ABSTRACT SUPPLEMENT: Oral Presentations
A mitochondrial ATP6 mutation causing a slowly progressive myeloneuropathy

Tanya Bardakjian, Steven Scherer

University of Pennsylvania, Philadelphia, PA, USA

We report on a 53-year-old man with an insidiously progressive myeloneuropathy that progressed over 20 years to more typical picture of CMT. The proband has had high arches for as long as he can remember, and developed hammertoes in his teens. He presented to a neurologist at the age of 32, and was found to have hammer toes and high arches, brisk reflexes in the arms and knees (but absent at the ankles) and prominent extensor planter reflexes. Nerve conduction studies and EMG showed a motor > sensory axonal neuropathy, with severe, chronic denervation in distal leg muscles. At age 52, he reported more difficult ambulating. His exam showed minimally worsened deficits, reflexes were less pronounced at the knees, vibration sense was absent at the toes, pinprick sensation was normal above the knees, and he had subtle extensor plantar responses. Nerve conductions and EMG showed little change from the prior study. His CMT neuropathy score was 17. His mother was examined at age 68. She had hammertoes, mild (4+) weakness in extensor hallicus longus and tibialis anterior. Vibration was absent in her toes, and she had bilateral extensor plantar responses. Her nerve conductions (showed reduced motor but not sensory amplitudes in the feet, and EMG showed moderate, chronic denervation in distal leg muscles. A hereditary neuropathy panel of the proband through GeneDx identified a VUS in the SCN9A. Reflex testing to whole exome sequencing with mitochondrial sequencing identified a homoplasmic pathogenic variant in MT-ATP6 - m.9176T>C/p.Leu217Pro in the proband and his mother. These findings add to the evidence that MT-ATP mutations can present as CMT or evolve into a CMT-like presentation. The presence of a mitochondrial disorder changes the genetic counseling and risk assessment for a patient. Therefore, mitochondrial sequencing should be a part of a complete CMT evaluation.

References:


Keywords: Human Genetics

Grant Support:

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Saturday, June 22, 2019 - 17:30 - 17:45
Adult Polyglucosan Body Disease Presenting With A Peripheral Neuropathy: Broadening The Clinical Spectrum
Jonathan De Winter¹, Willem De Ridder², Tine Deconinck³, Danique Beijer³, Martin Lammens¹, Jonathan Baets²

¹Antwerp University Hospital, Edegem, Belgium, ²Antwerp University Hospital, University of Antwerp, Edegem, Belgium, ³University of Antwerp, Edegem, Belgium

The purpose is twofold. First, to present a rare cause of a polyneuropathy with leukodystrophy as an initial presentation of a multisystemic polyglucosan body disease. Second, to expand the current knowledge concerning adult-onset GBE-deficiency phenotypes. A 49-year old man presented at our neuromuscular department with a two-year history of gait difficulties and distal sensory abnormalities. Distal weakness was noted as well as pedes cavi. Clinical examination was in keeping with a motor predominant peripheral neuropathy and mild pyramidal features. Nerve conduction studies showed a predominantly motor mixed axonal-demyelinating neuropathy. MR-imaging revealed confluent white matter lesions in both hemispheres. Family history revealed a younger brother with a similar albeit milder clinical phenotype. After five years of follow-up the patient had developed a progressively severe spasmodic gait with wheelchair use, neurogenic bladder dysfunction, discrete cerebellar ataxia and cognitive and behavioral changes. Progressive leukodystrophy and myelopathy were evident from serial MRIs. Distal weakness preceded proximal weakness in the lower limbs, analogous to a more pronounced motoric predominant neuropathy. Although mildly elevated serum CK levels (500-600U/L) were observed, muscle biopsy did not show primary myopathic abnormalities. Due to liver enzyme abnormalities, a liver biopsy was performed and showed a micro-macovesicular steatosis. Whole exome sequencing showed compound heterozygous mutations (p.Thr254Ala and Ile694Asn) in Glycogen Branching Enzyme 1 (GBE1) and segregation in the family was confirmed. In conclusion, we report a case of an adult polyglucosan body disease (APBD) with a peripheral nerve system impairment dominating the initial presentation. We broaden the current knowledge of GBE1-related disorders by representing a combination of the two allelic forms of GBE1 deficiency, namely GSD-IV (glycogen storage disease type 4) and APBD. The first is associated with involvement of the liver and skeletal muscles in childhood, the second with peripheral neuropathy and central nervous system involvement in adults.

References: None.

Keywords: Human Genetics

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Atypical Sensorimotor Neuropathy Related to Cutaneous Toxigenic Diphtheria Infection In A World Traveller

Penelope Spring1, Alice Powell1, Nilanthy Vigneswaran1, Stephen Reddel1, Genevieve McKew2, Verlaine Timms3, Min-Xia Wang4, Judith Spies4, John Pollard4

1Department of Neurology, Concord Repatriation General Hospital, Sydney, NSW, Australia, 2 Department of Infectious Diseases and Microbiology, Concord Repatriation General Hospital, Sydney, NSW, Australia, 3Centre for Infectious Diseases, Microbiology- Public Health, Westmead Hospital and University of Sydney, Sydney, NSW, Australia, 4Institute of Clinical Neurosciences, University of Sydney and Royal Prince Alfred Hospital, Sydney, NSW, Australia

Diphtheria is now an uncommon cause of peripheral neuropathy, however recent reports have related to pharyngeal infection1. Here we report a rare case of a sensorimotor neuropathy, initially resembling immunotherapy-resistant Guillain-Barre syndrome, following a highly toxigenic cutaneous diphtheria infection.

A 66-year-old Australian man, a frequent world traveller, presented in March 2018 with progressive sensorimotor neuropathy with neuropathic pain, maximal in the left foot (initial ONLS - Overall Neuropathy Limitations Scale-6). He had a resolving ulcer on the second left toe, present since January in Vanuatu, and treated with antibiotics; and recent Rhinovirus. Nerve conduction studies (NCS) suggested demyelination; lumbar puncture showed elevated protein-1.27g/L, normal cells. Intravenous immunoglobulin (IVIg) (2g/kg) and pregabalin induced minimal improvement.

Despite plasmapheresis (x5), pulsed methylprednisolone (Week 3), then oral steroid (1g/kg), and further IVIg, the patient progressed. Mild diplopia, facial weakness/paraesthesia, dysphonia, dysphagia, and moderate pulmonary restriction developed (Wk2-4). By Wk6 he had marked quadripareisis/proprionceptive loss (ONLS-11), and Raynaud-like fluctuating left foot cyanosis.

Autoimmune/serological investigations including ganglioside/paranodal antibodies were unremarkable apart from positive Ro60/52. MRI spine (Wk5) showed patchy cauda equina enhancement. Neurophysiology (Wk5) showed lower limb (LL) denervation, and somatosensory evoked potentials (SSEPs) were absent/delayed(LL/UL). Initial toe swab culture/PCR review showed a highly toxigenic strain of Corynebacterium diphtheriae, expressing A/B toxin beta subunits. IVIg was ceased due to deep vein thrombosis and likely diphtheritic neuropathy. Steroids were weaned during rehabilitation (Wk8-16), and there was dramatic functional improvement: ONLS-Wk10-9/Wk16-5/Wk24-2. Sural nerve/muscle biopsies (Wk7) showed a chronic active axonal neuropathy, and VEGF was normal. Neurophysiologically, improvement was mild by 5 months; substantial by 11 months.

This is the first report of generalised neuropathy related to cutaneous diphtheria infection since 19462,3. The axonal loss is atypical, with diphtheria usually associated with demyelinating neuropathy4. It is unclear whether timing, distal infection, antitoxin non-use, or organism strain affected the pathology.

References:

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Keywords: Inflammatory, Pain, Axonal Regeneration, Other

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Mutations in SCL25A46 cause a spectrum of clinical phenotypes including axonal CMT with optic atrophy.
Carolynne Doherty¹, Menelaos Pipis¹, Andrea Cortese¹, Roy Poh², James Polke², Robert Pitceathly¹, Alexander Rossor¹, Mary Reilly¹

¹MRC Centre for Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, United Kingdom of Great Britain and Northern Ireland, ²Neurogenetics unit, UCL Queen Square Institute of Neurology, London, United Kingdom of Great Britain and Northern Ireland

Introduction: Optic atrophy is seen in association with a number of inherited neuropathies, particularly those who have mutations associated with abnormalities of mitochondrial fusion. We present a case of axonal CMT with optic atrophy.

Clinical presentation: At age 6 this now 20-year-old female was noted to have atrophy of the distal legs with bilateral foot drop. A length dependent axonal motor and sensory neuropathy was demonstrated by neurophysiological evaluation. A deterioration of visual acuity 3/36 right and 6/60 left occurred at age 16 and the borderline visual evoked potentials were felt to be compatible with optic neuropathy. Her medical history includes attention deficit disorder. Clinical examination at age 18 showed pale optic discs. There was mild scoliosis. A positive knee bob sign was evident along with distal atrophy and weakness in a length dependent manner. Upper limb and knee reflexes were brisk except for absent ankle jerks and absent plantar responses. Pinprick was reduced below the ankle and vibration perception impaired at knee on Rydell testing.

Investigations: Mutations in OPA1, OPA3, POLG, ATPase 6 and 8 were sought but not identified. Whole Exome Sequencing identified compound heterozygous mutations in the SCL25A46 gene (p.A322D and p.Y386C), one of each was subsequently demonstrated in the parents.

Discussion: A spectrum of disorders of varying severity has been described associated with mutations in SCL25A46. These include CMT2 with optic atrophy (HMSN type 6B) sometimes with additional features including spasticity and ataxia, Leigh syndrome and pontocerebellar hypoplasia. The gene encodes a mitochondrial solute carrier protein and is thought to have a role in mitochondrial fusion/fission dynamics. We present a discussion of the literature alongside a case presentation of axonal neuropathy with optic atrophy.

References: None.

Keywords: CMTR, Human Genetics

Grant Support:
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Rare and Challenging case of Guillain- Barre syndrome associated with stroke secondary to spotted fever
Anomali Vidanagamage¹, Amila Chandrasekara¹, Chamali Aluwihare¹, Sujatha Pathirage², Anil De Silva¹, Arjuna Fernando¹

¹National Hospital, Colombo, Sri Lanka, ²Medical Research Institute, Colombo, Colombo, Sri Lanka

Introduction

Spotted fever is a rare disorder rarely manifesting as Guillain- Barre syndrome (GBS) and stroke.

A 44 years old man, from Deraniyagala, Sri Lanka presented with intermittent low-grade fever for two weeks with arthralgia, myalgia, and frontal headache. On admission to local hospital, bilateral lower limb weakness was noted and he went into respiratory paralysis requiring intubation and ICU care. On examination, there was global areflexia and the pupils were normal.

During the stay, a transient erythematous macular rash over the medial surface of the right elbow with a healed eschar on the right lower leg was noted.

His CSF study showed high protein of 86 g/dl with a cytoprotein dissociation compatible with GBS.

Nerve conduction study showed Sensory motor axonal polyneuropathy suggestive of AMSAN type GBS

Weil-Felix test was OXK 1:160, OX2- 1:160 and OX-19 Negative. IFA for spotted fever was positive supporting the diagnosis of spotted fever.

Persistent spiking fever did not respond to empirical antibiotics and showed marked improvement following doxycycline. With plasma exchange, he recovered from GBS and right hemiplegia became apparent. Following rehabilitation for the stroke, he had a good recovery with a modified ranking scale of 2.

Non-contrast imaging on admission revealed bilateral basal ganglia infarctions later confirmed by MRI and MRA suggestive of vasculitis. T2 FLAIR, DWI and ADC images demonstrate bilateral T2 FLAIR high signal intensities involving left internal capsule, right globus pallidus, and bilateral external capsules. These showed diffusion restriction suggesting acute infarcts.

The MRA demonstrate subtle irregularity of the right posterior cerebral arteries suggesting a vasculitic irregularity. No occlusions or luminal filling defects are noted.

Rest of the brain appears normal.

Conclusion

Although rare, rickettsia infection should be considered in febrile strokes as well as in atypical manifestations of Guillain- Barre syndrome

References: None.

Keywords: Inflammatory

Grant Support: None.
Peripheral Nervous System (PNS) Toxicity Induced by Immune-checkpoint Inhibitors in Cancer Patients: Single Centre Experience.

Silvia Bocci¹, Laura Insana¹, Riccardo Danielli², Liana Africa¹, Federica Ginanneschi¹, Laura Franci¹, Luana Calabrò², Anna Maria Di Giacomo², Michele Maio², Fabio Giannini¹

¹Department of Medicine, Surgery and Neurosciences - University of Siena, Siena, Italy, ²Medical Oncology and Immunotherapy, Istituto Toscano Tumori - University Hospital of Siena, Siena, Italy

Introduction. Immunomodulating monoclonal antibodies (ImAb) against cytotoxic T-lymphocyte antigen-4 (CTLA4), programmed death-1 (PD1) and its ligand (PDL1) have been approved for treatment of metastatic melanoma, lung and renal cancer. By unbalancing the immune system, ImAbs may generate several multi-organ immune-related Adverse Events (irAEs), including neuromuscular manifestations.

Case reports. We describe five patients suffering from metastatic melanoma and one from non-small- cell lung cancer that received ImAbs and experienced PNS-irAEs. Three patients presented sensory- motor axonal polyneuropathies, two of which with mild and slow course (CTCAE grade 1), onset 13 and 16 weeks after anti-CTLA4 and anti-CTLA4/anti-PD1 combination treatment and followed by spontaneous recovery in 4 and 12 months respectively. Third patient presented subacute course (grade 2) with associated myopathy, beginning after one week of anti-CTLA4/anti-PD1 therapy and partially improved after ImAb discontinuation and steroid therapy. Fourth patient presented rapidly ascending tetraparesis (grade 3) at week 6 of anti-PD1 therapy. Lab tests and EDX were diagnostic for AIDP. Full recovery was obtained after IVlg treatment. Fifth patient presented focal onset in left lower limb followed by severe spreading of signs and loss of deambulation (grade 3) after anti-CTLA4 and anti-PDL1 administration in sequence. EDX showed CIDP-like abnormalities. ImAb withdrawal and IVlg plus steroids administration caused moderate benefit. Last patient presented oftalmoparesis and diffuse limb weakness (grade 2) developing after 3 weeks of anti-PD1 therapy. EDX were consistent with CIDP plus ocular nerve involvement. Immunotherapy was discontinued and steroids were administered without benefit. Anti-gangliosides and anti-onconeural antibodies were negative in all patients.

Conclusions. PNS-irAEs are reported in less than 1% of patients. The current series quite represents the whole clinical spectrum of them, ranging from mild and spontaneously or treatment reversed to poorly responsive complications. Increasing use of ImAbs needs a joined specific alert by oncologists and neurologists, to promptly recognize and treat PNS-irAEs.

References:


Keywords: Inflammatory, Other

Grant Support: None.
Sunday, 23 June

Oral Abstract Presentations
Biallelic Neurofascin variants affect paranodal axoglial junctions causing neurodevelopmental impairment and central and peripheral demyelination
Stephanie Efthymiou¹, Vincenzo Salpietro¹, Jerome Devaux², Maria Nolano³, Henry Houlden¹
¹UCL Queen Square Institute of Neurology, London, United Kingdom of Great Britain and Northern Ireland, ²INSERM U1051, Institut de Neurosciences de Montpellier, Montpellier, France, ³Istituti Clinici Scientifici Maugeri, Naples, Italy

Axon pathfinding and synapse formation are essential processes for nervous system development and function. The assembly of myelinated fibres and node of Ranvier is mediated by a number of cell adhesion molecules of the immunoglobulin superfamily including the Neurofascin (NFASC) alternative isoforms Nfasc186 and Nfasc140, located in the axonal membrane at the node of Ranvier, and Nfasc155, a glial component of the paranodal axoglial junction. We identified 8 individuals from 5 unrelated families, exhibiting a neurodevelopmental disorder characterized a spectrum of central (intellectual disability, developmental delay, motor impairment, speech difficulties) and peripheral (early onset demyelinating neuropathy) involvement, who were found by exome or genome sequencing to carry one frameshift and four different homozygous non-synonymous variants in NFASC. Expression studies using immunostaining-based techniques identified absent expression of the Nfasc155 isoform as a consequence of the frameshift variant and a significant reduction of expression was also observed in association with two non-synonymous variants affecting the fibronectin type III domain. Cell aggregation studies revealed a severely impaired Nfasc155-CNTN1/CASPR1 complex interaction as a result of the identified variants. Immunofluorescence staining of myelinated fibres from two affected individuals showed a severe loss of myelinated fibres and abnormalities in the paranodal junction morphology. Our findings establish that recessive variants affecting the Nfasc155 isoform can affect the formation of paranodal axoglial junctions at the nodes of Ranvier. The genetic disease caused by biallelic NFASC variants includes neurodevelopmental impairment and a spectrum of central and peripheral demyelination as part of its core clinical phenotype. Our findings support possible overlapping molecular mechanisms of paranodal damage at peripheral nerves in both the immune-mediated and the genetic disease, but the observation of prominent central neurological involvement in NFASC biallelic variant carriers highlights the importance of this gene in human brain development and function.

References: None.

Keywords: Node Biology, Human Genetics

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The Polygenic Architecture of Carpal Tunnel Syndrome

Akira Wiberg, Akira Wiberg, Michael Ng, Annina Schmid, Georgios Baskozos, Robert Smillie, Michael Holmes, David Bennett, Dominic Furniss
University of Oxford, Oxford, United Kingdom of Great Britain and Northern Ireland

INTRODUCTION: Carpal tunnel syndrome (CTS) is the commonest entrapment neuropathy. Despite its high heritability of 0.46 [1], very little is known about the genetic aetiology of CTS. METHODS: We undertook a genome-wide association study (GWAS) of CTS using 12,312 cases and 389,344 controls of white British ancestry from the UK Biobank resource. We then performed RNA-Seq to investigate whether genes implicated in the GWAS were expressed in tenosynovium surgically resected from the carpal tunnels of CTS patients. We also performed a two-sample Mendelian randomisation analysis using height as the exposure and CTS status as the outcome, using 601 single nucleotide polymorphisms (SNPs) as instrumental variables for height, taken from a large meta-analysis of adult height GWAS [2]. RESULTS: We discovered genome-wide significant associations (p<5e-8) at 16 loci across the genome. Of the associated variants, rs72755233 (p=2.3e-15) is a missense variant in ADAMTS17, rs62621197 (p=7.5e-14) is a missense variant in ADAMTS10, and rs3791679 (p=2.0e-12) lies within an enhancer region of EFEMP1. All three genes are important in extracellular matrix modulation, and we demonstrated expression of these genes at high levels in surgically resected tenosynovium. These variants have been reported in previous GWAS to be associated with human height, and we found that on average, UK Biobank CTS patients are ~2cm shorter than controls. The Mendelian randomisation analysis demonstrated that a 1 S.D. increase in height is associated with an odds ratio of 0.79 for the development of CTS. CONCLUSIONS: We performed the first ever GWAS in CTS, and identified likely causal genes in its pathogenesis, all of which are highly expressed in carpal tunnel tenosynovium. Our findings support the idea that the genetic susceptibility to CTS may arise from aberrant connective tissue architecture or from altered musculoskeletal growth, and implicates an inverse causal role of height in the aetiology of CTS.

References:


Keywords: Human Genetics, CMTR, Pain

Grant Support:

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Mutations In The Small Heat Shock Proteins HSPB1 And HSPB8 Impair The Autophagic Flux

Angela Sisto, Mansour Haidar, Bob Asselbergh, Elias Adriaenssens, Vicky De Winter, Jean-Pierre Timmermans, Michaela Auer-Grumbach, Manisha Juneja, Vincent Timmerman

1 Pheripheral Neuropathy Research Group, University of Antwerp, Antwerp, Belgium, 2 Peripheral Neuropathy Research Group, University of Antwerp, Antwerp, Belgium, 3 VIB-UAntwerp Center for Molecular Neurology, Antwerp, Belgium, 4 Laboratory of Cell Biology & Histology, Antwerp Centre for Advanced Microscopy, University of Antwerp, Antwerp, Belgium, 5 Department of Orthopaedics, Medical University of Vienna, Vienna, Austria

Autophagy is a crucial process for the cellular homeostasis, as it promotes the removal and recycling of misfolded proteins and damaged organelles. Therefore, impairments of this pathway leads to the accumulation of toxic species and neurodegeneration. Indeed, mutations in HSPB1 and HSPB8, ubiquitously expressed molecular chaperones, cause axonal CMT (CMT2) or distal hereditary motor neuropathy (dHMN). HSPB1 knock-out cells reported impairments in autophagosome formation, which is rescued by the reintroduction of wild-type HSPB1. Through proteomic analysis, we revealed that HSPB1 directly interacts with sequestosome1 (P62/SQSTM1), main autophagy modulator, which mediates the recruitment of ubiquitinated proteins and promote the nucleation of autophagic vesicles. Mutations in HSPB1 lead to a decrease in the formation of p62 bodies, and subsequent impairment of phagophore formation, suggesting a regulatory role for HSPB1 in autophagy via interaction with SQSTM1. Moreover, in patient iPSC-derived motor neurons we confirmed impairment in the autophagic flux with reduction of P62 bodies and autophagosomes (1). The HSPB8 protein belongs to the chaperone-assisted selective autophagy (CASA) complex, composed of HSPB8-BAG3-HSP70, that recruits SQSTM1 and ubiquitinated chaperone-bound cargo. It was shown that depletion of HSPB8 impairs the formation of P62 bodies. We confirmed the autophagic impairment in our homozygous Hspb8K141N knock-in mouse model (2). Evidently mutant HSPB1 and HSPB8 share the same mechanism as their activities merges in SQSTM1 modulation, providing a scaffold for autophagosome formation.

References:


Keywords: Axonal Biology, CMTR

Grant Support: None.
Sunday, June 23, 2019 - 09:45 - 10:00
Targeting a core axonal degeneration program to treat vincristine and bortezomib-induced axonal degeneration
Stefanie Geisler¹, Ryan Doan², Shay Huang², Galen Cheng², Jeffrey Milbrandt², Aaron DiAntonio²
¹Washington University School of Medicine in St. Louis, St. Louis, MO, USA, ²Washington University School of Medicine in St. Louis, Saint Louis, MO, USA

Peripheral axonal polyneuropathy is a common side effect of many chemotherapeutic agents despite disparate mechanisms of action, suggesting that the axon destructive properties of various chemotherapies converge on a common axon degeneration (AxD) program. We discovered that genetic deletion of SARM1 protects axons from degeneration after axotomy and prevents neuropathy induced by the commonly used chemotherapeutic agent vincristine in a mouse model. It remains unknown whether the same upstream regulators and downstream effectors of SARM1 act in vincristine-induced AxD and axotomy, and whether the protective effects of SARM1 deletion are also realized by chemotherapeutics with different mechanisms of action. To address these questions, we used cultured mouse dorsal root ganglion neurons and the two chemotherapeutic agents vincristine and bortezomib (BTZ). Vincristine acts by stabilizing tubulin polymerization and interfering with intracellular trafficking, whereas BTZ inhibits the proteasome. We demonstrate that genetic deletion of SARM1 strongly decreases not only vincristine-induced AxD, but also AxD following administration of BTZ. In axotomy, SARM1 is activated by loss of NMNAT and acts through catastrophic decrease of NAD⁺. As in axotomy, AxD after vincristine and BTZ is preceded by loss of NAD⁺. Maintaining NAD⁺ levels by overexpression of nicotinamide riboside kinase and supplementation with nicotinamide riboside strongly protect from vincristine and BTZ-induced degeneration. Furthermore, as in axotomy, overexpressing cytNMNAT1 prevents degeneration following both vincristine and BTZ. However, while inhibiting the same MAP-kinase pathway that regulates SARM1 in axotomy protects from vincristine-induced AxD, it does not decrease BTZ-induced AxD. BTZ induced degeneration instead is transcriptionally regulated and mediated by axonal caspases. These findings indicate that different upstream pathways converge on SARM1. Excitingly, we are able to inhibit this program and, thus pathological AxD in vitro, by expressing a SARM1-dominant/negative mutant. We suggest that targeting SARM1 may have great therapeutic value in the prevention of multiple variants of chemotherapy-induced neuropathy.

