introduced (or not) with Schwann cells. The RNA expression profiles will created through Deep Sequencing followed by further analysis. I have great expectations that combining this two methods will reveal new genes and processes required for myelination and contribute to our understanding of PNS development.

As you can understand, establishing results of these sets of experiments will take time and therefor I will not have results to share in the upcoming Training course on PNS development, function, damage, regeneration and remyelination. However, I am certain that participating in the training course will contribute to my understanding of PNS development and myelination process, open my mined to new ideas and hopefully lead to fruitful collaborations to accelerate my work to meet new findings.

I will there for greatly appreciate your approval of my participance at the Training course on PNS development, function, damage, regeneration and remyelination.

Neutralization of Schwann cell-secreted VEGF is protective to diabetic neuropathy

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The pathogenetic role of vascular endothelial growth factor (VEGF) in long-term retinal and kidney complications of diabetes has been demonstrated. Conversely, little is known in diabetic neuropathy. We examined the modulation of VEGF pathway at mRNA and protein level on dorsal root ganglion (DRG) neurons and Schwann cells (SC) induced by hyperglycemia. Moreover, we studied the effects of VEGF neutralization on hyperglycemic DRG neurons and streptozotocin-induced diabetic neuropathy. Our findings demonstrated that DRG neurons were not affected by the direct exposition to hyperglycemia, whereas showed an impairment of neurite outgrowth ability when exposed to the medium of SC cultured in hyperglycemia. This was mediated by an altered regulation of VEGF and FLT-1 receptors. Hyperglycemia increased VEGF and FLT-1 mRNA without changing their intracellular protein levels in DRG neurons, decreased intracellular and secreted protein levels without changing mRNA level in SC, while reduced the expression of the soluble receptor sFLT-1 both in DRG neurons and SC. Bevacizumab, a molecule that inhibits VEGF activity preventing the interaction with its receptors, restored neurite outgrowth and normalized FLT-1 mRNA and protein levels in co-cultures. In diabetic rats, it both prevented and restored nerve conduction velocity and nociceptive thresholds. We demonstrated that hyperglycemia early affected neurite outgrowth through the impairment of SC-derived VEGF/FLT-1 signaling and that the neutralization of SC-secreted VEGF was protective both in vitro and in vivo models of diabetic neuropathy.

LOSS OF VIMENTIN CAUSES MOTOR NEUROPATHY

Authors: **Daniela Triolo1,7, Cristina Rivellini1,7, Ignazio Lopez2,3, Giorgia Dina2, Patrizia Dacci3, Ubaldo Del Carro3, Francesca Bianchi3, Maria Nolano4, Cristina Colombelli5, Laura Feltri5, Veronica Bianchi6, Patrizia D'Adamo6, Angelo Quattrini2,3 and Stefano Previtali1,3**

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Abnormal interactions between Schwann cells, axons and the surrounding extracellular matrix are a common cause of peripheral neuropathy. While the role of surface receptors and ligands has been thoroughly investigated, the involvement of downstream cytoskeleton components in both Schwann cells and neurons is still poorly understood.
Here we investigated the role of the intermediate filament vimentin, whose expression in Schwann cells and neurons is regulated during development and pathological conditions, suggesting a role for vimentin in nerve development and regeneration. Mice lacking vimentin present abnormal cytoskeleton architecture and hypermyelination due to increase expression of the neuregulin's pathway. Moreover they show progressive motor impairment and develop an age-and length-dependent peripheral motor neuropathy, characterized by motor action potential reduction in the neurophysiological tests, bad performances in motor behavioral tests, neuromuscular junctions denervation and delayed nerve regeneration after damage. On the other hand, sensory nerves and sensory behavioral tests are spared, except for some alterations in the epithelial sensory receptors.
All together our results reveal a novel role of vimentin in Schwann cell-axon interaction, and nerve formation and suggest Vimentin as a candidate gene for recessive motor neuropathies in humans.