References: None.

Keywords: Axonal Biology, Pre-clinical Studies, Other

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Rab35 GTPase is an inhibitor of mTORC1 and regulates myelination in the PNS

Federica Grandi¹, Linda Sawade², Marianna Mignanelli³, Roberta Di Guardo³, Genaro Patiño-López⁴, Steve Shaw⁵, Kerstin Klinkert⁶, Francina Langa Vives⁷, Arnaud Echard⁷, Volker Haucke², Alessandra Bolino¹

¹IRCCS Ospedale San Raffaele, Milano, Italy, ²Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin, Germany, ³IRCCS Ospedale San Raffaele, Milan, Italy, ⁴Hospital Infantil de México, Ciudad de México, Mexico, ⁵National Institutes of Health, 10 Center Dr., Bethesda, MD, USA, ⁶Institut Pasteur, 25-28 rue du Dr Roux, Paris., France, ⁷Institut Pasteur, 25-28 rue du Dr Roux, Paris, France

Introduction. Charcot-Marie-Tooth type (CMT) 4B1 and B2 are characterized by recessive inheritance, early onset, severe course, slowed nerve conduction, and myelin outfoldings, redundant loops of myelin. CMT4B1 and B2 neuropathies are caused by loss-of-function mutations in the MTMR2 and MTMR13, the Myotubularin related 2 and 13 genes, respectively. MTMR2 is a phosphatase which dephosphorylates PtdIns3P and PtdIns(3,5)P2 phosphoinositides, potent signaling molecules regulating endo-lysosomal trafficking. On the contrary, MTMR13, which is catalytically inactive, is thought to assist MTMR2 in its sub-cellular localization and to increase enzymatic activity. How decreased phospholipid levels cause CMT4B with myelin outfoldings still remains to be clarified. Interestingly, we found that MTMR2/MTMR13 interact with Rab35, which likely activates the myotubularin complex. Rab35 is a small GTPase known to recruit and activate effectors involved in vesicle formation or in cytoskeleton regulation. Methods. To examine the role of Rab35 in nerve development and myelination, we generated Rab35 conditional Knock-out mice with ablation of Rab35 specifically in Schwann cells (Rab35 cKO). Results. Interestingly, nerves of Rab35 cKO are characterized, in addition to myelin outfoldings, by the presence of abundant tomacula and myelin degeneration, which are not observed in nerves of either Mtmr2 or Mtmr13 mutant mice. Since myelin outfoldings and tomacula in the PNS could be associated with overactivation of mTORC1, we analyzed the mTORC1 pathway in Rab35 cKO nerves. Consistent with this, we found that mTORC1 signaling is up-regulated in Rab35 cKO nerves. Conclusions. Our data indicate that Rab35 is a novel regulator of myelination in the PNS. Of note, our data also suggest that PtdIns3P and PtdIns(3,5)P2 phospholipids, regulated by Rab35/Mtms, induce mTORC1 pathway activation in the nerve.

References: None.

Keywords: CMTR, Schwann Cell

Grant Support: None.
Dominant mutations of a Notch pathway component cause type 2 Charcot-Marie-Tooth disease

Jeremy Sullivan1, William Motley1, Janel Johnson2, William Aisenberg3, Katy Huh1, Meriel McEntagart4, Marie-Helene Marion5, Lucy Hicklin5, Hamid Modarres5, Emma Baple3, Aamir Zuberi6, Cathleen Lutz6, Rachelle Gaudet1, Bryan Traynor6, Andrew Crosby3, Charlotte Sumner1

1 Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2 National Institutes of Health, Bethesda, MD, USA, 3 RILD Wellcome Wolfson Centre, Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom of Great Britain and Northern Ireland, 4 St. George’s University of London, London, United Kingdom of Great Britain and Northern Ireland, 5 St. George’s Hospital, London, United Kingdom of Great Britain and Northern Ireland, 6 The Jackson Laboratory, Bar Harbor, ME, USA, 7 Harvard University, Cambridge, MA, USA, 8 National Institutes of Health, Johns Hopkins Hospital, Bethesda, MD, USA

The Notch pathway is a highly conserved cell-cell signaling mechanism with pleiotropic roles in normal tissue development and homeostasis. Dysregulated Notch signaling has also been implicated in the pathogenesis of a multitude of human diseases, including neurodegenerative disorders and cancer, and represents an emerging therapeutic target. Despite this pivotal pathogenic role, only a small number of monogenic disorders (e.g. CADASIL) have been shown to be caused by mutations in Notch pathway components. Here, we describe two missense mutations in a Notch pathway component, that segregate with Charcot-Marie-Tooth disease type 2 (CMT2) in two unrelated families with strikingly similar clinical features, including severe vocal fold paresis. In transfected cells, both mutant proteins exhibited impaired complex glycosylation and reduced plasma membrane expression. Knock-in mice harboring homozygous CMT2-associated mutations in the endogenous mouse gene exhibit embryonic lethality by mid-gestation, confirming a clear functional relevance of the amino acid substitution. Together, these findings broaden the spectrum of neurological disorders associated with alterations in the Notch pathway, and further point to a key role for Notch signaling in peripheral nerve function.

References:


Keywords: Human Genetics, Axonal Biology

Grant Support:

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Sunday, June 23, 2019 - 11:30 - 11:35
TOPIRAMATE PREVENTS OXALIPLATIN NEUROTOXICITY IN A RAT MODEL
PAOLA ALBERTI, CHIORAZZI ALESSIA, ANNALISA CANTA, POZZI ELEONORA, FUMAGALLI GIULIA, LAURA MONZA, CRISTINA MEREGALLI, ELISA BALLARINI, Virginia Rodriguez-Menendez, OGGIONI NORBERTO, PAOLA MARIROLI, CAVALETTI GUIDO
UNIVERSITY OF MILANO-BICOCCA, MONZA, Italy
PURPOSE. Oxaliplatin (OHP) Induced Peripheral Neurotoxicity (OIPN) is a long-lasting adverse event that burdens cancer survivors. OIPN consists of an acute and chronic syndrome; the first is related to axonal hyperexcitability and the latter to an actual nerve damage causing mainly sensory disfunctions. Acute OIPN is secondary to ion channel dysfunction and it is a predisposing factor to the detrimental chronic one. Thus, a tentative strategy to prevent chronic OIPN could be acting against acute one. We explored this hypothesis using topiramate (TPM). In a preliminary experiment we used Nerve Excitability Testing (NET) confirming the presence of acute OIPN in our chronic OIPN rat model. METHODS. Forty female Wistar rats were divided into 4 groups: control, OHP, OHP+TPM, TPM. OHP was administered iv (5mg/Kg, 2qws4ws) and TPM per os (daily, 100 mg/Kg). Standard neurophysiology was performed at the end of treatment as well as NET. All neurophysiological determinations were performed under deep anesthesia (isoflurane) and body temperature was kept constant (37±0.5° C). Dynamic test was also performed. Skin biopsy and caudal nerves were harvested. RESULTS. Standard neurophysiology and NET confirmed OIPN induction only in OHP group; OHP+TPM group standard neurophysiology was similar to controls and NET showed that the "channelopathy" was contained. IENF and caudal nerve morphology/morphometry showed statistically significant alterations only in OHP group. The same was observed for Dynamic test. CONCLUSION. All outcome measures showed neurotoxicity induction in OHP group, while OHP+TPM was preserved. This a true novelty and a translation in clinical practice can be not a distant future: TPM is a drug yet approved for clinical use with no detrimental interactions with OHP. In a scenario where no cure for OIPN is present this might be a promising evidence to modify OIPN natural history.

References: None.

Keywords: Axonal Biology, Pain, Pre-clinical Studies, Other

Grant Support: None.
molecular mechanisms of impaired sensory nerve regeneration in diabetes

Sung-Tsang Hsieh¹, Hung-Wei Kan², Chi-Chao Chao¹
¹National Taiwan University Hospital, Taipei, Taiwan, ²National Taiwan University, Taipei, Taiwan

Sensory neuropathy of the small fiber type is a major complication of diabetes and a critical factor leading to painless injury. The pathology hallmarks include skin denervation with reduced intraepidermal nerve fiber (IENF) density and degeneration of dermal nerve fibers. Although peripheral nerves are able to regenerate, this potential is impaired in diabetes and underlying mechanisms remain elusive. We aimed to address this issue by investigating the molecular mediators underlying extrinsic and intrinsic factors of nerve regeneration in 2 systems: (1) human skin biopsies and (2) a cell model of dorsal root ganglia (DRG) neurons co-cultured with human dermal fibroblast exposed to high-glucose medium. The expression of regenerating markers (growth-associated protein 43) was similar between diabetic patients and control subjects. The collagen composition was altered in diabetes and its expression was increased in diabetic skin compared with control skin due to upregulation of Sec31a, a key molecule in the transit between ER and Golgi apparatus for collagen synthesis. In the cell model, Sec31 was upregulated in human dermal fibroblast exposed to high-glucose medium. Under such molecular interactions, neurite outgrowth of cultured DRG neurons was retarded. The silencing of Sec31a reduced collagen protein expression and rescued neurite outgrowth of DRG neurons. The study established molecular mechanisms and scenario of poor nerve regeneration in diabetic neuropathy.


Keywords: Axonal Biology, Small Fibers, Diabetes

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The sensation of pain is essential for avoiding harmful risks and preserving the functional integrity of our body. We recently reported that mutations in PRDM12, an epigenetic regulator belonging to the PRDM (PRDI-BF1 and RIZ homology domain) family of putative histone-methyltransferases (HMTs), cause hereditary sensory and autonomic neuropathy type VIII resulting in the absence of pain sensation in affected individuals, yet the mechanistic insights on the causative deficits are missing.

Here we show that in a mouse model lacking PRDM12, nociceptors fail to be generated while other sensory neuron types (A-fiber low-threshold mechanoreceptors, A-LTMRs), remain unaffected. Our data further indicate that PRDM12 is required for initiation of neurogenesis and activation of a cascade of downstream transcription factors, including NEUROD1, BRN3A and ISL1 in the nociceptive lineage. In parallel, an enforced expression of PRDM12 in migrating neural crest cells biases them to localize in the sensory ganglia and to differentiate into sensory neurons. Importantly, while ectopic presence of PRDM12 is not sufficient to seal identity of presumptive pain neurons, it initiates nociceptor development by repressing markers of alternative sensory fates in postmitotic neurons. These data reveal that PRDM12 is necessary for the generation of pain initiating neuron types during development.

References: None.

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MRI and Triple Stimulation Technique to detect brachial plexus abnormalities in Multifocal Motor Neuropathy

Emilien Delmont¹, Thomas Le Corroller², Aude-Marie Grapperon¹, Giovanni Corazza¹, Shahram Attarian¹

¹Referral Centre for Neuromuscular diseases and ALS, Hôpital La Timone, Marseille, France, ²Department of Radiology, Institute for Locomotion, Hôpital Sainte-Marguerite, Marseille, France

Introduction

Conduction blocks (CB) are the diagnostic hallmark of multifocal motor neuropathy (MMN). Classical nerve conduction study cannot detect CB above the Erb’s point. Plexus MRI and motor evoked potential with triple stimulation (MEP-TST) detect abnormalities of the brachial plexus. The aim of this study was to compare the performance of MEP-TST and plexus MRI to detect brachial plexus abnormalities in MMN.Methods

Twenty-six patients underwent MEP-TST bilaterally performed on median and ulnar nerves and brachial plexus MRI using 3DT1-weighted and 3DT2-weighted short tau inversion recovery (STIR) imaging. Results

Median age was 54 years and median disease duration was 3.5 years. IgM anti GM1 antibodies were detected in 13/26 patients. Intravenous immunoglobulins were efficient in 23/26 patients. Seven patients never had CB on classical nerve conduction studies. MEP-TST detected proximal CB in 19/26 patients (73%). Plexus MRI showed T2 hyperintensity in 18/26 patients (69%) and nerve enlargement in 14/18 patients (54%). A combination of the two techniques increased significantly the sensitivity to 96% of the patients (25/26). Among patients without CB, MEP-TST and plexus MRI were respectively abnormal in 5/7 (71%) and 4/7 patients (57%). Conclusions

MEP-TST provides information on the nerve conduction and MRI on the morphology of the brachial plexus. The combination of both examinations adds value to the current diagnosis criteria of MMN especially in MMN without CB on conventional nerve conduction studies

References:

None.

Keywords:

Inflammatory, Other

Grant Support: None.
Role of Supportive Diagnostic Criteria in Chronic Inflammatory Demyelinating Polyradiculoneuropathy: Data from the Italian Database

Eduardo Nobile-Orazio1, Pietro Doneddu2, Dario Cocito3, Fiore Manganelli4, Chiara Brian5, Raffaella Fazio6, Massimiliano Filostot7, Luana Benedetti8, Anna Mazzeo9, Girolama Marfia10, Giovanni Antonini11, Laura Piccolo12, Giuseppe Cosentino13, Stefano Jann14, Maurizio Clerici15, Mariella Carpo16, Marco Luigetti17, Gabriele Siciliano18, Tiziana Rosso19, Giuseppe Lauria20, Guido Cavaletti21, Giuseppe Liberatore22, Erida Peci3, Lucio Santoro22, Marta Tronci10, Stefano Maggioni23, S Cotti Piccinelli24, Angela Schenone25, Antonio Toscano25, Giorgia Mataluni26, Luca Leonard11, Andrea Cortese27

1 Milan University, IRCCS Humanitas Clinica Institute, Milan, Italy, 2IRCCS Humanitas Clinical Institute, Rozzano, Italy, 3Presidio Sanitario Maggiore, Istituti Clinici Scientifici Maugeri, Udine, Italy, 4Università degli Studi di Napoli "Federico II", Naples, Italy, 5University of Padoa, Padova, Italy, 6IRCCS San Raffaele Hospital, Milan, Italy, 7University of Brescia, Spedali Civili Hospital, Brescia, Brescia, Italy, 8 Dipartimento di Neuroscienze, Università degli Studi di Genova, Genoa, Italy, 9Azienda Ospedaliera Universitaria "G. Martino," Messina, Italy, 10Dipartimento di Neuroscienze, Policlinico Tor Vergata, Roma, Italy, 11Santandrea Hospital, University of Rome, Rome, Italy, 12Neurologia Fondazione Mondino, Pavia, Pavia, Italy, 13Azienda Ospedaliera Universitaria Policlinico Paolo Giaccone, Palermo, Palermo, Italy, 14Ospedale di Niguarda, Milan, Italy, 15Fondazione Macchi Hospital, Varese, Italy, 16 Treviglio Hospital, Trevisiglio, Italy, 17Università Cattolica del Sacro Cuore, Roma, Italy, 18Azienda Ospedaliero Universitaria Pisana, Ospedale S. Chiara, Pisa, Italy, 19Azienda ULSS 8 Asolo, Castelfranco Veneto, Italy, 20IRCCS Carlo Besta Neurological Institute, Milan University, Milan, Italy, 21Milano Bicocca University, Monza, Italy, 22University of Padoa, Padova, Italy, 23A.O. "Spedali Civili" ed Università degli Studi di Brescia, Brescia, Italy, 24Department of Neuroscience, Genoa University, Genoa, Italy, 25Azienda Ospedaliera Universitaria "G. Martino," Messina, Italy, 26Dipartimento di Neuroscienze, Policlinico Tor Vergata, Rome, Italy, 27Neurologia, Fondazione Mondino, Pavia, Italy

Objective: A number of supportive criteria are considered by the EFNS/PNS to help in the diagnosis of CIDP even if their relative utility is not established.

Material and methods: We reviewed the data from the Italian CIDP database to determine the frequency and utility of supportive criteria in the diagnosis of CIDP.

Results: We enrolled 545 patients with a clinical diagnosis of CIDP including 437 with definite (405), probable (24), possible (6) CIDP or CISP (2) according to EFNS/PNS criteria. The diagnosis of definite CIDP was possible with only motor nerve conduction studies (NCS) in 346 patients (85%), with the addition of one supportive criteria in 20 (5%) patients and two in 39 (10%) patients. In five patients the diagnosis of probable CIDP was made with only NCS while 19 required one supportive criteria. Abnormality consistent with demyelination in at least two motor nerves were found on conduction velocity in 177 patients (51%), conduction block in 157 (45%), increased temporal dispersion in 122 (35%), in distal latency in 69 (20%) and in F-wave in 32 (9%). Among the 78 patients who improved the definition of their diagnosis, 51 (65%) had increased CSF proteins, 23 (29%) demyelinating features on sensory NCS or evoked potentials, while 3 had demyelinating findings on nerve biopsy and 6 nerve ultrasound/MRI abnormalities. The same abnormalities in the whole CIDP group, were 258/336 (77%) for increased CSF proteins, 150/394 (38%) for demyelinating sensory NCS/evoked potentials, 21/35 (60%) for nerve biopsy and 50/65 (77%) for US or MRI. In 38 patients (49%) response to immune therapy was necessary to improve diagnostic definition.

Discussion: In 82% of patients with CIDP the diagnosis can be made with only NCS while supportive diagnostic tests are often unnecessary to improve the diagnosis even if they are extensively made in the current practice.
References: None.

Keywords: Inflammatory

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Guillain-Barré Syndrome following Arboviral Infection in Northeast Brazil: a Case Series

Sonja Leonard1, Suzannah Lant2, Livia Brito Bezerra de Albuquerque3, Lais Cordero Diniz da França4, Vanessa Fragoso Cassiano4, Marcela Lopes Santos5, Roberta da Paz Melo4, Bart Jacobs1, Maria Pessoa Militão de Albuquerque5, Maria Brito Ferreira4

1Erasmus University Medical Center, Rotterdam, Netherlands, 2Institute of Infection and Global Health, University of Liverpool, Liverpool, United Kingdom of Great Britain and Northern Ireland, 3Federal University of Pernambuco (UFPE), Recife, Brazil, 4Hospital da Restauração, Recife, Brazil, 5Fiocruz, Recife, Brazil

Introduction

Zika virus (ZIKV) has been associated with the Guillain-Barré syndrome (GBS). Other arthropod-borne viruses (arboviruses), including Chikungunya (CHIKV) and Dengue virus (DENV), have also been linked to GBS. We describe a large cohort of GBS patients with evidence of a preceding arbovirus infection in Northeast Brazil.

Methods

Adult GBS patients with preceding arboviral symptoms were recruited in our center between December 2014 and January 2017. Diagnostic evidence of recent ZIKV, CHIKV, or DENV infection was defined as presence of viral RNA or specific IgM in serum or cerebral spinal fluid (CSF).

Results

Of the 73 included patients, 51 (70%) had a recent arboviral infection: 53% with ZIKV, 33% with CHIKV, and 11% with DENV. Recent infection with multiple arboviruses was found in 20 patients. Median time between start of systemic and neurological symptoms was 8 days (IQR 5-24). Clinical features included: limb weakness (97%), sensory signs (85%), facial palsy (58%), dysautonomia (26%), and respiratory insufficiency (16%). Nerve conduction studies showed a demyelinating neuropathy in 14 (64%) and an axonal neuropathy in 6 (29%) of 21 tested cases. Fourteen cases (19%) were admitted to the intensive care unit (ICU). Sensory signs during admission and complete recovery at last follow-up were significantly more frequent in arbovirus-positive compared to -negative cases.

Conclusion

Our results suggest that besides infection with ZIKV, infection with CHIKV, DENV, or recent sequential or co-infection with more than one of these three viruses, may trigger GBS. Post-infectious instead of (para) infectious pathophysiology is most likely in the majority of cases. GBS related to arbovirus infection is characterized by a classic sensorimotor demyelinating neuropathy with facial palsy, respiratory insufficiency and dysautonomia, and has a relatively favorable outcome. It is advised to consider multiple arboviruses in GBS patients in endemic regions and to be aware of complications that necessitate ICU admission.

References: None.

Keywords: Inflammatory, Other

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Unravelling the Role of Niaspan in Peripheral Nerve Regeneration

Stefano Previtali, Alessio Gioia, Silvia Cipriani, Emanuela Porrello, Valeria Alberizzi, Roberta Di Guardo, Francesca Bianchi, Ubaldo Del Carro, Alessandra Bolino
Institute of Experimental Neurology (InSpe), IRCCS Ospedale San Raffaele, Milano, Italy

Introduction: Charcot-Marie-Tooth (CMT) neuropathies are highly heterogeneous disorders caused by mutations in more than 100 genes, with no available treatment. Thus, it is difficult to envisage a single suitable treatment for all pathogenetic mechanisms. Axonal Neuregulin-1 type III (Nrg1t3) drives Schwann cell myelination and determines myelin thickness. Nrg1t3 is inhibited by the α-secretase TACE, which negatively regulates PNS myelination. We hypothesized that modulation of Nrg1 levels and/or secretase activity may constitute a unifying treatment strategy for CMT neuropathies with focal hypermyelination as it could restore normal levels of myelination. We recently reported that in vivo delivery of Niaspan, a FDA approved drug known to enhance TACE activity, efficiently rescues myelination in two models of CMT neuropathy: the Mtmr2 KO mouse, a model of CMT4B1 with myelin outfoldings, and in the Pmp22 +/ - mouse, which reproduces HNPP with tomacula. As Nrg1 may also influence axonal regeneration and remyelination, which are key events in the progression of CMTs, we investigated whether Niaspan can influence peripheral nerve regeneration in CMT mouse models.

Methods: We performed nerve crush injury in adult Pmp22+/- and Mtmr2 KO mice. Niaspan was administered once a day starting the day after the crush. We carried out morphologic, morphometric, and histochemical analyses to evaluate the regeneration status (T21; 21 days post injury) and the achievement of both structural and functional repair (T45). Results: Preliminary results obtained in the Pmp22+/- model suggest that Niaspan treatment does not negatively influence nerve regeneration/remyelination, but rather it may ameliorate neurophysiology. We are evaluating Niaspan effect also in Mtmr2-KO mice. Of note, we observed defects in regeneration in both Pmp22+/- and Mtmr2-KO mice, which have not previously reported. Conclusions: Understanding the role of Niaspan in peripheral nerve regeneration will provide new insights to the use of this drug for the therapy of CMT neuropathy.

References: None.

Keywords: Axonal Regeneration, Pre-clinical Studies, Schwann Cell, Axonal Biology, CMTR

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Neuropathy related genes as possible modifiers of PMP22 related neuropathies
Anneke van der Kooi1, Barbara van Paassen2, Fred van Ruissen1, Marianne de Visser1, Camiel Verhamme1, Frank Baas3
1Amsterdam University Medical Center, location Academic Medical Center, Amsterdam, Netherlands, 2Erasmus Medical Center, Rotterdam, Netherlands, 3Leiden University Medical Center, Leiden, Netherlands

Charcot-Marie-Tooth disease type 1A (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP) are autosomal dominantly inherited peripheral neuropathies caused by copy number variation of the PMP22 gene. Considerable phenotypic variation is known for both disorders, suggesting modifiers.

We undertook a search for genetic modifiers of PMP22 related neuropathies by selecting the extremes of the spectrum of CMT1A and HNPP patients, based on disability assessed by the Overall Neuropathy Limitation Scale (ONLS). The ONLS data of 287 patients (184 CMT1A and 103 HNPP patients) showed a Gaussian distribution for both disorders. The median score for CMT1A patients was 4 and for HNPP patients 3. Twenty-one mild CMT1A (ONLS <2), 26 severe CMT1A (ONLS >5), 25 mild HNPP (ONLS <2) and 25 severe HNPP patients (ONLS >4) were clinically evaluated to further characterize disease severity. A next generation sequencing gene panel containing 147 genes related to neuropathies and hereditary motor syndromes was tested in this selection of patients.

Missense, frameshift, nonsense and intronic mutations possibly affecting splicing were selected. No significant difference was found in the mean number of variants (mutation burden) per patient between the mild and severe groups. Further selection of variants with an allele frequency of ≤4% in a control population was done. A significant difference between the mild and the severe HNPP group was found. In several patients double trouble was found (another likely pathogenic mutation in a neuropathy related gene). Replication of these results in a larger cohort are needed.

References: None.

Keywords: CMTR, Human Genetics

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Genetic and clinical finding in a cohort of 341 Chinese patients with Charcot-Marie-Tooth disease

Ruxu Zhang¹, Zhiqiang Lin¹, Beisha Tang², Stephan Zuchner³

¹the third Xiangya Hospital, Central South University, Changsha, Hunan, China, ²Xiangya Hospital, Central South University, Changsha, Hunan, China, ³John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL, USA

We are reporting the genetic and clinical profile of a cohort of 341 Chinese CMT patients and analyze the mutational distributions according to geographic location and age of participants. CMT patients were enrolled between 2006 and 2018. MLPA, Sanger sequencing or gene panel testing was applied to detect sequence variants. Genotype-phenotype analysis was carried out in the cohort, where 159 patients live in Hunan province. The percentage of CMT1 and CMT2 patients were 50.1% and 49.9%, respectively. The genetic diagnosis rate was 54.0% and the most common four genes accounted for 85% of diagnosed patients. EGR2 and NEFL were relatively common in CMT1. GDAP1, IGHMBP2 and HSPB1 were commonly observed in CMT2 patients. The CMT1 genetic diagnosis rate was 59.6 % in children and 66.2% in adults. PMP22, GJB1 and MPZ were the most frequently mutated genes. The CMT2 genetic diagnosis rate was 66.2% in children and 27.5% in adults. Twenty-five previously unreported pathogenic variants in ten CMT genes were identified. Three simultaneous heterozygous mutations in MFN2 and GDAP1 were detected and related to severe CMT2 phenotypes with early age of onset. This study represents the largest cohort of Chinese CMT patients to date. We are significantly expanding the phenotype and genotype spectrum of CMT.

References: None.

Keywords: Human Genetics

Grant Support: National Natural Science Foundation of China (81771366), the Hunan Provincial Natural Science Foundation (2017JJ2365), the Science Foundation of Health and Family Planning Commission of Hunan Province (A2017001)
Diphtheria is now an uncommon cause of peripheral neuropathy, however recent reports have related to pharyngeal infection. Here we report a rare case of a sensorimotor neuropathy, initially resembling immunotherapy-resistant Guillain-Barre syndrome, following a highly toxigenic cutaneous diphtheria infection.

A 66-year-old Australian man, a frequent world traveller, presented in March 2018 with progressive sensorimotor neuropathy with neuropathic pain, maximal in the left foot (initial ONLS-Overall Neuropathy Limitations Scale-6). He had a resolving ulcer on the second left toe, present since January in Vanuatu, and treated with antibiotics; and recent Rhinovirus. Nerve conduction studies (NCS) suggested demyelination; lumbar puncture showed elevated protein 1.27g/L, normal cells. Intravenous immunoglobulin (IVIg) (2g/kg) and pregabalin induced minimal improvement.

Despite plasmapheresis (x5), pulsed methylprednisolone (Week 3), then oral steroid (1g/kg), and further IVIg, the patient progressed. Mild diplopia, facial weakness/paraesthesia, dysphonia, dysphagia, and moderate pulmonary restriction developed (Wk2-4). By Wk 6 he had marked quadriparesis/proprioceptive loss (ONLS-11), and Raynaud-like fluctuating left foot cyanosis.

Autoimmune/serological investigations including ganglioside/paranodal antibodies were unremarkable apart from positive Ro60/52. MRI spine (Wk5) showed patchy cauda equina enhancement. Neurophysiology (Wk5) showed lower limb (LL) denervation, and somatosensory evoked potentials (SSEPs) were absent/delayed(LL/UL). Initial toe swab culture/PCR review showed a highly toxigenic strain of Corynebacterium diphtheriae, expressing A/B toxin beta subunits. IVIg was ceased due to deep vein thrombosis and likely diphtheritic neuropathy. Steroids were weaned during rehabilitation (Wk8-16), and there was dramatic functional improvement : ONLS-Wk10-9/Wk16-5/Wk24-2. Sural nerve/muscle biopsies (Wk7) showed a chronic active axonal neuropathy, and VEGF was normal. Neurophysiologically, improvement was mild by 5 months; substantial by 11 months.

This is the first report of generalised neuropathy related to cutaneous diphtheria infection since 1946. The axonal loss is atypical, with diphtheria usually associated with demyelinating neuropathy. It is unclear whether timing, distal infection, antitoxin non-use, or organism strain affected the pathology.

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CMTR Platform Presentation: Schwann Cells & Myelination
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&

CMTR Platform Presentations: Pathomechanisms
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Neddylation plays a critical role for formation, maturation and maintenance of Schwann cell myelin sheaths

Ashwin Woodhoo\textsuperscript{1}, Miguel Tamayo\textsuperscript{2}, Marta Palomo–Irigoyen\textsuperscript{2}, Encarnacion Perez-Andres\textsuperscript{2}, Marta Varela-rey\textsuperscript{3}

\textsuperscript{1}CIC bioGUNE, Ikerbasque Research Foundation, Derio, Spain, \textsuperscript{2}CIC bioGUNE, Derio, Spain, \textsuperscript{3}CIC bioGUNE, Ciberehd, Derio, Spain

Myelinating Schwann cells play a critical role for neuronal function and health. Defective myelination is responsible for the morbidity of a number of peripheral neuropathies, including Charcot-Marie-Tooth disease and diabetic neuropathy. Decades of research has uncovered a complex transcriptional and post-transcriptional program that drives the formation and maintenance of the myelin sheath. In contrast, much less is known about the functional role of post-translational modification (PTM) of proteins in this remarkable biogenic process.

Neddylation, a PTM that involves the conjugation of the ubiquitin-like protein Nedd8 to protein targets, has recently emerged as a central and versatile regulator of many cellular processes, including ubiquitination, protein transcription and signalling transduction. In Schwann cells, a functional role for neddylation has so far not been defined.

In this study, using various models of genetic and pharmacological inhibition of neddylation \textit{in vivo}, we show that this PTM has complex and extensive regulatory functions in Schwann cells. For instance, genetic inactivation of \textit{NAE1}, the enzyme that catalyses neddylation reactions, specifically in developing Schwann cells, leads to striking nerve defects that exhibit all the hallmarks of a severe neuropathy, including gait abnormalities, muscle weakness, and hindlimb clashing. Strikingly, \textit{NAE1}-deficient mice lack peripheral myelin and exhibit active myelin breakdown of the few formed myelin sheaths. Mechanistically, this severe block of myelination is due to a deficiency in the ubiquitin-mediated degradation of negative regulators of myelination in perinatal nerves, which remain artificially elevated, thus blocking myelination. Notably, we also found an important function of neddylation in maturation and maintenance of myelin sheaths, and in the Schwann cell responses to nerve injury.

In summary, our study reveals that PTMs can play a central role in nerve development, and identifies neddylation as a tractable target for the development of new therapies in demyelinating disorders and for nerve regeneration.

\textbf{References:} None.

\textbf{Keywords:} Schwann Cell, Axonal Regeneration, Other

\textbf{Grant Support:}

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During myelination, Schwann cells up-regulate lipid biosynthesis to supply the lipid-rich (70 - 85%) mature myelin sheath. Several of these lipids are directly derived from choline-dependent metabolism, whereas the synthesis pathways of others intersect with choline derivatives. Choline-derived lipids are important both as structural components and as reservoirs of signaling molecules that have a direct implication on the initiation of myelination, the compaction and the maintenance of the myelin sheath. Little is known about the molecular mechanisms that regulate the lipid synthesis during Schwann cell myelination. It is likely, however, that axo-glial interactions are involved. Recently we identified in Schwann cells a protein complex that incorporates cell adhesion molecule Nectin-like 4 (Necl4) and Choline Transporter-Like protein 1 (CTL1). We have shown that intracellular choline homeostasis, as well as choline-dependent lipid biogenesis of phosphatidylcholine and phosphatidylinositol are disrupted in Necl4-deficient Schwann cells. To expand our in vitro data to an in vivo system, we generated a Schwann cell-specific CTL1 knockout mouse (dhhCre;CTL1fl/fl). EM analyses of developing sciatic nerves reveals PN myelination defects including delayed myelination, thinner myelin sheaths as well as myelin in-folding and out-folding. The mice also exhibit impaired motor behavioral performance and reduced nerve conduction thresholds. While choline is a vital nutrient that must be acquired though the diet, cells do have the ability to produce choline de novo through the PEMT pathway. Thought to have little to none biological role in non-hepatic tissues, we show that the PEMT enzymes are strongly expressed in developing sciatic nerves at the onset of myelination. Similar to the CTL1 knockout mice, PEMT knockout mice present numerous myelin abnormalities in the PNS. These results suggest that the regulation of choline metabolism through CTL1 and PEMT pathways is an important parameter to myelin formation.

References: None.

Keywords: Schwann Cell, CMTR

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NRG1 type I dependent autoparacrine stimulation of Schwann cells in onion bulbs of peripheral neuropathies
Ruth Stassart¹, Robert Fledrich², Dagmar Akkermann³, Vlad Schütza⁴, Tamer Abdelaal⁵, Doris Hermes⁶, Maria Soto-Bernardini⁷, Tilmann Götze⁶, Theresa Kungl⁶, Michael Sereda⁶, Markus Schwab⁸, Klaus-Armin Nave⁶

¹Department of Neuropathology University Clinic Leipzig, Department of Neurogenetics MPI of Experimental Medicine, Leipzig, Germany, ²Institute of Anatomy, University of Leipzig, Leipzig, Germany, ³Department of Neuropathology, Leipzig, Germany, ⁴Department of Neuropathology, University Clinic Leipzig, Leipzig, Germany, ⁵Department of Neurogenetics, Max-Planck-Institute of Experimental Medicine, Göttingen, Germany, ⁶Department of Neurogenetics, Max-Planck-Institute of Experimental Medicine, Göttingen, Germany, ⁷Department of Neurogenetics, Max-Planck-Institute of Experimental Medicine, Göttingen, Germany, ⁸Hanover Medical School, Hanover, Germany

In contrast to acute peripheral nerve injury, the molecular response of Schwann cells in chronic neuropathies remains poorly understood. Onion bulb structures are a pathological hallmark of demyelinating neuropathies, but the nature of these formations is unknown. Here, we show that Schwann cells induce the expression of Neuregulin-1 type I (NRG1-I), a paracrine growth factor, in various chronic demyelinating diseases. Genetic disruption of Schwann cell-derived NRG1 signalling in a mouse model of Charcot-Marie-Tooth Disease 1A (CMT1A), suppresses hypermyelination and the formation of onion bulbs. Transgenic overexpression of NRG1-I in Schwann cells on a wildtype background is sufficient to mediate an interaction between Schwann cells via an ErbB2 receptor- MEK/ERK signaling axis, which causes onion bulb formations and results in a peripheral neuropathy reminiscent of CMT1A. We suggest that diseased Schwann cells mount a regeneration program that is beneficial in acute nerve injury, but that overstimulation of Schwann cells in chronic neuropathies is detrimental.

References: None.

Keywords: CMTR, Schwann Cell

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Hyperglycosylation of Myelin Protein Zero: from pathogenesis to therapeutic options.

Marina Grandis¹, Francesca Veneri², Valeria Prada², Rosa Mastrangelo³, Cinzia Ferri³, Alessandro Geroldi¹, Francesca Bianchi³, Ubaldo Del Carro³, Angelo Schenone¹, Maurizio D’Antonio³

¹University of Genova, Genova, Italy, Genova, Italy, ²University of Genova, Genova, Italy, Genova, Italy, ³San Raffaele Scientific Institute, Milano, Italy, Milano, Italy

Introduction: mutations in the Myelin Protein Zero gene (MPZ), encoding P0, the major structural glycoprotein of peripheral nerve myelin, are found in 5% of Charcot-Marie-Tooth (CMT) patients. P0 variants may cause different gain of function including misglycosylation (either gain-of-glycosylation or loss-of-glycosylation), a novel pathomechanism encompassing several genetic disorders. We decided to establish a mouse model carrying the D61N mutation, a MPZ variant causing hyperglycosylation of P0 (1). Methods: the knock-in mouse model was generated using the Crispr/Cas9 system. The phenotype was characterized by behavioral, electrophysiological and neuropathological tests, at different time points. We evaluated the presence of tremor, the motor capacity, the sciatic motor nerve conduction velocity and cMAP. We performed light microscopy morphological observations, analyzing sciatic nerve semithin sections. We also decided to establish myelinating DRG cultures from wild type (WT) and Mpz D61N/+ embryos. Results: at one and three months of age, all evaluations, showed significant differences in Mpz D61N/+ mice compared with WT animals. Tremor was evident in all Mpz D61N/+ mice and correlated with a significant motor impairment on the accelerating Rotarod where mutant mice showed a reduced average latency to fall. Electrophysiological parameters also differed between the two groups: at one month of age, the average MCV was 11.8m/s ±1.2 in Mpz D61N/+ as compared with 32.88m/s ±1.3 in controls. The pathological analysis confirmed a demyelinating phenotype with a significant increase of the G-ratio in Mpz D61N/+ animals. In myelinating DRG cultures we detected fewer internodes carrying remarkable myelin abnormalities; when DRGs were treated with NB-DNJ, an immuno-sugar potentially able to reverse hyperglycosylation, the myelin defects seemed to decrease. Conclusions: we obtained an animal model expressing the P0D61N variant, a mutation causing gain-of-glycosylation in P0 which recapitulates the human phenotype of this variant and we are testing possible therapeutic strategies.

References:


Keywords:CMTR, Schwann Cell, Human Genetics

Grant Support:

AFM-Telethon (20572) to Marina Grandis and Maurizio D'Antonio.
Role of the ER stress transcription factor XBP1 in Charcot-Marie-Tooth disease type 1B

Thierry Touvier¹, Rosa Mastrangelo¹, Francesca Veneri¹, Cinzia Ferri¹, Francesca Bianchi², Ubaldo Del Carro², Laurie Glimcher³, Christina E. Barkauskas⁴, Lawrence Wrabetz⁵, Maurizio D’Antonio¹

¹Division of Genetics and Cell Biology San Raffaele Scientific Institute, Milan, Italy, ²Division of Neuroscience San Raffaele Scientific Institute, Milan, Italy, ³Dana-Farber Cancer Institute, Harvard Medical School and Brigham and Women’s Hospital, Boston, USA, ⁴Duke University Medical Center, Durham, USA, ⁵HJKRI-University of Buffalo, Buffalo, USA

Myelin protein zero (Mpz) protein is the most abundant protein in the myelin of peripheral nerves. The mutant MpzS63del causes Charcot-Marie-Tooth (CMT) 1B disease in humans and a similar demyelinating neuropathy in transgenic mice. MpzS63del protein provokes an endoplasmic reticulum (ER) stress in myelinating Schwann cells, resulting in an unfolded protein response (UPR) characterized by activation of PERK, ATF6 and XBP1 pathways. We have previously reported that activation of CHOP and GADD34, two mediators downstream of PERK, is pathogenetic in MpzS63del mice, but the role of the other UPR branches remains to be investigated.

To unravel the role of the XBP1 pathway in CMT1B, we generated new models of CMT1 mice in which XBP1 gene is deleted or overexpressed specifically in Schwann cells and, in parallel, we exploited MpzS63del dorsal root ganglia (DRG) explant cultures in which XBP1 signaling is modulated by gain/loss of function approaches.

We have observed that the absence of XBP1 dramatically worsens hypomyelination and electrophysiological/locomotor parameters in young and adult S63del neuropathic animals. Interestingly, we observed strong upregulation of PERK and IRE1-mediated RIDD signalings in neuropathic animals lacking XBP1. This suggests that the activation of XBP1 targets plays a critical role in limiting MpzS63del toxicity, which cannot be compensated by other stress responses. In addition, we demonstrated in S63del DRG cultures that inhibition of XBP1 pathway impairs myelination while activation of XBP1 signaling ameliorates myelination.

Overall, these data demonstrate that the XBP1 pathway has a essential adaptive role in MpzS63del neuropathy and suggest that activation of this pathway is beneficial for CMT1B and possibly for other neuropathies characterized by UPR activation.

References: None.

Keywords: Schwann Cell

Grant Support: Telethon GGP14147
Finely tuned calcium dynamics are essential for normal neuronal function, and excessive calcium flux has been repeatedly implicated in the pathogenesis of neurodegenerative diseases. The mechanisms that lead to neuronal dysfunction and degeneration downstream of calcium entry remain poorly defined. Mutations in the non-selective cation channel TRPV4 cause motor predominant peripheral neuropathies, including Charcot Marie Tooth disease subtype 2C (CMT2C). To investigate the role of calcium in neurodegeneration and pathological mechanisms involved in CMT2C, we explored the consequences of CMT2C-causing mutant TRPV4 expression in primary mammalian neurons and in Drosophila.

Expression of mutant TRPV4 causes neuronal dysfunction and axonal and dendritic degeneration that can be prevented by genetically or pharmacologically inactivating the TRPV4 ion channel pore. While activation of both wild-type and mutant TRPV4 increases intraneuronal calcium, we demonstrate that mutant TRPV4 is more sensitive to stimulation than wild-type TRPV4 in neurons. Additionally, mutant TRPV4 causes neuronal dysfunction manifested as hyperexcitability and impaired mitochondrial transport in the absence of TRPV4 stimulation. Interestingly, acute pharmacologic activation of wild-type TRPV4 also disrupted mitochondrial transport, suggesting mitochondrial transport is regulated by TRPV4 mediated calcium influx. To investigate signaling mechanisms involved in mutant TRPV4 mediated toxicity, we performed a genetic modifier screen in the fly and identified CaMKII as a potent genetic modifier of mutant TRPV4. RNAi silencing of CaMKII prevents neuronal dysfunction and neurodegeneration. Remarkably, pharmacologic inhibition of CaMKII substantially suppresses TRPV4 mediated calcium influx, suggesting CaMKII potentiates TRPV4 activity and operates at the level of calcium entry in our models. Our data suggest that neuropathy-causing mutants sensitize the TRPV4 ion channel, resulting in CaMKII dependent calcium influx and subsequent calcium-dependent disruption of mitochondrial transport and neurodegeneration. Furthermore, they suggest that TRPV4 selective antagonists warrant further investigation as potential therapeutics for TRPV4-mediated peripheral neuropathies.

References: None.

Keywords: CMTR, Axonal Biology

Grant Support: None.
The Integrated Stress Response Contributes to Charcot-Marie-Tooth Type 2D Peripheral Neuropathy in Mice

Emily Spaulding, Robert Burgess

The Jackson Laboratory, The University of Maine, Bar Harbor, ME, USA

Dominant mutations in glycyl-tRNA synthetase (GARS) cause CMT type 2D (CMT2D). How mutations in GARS cause neurodegeneration is unclear, but impaired translation has emerged as a potential toxic gain-of-function mechanism based on work with Drosophila. To test this mechanism in mice, we have profiled translation in motor neurons of mice with mutations in Gars that are validated as CMT2D models. In vivo, cell type-specific, fluorescent non-canonical amino acid-tagging (FUNCAT) has revealed reduced translation in motor neuron cell bodies of mutant Gars mice. To complement the protein analysis, in vivo ribosome-tagging from mutant Gars motor neuron cell bodies was used to identify mRNAs undergoing translation. This revealed an upregulation of transcripts associated with the integrated stress response, including ATF4 and several of its gene targets. Using RNAscope in situ hybridization, we show that (1) activation of the stress response occurs in approximately 70% of mutant motor neurons, (2) most gamma motor neurons do not show this response, (3) a subset of sensory neurons in dorsal root ganglia also upregulate the stress response, and (4) no other cell types in the spinal cord or dorsal root ganglia activate this response. We also find evidence of stress response activation in the spinal cords of mutant Yars-E196K mice, a model of dominant intermediate CMT type C. Genetic experiments reveal that removing GCN2, a kinase that activates the stress response, from mutant Gars mice prevents expression of the most highly upregulated ATF4 gene targets. Removing GCN2 also significantly alleviates neuropathy, resulting in increased body weight, improved grip strength, less denervation at the neuromuscular junction, increased nerve conduction velocity, and less motor axon loss. Because chronic stress response activation is detrimental to motor neurons in this disease context, inhibiting GCN2 in human patients with mutations in tRNA synthetase genes may be beneficial.

References: None.

Keywords: CMTR, Axonal Biology

Grant Support: Ruth L. Kirschstein NRSA Individual Predoctoral Fellowship F31NS100328 to ELS and RO1 NS054154 to RWB.
INC Oral Abstracts
Sunday, June 23, 2019 - 15:30 - 16:00
&
INC Oral Abstracts
Sunday, June 23, 2019 - 17:30 - 18:30
The Association of Dengue Infection and Guillain-Barré Syndrome in Malaysia: A Case Control Study
Cheng-Yin Tan, Siti Nur Omaira Razali, Khean-Jin Goh, I-Ching Sam, Nortina Shahrizaila
University of Malaya, Kuala Lumpur, Malaysia

Introduction: In Guillain-Barré syndrome (GBS), two thirds of patients are reported to have antecedent infections up to four weeks prior to the onset of weakness. The profiles of antecedent infections vary geographically. In Southeast Asia, arthropod-borne viruses are common and dengue, specifically, is hyperendemic. In this case-control study, we aim to determine the association of a recent dengue infection and GBS in a Malaysian population.

Methods: Consecutive patients presenting with features supportive of GBS were recruited between 2010 and 2018. The frequency of dengue virus infections was determined by dengue IgM antibodies. The sera of neurological controls with a similar distribution in age, gender, and period of sampling were obtained. Sera from patients with GBS were obtained before treatment.