TITLE: Role of LGI proteins in juxtaparanodal membrane organization in the peripheral nervous system

Several proteins, among them voltage-gated potassium channels (Kv1), Contactin Associated Protein 2 (CASPR2), Transient Axonal Glycoprotein 1 (TAG-1) and A Disintegrin And Metalloproteinase-22 (ADAM) are found highly enriched and co-localized in juxtaparanodal regions of myelinated axons in the peripheral nervous system. The role of this complex remains largely unknown, as do the mechanisms behind the targeting of these proteins and their reciprocal interactions. In absence of any one of these proteins, the juxtaparanodal complex is not formed properly and the involved proteins are found either dispersed in the internodal axonal membrane or abnormally localized. LGI proteins play important roles in the molecular organization of axonal and myelin membranes through interaction with ADAM receptors. In this project we aim to elucidate the molecular mechanism of LGI protein function in axonal membrane organization and clustering of macromolecular complexes during normal development and following trauma.

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Affiliation: Centre for Neuroregeneration, University of Edinburgh

TITOLO: LIPIDS AMOUNT IN DEMYELINATING DISEASES: A TOOL TO MONITOR MYELINATION

Autore: Davide Visigalli

Università o Scuola di afferenza: DINOGMI - Università di Genova

Remyelination is a major issue for physicians working on myelin disorders but fast, accurate and reliable methods to quantify the amount of myelin still lack, both in humans and experimental models. We focused on the lipid component of the myelin sheath due to the higher lipid to protein ratio and unique lipid composition compared to other cell membranes. In particular, we quantified sphingomyelin and cholesterol content in myelinated tissues and DRG cultures by a fluorescence-based assay. This method allowed us to assess the myelin content of different experimental conditions, i.e., rat and mouse tissues, central and peripheral nervous system, genetic and inducible injury, where the myelin sheath is different both in structure and chemical composition. We found that sphingomyelin and cholesterol are progressively enriched in wild type tissues and cultures in a time-dependent manner and that dys/demyelinated samples, derived from CMT1A rat and EAE mouse models, showed a significant decrease of lipids content compared to the wild type ones. Interestingly, in wild type DRG cultures treated with forskolin, a reversible demyelinating compound, our assay is able to detect remyelination occuring after the drug removal. To validate our sphingomyelin and cholesterol dosage as myelin biomarkers, we developed an 'ad hoc' macro to perform a detailed morphological and morphometrical analysis on myelinated tissues. A positive correlation between total myelinated area and lipids amount was found. Thus, we are confident that our assay is sensitive, specific and reliable enough to be translated to the study of human myelin disorders.

The role of the ERAD pathway in the pathophysiology of PNS myelination

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P0, the most abundant glycoprotein in PNS myelin, is produced by Schwann cells and acts as an adhesive molecule in the myelin membrane. In Humans, mutations in P0 result in different types of peripheral hereditary neuropathies. In particular the deletion of serine 63 (P0S63del) causes the demyelinating CMT1B disease, similarly manifested in transgenic mice expressing P0S63del protein. P0S63del protein is misfolded and retained in the ER causing a chronic ER stress that triggers an unfolded protein response (UPR). The UPR is a cellular response aimed at reducing the level of ER stress by attenuating protein translation, increasing the folding machinery and stimulating protein degradation via the ER-Associated-Degradation (ERAD) pathway. Microarray analysis revealed that several ERAD genes, such as *derlins*, are strongly upregulated in S63del nerves. Moreover Derlin-1 and -2 coimmunoprecipitate with P0S63del protein, suggesting a potential adaptive role of ERAD in the neuropathy. To analyze the role of ERAD in myelination, we generated Schwann-cell specific Derlin-2 KO mice. These mice show no defects in myelin formation, but appear mildly hypomyelinated at later stages with a small reduction in NCV. In S63del mice the deletion of Derlin-2 clearly worsens the severity of the disease starting as early as P15. S63del/Derlin-2 KO sciatic nerves display more severe hypo- and de-myelination and a reduction in NCV as compared to S63del. These observations indicate that Derlin-2 (and ERAD) may be part of the Schwann cell adaptive response that attempts to cope with chronic ER stress, being a potential therapeutic target to treat CMT1B.

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