Results: A total of 95 patients with GBS were recruited. Evidence of recent dengue infection was present in 20.0% of GBS patients compared to 7.4% of neurological controls (19/95 vs 5/68, OR 3.2, 95% CI 1.1-8.9, p = 0.025). On univariate analysis, GBS patients with dengue IgM were associated with diarrheal symptoms (p = 0.027), severe disease at nadir (Medical Research Council sum score: p = 0.009; GBS disability score: p = 0.018), need for ventilation (p = 0.002), facial palsy (p = 0.004), absence of anti-ganglioside antibody (p = 0.022) and acute inflammatory demyelinating polyneuropathy (AIDP) subtype on electrodiagnosis (p < 0.001). AIDP subtype (p = 0.008) was the only independent associated factor on multivariate analysis. The presence of dengue IgM antibodies in patients with GBS was not associated with age, gender, disease progression, sensory deficits, cerebrospinal fluid albuminocytological dissociation or clinical outcome at 6 months.

Conclusions: A recent dengue infection is significantly associated with GBS in Malaysia. Dengue-associated GBS patients were more likely to have AIDP on electrophysiology.

References:

Keywords: Inflammatory

Grant Support:

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INTRODUCTION. The Medical Research Council (MRC) scale is an outcome measure of strength, routinely used in neurological examinations. Previous research reported limitations of the MRC scoring system, proposing a collapsed 0-to-3-point scale. We aimed to assess the clinimetric properties of the original 0-to-5-point MRC scoring system and its sensitivity for GBS patients, using data from the International GBS Outcome Study (IGOS)-1300 cohort. METHODS. MRC scores were assessed at entry bilaterally for: shoulder abduction, elbow flexion, wrist extension, hip flexion, knee extension and foot dorsiflexion. Rasch analysis was conducted to assess the suitability of the MRC 0-to-5-option response format, individual item fit, local dependency and dimensionality. Discriminative ability was determined by comparing scores to disability level [GBS Disability Score (GBS-DS)]. RESULTS. Data were available from 1099 patients (mean age 49±19 years, 40% female). The cohort comprised 76% (n=838) severely affected patients (GBS-DS>3). No disordered thresholds were observed, supporting use of the 0-to-5 point response format. It was necessary to combine bilateral measurements to overcome local dependency caused by high inter-item correlations (>0.8). The foot dorsiflexion item required removal from the sum-score to achieve fit to the Rasch model. Rasch-derived MRC sum-scores (0-100, higher values indicate increasing strength) were generated for patients (mean 56.7±25.5). Sum-scores differentiated between patients with mild (mean score 79.3±17.3) versus severe (mean score 49.8±23.6, p<0.001) disability levels. Foot dorsiflexion raw scores (bilateral measures, out-of-10) independently discriminated between disease severity (mild: mean score 8.8±1.5 vs. severe: 5.7±3.4). CONCLUSION. For clinical use, MRC scores in their original format require no adjustment for GBS patients. For research purposes, Rasch-derived MRC sum-scores should be generated, and bilateral measurements combined to account for the symmetrical nature of GBS. Further, we recommend to assess foot dorsiflexion separately, as it may be an important individual indicator of disease severity that cannot be summarized by sum-scores.

References: None.

Keywords: Inflammatory

Grant Support: None.
In the acute motor axonal neuropathy (AMAN) variant of Guillain-Barré syndrome (GBS), autoantibodies against gangliosides damage peripheral nerve axons by activating the classical complement cascade. In mouse models, anti-ganglioside antibodies (AGAbs) plus a complement source are used to target the distal motor nerve terminal (mNT), outwith the blood-nerve barrier. In large nerve bundles (spinal roots, nerve trunks), macrophage infiltration into the periaxonal space is a key early feature of AMAN, potentially acting as both executors of axonal injury and assisting in essential debris clearance. At the presynaptic mNT, perisynaptic Schwann cells (pSCs) overlying the terminal axonal membranes respond rapidly to mNT injury, and have been implicated in debris clearance. In this study, we have evaluated the relative responses of macrophages and pSCs to distal nerve injury in our AMAN model.

Three days after AGAb and complement-mediated injury, injured and control diaphragms from MacGreen mice (expressing EGFP in monocytes and macrophages) were analysed for macrophage content and activation state. In parallel, ex vivo nerve-muscle preparations were used to investigate the role of pSCs in mNT injury. In vivo, we show macrophage numbers are not elevated in the diaphragm, nor do they shift to pro- or anti-inflammatory phenotype. However, there is a redistribution of macrophages towards the vicinity of the mNT, indicating that tissue-resident macrophages are attracted to the injury. As the mNT regenerates rapidly, these macrophages are unlikely involved in continued nerve damage. We are currently investigating whether they are critical for debris clearance to allow regeneration. In contrast, observations from ex vivo mNT injury studies demonstrate pSCs do rapidly become phagocytic and engulf axonal debris from the injured mNT.

In conclusion, pSCs are important for clearance of debris and subsequent axonal regeneration. Tissue-resident macrophages are also attracted towards the mNT after localised injury, and their role is being investigated.

References: None.

Keywords: Axonal Biology, Axonal Regeneration, Inflammatory, Schwann Cell

Grant Support: Wellcome Trust
Clinical and serological investigations in CIDP patients with antibodies against CNTN1/Caspr1 complex.

Elba Pascual-Goñi1, Janev Fehmi2, Lorena Martín-Aguílar1, Cinta Lleixà1, Aleksandar Radunovic3, Alejandra Carvajal4, Yusuf A. Rajabally5, Romana Höftberger6, Shane Smyth7, Laura Williams7, Veronika Potocková8, Nigel Hinds9, Isabel Illa10, Simon Rinaldi2, Luis Querol11

1Neuromuscular Diseases Unit, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain, 2Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Oxford, United Kingdom of Great Britain and Northern Ireland, 3Department of Neurology, Barts Health NHS Trust, London, United Kingdom of Great Britain and Northern Ireland, 4Hospital Universitario Virgen de las Nieves, Granada, Spain, 5Regional Neuromuscular Clinic, Queen Elizabeth Hospital, University Hospitals of Birmingham, Birmingham, United Kingdom of Great Britain and Northern Ireland, 6Institute of Neurology, Medical University of Vienna, Vienna, Austria, 7Mater Misericordiae University Hospital, Dublin, Ireland, 8Motol University Hospital, Prague, Czechia, 9Abertawe Bro Morgannwg University Health Board, Swansea, Wales, United Kingdom of Great Britain and Northern Ireland, 10Neuromuscular Diseases Unit, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain, 11Neuromuscular Diseases Unit, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, Barcelona, Spain

Introduction:

Autoantibodies against paranodal proteins are useful biomarkers for diagnosis and treatment decision-making in patients with CIDP. Among them, antibodies against contactin-1 (CNTN1) and against contactin-associated protein-1 (Caspr1) were described in small subsets of patients with CIDP. Also, antibodies targeting the paranodal CNTN1/Caspr1 complex (but not CNTN1 alone) were described in one patient with an aggressive CIDP. However, the clinical-immunological features associated with antibodies against the CNTN1/Caspr1 complex have never been described.

Methods:

Eight CIDP patients with antibodies against CNTN1/Caspr1 complex were enrolled for characterization. Antibodies were tested by cell-based assays using HEK293 cells cotransfected with CNTN1 and Caspr1, or transfected with CNTN1 alone. We collected clinical, neurophysiological, laboratory and treatment response data.

Results:

We identified eight patients (5M, 3F) aged between 40 and 75. Patients’ sera showed reactivity only when CNTN1 and Caspr1 were cotransfected, but not when CNTN1 was transfected alone. All patients fulfilled EFNS/PNS definite diagnostic criteria for CIDP. They presented with an aggressive CIDP, with predominantly motor involvement. Half of them were initially diagnosed of Guillain-Barré syndrome due to a subacute onset. Neurophysiological studies showed findings of acquired demyelination in all patients, and acute denervation in at least two of them. Complete response to IVlg or steroids was not observed in any patient, while the response to rituximab in four treated patients was excellent.

Conclusion:

Antibodies against CNTN1/Caspr1 complex are present in a subset of patients with aggressive CIDP with poor response to first line treatments. We recommend screening antibodies against the CNTN1/Caspr1 complex as they will help guide the management. Experiments to elucidate the specific target of the autoantibodies of these patients are undergoing.
References:


Keywords: Inflammatory, Node

Grant Support: None.
Sunday, June 23, 2019 - 17:40 - 17:50
Treatment Status Following Corticosteroid And Immunoglobulin Treatment In The International CIDP Outcome Study (ICOS)
Sander Bus¹, Merel Broers², Carina Bunschoten², Ilse Lucke¹, Gwen van Lieverloo¹, Max Adrichem¹, Stephan Goedee⁹, Ludo van der Pol³, Bart Jacobs², Filip Eftimov¹

¹Amsterdam UMC, University of Amsterdam, Department of Neurology, Amsterdam Neuroscience, Amsterdam, Netherlands, ²Erasmus MC, University Medical Center, Department of Neurology, Rotterdam, Netherlands, ³University Medical Center Utrecht, Department of Neurology, Brain Center Rudolf Magnus, Utrecht, Netherlands

**Background:** Treatment in chronic inflammatory demyelinating polyneuropathy (CIDP) mainly consists of corticosteroids or intravenous immunoglobulins (IVIg). Remission is an important long term outcome. Corticosteroid therapy possibly increases the chance of long term remission. We aim to assess duration of treatment and compare remission rates following treatment with corticosteroids (monotherapy or in combination with IVIg) and IVIg.

**Methods:** Patients fulfilling the EFNS/PNS 2010 criteria for CIDP were prospectively enrolled in the ICOS and treatment data were systematically collected. A preliminary analysis was conducted in treatment naïve incident cases from two tertiary centers with a follow-up period of at least one year. For comparison, we grouped patients based on treatment type: corticosteroids (monotherapy or with IVIg) or IVIg monotherapy. We assessed treatment persistence at one year. In addition we assessed remission rates, defined as sustained improvement after discontinuation of treatment.

**Results:** 31 patients were included of which 22 (71%) received corticosteroids and nine (29%) IVIg monotherapy at baseline. In the corticosteroid group, six received pulsed high-dosed dexamethasone (during six months) and 16 received a combination of IVIg and methylprednisolone (every three weeks during four months). At one year nine (41%) patients in the corticosteroid group were still treated and five (56%) in the IVIg group. In the IVIg group, withdrawal was attempted in seven patients. Remission at one year was achieved in 13 patients (59%) patients treated with corticosteroids compared to four (44%) patients treated with IVIg.

**Conclusion:** Overall about half of patients were treated at one year. Based on this preliminary data, 59% of patients who received corticosteroid monotherapy or the combination therapy were in remission at one year compared to 44% of patients receiving IVIg. At the conference we will present the treatment results of all incident cases in ICOS, including a cohort of patients from a third tertiary center.

**References:**None.

**Keywords:**Inflammatory

**Grant Support:** None.
Diagnostic Delay and Work-Up of CIDP in the International CIDP Outcome Study (ICOS) cohort

Carina Bunschoten, Ilse Lucke, Merel Broers, Bart Jacobs, Gwen van Lieverloo, Max Adrichem, Ludo van der Pol, Stephan Goedee, Sander Bus, Filip Eftimov

1 Erasmus MC, University Medical Center, Rotterdam, Netherlands, 2 Amsterdam University Medical Center, Amsterdam, Netherlands, 3 Brain Center Rudolf Magnus, University Medical Center, Utrecht, Netherlands

Introduction

The diagnostic challenges in the spectrum of chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) are related to the variation in clinical presentation, electrophysiology, treatment response, and differential diagnosis of CIDP. CIDP is a treatable disorder and an early and correct diagnosis is essential to prevent secondary axonal damage and improve clinical outcome. We aim to describe the diagnostic work-up, identify factors related to diagnostic delay, and investigate the impact on clinical outcome in CIDP.

Methods

A preliminary analysis was conducted in 179 patients included in the International CIDP Outcome Study (ICOS). For comparisons the median duration to CIDP diagnosis was used to separate patients in an ‘early’ and a ‘late’ diagnosis group.

Results

The time from onset of first symptoms until CIDP diagnosis was median 9 months (interquartile range 4-24, range 1-233 months) and more than 12 months in 70 patients (41%). The diagnostic work-up included nerve conduction studies (100%), lumbar puncture (82%), nerve biopsy (3%), nerve ultrasound (30%), magnetic resonance imaging (33%) and somato-sensory evoked potential (1%). Patients with a late diagnosis more frequently had asymmetric CIDP variants (26% vs 8%) and nerve ultrasound examinations (39% vs 19%). Patients with an early diagnosis more frequently had cerebrospinal fluid examinations (94% vs 74%) and more often had elevated CSF protein levels (92% vs 75%).

Conclusions

Based on preliminary analysis, this study confirms the presence of diagnostic delay in CIDP and the first possible related clinical and diagnostic factors have been identified. At the conference, ICOS will be expanded by an additional cohort of treatment naïve CIDP patients and results of the full CIDP cohort regarding diagnostic delay in CIDP, including data on (initial) misdiagnosis, EFNS/PNS classification and possible related impact on clinical outcome.

References:


Keywords: Clinical Trials, Inflammatory
Grant Support: None
Sunday, June 23, 2019 - 18:00 - 18:10

Pure Motor Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) in 17 Patients: Clinical Characteristics, Electrophysiologica

Antoine Pegat1, William Boisseau2, Thierry Maisonobe2, Rabab Debs2, Timothée Lenglet2, Karine Viala3


Introduction: Pure motor Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) is a rare and poorly described form of CIDP.

Methods: Patients with definite or probable CIDP with pure motor clinical form, in a polyneuropathic distribution with abnormalities in sensory conduction studies in electrodiagnostic (EDX) studies (Pure Clinical Motor CIDP, PCM-CIDP) or without (Pure Motor CIPD, PM-CIDP) were included.

Results: 17 patients (prevalence of 2%) were included, with male predominance (71%), and a median age at onset of 48 years. At peak of severity, patients had upper and lower limb weakness (94%), with distal and proximal weakness in four limbs in 8 patients (53%). Clinical course was progressive in 12 patients. 6 patients had an associated disease including 3 patients with paraneoplastic CIDP (B-cell lymphoma, lung cancer and palate cancer), one patient with HIV, one patient with HCV and Sjogren syndrome and one patient with Inflammatory bowel disease. 12/16 and 4/5 patients had response to intravenous immunoglobulin (IVIg) and corticosteroids respectively. In EDX study, conduction block (CB) (82% of patients) and F abnormalities (88%) were frequent. Anti-ganglioside antibodies were positive in 3 patients (20%) including 2 patients with GM1+. The CSF protein was mildly elevated (>50mg/dl) in 11 patients (79%). During the follow-up, 4 of 10 patients in PCM-CIDP developed mild sensory symptoms, none in PM-CIPD group. Patients with PM-CIDP seem to have poorer outcome at the last follow up (median ONLS 4 versus 2, p = 0.03).

Conclusion: Beyond the previously reported features of pure motor CIDP including the low prevalence, response to IVIg, frequent CBs and F waves abnormalities in EDX study; our study revealed progressive clinical course in majority of patients, frequent associated paraneoplastic disorders and sensibility to corticosteroid therapy. In contrast to PCM-CIDP patients, PM-CIDP patients seem to have poorer outcome and did not develop sensory symptoms during follow-up.

References: None.

Keywords: Inflammatory

Grant Support: None.
Serum Contactin-1 Levels In Chronic Inflammatory Demyelinating Polyneuropathy – A Pilot Study

Luuk Wieske¹, Gwen van Lieverloo¹, Camiel Verhamme¹, Marleen Koel-Simmelink², Ivo van Schaik¹, Filip Eftimov¹, Charlotte Teunissen³

¹Department of Neurology, Amsterdam Neuroscience, Amsterdam UMC, Amsterdam, Netherlands, ²Department of Clinical Chemistry, Amsterdam Neuroscience, Amsterdam UMC, Amsterdam, Netherlands, ³Department of Clinical Chemistry, Amsterdam Neuroscience, Amsterdam UMC, Amsterdam, Netherlands

Purpose:

Biomarkers assessing disease activity in CIDP are needed to make informed treatment decisions in everyday care. Contactin-1 (CNTN1) is a paranodal axonal adhesion protein. Paranodal and juxtaparanodal proteins, including CNTN1, are targets for autoimmunity in subsets of CIDP patients. We hypothesized that serum CNTN1 levels reflect disease activity in CIDP.

Methods:

Three prospective cohorts of CIDP patients were studied: 1) patients starting induction treatment (IT cohort, N:27) measured at baseline and six months after starting treatment; 2) patients on maintenance treatment starting IVIg withdrawal (MT cohort, N:24) measured at baseline and six months after IVIg withdrawal or at time of relapse and 3) patients in long-term remission without treatment (N:26). Serum CNTN1 was measured using Luminex® assay. Age matched healthy controls (N:33) were used for comparison. Treatment response was defined as improvement by at least the minimal clinical important difference (MCID) on the I-RODS; and/or an increase of ≥8 kPa on grip strength. Relapse was defined as any deterioration requiring retreatment.

Results:

CNTN1 levels were lower in the IT cohort (median 10.2 ng/ml; IQR 8.9-12.5) compared to the MT cohort (12.0; IQR 10.4-15.0), patients in remission (14.2; IQR 11.3-17.7) and healthy controls (13.0; IQR 11.2-14.9; p<0.01 overall). After induction treatment, 2/8 (25%; follow-up sample missing for 1 patient) of the non-responders showed an increase in CNTN1 compared to 12/18 (67%) of the responders (p:0.09). After treatment withdrawal, 5/15 (33%) of patients with a relapse showed a decrease in CNTN1 compared to 7/9 (78%) of the patients without relapse (p:0.09).

Conclusion:

In this pilot study, CNTN1 levels in untreated CIDP patients with active disease were lower compared to patients stable on maintenance treatment, patients in remission and healthy controls. Changes in CNTN1 levels in relation to treatment response should be investigated in a larger cohort.

References: None.

Keywords: Inflammatory

Grant Support: None.
**Introduction:** CIDP is a heterogeneous autoimmune disease affecting the peripheral nerves. IgG4 antibodies to contactin-1 (CNTN1) are associated with a specific CIDP subtype. Risk factors associated with the appearance of these antibodies have not been described. HLA class II haplotypes strongly associate with several IgG4-mediated diseases, including anti-NF155-associated CIDP. This study describes the human leukocyte antigen (HLA) class II allele frequencies in chronic anti-CNTN1 positive patients.

**Methods:** 15 anti-CNTN1 positive and 51 anti-CNTN1 negative CIDP patients were included in the study. The frequencies of the HLA-DRB1 and HLA-DQ alleles were analyzed in all patients and compared with the allele frequencies of the general population obtained from the Allele frequencies database. In silico HLA-peptide binding and CNTN1 antigenicity predictions were performed to analyze overlap between presented peptides and antigenic regions.

**Results:** When comparing anti-CNTN1+ patients with the normal population: DRB1*11:01 alleles were present in 5 of the 15 anti-CNTN1+ (33.3 vs 14.6%; OR = 3.8, CI = 1.26 to 11.47); DRB1*11:02 alleles were founded in 2 anti-CNTN1+ (13.3 vs 2.84%; OR = 5.87, CI = 1.27 to 27.02) and 1 anti-CNTN1+ patient had the DRB1*11:03 allele (6.67 vs 1.97%; OR = 6.7, CI = 0.82 to 53.63); in contrast, none of the anti-CNTN1+ patients presented the DRB1*11:04 allele (0 vs 9.4%; OR = 1.2, CI = 0.07 to 20.98).

Overall, DRB1*11 alleles appeared in significantly higher proportions in anti-CNTN1+ patients than in normal population (53.33 vs 28.8%; OD = 3.3, CI = 1.14 to 9.56); even though the DRB1*11:04 is more frequently expressed in the general population. DRB1*11 alleles were predicted to present the same peptides and can be considered functionally homologous.

There were no statistically significant differences between the HLA II alleles in anti-CNTN1+ patients and seronegative CIDP patients.

**Conclusion:** HLA-DRB11 alleles are associated with CNTN1-antibodies in CIDP patients.

**References:** None.
Keywords: Human Genetics

Grant Support: None.
CMTR Oral Poster Session: iPSC's
Sunday, June 23, 2019 - 15:45 - 16:00
Modeling Axonal Degeneration in CMT2E using Human Motor Neurons

Mario Saporta, Renata Maciel, Renata Correa, Juliana Taniguchi, Igor Araujo
University of Miami Miller School of Medicine, Miami, FL, USA

Mutations in neurofilament light chain (NEFL) cause autosomal dominant, axonal Charcot-Marie-Tooth disease (CMT2E). Despite advances in understanding its pathophysiology, there is no disease-modifying therapy for CMT2E. This is partially due to lack of translational models suitable for drug discovery. Our previous work using iPSC-derived motor neurospheres have identified neurofilament deposits in the axons of motor neurons from three N98S CMT2E patients, similar to what has been shown in nefl$^{N98S}$ knock in mice. We have also identified two kinase inhibitors that promoted 50% reduction in the number and area of NEFL deposits. The aim of this study was to determine whether evidence of axonal degeneration could be found in N98S CMT2E motor neurons in order to further validate this platform as a reliable disease model and to identify potential in vitro biomarkers of axonal degeneration for use in drug discovery.

Morphological analysis of axons and measurement of neurofilament light chain protein (NEFL) in the culture supernatant of control and N98S CMT2E motor neuron cultures were performed at baseline conditions and after ascending doses of a known axonotoxic compound (Vincristine) in doses ranging from 1 to 10 nM. Despite normal axonal morphology at baseline, N98S CMT2E demonstrated increased levels of NEFL in culture supernatant when compared to controls. Furthermore, N98S CMT2E motor neurons were more susceptible to vincristine-induced axonal degeneration, as demonstrated by both increased supernatant NEFL levels as well as increased axonal beading and breakdown when compared to controls. Taken together, these findings demonstrate that iPSC-derived N98S CMT2E motor neurons are more susceptible to axonal degeneration both at baseline conditions and in response to exposure to vincristine, suggesting that this platform can reliably model CMT-associated axonal degeneration in vitro and could be used to identify modulators of axonal degeneration with therapeutic potential for CMT2E and other axonopathies.

References: None.

Keywords: CMTR, Axonal Biology, Human Genetics, Pre-clinical Studies

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Modelling dHMNX and CMTX6 using patient derived iPSC motor neurons.

Gonzalo Perez Siles¹, Anthony Cutrupi², Jakob Kuriakose³, Rebecca Screnci³, Melina Ellis⁴, Garth Nicholson⁴, Marina Kennerson¹

¹Northcott Neuroscience Laboratory (ANZAC Research Institute), Sydney Medical School (University of Sydney), Sydney, Australia. ²Northcott Neuroscience Laboratory (ANZAC Research Institute), Sydney Medical School (University of Sydney), Sydney, Australia. ³School of Life Sciences (University of Technology Sydney), Sydney, Australia. ⁴Northcott Neuroscience Laboratory (ANZAC Research Institute), Sydney, Australia

INTRODUCTION  Mutations in the copper (Cu) transporter ATP7A and in the pyruvate dehydrogenase kinase 3 (PDK3) genes cause X-linked hereditary distal motor neuropathy (dHMNX) and X-linked Charcot-Marie-Tooth type 6 neuropathy (CMTX6), respectively. Our investigations using dHMNX and CMTX6 patient fibroblasts have shed light on the pathomechanisms underlying these diseases:

- Fibroblasts harbouring the p.T994I ATP7A mutation show defective retrograde trafficking of mutant ATP7A leading to intracellular Cu dysregulation which has been reproduced in embryonic fibroblasts of a conditional knock in Atp7a mouse model for dHMNX. - CMTX6 patient fibroblasts with the p.R158H mutation show hyperactivity of PDK3 and hyperphosphorylation of the E1 subunit of the pyruvate dehydrogenase complex, a critical regulator of the energy producing Krebs cycle, leading to mitochondrial abnormalities, lactate acidosis and reduced ATP production.

METHODS  To investigate how defective ATP7A trafficking and PDK3 kinase hyperactivity leads to axonal degeneration we have established two lines of induced pluripotent stem cells by re-programming fibroblasts from a dHMNX patient with the ATP7A p.T994I mutation (iPSC_dHMNX) and a CMTX6 patient harbouring the PDK3 p.R158H substitution (iPSC_CMTX6). RESULTS  Our data demonstrates the iPSC_dHMNX and the iPSC_CMTX6 lines retain pathogenic molecular phenotypes found in the dHMNX and CMTX6 patient fibroblasts, respectively, iPSC_dHMNX cells show altered ATP7A intracellular distribution. iPSC_CMTX6 cells maintain the E1-hyperphosphorylation signature and treating the patient cells with the PDK inhibitor dichloroacetate reduces the levels of phosphorylation, suggesting PDK3 is an ideal pharmacological target for the development of treatment therapies. We have successfully differentiated spinal cord motor neurons from the iPSC_dHMNX and the iPSC_CMTX6 lines and shown the patient derived motor neurons (MN_dHMNX and MN_CMTX6) display disease specific pathological features.

CONCLUSIONS  Patient MN_dHMNX and MN_CMTX6 motor neurons are an ideal neuronal system to model axonal degeneration in dHMNX, CMTX6 and other neurodegenerative diseases in which Cu dysregulation and mitochondrial abnormalities occur.

REFERENCES:


KEYWORDS: Axonal Biology, Human Genetics, CMTR, Metabolic
Grant Support: None.
Human iPSC-derived motor neuron model of CMT2A from MFN2 mutations

Robert Baloh, Yueqin Zhou, Shaughn Bell, Michael Guerrero
Cedars-Sinai Medical Center, Los Angeles, CA, USA

Mutations in MFN2 are the most commonly identified genetic cause of Charcot-Marie-Tooth disease type 2A. While long sensory and motor peripheral nerves are the most susceptible structures to MFN2 mutations, a variety of additional phenotypes have been reported including optic atrophy, spastic paraparesis, developmental delay, myopathy, and lipodystrophy. MFN2 is an outer mitochondrial membrane protein that regulates a variety of functions including mitochondrial fusion, transport, ER interactions, and mitophagy. However, the mechanism by which the primarily dominantly inherited point mutations in MFN2 promote mitochondrial and axonal injury remains unknown. We generated induced pluripotent stem cells from two patients with CMT2A (T105M, H361Y). Additionally, we used CRISPR/Cas9 combined with single stranded oligonucleotide donors to generate isogenic control lines using homologous recombination for the T105M and H361Y lines. Subsequently we differentiated CMT2A patient iPSCs into motor neurons using established protocols, fluorescently labeled mitochondria, and used live cell imaging to examine mitochondrial dynamics in axons. Mitochondrial size was found to be smaller in iPSC-MNs from CMT2A patients compared to normal and isogenic controls. Mitochondrial also spent a greater percentage of time paused, and displayed less anterograde movement in CMT2A iPSC-MNs compared to normal or isogenic controls. Ongoing work focuses on examining diverse MFN2 functions in these models, and whether MFN1 augmentation can mitigate phenotypes observed.

References: None.

Keywords: Axonal Biology

Grant Support: None.
Sensory Neuron-derived IGF-1 Augments Neurite Outgrowth And This Autocrine/paracrine Pathway Is Suppressed in Diabetes
Reza Aghanoori, Mohamad-Reza Aghanoori, Darrell Smith, Prasoon Agarwal, Vernon Dolinsky, Vinith Yathindranath, Donald Miller, Paul Fernyhough

1University of Manitoba, Winnipeg, Canada, 2St. Boniface Hospital Albrechtsen Research Centre, Winnipeg, Canada

The level of insulin-like growth factor 1 (IGF-1) in serum of diabetic patients and animal models with type 1 and 2 diabetes declines significantly as the disease progresses. Recently, IGF-1 has been used for treatment of neurodegenerative disorders including Alzheimer’s disease and amyotrophic lateral sclerosis. We hypothesized that impaired autocrine/paracrine IGF-1 in dorsal root ganglia (DRG) was a contributing factor to progressive neurodegeneration and impaired nerve regeneration in diabetic sensory neuropathy. DRG neuron cultures and tissues from age-matched control or streptozotocin (STZ)-induced type 1 diabetic rats were used for in vitro and in vivo studies. Despite no difference in IGF-1 receptor level, IGF-1 protein and mRNA levels in liver and DRG tissues were significantly (P<0.05) lower in type 1 diabetic rats vs age-matched control rats. DRG neurons derived from control rats secreted a higher amount of IGF-1 into the culture media compared to cultures from diabetic rats (P<0.05). IGF-1 mRNA was expressed in neurons of the DRG and brain rather than in glial cells or sciatic nerve tissue as determined by RNA-FISH and Northern blot analysis. The hyperglycemic state suppressed IGF-1 mRNA expression in DRG neurons after 2 days which was relieved by treatment with (10nM) IGF-1 or an aldose reductase inhibitor, Sorbinil (blocks polyol pathway activity under high [glucose]). Bioinformatic screening and chromatin immunoprecipitation assay revealed NFAT1 and CEBP-β functional binding sites on the IGF-1 gene promoter in rat DRG neurons. In growth factor-free media, either IGF-1 neutralizing antibody or two IGF-1-targeting encapsulated siRNAs (in cationic nanoparticles) downregulated IGF-1 receptor and Akt S473 phosphorylation, and lowered background neurite outgrowth in cultured DRG neurons. In conclusion, downregulation of endogenous IGF-1 in DRG neurons in diabetes may contribute to pathogenesis of progressive distal dying-back neurodegeneration and up-regulation of neuronal IGF-1 at the mRNA level may be a promising target for therapy.

References: None.

Keywords: Axonal Biology, Axonal Regeneration, Other
Grant Support:

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Deletion of SARM1 has a Protective Effect for High-fat Diet-induced Peripheral Neuropathy and Glucose Intolerance
Ahmet Hoke, Junsoon Kim, Aysel Fisgin  
Johns Hopkins University, Baltimore, MD, USA

Introduction: Recently, SARM1 has been highlighted as a critical factor causing axon degeneration both in acute neuronal injury and subacute/chronic conditions such as chemotherapy-induced peripheral neuropathy. Here, we evaluated the effect of SARM1 deletion for the development of distal neuropathy and glucose intolerance after the high-fat diet (HFD).

Methods: SARM1 knockout (KO) mice and wild-type (WT) littermates were fed with normal diet or HFD for 12 weeks. Analysis of thermal test, sensory nerve conduction study (NCS) and intra-epidermal nerve fiber density (IEND) at the hind paws were carried out after diet for the assessment of distal neuropathy. We also measured fasting blood glucose level every week and performed glucose tolerance test, insulin tolerance test, insulin secretion test and innervation of pancreas at the end-point for evaluation of glucose/insulin homeostasis.

Results: WT mice with HFD developed thermal hypoalgesia and significant loss of IEND compared to WT with the normal diet. However, in SARM1 KO mice with HFD, the development of thermal hypoalgesia and the loss of IEND were prevented. There were no significant differences for sensory NCS between groups. Although both SARM1 KO mice and WT littermates showed increased fasting blood glucose level when fed HFD, SARM1 KO mice had significantly lower fasting blood glucose level than WT at the end of the study. Moreover, SARM1 KO mice fed HFD showed less insulin resistance compared to WT on the insulin secretion test and had preserved innervation of the pancreatic islet cells.

Conclusions: This study reveals SARM1 as a potent regulator of distal axonal degeneration and dysfunctional glucose/insulin metabolism induced by HFD. The further research uncovering precise molecular mechanisms will be needed.

References: None.

Keywords: Pre-clinical Studies, Axonal Biology

Grant Support:
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Saturated and Monounsaturated Fatty Acids Differentially Regulate Nerve Function in Murine Models of Obesity

Amy Rumora, Giovanni LoGrasso, John Hayes, Faye Mendelson, Maegan Tabbey, Julia Haidar, Stephen Lentz, Eva Feldman

University of Michigan, Ann Arbor, MI, USA

Neuropathy is the most prevalent complication of type 2 diabetes (T2D) and prediabetes. The progression of neuropathy in prediabetic and T2D patients correlates with dyslipidemia characterized by elevated levels of circulating saturated fatty acids (SFAs). Recent studies indicate that dietary replacement of SFAs with monounsaturated fatty acids (MUFAs) improves the metabolic health of prediabetic and T2D patients; however, the differential effect of dietary SFAs and MUFAs on neuropathy is unknown. This study examined the impact of SFAs and MUFAs on nerve function.

Three groups of mice were fed diets with varying fatty acid composition from 6 to 24 weeks including a standard diet (SD), a SFA-rich high fat diet (HFD), and a SFA-rich HFD until 16 weeks followed by a MUFA-rich HFD (HFD-MUFA) until 24 weeks. At 24 weeks, both HFD and HFD-MUFA groups exhibited impaired glucose tolerance, increased body weight, and higher body fat mass compared to the SD group. Despite equivalent metabolic dysfunction in HFD and HFD-MUFA groups, the HFD-MUFA mice exhibited a complete restoration in sural and sciatic nerve conduction velocity. In parallel, intraepidermal nerve fiber density was significantly increased in HFD-MUFA mice compared to HFD mice.

To identify molecular changes underlying the restoration of sensory function in HFD-MUFA mice, we next evaluated the effect of SFA palmitate and MUFA olate on mitochondrial dynamics in cultured dorsal root ganglion (DRG) sensory neurons. Diabetic concentrations of palmitate impaired mitochondrial transport and function in DRG axons. Supplementation of palmitate treatments with olate prevented the impairment of axonal mitochondrial transport and restored mitochondrial membrane potential and ATP production in DRG neurons.

Together, these results support the contention that the development of neuropathy in prediabetes is related to mitochondrial dysfunction induced by SFAs, and that MUFAs reverse the progression of neuropathy by protecting mitochondrial function and dynamics in DRG neurons.

References: None.

Keywords: Metabolic, Diabetes, Axonal Biology

Grant Support: This study was supported by U.S. National Institutes of Health (NIH) National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Grants R24 DK082841 and R01 DK107956 (to E.L.F.) and F32 1F32DK112642 and T32 1T32DK101357 (to A.E.R.); the NIDDK DiaComp Award DK076169 (to E.L.F.); Novo Nordisk Foundation Grant NNF14OC0011633 (to E.L.F.); the Milstein, Nathan and Rose Research Fund; the American Diabetes Association, the Program for Neurology Research and Discovery; and the A. Alfred Taubman Medical Research Institute. Confocal microscopy and image analysis were completed at the Michigan Diabetes Research Center’s Microscopy and Image Analysis Core, supported by NIH NIDDK Grant P60DK020572.
Distal Symmetric Polyneuropathy is Likely the First Neurologic Complication of Obesity

Ericka Chant, Evan Reynolds, Emily Villegas-Umana, Kristen Votruba, Bruno Giordani, Thomas Gardner, Mousumi Banerjee, Eva Feldman, Brian Callaghan
University of Michigan, Ann Arbor, MI, USA

Previous studies suggest that the metabolic syndrome (MetS) is associated with distal symmetrical polyneuropathy (DSP), and that diabetes and obesity are the main metabolic drivers. The aim of this study is to investigate the association of MetS components with retinal and cognitive function in a bariatric surgery cohort prior to surgery.

Patients were recruited from the Bariatric Surgery Clinic at the University of Michigan and lean controls from a research website (no MetS components based on NCEP/ATPIII definition). Participants underwent extensive metabolic phenotyping including a glucose tolerance test and fasting lipid profile. DSP was defined using the Toronto consensus definition of probable clinical neuropathy. Retinal function was measured with frequency doubling technology perimetry (mean deviation), and cognitive function with the NIH Toolbox (composite score).

Multivariable linear regression models were used to evaluate the association between MetS components and retinal/cognitive function. We recruited 138 bariatric surgery participants and 46 lean controls. The DSP prevalence was 2.2% in lean controls, 12.1% in normoglycemic, 7.1% in pre-diabetic, and 40.8% in diabetic bariatric participants (p<0.01 for trend). Retinal function was -0.4 (2.8), -0.4 (2.7), -2.1 (4.02), and -1.4 (4.4) (p=0.04 for trend), and cognitive function was 116.9 (13.7), 105.0 (17.4), 105.1 (17.8), 101.6 (18.7) (p<0.01 for trend) for these same groups. Pre-diabetes (-1.8, 95%CI -3.6, 0.0) was the only MetS component associated with retinal function. Systolic blood pressure (2.2, 95%CI 0.1, 4.3) and waist circumference (-1.4, 95%CI -2.3, -0.5) were associated with cognitive function. Obesity alone may be sufficient to cause DSP and cognitive decline. Similar to previous data for DSP, pre-diabetes and obesity are associated with retinal and cognitive function respectively. Interestingly, while clinical DSP is common in this population, clinical retinopathy and dementia are not, indicating that DSP may be the first metabolic complication in the morbidly obese.

References: None.

Keywords: Metabolic, Diabetes

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Evidence of Altered Peripheral Nerve Function in a Rodent Model of Pre-diabetes

Md Jakir Hossain, Brandon Wild, Michael Kendig, Margaret Morris, Ria Arnold
School of Medical Sciences, UNSW Sydney, Sydney, Australia

Objective: Peripheral neuropathy (PN) is one of the major microvascular complications of diabetes, affecting >50% of diabetic patients. While the pathophysiology remains unclear, recent evidence has linked obesity and pre-diabetes to PN risk prior to development of overt hyperglycemia. We utilised a translationally relevant rodent model of obesity to examine early changes in peripheral nerve electrophysiology.

Methods: Thirty adult male Sprague-Dawley rats were randomised to control (n=15) or cafeteria style (n=15) diet (pre-diabetes). After 12 weeks of diet, nerve conduction studies were undertaken in caudal (sensory) and tibial (motor) nerves. Nerve excitability, an indirect measure of ion channel function and membrane potential, was undertaken in the tibial nerve. Body composition, fasting blood glucose (FBG), triglycerides and HDL cholesterol were measured 1 week later prior to intra-peritoneal glucose tolerance test (ip-GTT). Fasting plasma insulin, IL-1β and IL-6 were measured by ELISA at cull (15 weeks of diet).

Results: The pre-diabetes group had significantly higher body weight, FBG, fasting insulin and adiposity. Pre-diabetic rats also showed dyslipidemia (higher triglycerides and lower HDL cholesterol), impaired glucose tolerance, insulin resistance (HOMA-IR) and increased IL-1β (all p<0.05) although IL-6 remain unchanged. Standard nerve conduction parameters including amplitude and latency of motor or sensory nerves were not different between groups. However, nerve excitability measures demonstrated a significant difference in superexcitability (control: 2.18±0.77 [SEM], pre-diabetic: -0.54±0.64; p<0.05), which was significantly positively correlated with fasting HDL and body composition (rs=0.4; p<0.05). This suggests abnormal fast potassium conductances at the node of Ranvier. While sensory nerve amplitudes did not differ between groups, there was a significant negative correlation between FBG and sensory amplitudes (r=-0.48; p=0.008).

Conclusion: This dietary pre-diabetes model demonstrates dyslipidemia, metabolic impairment, elevated IL-1β and changes in peripheral nerve function, thus providing a platform to investigate pathophysiological mechanisms and relationships between metabolic parameters and peripheral nerve damage.

References: None

Keywords: Diabetes, Metabolic, Inflammatory, Pre-clinical Studies, Axonal Biology

Grant Support:

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Distribution of obesity is a key differentiator of neuropathy status
Brian Callaghan, Evan Reynolds, Mousumi Banerjee, Ericka Chant, Emily Villegas-Umana, Eva Feldman

University of Michigan, Ann Arbor, MI, USA

While much is known about diabetes and neuropathy, much less is known about the relationship between obesity and neuropathy. Therefore, we aimed to determine the prevalence of neuropathy stratified by glycemic status to evaluate whether obesity alone is a potential cause of neuropathy. We also aimed to determine the association between the distribution of obesity, using extensive anthropometric measurements, and neuropathy. We performed a cross sectional, observational study in patients attending a bariatric surgery clinic prior to intervention. We also recruited lean controls from a research website. Neuropathy was defined by the Toronto consensus definition of probable neuropathy. Diabetes and pre-diabetes were defined according to the Expert Committee on the diagnosis and classification of diabetes mellitus, and the metabolic syndrome by NCEP/ATPIII criteria. We compared nine anthropometric measurements between obese participants with and without neuropathy. We used multivariable logistic regression to explore associations between these measures, and other metabolic risk factors, and neuropathy. We recruited 138 obese individuals and 46 lean controls. The mean age (SD) was 45.1 (11.3) in the obese population (76% female) and 43.8 (12.1) in the lean controls (82% female). The prevalence of neuropathy was 2.2% in lean controls, 12.1% in obese participants with normoglycemia, 7.1% in pre-diabetes, and 40.8% in diabetes (p=<0.01). Waist circumference was the only anthropometric measure that was larger in those with neuropathy (139.3 cm vs. 129.1 cm, p=0.01). Hip-thigh (71.1 cm vs. 76.6 cm, p<0.01) and mid-thigh (62.2 cm vs. 66.3 cm, p=0.03) circumferences were smaller in those with neuropathy. Waist circumference (OR=1.07, 95%CI 1.02-1.12), systolic blood pressure (OR=2.89, 95%CI 1.49-5.61), and triglycerides (OR=1.31, 95%CI 1.00-1.70) were significantly associated with neuropathy. Normoglycemic obese patients have a high prevalence of neuropathy indicating that obesity alone may be sufficient to cause neuropathy. Central obesity, but not general obesity, is significantly associated with neuropathy.

References: None.

Keywords: Metabolic, Diabetes

Grant Support: The project described was supported by Grant Number P30DK020572 (MDRC) from the National Institute of Diabetes and Digestive and Kidney Diseases.
Sunday, June 23, 2019 - 17:30 - 17:35
Plasma Deoxydihydroceramides are Elevated in People with Diabetic Neuropathy and Correlate with Neuropathy Severity

Vera Fridman¹, Simona Zarini², Stefan Sillau¹, Bryan Bergman², Eva Feldman³, Brian Callaghan³, Jane Reusch²

¹University of Colorado Anschutz Medical Campus Department of Neurology, Aurora, CO, USA, ²University of Colorado Anschutz Medical Campus Division of Endocrinology, Metabolism, and Diabetes, Aurora, CO, USA, ³University of Michigan Department of Neurology, Ann Arbor, MI, USA

Diabetic neuropathy (DN) is a debilitating condition that affects up to 50% of people with diabetes. Altered sphingolipid metabolism in diabetes may lead to an accumulation of atypical, neurotoxic deoxysphingolipids (dSLs) associated with neuropathy. dSLs can arise from reduced availability of the amino acid L-serine and/or the presence of excessive L-alanine. Studies have not investigated which dSL molecules are relevant to DN. We hypothesized that dSLs are elevated with DN. We examined dSL species using LC/MS/MS in plasma samples from a university based weight management program from age and HbA1C matched subjects with obesity (0, n=19), obesity with Type 2 Diabetes (T2D) (OD, n=18), obesity with T2D and DN (ODN, n=19), and lean controls (LC n=19). Sample means for the majority of deoxydihydroceramides (1-deoxyDHCer) for the four groups followed the same order from lowest to highest: LC, O, OD, ODN. Adjusting for pair-wise comparisons, mean levels of most 1-deoxyDHCer species were higher in the ODN as compared to the LC groups (0.527 vs. 0.226 pmol/100 µl for C16 1-deoxyDHCer, p=0.0021; 1.129 vs. 0.385 pmol/100 µl for C18 1-deoxyDHCer, p=0.0002; 1.136 vs. 0.492 pmol/100 µl for C20 1-deoxyDHCer, p<0.0001; 2.461 vs 1.585 for C22 1-deoxyDHCer, p=0.0496; 0.181 vs. 0.932 pmol/100 µl for C24:1 1-deoxyDHCer, p=0.0073). Mean levels of C20 1-deoxyDHCer were higher in the ODN group than the O group (1.136 vs. 0.733 pmol/100 µl, p=0.0350). No significant difference was observed between OD and ODN groups. Further analysis demonstrated that L-alanine was higher and L-serine lower in ODN versus LC (326.2 vs. 248.0, p=0.0086 and 70.2 vs. 89.8, p=0.0110), consistent with a causal contribution to the observed dSL profiles. 1-deoxyDHCer correlated inversely with nerve fiber density across all groups. These novel findings indicate that 1-deoxyDHCer are elevated in individuals T2D and DN and may be important biomarkers and/or mediators of DN.

References: None.

Keywords: Diabetes, Metabolic

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Early Parallel Progression Of Peripheral And Cardiac Autonomic Nerve Dysfunction In Recent-Onset Type 1 Diabetes

Gidon Bönhof1, Alexander Strom2, Karsten Müßig3, Oana-Patricia Zaharia2, Julia Szendroedi3, Michael Roden3, Dan Ziegler3

1German Diabetes Center, Division of Endocrinology and Diabetology at HHU, Düsseldorf, Germany, 2German Diabetes Center, German Center for Diabetes Research (DZD), Düsseldorf, Germany, 3German Diabetes Center, German Center for Diabetes Research (DZD), Division of Endocrinology and Diabetology at HHU, Düsseldorf, Germany

Purpose: We previously demonstrated an early parallel involvement of small and large fibers in recent-onset type 2 diabetes. Here we hypothesized that this pattern may also be pertinent to type 1 diabetes (T1D).

Methods: Motor and sensory nerve conduction velocity (MNCV, SNCV), vibration perception thresholds (VPT), thermal detection thresholds (TDT), intraepidermal nerve fiber density (IENFD), and heart rate variability (HRV) were assessed in participants with T1D from the German Diabetes Study (GDS) at baseline (diabetes duration £1 year) and in glucose-tolerant controls: CON/T1D-B: n=96/360; age [median (1st; 3rd quartile)]: 34.5 (26.0; 46.8)/34.6 (26.5; 45.3) years; male: 72/58%; BMI: 25.0 (22.9; 28.3)/24.0 (22.0 (22.0; 27.0) kg/m²; diabetes duration: -/173 (114; 173) days; HbA1c: 5.1 (5.0; 5.3)/6.4 (5.9; 7.2)%; M-value (hyperinsulinemic-euglycemic clamp): 11.6 (9.1; 13.6)/8.2 (6.5; 10.4) mg*kg⁻¹*min⁻¹.

Results: T1D-B showed lower peroneal MNCV ([mean±SEM]: 45.8±0.2 vs 47.0±0.3 m/s), median MNCV (55.1±0.2 vs 56.0±0.7 m/s), and IENFD (10.0±0.5 vs 11.2±0.5 fibers/mm) than CON (P<0.05). In T1D, a deterioration from baseline to 5 years (n=151) was noted for ulnar and median MNCVs and SNCVs (e.g. ulnar MNCV: 57.4±0.4 vs 56.3±0.3 m/s), malleolar VPT (0.76±0.07 vs 1.12±0.12 µm), and HRV indices (e.g. standard deviation of normal RR intervals (SDNN): 69.3±2.4 vs 62.0±2.1 ms; root mean square of successive differences (RMSSD): 42.8±2.3 vs 34.7±2.0 ms) (all P<0.05). Peroneal MNCV, sural SNCV, and TDT remained unchanged. The decline in MNCV was associated with an increase in HbA1c (e.g. median nerve: β=-0.316, P=0.004) and the deterioration in HRV with decreasing M-value (e.g. SDNN: β=0.246, P=0.041).

Conclusions: Within the first 5-6 years of type 1 diabetes despite good glycemic control, the deterioration in median and ulnar MNCV was related to worsening HbA1c levels, while cardiac autonomic dysfunction progressed in relation to increasing insulin resistance.

References:None.

Keywords:Small Fibers

Grant Support: This work was supported by the Ministry of Culture and Science of the State of North Rhine-Westphalia and the German Federal Ministry of Health. This study was supported in part by a grant from the German Federal Ministry of Education and Research to the German Center for Diabetes Research (DZD).
Diabetic Neuropathy (DN) is a common complication of diabetes. The underlying pathophysiological mechanisms of DN are not clear. However, reactive oxygen species (ROS) appear to play a key role in the cellular and molecular injury observed in DN. NADPH oxidase (NOX) enzymes generate ROS and of the 5 isoforms of NOX (1-5), NOX5 is present only in man. The aim of this study was to investigate a role for NOX5 in DN in cutaneous nerve fibers and sural nerve biopsies of subjects with DN.

Cellular localization of NOX5, myelin basic protein (MBP) and protein gene product (PGP) 9.5 were determined in cutaneous nerve fibers of non-diabetic controls and subjects with DN. NOX5 methylation status, gene expression and protein levels were assessed in subjects with DN that were divided into two groups based on changes in sural nerve myelinated fiber density: regenerators (showing significant nerve regeneration) and degenerators (showing significant nerve degeneration).

Our preliminary findings show that NOX5 is present in diabetic myelinated cutaneous nerve fibers, but absent in control fibers. Genome-wide DNA methylation analysis revealed that the NOX5 promoter, enriched with CpG sites, is hypomethylated in sural nerve biopsies of the degenerator cohort compared to the regenerator. Focused qPCR array revealed alteration of gene profiles in the oxidative and antioxidative pathways of the degenerator sural nerves compared to regenerators. In particular, NOX5 was increased at both the mRNA and protein levels in the degenerator cohort. The increase in NOX5 protein expression in degenerator sural nerves was accompanied with a decrease in MBP levels relative to the regenerator group.

Overall, our results point to a potential epigenetic and mechanistic role for NOX5 in DN, although further mechanistic studies are needed to provide more insight into the contribution of NOX5 to DN pathogenesis.

References: None.

Keywords: Diabetes

Grant Support: None.
Mutation Burden and Oligogenic Inheritance in a large Inherited Axonopathy Cohort

Stephan Zuchner\textsuperscript{1}, Dana Bis-Brewer\textsuperscript{1}, Feifei Tao\textsuperscript{2}, Ziv Gan-Or\textsuperscript{3}, Lisa Abreu\textsuperscript{2}, Patrick Sleiman\textsuperscript{4}, Hakonarson\textsuperscript{4}, Guy Rouleau\textsuperscript{3}

\textsuperscript{1}University of Miami Miller School of Medicine, Miami, FL, USA, \textsuperscript{2}University of Miami Miller School of Medicine, Miami, USA, \textsuperscript{3}McGill University, Montreal, Canada, \textsuperscript{4}Children's Hospital of Philadelphia, Philadelphia, USA.

Inherited axonopathies include the clinically distinct phenotypes, Charcot-Marie-Tooth (CMT) and Hereditary Spastic Paraplegia (HSP), which both cause slowly-progressing, length-dependent axonal degeneration. Both phenotypes are genetically and phenotypically diverse with close to 100 Mendelian genes involved for each thus far. Whole-exome sequencing of axonopathy patients may identify more than one rare variant within known disease genes. Occurrence of additional rare variation, also referred to as a ‘mutation burden’, has been reported in two independent CMT cohorts (n \leq 40) supported by functional zebrafish assays. The data indicate that mutation burden may influence clinical heterogeneity and severity of disease. We sought to replicate a mutation burden across inherited axonopathies in a WES cohort 10-fold larger than the original observations (CMT cases = 357, HSP cases = 515, controls = 931). We tested the mutation burden in cases compared to controls for both non-synonymous and loss-of-function variants at ExAC MAF \leq 0.1% and 1%. For each tested variant set, cases harbored a higher average number of qualifying variants (Mann-Whitney, p-value \leq 0.05). The significance of this difference was further evaluated by permuting case/control status over 10,000 iterations (p-value \leq 0.05). Next, we evaluated the possibility of di- and oligogenic inheritance within each cohort. Cases carrying a qualifying variant in \geq 2 genes were classified as di/oligogenic and in \geq 3 genes as oligogenic. We observed a difference in the proportion of cases and controls carrying variants for both di/oligogenic and oligogenic inheritance for non-synonymous variation (Chi-squared, p-value \leq 0.05). Neither HSP nor CMT showed evidence of oligogenic inheritance for loss-of-function variation; however, HSP cases were enriched for digenic inheritance (Chi-squared, p-value \leq 0.05). In this study, we provide further evidence of a mutation burden in CMT cases, demonstrate a mutation burden in HSP cases, and explore potential oligogenic inheritance patterns in a large cohort.

References: None.

Keywords: CMTR, Human Genetics

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Sunday, June 23, 2019 - 18:05 - 18:10
Mutations in Cell Adhesion Molecules Belonging to the CADM Family Cause Charcot-Marie-Tooth Disease
Adriana Rebelo¹, Andrea Cortese², Lisa Abreu³, Steve Courel³, Elior Peles⁴, Chelsea Bacon⁵, Shawna Feely⁶, Diane Castro⁶, Michael Shy⁵, Mary Reilly⁷, Stephan Zuchner³
¹Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL, USA, ²UCL Institute of Neurology Centre for Neuromuscular Diseases, UCL Institute of Neurology, London, United Kingdom of Great Britain and Northern Ireland, ³Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, USA, ⁴Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel, ⁵Department of Neurology, Carver College of Medicine, University of Iowa, Iowa, USA, ⁶Departments of Pediatrics, Neurology and Neurotherapeutics, University of Texas Southwestern Medical Center, Dallas, USA, ⁷MRC Centre for Neuromuscular Diseases, UCL Institute of Neurology, London, United Kingdom of Great Britain and Northern Ireland

CADM family of proteins consists of four neuronal specific adhesion molecules (CADM1, CADM2, CADM3 and CADM4) that mediate the contact interaction between axons and glia. In the peripheral nerve, axonal-Schwann cell interaction is essential for the structural organization of myelinated fibers and it is primarily mediated by the binding of CADM3, expressed in axons, to CADM4, expressed by myelinating Schwann cells. We have identified by whole exome sequencing three families with axonal Charcot-Marie-tooth disease (CMT2) sharing the same private variant in CADM3 and one autosomal dominant CMT2 family with a private variant in CADM4. Although all families have CMT2, CADM3 and CADM4 families developed distinct subclinical features. The CADM3 families share the same peculiar phenotype consisting of axonal motor neuropathy affecting mainly the upper limbs with pyramidal features. In contrast, the CADM4 family developed a more typical length dependent CMT. The variant identified in CADM3, Y138C, was found to be de novo in two families, while in the third family the variant shows dominant segregation. High resolution mass spectrometry analysis coupled with nanoflow UPLC detected disulfide bonds modifications in the mutant CADM3 potentially modifying the native protein conformation. In addition, we observed a significant increased protein retention of the mutant in the endoplasmic reticulum leading to activation of the unfolded protein response (UPR). Interestingly, ablation of CADM4, but not CADM3, in mouse results in myelination abnormalities and impaired motor function. We are currently studying a CADM3 knockin (KI) mouse carrying the same variant observed in our patients to better mimic the human phenotype and its pathological mechanism. Preliminary results indicate increased muscle weakness in the CADM3-KI mouse compared to the wild-type. Our findings indicate a novel molecular pathway involving axon-glial interaction abnormalities in patients with CMT.

References:None.

Keywords:CMTR, Axonal Biology, Human Genetics, Schwann Cell

Grant Support: None.
Charcot-Marie-Tooth diseases (CMT) are a heterogeneous group of hereditary genetic neuropathies. CMT1b is a rare form of CMT caused by mutations in the myelin protein zero (MPZ) gene. Phenotype is variable and heterogeneous as is the age of onset.

Our purpose is to characterize genotype–phenotype correlations and establish baseline clinical data for peripheral neuropathies caused by mutations in the MPZ gene in France. It is important to make clinical trials for patients with MPZ mutations a realistic possibility, in order to reduce misdiagnosis.

We present retrospective data to define the phenotypic spectrum and clinical baseline of patients with these mutations. A cohort of patients with MPZ gene mutations was identified in 11 French reference centers for neuromuscular diseases. Patient phenotypes were quantified by the Charcot–Marie–Tooth disease examination score (CMTES). Genetic testing was performed in all patients to document mutation in MPZ gene indicating diagnosis of CMT1B. There were 80 patients with 44 different MPZ mutations with a mean age of 56 years (range 20–86 years). Childhood onset represented 7%.

Twenty patients wore orthoses, twenty-six required walking assistance or support, and six required wheelchairs. There was hearing loss in seven patients, scoliosis in sixteen patients, optic atrophy in twelve patients and five patients presented with respiratory failure. Hip dysplasia was noted in one patient.

Preliminary data didn’t reveal any significant correlation between CMTES and age of onset, nor between CMTES and age.

These results demonstrate that MPZ mutations can be associated with heterogeneous phenotypes, which is consistent with previous studies. Adult forms appear to be moderately severe with an average onset age of 32 and an average CMTES of 9.

Data obtained from the French cohort is useful as a baseline for future clinical trials of patients with CMT1b.

References: Sanmaneechai et al, Genotype-phenotype characteristics and baseline natural history of heritable neuropathies caused by mutations in the MPZ gene, Brain, 2015

Keywords: CMTR, Human Genetics

Grant Support: None.
The Italian Registry for Charcot-Marie-Tooth disease

Davide Pareyson1, Daniela Calabrese1, Giuseppe Vita2, Anna Mazzeo2, GianMaria Fabrizi3, Angelo Schenone4, Tiziana Cavallaro3, Marina Grandis4, Stefano Previtali5, Isabella Allegri6, Luca Padua7, Costanza Pazzaglia8, Aldo Quattrone9, Isabella Moroni1, Stefano Tozza10, Fiore Manganelli10, Chiara Pisciotta1, Lucio Santoro10

1Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy, 2University of Messina, Messina, Italy, 3University of Verona, Verona, Italy, 4University of Genoa, Genoa, Italy, 5Ospedale San Raffaele, Vita Salute San Raffaele University, Department of Neurology and INSPE, Milan, Italy, 6UOC Neurologia Azienda Ospedaliera di Parma, Parma, Italy, 7IRCCS Fondazione Don Carlo Gnocchi, Catholic University of the Sacred Heart, Rome, Italy, 8Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy, 9Magna Graecia University, Catanzaro, Italy, 10Federico II University, Naples, Italy

The Italian Charcot-Marie-Tooth disease (CMT) Registry is fully operative at the https://www.registeronmd.it website. It is a dual registry where the patient registers herself/himself, chooses a reference centres among nine spread all over Italy, where the attending clinician, in an ad hoc visit, collects a minimal dataset of information and administers the CMT Examination/Neuropathy Score, CMTES/CMTNSv2, for adults, or the CMT Pediatric Scale, CMTPeds, for children; data are entered in the Registry and encrypted.

796 CMT patients have registered thus far and have chosen one of the 9 reference centers; information has been entered in the Registry for 649 of them (337 females; mean age 47.1, range 7-89). Diagnoses are the following: 407 CMT1, 95 CMT2, 21 CMT4, 8 dHMN, 32 HNPP, 1 HSAN, 85 still unclassified. Genetic diagnosis, achieved in 556 cases, show that the most frequently mutated genes are: PMP22 (295 CMT1A, 32 HNPP, 3 CMT1E), GJB1 (66 CMTX1, 30 females), MPZ (33 CMT1B, 32 CMT2I/J), GDAP1 (7 dominant and 8 recessive cases), MFN2 (17 CMT2A), NEFL (12 cases), and SH3TC2 (12 CMT4C).

Clinical score are the following: CMTES (n = 633) mean 8.4 +/- 5.2, range 0-27; CMTNS (n = 190) mean 12.5 +/- 6.5, range 0-31. Ninety-four subjects have at least one follow-up visit; 472 complain of gait difficulties, 294 use orthotics aids, 70 need support for walking (44 unilateral, 26 bilateral) or use a wheelchair (23); 123 patients have scoliosis (9 requiring surgery, 39 bracing), 11 hip dysplasia, 5 optic atrophy and 2 profound hearing loss.

Conclusions: analyses of data from the Italian CMT Registry are giving results which are important for: a) epidemiology of CMT across Italy, b) assessing disease burden to develop standards of care, c) recruiting patients in forthcoming clinical trials. The Registry will be linked to the CMT International Database.

References: None.

Keywords: CMTR

Grant Support:

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Genotype and phenotype in Thai children with Charcot-Marie-Tooth Disease.
Oranee Sanmaneechai¹, Theeraphong Pho-iam¹, Chanin Limwongse¹, Byung-Ok Choi², Ki Wha Chung³

¹Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand, ²Samsung Medical Center, Seoul, Korea (Republic of), ³Kongju National University, Gongju, Korea (Republic of)

Introduction
Charcot-Marie-Tooth disease (CMT) is a group of genetically heterogeneous, despite the clinical similarities. So far, more than 100 gene mutations have been reported to be associated with different types of CMT. However, with the limitation of next generation sequencing in our country, very few studies have been done for genetic identification in CMT patients. The aim of this study is to investigate the CMT genotype and phenotype in Thai children.

Methods
We prospectively evaluate children with the clinical and neurophysiological diagnosis of CMT from January 2017 to October 2018. Clinical presentation, CMTNS or CMTPeds and neurophysiologic studies were documented. DNA samples were sent to Samsung Medical Center and Kongju National University in South Korea for genetic identification with next generation sequencing (NGS) technique.

Results
A total of 25 patients, 19 have axonal CMT and 6 have demyelinating CMT. Mutation analysis by whole exome sequencing (WES) was performed in 25 cases from 24 families. The result of WES revealed genetically confirmed in 21 patients. Patients have mutation in MFN2 (5 patients), PMP22 (3 patients), NEFL (3 patients), GDAP (3 patients), SETX (2 patients), MPZ (1 patients), GJB1 (1 patients), IGHMBP2 (1 patients), EGR2 (1 patients, HK (1 patients). For the pathogenic or likely pathogenic mutations, the mutations were confirmed by Sanger’s sequencing method.

Conclusion
Whole exome sequencing give a higher yield to identify genetic mutation in CMT, especially in pediatric population with higher percentage of axonal subtype CMT and also for the resource-limited country. Future collaboration is crucial for genetic function testing. Further prognosis and genetic counseling can be done after genetic abnormalities had been identified.

References:
Keywords: CMTR

Grant Support: None
Implications of Disease Progression During Childhood and Adolescence on Walking Speed in Charcot-Marie-Tooth Disease

Sylvia Ounpuu¹, Gyula Acsadi¹, Kristan Pierz¹, Kelly Pogemiller², Tishya Wren³
¹Connecticut Children’s Medical Center, University of Connecticut School of Medicine, Farmington, CT, USA, ²Connecticut Children’s Medical Center, University of Hartford, Farmington, CT, USA, ³Children’s Hospital of Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA, USA

The purpose of this study was to examine differences in preferred walking speed as a function of development and CMT type in youth with CMT. The preferred walking speed of 22 youth with CMT1 (12.2 ± 3.1 years), 12 youth with CMT2 (9.8 ± 4.6 years), and 54 age matched typically developing (TD) peers (9.6 ± 3.4 years) was measured using gait analysis. Some patients were tested more than once resulting in 29 total CMT1 and 22 total CMT2 observations. Changes in walking speed with age were compared among groups using linear mixed effect models including a random intercept term to model the repeated measures for some participants. Walking speed increased with age in controls (2.2 cm/sec/year; 95% CI: 0.7 to 3.6; p=0.004), however, changed at a significantly lower rate and tended to decrease with age in CMT1 (-2.2 cm/sec/year; 95% CI: -4.9 to 0.4; p=0.097) and CMT2 (-2.4 cm/sec/year; 95% CI: -5.0 to 0.3; p=0.085). The differences in walking speed among groups were primarily due to stride length which increased with age in TD peers (4.4 cm per year; 95% CI: 3.5 to 5.4; p<0.001) but decreased with age in CMT1 (-2.1 cm/year; 95% CI: -3.8 to -0.3; p=0.02) and CMT2 (-1.8 cm/year; 95% CI: -3.4 to -0.1; p=0.38). Youth with CMT show a decline in walking speed with age compared to TD peers. This appears to be more severe and starts earlier for those with CMT2 vs. CMT1. The decline in walking speed resulted from reduced stride length, which is likely caused by reduced plantar flexor strength and increased ankle instability. Treatments that increase step length such as plantar flexor strengthening and bracing, which can also improve ankle stability in stance, are likely to improve walking speed and associated function such as keeping up with peers.

References: None.

Keywords: Other, Other, Other, Other, Other

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Monday, 24 June

Oral Abstract Presentations
Analysis of 193 whole genome sequencing data to understand neuropathic pain disorders.

Andreas Themistocleous¹, Karyn Megy², Rutendo Mapeta², Sri Deevi², Iulia Blesneac¹, Tom Vale¹, Natalie van Zuydam¹, David Bennett¹

¹University of Oxford, Oxford, United Kingdom of Great Britain and Northern Ireland, ²University of Cambridge, Cambridge, United Kingdom of Great Britain and Northern Ireland

Extreme pain phenotypes, such as erythromelalgia and insensitivity to pain, caused by rare high impact genetic variants, offer us insight into mechanisms that may apply to more common causes of neuropathic pain. The aim of our study was to identify singleton patients and families with extreme pain phenotypes to determine whether variants/mutations were present in genes known to cause neuropathic pain. We included participants with: congenital insensitivity to pain; painless sensory neuropathy; chronic pain caused by erythromelalgia, small fibre neuropathy, and sensory neuropathy. NeuPSIG grading criteria for neuropathic pain were used to stratify the cohort. A total of 219 participants were recruited from secondary care clinics across the UK. Neuropathic pain was classified as: not present (n=9, 4.1%), definite (n=125, 57.1%), probable (n=62, 28.3%), possible (n=12, 5.5%) or unlikely (n=1, 0.5%). Ten participants were unaffected family members. Whole genome sequencing data, acquired using next generation sequencing technology, were available for 193 participants. Previously characterised pathogenic variants in SCN9A, the gene encoding the sodium channel (Nav) 1.7, were identified in 11 participants. For example, the SCN9A pathogenic variant, c.2543T>C (p.Ile848Thr), was identified in a pair of sisters diagnosed with erythromelalgia. Novel uncharacterised variants, predicted through in silico analysis to be pathogenic and confirmed in multi-disciplinary team meetings, were identified in SCN9A, SCN10A, SCN11A and SPTLC1 genes. We have demonstrated that a meticulous phenotyping approach combined with next generation sequencing provides a powerful platform to explore pathophysiological mechanisms of chronic neuropathic pain.

References: None.

Keywords: Pain, Human Genetics, Small Fibers

Grant Support:

NIHR Bioresource

Wellcome Trust
Late-onset ataxia is a common reason for neurological consultation, but its cause often remains idiopathic. Cerebellar dysfunction, but also proprioceptive or vestibular impairment, can lead to ataxia. When in combination, this more severe type of ataxia is termed cerebellar ataxia, neuropathy, vestibular areflexia syndrome (CANVAS). Both sporadic and familial cases of CANVAS have been reported, suggesting the possibility of a recessive transmission of the disease. The aim of this study was to identify the genetic cause of CANVAS. We performed non-parametric linkage analysis and genome sequencing. We identified an intronic recessive pentanucleotide repeat expansion in RF gene as the cause of CANVAS and a common cause of late-onset sensory ataxia. The presence of the repeat expansion was confirmed by repeat-primed PCR, long-range PCR and southern blot. Functional studies were performed to assess the effect of the repeat expansion on the expression of the repeat-hosting gene. The recessive repeat expansion, ranging in patients from 400 to several thousand repeats, showed full segregation in 23 cases from 11 families. Additionally, 33 (22%) out of 150 sporadic cases with late-onset ataxia from a single-centre carried the recessive repeat expansion. The percentage raised to 62% in patients with sensory neuropathy and cerebellar involvement and 92% in full-blown CANVAS disease. Screening of additional cases with late-onset ataxia or CANVAS from Australia, New Zealand and France identified another 18 positive cases from seven unrelated families. Notably, the pentanucleotide repeat expansion does not affect expression of the repeat-hosting gene at mRNA and protein levels in patient fibroblasts. These data, together with the observation of an allelic carrier frequency of the expanded repeat of 0.7% in the European population, suggests that this biallelic pentanucleotide repeat expansion represents a frequent cause of late-onset ataxia and identifies an unconventional disease-causing mechanism in this late-onset recessive disorder.

References:None.
**Keywords:** CMTR, Human Genetics

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Whole Genome Sequencing in CMT cases from the 100,000 Genome Project

Menelaos Pipis, James Polke, Mariola Skorupinska, Jana Vandrovcova, Carolynne Doherty, Matilde Laura, Henry Houlden, Alexander Rossor, Mary Reilly

1MRC Centre for Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, United Kingdom of Great Britain and Northern Ireland, 2Neurogenetics Unit, UCL Queen Square Institute of Neurology, London, United Kingdom of Great Britain and Northern Ireland, 3MRC Centre for Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, United Kingdom of Great Britain and Northern Ireland, 4MRC Centre for Neuromuscular Diseases, Neurogenetics Unit, UCL Queen Square Institute of Neurology, London, United Kingdom of Great Britain and Northern Ireland

Whole genome sequencing (WGS) as a single molecular genetic test is very appealing with the ability to simultaneously sequence both nuclear and mitochondrial genomes. It can reliably detect splice-site and non-coding single nucleotide variants (SNV) as well as large balanced and unbalanced structural variants. As part of the 100,000 Genome Project which was launched in 2012, we have recruited 290 pedigrees for WGS, who had genetically undiagnosed CMT despite gene panel and other molecular genetic testing.

We present the phenotypic and initial WGS analysis of our first 40 CMT cases, with a provisional genetic diagnosis achieved in 10 pedigrees (25%) to date. Analysis of SNV and small insertions/deletions (indels) in all genes known to cause monogenic Mendelian disease (mini-exome) has been carried out and we identified variants in MORC2, HINT1, MT-ATP6, IGHMBP2, POLG, VRK1, MME and WARS. The avalanche of sequence data that accompanies WGS necessitates effective filtering of variants and this can be aided by setting a maximum credible population allele frequency for pathogenic variants in dominant and recessive CMT genes. As illustrated by some of our cases, the inclusion of trio-based WGS studies (affected proband and unaffected parents) allows the identification of de novo variants and the determination of variant phase in cases of compound heterozygosity. Furthermore, in more complex pedigrees, the recruitment of multiple affected and unaffected individuals from the same pedigree allows linkage through genome-wide genotyping of a large number of single nucleotide polymorphisms.

Incorporating WGS as a first-line test in CMT clinical practice will be normal practice in the future. Our caseload illustrates the multiple advantages of this approach as current gene panel-based and other molecular genetic testing does not achieve a diagnosis in 40% of our CMT cases.

References: None.

Keywords: CMTR, Human Genetics

Grant Support: This research was made possible through access to the data and findings generated by the 100,000 Genomes Project.
Monday, June 24, 2019 - 09:45 - 10:00
Depleted Systemic Markers of Neuroinflammation And Growth Factors In Type 2 Diabetes Patients With Polyneuropathy
Gidon Bönhof1, Alexander Strom2, Julia Kannenberg2, Margit Heier3, Wolfgang Rathmann2, Annette Peters4, Christa Meisinger5, Michael Roden6, Barbara Thorand4, Christian Herder6, Dan Ziegler6

1German Diabetes Center, Division of Endocrinology and Diabetology at HHU, Düsseldorf, Germany, 2German Diabetes Center, German Center for Diabetes Research (DZD), Düsseldorf, Germany, 3Helmholtz Zentrum München, Munich, Germany, 4Helmholtz Zentrum München, German Center for Diabetes Research (DZD), Munich, Germany, 5Helmholtz Zentrum München, German Center for Diabetes Research (DZD), München, Germany, 6German Diabetes Center, German Center for Diabetes Research (DZD), Division of Endocrinology and Diabetology at HHU, Düsseldorf, Germany

Purpose: The determinants and mechanisms contributing to diabetic sensorimotor polyneuropathy (DSPN) remain unclear. Since inflammation and altered nerve regeneration have been implicated in the pathogenesis of both DSPN and neuropathic pain, we hypothesized that the corresponding biomarkers could be associated with DSPN and may have the potential to discriminate between the painful and painless DSPN entities.

Methods: We measured 92 serum biomarkers including pro- and anti-inflammatory cytokines, chemokines, and growth factors (GF) using the Proseek Multiplex INF I assay (OLINK Proteomics) in 304 individuals with type 2 diabetes and polyneuropathy (DSPN+), defined by the Toronto Consensus Criteria (2011), as well as in 158 individuals with type 2 diabetes without DSPN (DSPN-) and 354 individuals with normal glucose tolerance and without DSPN (NGT). (DSPN+/DSPN-NGT [mean±SD]: age: 68±9/71±6/69±5 years; male: 76/59/41%; BMI: 30.8±5.3/30.8±4.4/26.9±3.7 kg/m²; diabetes duration: 13.5±9.6/7.6±5.8/– years; HbA1c: 7.4±1.3/6.6±1.0/5.5±0.3%). Within DSPN+, 161 participants suffered from neuropathic pain.

Results: After adjustment for multiple testing and sex, age, BMI, HbA1c, and smoking, the serum levels [normalized protein expression values] of 17 biomarkers including four cytokines (e.g. tumor necrosis factor ligand superfamily-12 (TNFSF12): 9.06±0.35 vs 9.30±0.32 and 9.45±0.29), five chemokines (e.g. C-C motif ligand-4 (CCL4): 7.93±0.68 vs 8.36±0.64 and 8.18±0.57), and four growth factors (e.g. Neurotrophin-3: 0.87±0.40 vs 1.00±0.33 and 1.03±0.34) were lower, while the level of one chemokine was higher in DSPN+ (CCL20: 5.79±1.23 vs 5.24±1.11 and 4.91±1.18) compared to DSPN- and NGT (P<0.05). No differences in biomarker levels were found in DSPN+ individuals with or without neuropathic pain. In diabetes, six biomarkers were associated with measures of peripheral nerve function (e.g. TNFSF12 with sural sensory nerve conduction velocity: r=0.259, P<0.0001).

Conclusions: Deficits in systemic cytokines, chemokines, and growth factors promoting nerve regeneration in type 2 diabetes are linked to polyneuropathy in general but not specifically to the painful or painless entity.

References: None.

Keywords: Inflammatory, Pain
Grant Support: The study was funded in part by grants from the European Union Seventh Framework Programme FP7/2007-2013 (PROPANE consortium; grant no. 602273), the German Center for Diabetes Research, and from the German Diabetes Association (Deutsche Diabetes-Gesellschaft, DDG). This work was also supported by the Ministry of Culture and Science of the State of North Rhine-Westphalia and the German Federal Ministry of Health. This study was supported in part by a grant from the German Federal Ministry of Education and Research to the German Center for Diabetes Research (DZD). Helmholtz Zentrum München – German Research Center for Environmental Health is funded by the German Federal Ministry of Education and Research and by the State of Bavaria. Research was supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ.
Monday, June 24, 2019 - 10:00 - 10:15
Protective Effects Of Endogenously Expressed Calpain Inhibitor In A Mouse Model Of Guillain-Barré Syndrome.
Rhona McGonigal¹, Madeleine Cunningham¹, Kathryn Saatman², Hugh Willison¹
¹University of Glasgow, Glasgow, United Kingdom of Great Britain and Northern Ireland, ²University of Kentucky, Lexington, KY, USA

Axon degeneration accounts for poor recovery in patients with Guillain-Barré syndrome (GBS), but there are no first-line treatments to target this key stage in pathogenesis. Animal models of the acute motor axonal neuropathy (AMAN) variant have demonstrated that injury to the nerve is caused by autoantibodies to axonal antigens activating the complement cascade, culminating in the formation of a pore. Uncontrolled influx of water and ions, including calcium, through the pore results in conduction block and structural disruption through activation of the calcium-dependent cleavage enzyme calpain. We assessed the potential of calpain inhibition as an axon protective therapy using transgenic mice that over-express the endogenous calpain inhibitor calpastatin (hCAST). Axonal integrity was compared between wild type (WT) and hCAST mice (n=4/group) in our established ex and in vivo injury models of AMAN. Immune-mediated injury was induced at distal axons by administering monoclonal anti-ganglioside antibodies and complement. Neurofilament, a known calpain substrate, was used as a marker of axonal structural integrity. As the diaphragm is the target in our in vivo model, respiratory function was measured by whole-body plethysmography as a functional output. Axon integrity (neurofilament immunolabeling) is significantly protected in ex vivo injury preparations from hCAST compared to WT mice, while nodal integrity is partially protected. In vivo, both WT and hCAST mice acutely develop weakness, and respiratory dysfunction. Distal axonal neurofilament immunolabeling was significantly reduced in WT mice, and in contrast was protected in hCAST mice. In summary, calpain inhibition can protect the axonal integrity of the nerve in an in vivo injury paradigm, but not the acute loss of function, as expected from the effects of uncontrolled ion flux. These studies provide proof of principle that calpain inhibition can protect axons in vivo and lays the foundation for further animal and clinical study using exogenous calpain inhibitors.

References: None.

Keywords: Axonal Biology, Node Biology, Pre-clinical Studies, Inflammatory

Grant Support: Wellcome Trust
Background: Diagnosis of chronic inflammatory demyelinating polyneuropathy (CIDP) is based on consensus guidelines in which interpretations of nerve conduction studies (NCS) have a major role. Automated analysis could improve interpretation of findings to fit into the current electrodiagnostic criteria, which could lead to better diagnostic accuracy. In addition, machine learning is able to detect complex relationships in datasets, which are not visible to the human eye or simple statistical models.

Objectives: to evaluate the diagnostic accuracy of the current EFNS/PNS electrodiagnostic criteria with (out) adaptations and to explore the diagnostic accuracy of machine learning algorithms.

Methods and results: NCS data from patients suspected of subacute or chronic immune mediated neuropathies were extracted from our NCS database in the period between 2009 and 2018. Automating the electrodiagnostic criteria (EFNS/PNS 2010) (85 CIDP patients and 180 patient controls) showed a relatively high sensitivity and moderate specificity. Adaptations to the electrodiagnostic criteria were implemented to explore changes in diagnostic accuracy, such as: exclusion of known pressure segments, the allowed amount of temporal dispersion in the leg nerves, the implementation of CMAP area instead of amplitude, and defining minimal distal CMAP amplitude to allow the criteria to be valid. Especially exclusion of the distal CMAP duration criterion led to an improved specificity. Subsequently, we explored a machine learning model: a Random Forest Classifier algorithm. This algorithm uses the input parameters to create random dichotomous decision trees and then averages the outcomes, thereby creating a robust model for classification. Preliminary results showed that the performance of this random forest classifier was higher than the current EFNS/PNS electrodiagnostic criteria.

Conclusions: Implementing adaptations of the current consensus-based electrodiagnostic guidelines for CIDP improve the diagnostic accuracy in our hospital. A data-driven approach using machine learning algorithms may further improve diagnostic performance.

References: None.

Keywords: Inflammatory

Grant Support: None.
PNS Oral Poster Session II
Monday, June 24, 2019 - 11:30 - 12:30
Monday, June 24, 2019 - 11:30 - 11:35
Acute small fibre neuropathy: a neglected condition?
Thierry Gendre1, Abir Wahab1, Julie Bismuth2, Jean-Pascal Lefaucheur2, Alain Créange1
1Department of Neurology, Hôpital Henri Mondor, AP-HP, Université Paris-Est, Créteil, France, 2
Department of Neurophysiology, Hôpital Henri Mondor, AP-HP, Université Paris-Est, Créteil, France

**Background:** Small fibre neuropathies (SFN) constitute a disorder involving thinly myelinated Aδ-fibres and unmyelinated C-fibres. Causes are various and often remain unknown. SFN are typically chronic length-dependent polyneuropathies. However, some patients report an acute onset.

**Aim:** To describe a series of patients with an acute small fibre neuropathy (ASFN).

**Methods:** To be included, patients must present with sensory manifestations involving at least two limbs, an exclusive impairment of pain and/or heat sensation on clinical examination and neurophysiological investigation, and a progression phase of less than 4 weeks. Patients with associated large fibre involvement were excluded. We collected their clinical, neurophysiological, and biological data.

**Results:** From November 2017 to February 2019, we prospectively included 11 patients with ASFN (7F:4M, median age: 43.7 [23.5-59.2]). Ten patients reported neuropathic pains. Nine patients presented a non-length-dependent profile. Orthostatic hypotension was present in 3 patients. Ten patients had at least abnormal results of laser-evoked potentials, warm detection thresholds or electrochemical skin conductance. Standard immunological blood tests were normal. AntiFGFR-3 antibodies were positive in 3 patients (6 tested). Cerebrospinal fluid was normal in 4 patients. A precipitating potential event was present in 7 patients: 3 infections, 2 vaccinations, and 2 treatment intolerances. Disease course was characterized by a complete remission in 2 cases, recurrent episodes in 5 cases, and chronicity in 4 patients.

**Discussion:** Few cases of ASFN have been reported, usually with a favourable prognosis. However, the present series shows a variable disease course. The evidence of a precipitating factor in two-thirds of cases and the presence of antiFGFR-3 antibodies support an immune dysfunction, as it was previously suggested with the transient detection of antibodies directed against small fibres. Early immunomodulating treatment is worth to be discussed.

**Conclusion:** ASFN appears as a potential inflammatory neuropathy with an important clinical impact and a variable disease course.

**References:**


**Keywords:** Small Fibers, Inflammatory, Pain

**Grant Support:** None in relation to this work.
Monday, June 24, 2019 - 11:35 - 11:40

Ultrastructural Mechanisms of Macrophage-Induced Demyelination in Guillain-Barré Syndrome

Haruki Koike, Yuki Fukami, Ryoji Nishi, Yuchi Kawagashira, Masahiro Iijima, Masahisa Katsuno, Gen Sobue
Nagoya University Graduate School of Medicine, Nagoya, Japan

Introduction: Although recent advances in the identification of anti-ganglioside antibodies have significantly contributed to clarifying the pathogenesis of Guillain-Barré syndrome, particularly acute motor axonal neuropathy, the mechanism of classical macrophage-induced demyelination in acute inflammatory demyelinating polyneuropathy (AIDP) remains unclear.

Methods: Longitudinal sections of sural nerve biopsy specimens from 11 patients with AIDP exhibiting macrophage-associated demyelinating lesions were examined using electron microscopy. A total of 1205 nodes of Ranvier, with middle sections that were cut perpendicularly, were examined to determine the relationship of the macrophage-associated demyelinating lesions with the nodal regions.

Results: Overall, 252 macrophage-associated demyelinating lesions were identified in the longitudinal sections. Of these, 40 lesions exhibited complete demyelination with no association with the lamellar structures of myelin. In 183 lesions, the macrophage cytoplasm was located at the internodes without association with the nodes of Ranvier or paranodes. In particular, these internodal lesions were more frequent in one patient (152 lesions). Focal unraveling of the myelin layers apposed to the macrophage cytoplasm was frequently seen at the Schmidt-Lanterman incisures, where uncompaction of the myelin lamellae is observed under normal conditions as well. In the remaining 29 lesions, the involvement of nodal regions was obvious. Invasion of the macrophage cytoplasmic processes into the space between the paranodal myelin terminal loops and axolemma from the node of Ranvier was observed in three patients. Immunohistochemistry suggested complement C3d deposition in the areas with initial macrophage-associated demyelinating lesions in patients who underwent biopsy during early disease phase.

Conclusions: The initial macrophage-associated demyelinating lesions were located not only at the internodes but also at the paranodes. The sites at which the macrophages initiated phagocytosis of myelin might be associated with the location of complement deposition in certain patients with AIDP.

References: None.

Keywords: Inflammatory, Node, Schwann Cell

Grant Support: None.
Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a heterogeneous disorder considered an autoimmune disease involving both cellular and humoral immunity. IgG fixation on the outer surface of the Schwann cell has been described in patients' nerve biopsies, suggesting that autoantibodies may be implicated in the demyelination process, and antibodies against specific nodal and paranodal junction components have been recently identified. In a cohort of 178 French CIDP patients we found that 31% of patients' sera presented an IgG reactivity toward the node and paranode of mouse sciatic nerve. Interestingly, 18% of the patients presented a strong IgG or IgM reactivity against the internodal compact myelin. We report here the clinical, electrophysiological, immunological, and microscopic features of six of these CIDP patients in whom sural nerve biopsies were available. Five over six patients fulfilled the EFNS/PNS electrophysiological criteria for definite CIDP. These five patients showed increased (1.6 to 5.1 times) duration of proximal compound muscle action potential in at least two nerves. Electron microscopy of sural nerve biopsies showed normal paranodes and nodes, but demonstrated the presence of macrophage-mediated demyelination restricted to the internode. Immunolabeling for Nav channels, MPZ, and neurofilament-H confirmed the presence of segmental demyelination and remyelination. However, the nodal region appeared unaffected in these patients. Altogether these results indicate that CIDP patients with antibodies to internodal myelin or nodal/paranodal components show differential morphological features and pathogenic mechanisms.

References: None.

Keywords: Schwann Cell, Inflammatory, Other, Other, Other

Grant Support:

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Monday, June 24, 2019 - 11:45 - 11:50
Prognostic features for death and progression in patients with POEMS syndrome
Stephen Keddie1, Janev Fehmi2, Mahima Kapoor1, David Foldes3, Aisling Carr1, Mary Reilly1, Shirley D’sa1, Simon Rinaldi2, Michael Lunn1

1MRC Centre for Neuromuscular DiseasesNational Hospital for Neurology and Neurosurgery, London, United Kingdom of Great Britain and Northern Ireland, 2Nuffield department of Clinical Neurosciences, Oxford, United Kingdom of Great Britain and Northern Ireland, 3Cancer Division, Department of HaematologyUniversity College London Hospitals NHS Foundation Trust, London, United Kingdom of Great Britain and Northern Ireland

Background

POEMS syndrome is a rare multisystem disorder with favourable long term prognosis if appropriately treated. A proportion of patients appear resistant to treatment, some relapse and few die. Ascertaining prognostic factors will assist with identifying high risk patients likely to require more frequent monitoring or more aggressive or alternative therapeutics.

Aim

To evaluate individual risk factors and produce a predictive model for risk of progression or death in POEMS syndrome.

Methods

We retrospectively analysed 100 patients with newly diagnosed POEMS syndrome at our institute from 1998 to present day. We performed univariate and multivariate regression analysis to identify statistically significant risk factors leading to poor outcome.

Results

A binomial logistic regression was performed which ascertained the effect of Haematological non-response (hNR), VEGF non-response (vNR), low glomerular filtration rate (GFR), low albumin at presentation and treatment with autologous stem cell therapy (vs other forms of treatment) on the likelihood of progression or death. The regression model was statistically significant, $X^2 = 50.117$ (p<0.005). The model explained 54% (Nagelkerke $R^2$) of the variance in progression or death and correctly classified 87% of cases. Sensitivity was 70%, specificity 95%, positive predictive value was 88% and negative predictive value 86%.

Conclusion

Haematological non-response (hNR), VEGF non-response (vNR), low glomerular filtration rate (GFR), low albumin at presentation and treatment with autologous stem cell therapy (vs other forms of treatment) are significant risk factors in outcome (progression or death) in POEMS syndrome.

References: None.

Keywords: Inflammatory

Grant Support: Dr Keddie is funded by ABN and Guarantors of Brain
Consequences of SAC3/FIG4 deficiency to phosphoinositides in fibroblasts of patients with CMT4J

Jun Li, Assia Shisheva, Diego Sbrissa, Bo Hu
Wayne State University School of Medicine, Detroit, MI, USA

Introduction: Charcot-Marie-Tooth disease type-4J (CMT4J) is autosomal recessively inherited peripheral neuropathy caused by compound heterozygous mutations in FIG4 (also known as SAC3) gene, to result in severe loss/absence of the SAC3/FIG4 protein, which triggers neuronal degeneration, segmental demyelination, sensory disorder and limb muscle weakness. In mouse fibroblasts, the absence of the phosphatidylinositol (PtdIns) 3,5P2 5-phosphatase SAC3/FIG4 leads to reduced PtdIns(3,5)P2 due to disassembly of PtdIns(3,5)P2-metabolizing machinery, composed of the PIKfyve kinase, the ArPIKfyve scaffold and the SAC3/FIG4 phosphatase. Decreases of PtdIns(3,5)P2 over 70% is incompatible with life as shown in genetically modified mouse models deficient in either of the three genes. However, phosphoinositides (PIs) in humans with loss-of-function CMT4J mutations have never been evaluated. How changes in PIs relate to lysosomal phenotypes is also unclear.

Methods: De-identified fibroblasts were obtained as previously described (Hu et al, 2016). Fibroblasts were labeled with myo-[2-3H] inositol to equilibrium. Extracted PIs were quantified by HPLC. Results: Compared to fibroblasts from normal human controls (n=9), both PtdIns(3,5)P2 and PtdIns5P levels were significantly decreased in CMT4J fibroblasts (n=13) by 36.4±3.6% and 43.1±4.4%, respectively (mean±SEM; p<0.0001). Whereas mean values for PtdIns3P levels remained unchanged vs. controls, there were high variations in PtdIns3P among individual patients. Morphological alterations in the form of multiple endolysosomal vacuoles, typically seen under PtdIns(3,5)P2 reduction, were apparent but not in fibroblasts from all CMT4J patients. Patients who failed to display aberrant cytoplasmic vacuolation exhibited significantly low levels of PtdIns3P vs. controls. Conclusions: 1). Our study assesses for the first time the PI profiles in humans with CMT4J. 2). The phenotypes in CMT4J patients may not be solely due to reduction of PtdIns(3,5)P2, but also to that of PtdIns5P, known to be involved in non-canonical autophagy. 3). Lack of vacuoles in fibroblasts from some CMT4J patients may be related to low PtdIns3P levels.

References: None.

Keywords: CMTR, Human Genetics

Grant Support: U.S. Department of Veterans Affairs (IBX003385A)
Adult Polyglucosan Body Disease Presenting With A Peripheral Neuropathy: Broadening The Clinical Spectrum
Jonathan De Winter¹, Willem De Ridder², Tine Deconinck³, Danique Beijer³, Martin Lammens¹, Jonathan Baets²

¹Antwerp University Hospital, Edegem, Belgium, ²Antwerp University Hospital, University of Antwerp, Edegem, Belgium, ³University of Antwerp, Edegem, Belgium

The purpose is twofold. First, to present a rare cause of a polyneuropathy with leukodystrophy as an initial presentation of a multisystemic polyglucosan body disease. Second, to expand the current knowledge concerning adult-onset GBE-deficiency phenotypes. A 49-year old man presented at our neuromuscular department with a two-year history of gait difficulties and distal sensory abnormalities. Distal weakness was noted as well as pedes cavi. Clinical examination was in keeping with a motor predominant peripheral neuropathy and mild pyramidal features. Nerve conduction studies showed a predominantly motor mixed axonal-demyelinating neuropathy. MR-imaging revealed confluent white matter lesions in both hemispheres. Family history revealed a younger brother with a similar albeit milder clinical phenotype. After five years of follow-up the patient had developed a progressively severe spasmodic gait with wheelchair use, neurogenic bladder dysfunction, discrete cerebellar ataxia and cognitive and behavioral changes. Progressive leukodystrophy and myelopathy were evident from serial MRIs. Distal weakness preceded proximal weakness in the lower limbs, analogous to a more pronounced motoric predominant neuropathy. Although mildly elevated serum CK levels (500-600U/L) were observed, muscle biopsy did not show primary myopathic abnormalities. Due to liver enzyme abnormalities, a liver biopsy was performed and showed a micro-macrovesicular steatosis. Whole exome sequencing showed compound heterozygous mutations (p.Thr254Ala and Ile694Asn) in Glycogen Branching Enzyme 1 (GBE1) and segregation in the family was confirmed. In conclusion, we report a case of an adult polyglucosan body disease (APBD) with a peripheral nerve system impairment dominating the initial presentation. We broaden the current knowledge of GBE1-related disorders by representing a combination of the two allelic forms of GBE1 deficiency, namely GSD-IV (glycogen storage disease type 4) and APBD. The first is associated with involvement of the liver and skeletal muscles in childhood, the second with peripheral neuropathy and central nervous system involvement in adults.

References: None.

Keywords: Human Genetics

Grant Support: None.
Charcot-Marie-Tooth disease (CMT) is most commonly caused by duplication of a chromosomal segment surrounding Peripheral Myelin Protein 22, or PMP22 gene, which is classified as CMT1A. Several candidate therapies reduce Pmp22 levels in CMT1A rodent models, but development of biomarkers for clinical trials in CMT1A is a challenge given its slow progression and the difficulty in obtaining nerve samples. Quantitative PCR measurements of PMP22 in dermal nerves have been performed using skin biopsies in human clinical trials for CMT1A, but this approach does not show increased PMP22 mRNA in CMT1A patients compared to controls. One complicating factor is the variable amounts of Schwann cells (SC) in skin. The objective of the study was to develop a novel method for precise evaluation of PMP22 levels in skin biopsies that can discriminate CMT1A patients from controls. To accomplish this, we have developed methods to normalize PMP22 transcript levels to SC-specific genes that are not altered by CMT1A status. Several CMT1A-associated genes were assembled into a custom Nanostring panel to enable precise transcript measurements that can be normalized to variable Schwann cell content. Nanostring technology enables direct detection of transcripts without cDNA synthesis and amplification. The digital expression data from Nanostring analysis showed reproducible elevation of PMP22 levels in CMT1A vs. control skin biopsies, particularly after normalization to SC-specific genes. This platform should be useful in clinical trials for CMT1A as a measure of target engagement that can be used to optimize dosing, and the same normalization framework is applicable to other types of CMT.

References: None.

Keywords: CMTR, Schwann Cell, Other

Grant Support:

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Unravelling hallmarks of axonal degeneration in Charcot-Marie-Tooth type 2 using induced pluripotent stem cells

Jonas Van lent, Manisha Juneja, Peter Verstraelen, Lotte Conings, Bob Asselbergh, Vicky De Winter, Winnok De Vos, Vincent Timmerman

1University of Antwerp, Antwerp, Belgium, 2University of Antwerp, VIB, Antwerp, Belgium

Introduction: Our knowledge in the disease mechanisms of Charcot-Marie-Tooth (CMT) has significantly increased due to the successful generation of transgenic mouse models recapitulating the neuropathy phenotype. The human induced pluripotent stem cell (hiPSC) technology seems promising to substitute animal models of disease and may facilitate the identification and validation of reliable molecular therapies. We aimed to obtain an in vitro cellular model to study the hallmarks of axonal degeneration among different CMT2 subtypes.

Methods: We reprogrammed fibroblasts derived from five CMT2 patients with different causal mutations in the MFN2, NEFL, HSPB8 and HSPB1 genes using Sendai-virus transductions. We differentiated these hiPSC lines, along with healthy controls, into spinal motor neurons using an established protocol [1]. We investigated: 1. signs of neurodegeneration using high content phenotypic screening, 2. cytoskeletal abnormalities disturbing axonal transport, 3. axonal transport deficits in mitochondria and 4. abnormalities in neuronal excitability using calcium imaging.

Results: We successfully generated spinal motor neurons from hiPSCs with an efficiency of almost 95% from both patients and controls. A decrease in neurite area, length and branching for the MFN2, NEFL and HSPB1 patient lines indicated an altered neurite network. Deficits in mitochondrial trafficking and morphology were found in neurons from MFN2 and NEFL patient iPSC lines. Preliminary data revealed electrophysiological abnormalities based on calcium imaging, such as a shorter recovery time after stimulation in motor neurons derived from patients.

Conclusions: Our findings provide insights into the molecular and cellular phenotypes of hiPSC-derived models for axonal CMT neuropathies.


Keywords: Axonal Biology

Grant Support: None.
Monday, June 24, 2019 - 12:10 - 12:15

Altered Nerve Triglycerides in Mouse Models of Diabetes with Neuropathy.

Phillipe O'Brien¹, Kai Guo², Stephanie Eid¹, Amy Rumora¹, Lucy Hinder¹, John Hayes¹, Faye Mendelson¹, Hur Junguk², Eva Feldman¹

¹University of Michigan Medical School, Ann Arbor, MI, USA, ²University of North Dakota, Grand Forks, ND, USA

Lifestyle changes, including dietary reversal (DR), can ameliorate peripheral neuropathy (PN) in patients with prediabetes and type 2 diabetes (T2D) however the mechanisms remain unclear. Our objective was to identify the contributions of altered nerve lipid profiles in PN development using mouse models of diabetes that underwent DR. 5wk old mice were fed a standard (10% kcal fat; SD) or high fat diet (60% kcal fat; HF) and at 12wk, a subset of HF mice were injected with STZ to induce a more diabetic-like phenotype. To simulate DR, subsets of HF and HF-STZ mice were placed on the SD from 16-24wk. At study conclusion, untargeted and targeted lipidomic profiling, complemented with RNAseq gene expression analysis, was performed on sciatic nerve (SCN) tissue. Compared to HF and HF-STZ mice, PN was corrected in HF-DR and HF-STZ-DR animals. Untargeted lipidomics revealed that triglycerides were increased in HF and HF-STZ SCN tissue but decreased in SD and DR groups. Quantitative targeted lipidomics validated these findings with an increase in triglycerides containing saturated fatty acids being observed in HF mice. Gene expression analysis revealed numerous differentially expressed genes that were dysregulated in HF and HF-STZ SCN but reversed by DR while KEGG enrichment indicated that lipid metabolism pathways were enriched. The lipidome and transcriptome datasets were integrated and identified a biologically relevant correlation between lipid levels and genes involved in triglyceride regulation. In parallel, DGAT2 expression, the enzyme required for triglyceride synthesis, was increased in sural nerve biopsies from hyperlipidemic diabetic patients with PN. We demonstrate that DR in HF and HF-STZ mice can restore PN that coincides with restoration of nerve lipid homeostasis. Collectively, these findings strengthen the hypothesis that abnormal nerve-lipid signaling is a key player in peripheral nerve dysfunction and suggest that lipid centric therapeutic interventions are needed for PN.

References: None.

Keywords: Diabetes, Pre-clinical Studies, Metabolic, Other, Other

Grant Support: This work was supported by the National Institutes of Health (1DP3DK094292, 1R24082841 to E.L.F.); Novo Nordisk Foundation Center for Basic Metabolic Research (NNF14° C0011633 to E.L.F.); Nathan and Rose Milstein Research Fund; Sinai Medical Staff Foundation Neuroscience Scholar Fund 2; Robert C Graham Fund; Walbridge Aldinger Graduate Fellowship Fund (Post-doctoral Fellowship to P.D.O.); American Diabetes Association (7-12-BS-045); Program for Neurology Research & Discovery; and the A. Alfred Taubman Medical Research Institute; the University of North Dakota Post-Doc Pilot Grant (to K.G.). Research reported in this publication was also supported by Core Services supported by the National Institute of Diabetes and Digestive Kidney Diseases (NIDDK) of the National Institutes of Health (NIH) under award number U2CDK110768 (MMPC). Plasma insulin measurements were performed at The Vanderbilt MMPC (supported by NIH grants DK059637 and DK020593) and lipid measurements were performed by the Cincinnati MMPC (supported by NIH grant DK059630).
Risk Factors for the Development of Chemotherapy Induced Peripheral Neuropathy: A Retrospective Study
Noah Kolb¹, John Singleton², Joan Skelly³, Summer Karafaith², Alpert Smith⁴
¹Robert Larner College at Medicine at the University of Vermont, Burlington, VT, USA, ²University of Utah, Salt Lake City, UT, USA, ³University of Vermont, Burlington, VT, USA, ⁴Virginia Commonwealth University, Richmond, VA, USA

Introduction: The objective of this study was to assess frequency, severity and risk factors for chemotherapy induced peripheral neuropathy associated (CIPN) with paclitaxel treatment.

Methods:

A natural language processing tool was utilized to perform retrospective chart review on paclitaxel treated breast cancer patients at the University of Utah between 1999 and 2015. CIPN risk factors were determined via time stamped ICD9/10 records while progress notes were reviewed for CIPN diagnosis and NCI CTCAE severity. Stepwise logistic modeling was used to determine the significant risk factors for the development of CIPN.

Results:

The mean age of the 549 patients was 52±12 with 99.6% female, and 57.9% weekly and 42.1% dose dense paclitaxel. Mean total paclitaxel dose (mg/m²) of those with vs. those without CIPN was 754±231 vs. 738±256 p=0.49.

At the conclusion of chemotherapy: 74% had CIPN: 32% grade 1, 35% grade 2, 4% grade 3, <1% grade 4 or 5 neuropathy. At 2 years after chemotherapy: 36% had CIPN: 20% grade 1, 15% grade 2, <1% grade 3, 4 or 5, 14% CIPN still present but unable to determine severity. There was no significant difference in the percent with CIPN between dose dense and weekly dosing (75.5% vs 71.9%, p=0.31)

Result of stepwise regression modeling:

Significant risk factors for the development of CIPN: pre-existing non-diabetic/non- hereditary neuropathy (OR=12.0 (95%CI: 5.72-25.28)) and hyperlipidemia (OR=1.91 (1.13-3.21)). Risk factors for persistent CIPN at 2 years: Non-diabetic/non-hereditary neuropathy (OR=4.42 (2.94-6.63)), diabetic polyneuropathy (OR=3.46 (1.11-10.79)) and hypertension (OR=1.95 (1.33-2.86)).

Conclusions:

Approximately 75% of breast cancer patients treated with paclitaxel develop CIPN and it frequently persists 2 years later. Regression modeling demonstrates that pre-existant neuropathy, hyperlipidemia and hypertension may represent important risk factors for CIPN development and persistence.

References: None.
Keywords: Other

Grant Support: None.
BROADENING THE SPECTRUM OF BIALLELIC ADPRHL2 MUTATIONS INTO COMPLEX EARLY-ONSET MOTOR NEUROPATHY PHENOTYPES


1University of Antwerp, Antwerp, Belgium, 2Oxford University, Oxford, United Kingdom of Great Britain and Northern Ireland, 3Antwerp University Hospital, Antwerpen, Belgium, 4University of Belgrade, Belgrade, Serbia, 5University of Antwerp, Antwerp University Hospital, Antwerp, Belgium

ADP-ribosylation is a process of posttranslational modification of proteins and is mediated by several factions of enzymes: poly-ADP-ribose polymerases, like PARP1, which catalyze the attachment of poly-ADP-ribose (PAR) units to proteins using NAD+ as a donor for ADP-ribose. Subsequently, mono- or poly-ADP hydrolases, like PARG, catalyze the removal of single or strings of ADP-ribose units. A stress response initiates a rapid breakdown of PAR, preventing excessive PAR accumulation that would result in cell death via the parthanatos pathway. The ADP-ribosylation pathway is an ubiquitously expressed pathway controlling cellular stress. However, impairment in this pathway seems to primarily affect neuronal tissues in a neurodegenerative fashion. Recently, recessive ADPRHL2 mutations were shown to cause a neurodegenerative stress-induced epileptic ataxia syndrome with early pediatric onset, and neurodegeneration with developmental delay, ataxia, and axonal neuropathy. In this study, we present two families with homozygous mutations in ADPRHL2 with a complex juvenile-onset neurodegenerative peripheral neuropathy phenotype, further validating the neuronal vulnerability for dysregulation of the ADP-ribosylation pathway. In vitro studies of the identified ADPRHL2 missense mutations were performed in order to assess the expression levels, stability and localization of the mutant and wild-type proteins. In addition, patient fibroblast cells were investigated to validate the in vitro experiments.

References: None.

Keywords: Human Genetics

Grant Support: None.
Neuromuscular disease (NMD) researchers are faced with the challenge of discerning pathophysiological mechanisms and treatment opportunities for hundreds of disease genes identified to date. At the same time, a large fraction of NMD-genes remains to be discovered – a daunting task, as variants in any particular disease gene are often exceedingly rare. Leveraging advanced deep learning algorithms, we have developed a high-content, unbiased, and scalable method to rapidly identify disease-associated phenotypes in high-resolution, multiplexed, fluorescent microscopy images of primary, NMD-patient derived cells. Our approach outperforms human-experts and traditional image-analysis methods by at least 5-fold in single-cell classification accuracy, improves image-analysis throughput by orders of magnitude, and allows for the efficient generation of a standardized, deep, and quantitative map of cellular phenotypes across NMD-subtypes, that facilitates the identification of shared disease pathways. At the same time, our method maintains patient-specific granularity, and efficiently identifies disease-associated cellular phenotypes in NMD-patients with unknown genetic etiology. The identified phenotypes (a) can be functionally interpreted by their similarity to profiles of cells with established genotype-phenotype associations, and (b) enable direct experimental validation of candidate variants in NMD-cases with pending genetic diagnosis. Moreover, (c) the high-content nature and cost-efficiency of our approach compared to orthogonal methods such as RNA-Seq, renders it suitable for small-molecule screens on a patient-specific, cellular level.

References: None.

Keywords: Human Genetics, CMTR, Pre-clinical Studies, Other

Grant Support:

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Monday, June 24, 2019 - 15:30 - 15:45
Long-term Safety and Efficacy of Patisiran in Patients with hATTR Amyloidosis: Global OLE Study

Michael Polydefkis1, Alejandra González-Duarte2, Teresa Coelho3, Jonas Wixner4, Arnt Kristen5, Hartmut Schmidt6, John Berk7, Quinn Dinh8, Erhan Berber9, Marianne Sweetser9, Matthew White9, Jing Jing Wang9, David Adams9

1Johns Hopkins University, Baltimore, MD, USA, 2Instituto Nacional de Ciencias Médicas y Nutrición, Salvador Zubirán, Mexico City, Mexico, 3Hospital de Santo António, Porto, Portugal, 4Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden, 5Heidelberg University Hospital, Heidelberg, Germany, 6University Hospital Muenster, Muenster, Germany, 7Amyloid Treatment and Research Program, Boston University, Boston, MA, USA, 8Alnylam Pharmaceuticals, Cambridge, MA, USA, 9National Reference Center for FAP (NNERF)/ APHP/ INSERM U 1195/ CHU Bicêtre, Le Kremlin Bicêtre, France

Introduction: Hereditary transthyretin-mediated (hATTR) amyloidosis is a progressive, life-threatening disease; majority of patients develop a mixed phenotype including polyneuropathy and cardiomypathy. Patisiran’s efficacy and safety over 18-24 months have been demonstrated in Phase 2 and Phase 3 (APOLLO) studies in hATTR amyloidosis with polyneuropathy. Safety and efficacy from an interim analysis of the ongoing Global Open-Label extension (OLE) study are presented.

Methods: Multicenter, international, OLE, safety and efficacy study (NCT02510261) in eligible patients who completed parent studies, including APOLLO patients randomized to placebo (APOLLO/placebo, n=49) or patisiran (APOLLO/patisiran, n=137) and Phase 2 OLE patients (n=25).

Results: 211 patients enrolled into Global OLE; 189 had 12-month assessments by September 24, 2018. Safety profile remained consistent with previous studies. After 12 months of additional patisiran treatment in the Global OLE, durable improvement was seen for mNIS+7 (mean change [SEM]) in APOLLO/patisiran (-4.0 [1.9]) and Phase 2 OLE (-4.7 [3.5]) groups compared to their parent study baselines. Norfolk QOL-DN (only measured at parent study baseline in APOLLO) also showed durable improvement in APOLLO/patisiran patients (-3.9 [2.1]) following additional 12-months treatment in OLE. In the Global OLE, APOLLO/placebo patients experienced improvement on average after 12 months of patisiran (mNIS+7: -1.4 [2.4], Norfolk QOL-DN: -4.5 [2.5]), although they had progressed relative to APOLLO baseline (mNIS+7: +24.0 [4.2], Norfolk QOL-DN: +15.0 [3.4]) given the progression while on placebo in APOLLO.

Conclusions: Long-term patisiran treatment continues to show a positive benefit:risk profile, including patients dosed for 4 years or more. Overall, patients with longer-term exposure to patisiran demonstrated durability of effect. Despite marked progression on placebo during the 18-month APOLLO study, previously untreated patients exhibited halting of disease progression and QOL improvement following 12 months of patisiran. However, delay in treatment resulted in these patients accumulating greater disease burden compared to patients treated earlier with patisiran.

References: None.

Keywords: Amyloidosis

Grant Support: None.
Wildtype and Familial Transthyretin Amyloid Polyneuropathy: Distinct Cutaneous Biomarkers at the Distal Limb

Gigi Ebenezer, Mohammad Khoshnoodi, Daniel Judge, Ashley Marascalchi, Kelly Wagner, Boahan Pan, Michael Polydefkis
Johns Hopkins School of Medicine, Baltimore, MD, USA

We examined clinical and pathological peripheral nerve features among hATTR and wtTTR patients including (1) pathogenic TTR carriers with peripheral neuropathy (PN): hATTR-PN (n=19), (2) hATTR pathogenic carriers without PN: hATTR-noPN (n=9), (3) patients with ATTRwt presenting with cardiomyopathy (ATTRwt-CM, n=7), (4) PN patients found to have TTRwt amyloid on biopsy (TTRwt-PN, n=4), (5) 40 age/gender matched disease controls, and (6) non-TTR amyloidosis (AL (N=2), gelsolin (N=2)). Patients underwent examination (NIS), electrophysiology and 3mm skin biopsies at the distal limb. 50µM skin sections were assessed for IENFD, SGNFD (PGP9.5), and TTR amyloid by anti-misfolded TTR immunohistochemistry and Congo red.

Amyloid was detected in d.leg skin with higher sensitivity by anti-misfolded TTR-immunohistochemistry than with Congo red staining (in hATTR: 57%vs.93%, hATTR-PN: 58%vs.100%) with no controls or AL/gelsolin patients being positive (100% specificity). Amyloid burden was higher in hATTR-PN vs hATTR-noPN (% mean±SD, hATTR-PN: 17.5±25.8, TTR-noPN: 7.1±13.2) and inversely correlated with d.leg IENFD (p=0.02, r=-0.45). While amyloid was detected in 100% of hATTR-PN subjects in least at one leg site, the amyloid burden varied greatly with dense deposits in V30M patients to mild in V122I. IENFD correlated inversely with NIS-LL (d.leg: p<0.0001, r=-0.70; p.thigh: p<0.001, r=-0.63), and positively with sural (p=<0.001, r=0.69) and peroneal nerve (p<0.001, r=0.68) amplitudes. TTRwt-PN and TTRwt-CM patients had scanty amyloid deposits, primarily on ascending sweat ducts and nerve fibers while amyloid deposited in many dermal adenexal structures in hATTR.

3mm skin biopsies have high diagnostic yield in detecting amyloid of different etiologies. Immunohistochemistry against anti-misfolded TTR is more sensitive than Congo red in detecting amyloid and differentiates hATTR from TTRwt. TTRwt may represent a rare unrecognized etiology for peripheral neuropathy analogous to TTRwt-CM. The pattern of amyloid deposition in hATTR is distinct from ATTRwt.

References:


Keywords: Amyloidosis, Small Fibers

Grant Support: None.
Plasma NfL concentration is increased in patients with ATTRm and correlates with clinical severity scores

Mahima Kapoor¹, Julian Gillmore², Michael Lunn³, Andrea Malaspina⁴, Amanda Heslegrave⁵, Henrik Zetterberg⁶, Alex Rossor¹, Mary Reilly¹

¹MRC Centre for Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, United Kingdom of Great Britain and Northern Ireland, ²National Amyloidosis Centre, University College London (Royal Free Campus), London, United Kingdom of Great Britain and Northern Ireland, ³National Hospital for Neurology and Neurosurgery, London, United Kingdom of Great Britain and Northern Ireland, ⁴Centre for Neuroscience and Trauma, Blizard Institute, Queen Mary University of London, London, United Kingdom of Great Britain and Northern Ireland, ⁵Department of Neurodegenerative Disease, UK Dementia Research Institute, UCL Queen Square Institute of Neurology, London, United Kingdom of Great Britain and Northern Ireland, ⁶UCL QS ION, Inst. Neuroscience & Physiology, Uni of Gothenburg, Clinical Neurochemistry Lab, Sahlgrenska Uni Hosp, London, United Kingdom of Great Britain and Northern Ireland
Background

The success of gene silencing therapies in hereditary transthyretin amyloidosis (ATTRm) is a breakthrough for adult-onset, neurodegenerative diseases. A biomarker for peripheral nerve damage would be useful in detecting early peripheral nerve involvement and monitoring response to therapy. Neurofilament light chain (NFL) has been studied in several neurological disorders.

Aim

1) Evaluate whether plasma NFL (pNFL) concentration is elevated in patients with ATTRm compared to controls; 2) Investigate the association between pNFL and Neuropathy Impairment Score (NIS); 3) Assess the correlation between NIS and weighted examination score of the CMT Neuropathy Score (CMTES-R).

Methods

Blood samples were collected in 15 healthy controls and 85 patients with genetically-confirmed ATTRm, 42 (49.5%) had a neurological examination within four months of venepuncture. PNFL concentration was measured using a commercially available Simoa assay (Quanterix, Lexington, MA).

Results

Of the 85 patients with ATTRm, the most common genotypes were ATTRV30M (57.6%), ATTRS77Y (25.6%) and ATTTRT60A (22.9%). The control group was significantly older than the ATTRm group (70.7 years vs. 56.7 years, p= 0.04). NFL concentration was significantly higher in patients with ATTRm compared to healthy controls (44.3 pg/ml vs. 16.8 pg/ml, p< 0.001). Disease severity measured by NIS was a significant predictor of pNFL (β = 1.167, p = 0.006), whereas age was not (β = 0.04, p = 0.94). There was a tight correlation between NIS and CMTES-R (r = 0.89, p = <0.0001). 10 patients had received treatment for ATTRm prior to venepuncture; 8 patients had or were currently taking Diflunisal, 2 patients had undergone liver transplantation, 2 patients received patisiran and 1 patient was taking tafamidis.

Conclusion

NFL is a biomarker that is significantly raised in patients with ATTRm and correlates with the NIS. Further work is required to assess its clinical value in monitoring response to treatment.

References: None.

Keywords: Amyloidosis, Axonal Biology

Grant Support: None.
Monday, June 24, 2019 - 16:15 - 16:30
Identification of TMPRSS5/Spinesin, a Novel Schwann Cell Derived Plasma Biomarker for CMT1A
Matthew Davison1, Hongge Wang1, Kathryn Wang1, Katherine Call1, Xingyao Wu2, Riccardo Zuccarino2, Chelsea Bacon2, Yunhong Bai2, Laurie Gutmann2, Daniel Anderson2, Alexander Rosser3, Mary Reilly3, John Svaren4, Michael Shy2

1Sanofi, Framingham, MA, USA, 2University of Iowa, Iowa City, IA, USA, 3University College London, Queen Square Institute of Neurology, London, United Kingdom of Great Britain and Northern Ireland, 4University of Wisconsin, Madison, WI, USA

The development of biomarkers for Charcot-Marie-Tooth disease is critical for implementing effective clinical trials. A recent report1 identified neurofilament light (NEFL) as upregulated in the plasma of CMT disease patients (including CMT1A, SPTLC1, CMT2, CMTX1 and other CMT disease subtypes) compared to healthy controls, and NEFL correlated with disease severity clinical scores. However, no plasma/serum biomarker has been identified that is specific to the Schwann cells of peripheral nerve, the most directly affected cells in CMT1A.

We have used the new Olink immunoPCR platform, which provides high multiplex, good sensitivity detection, to profile CMT1A patient (n= 47, 2 cohorts) and normal control plasma (n= 41, two cohorts). Using five different Olink panels, each profiling 92 different proteins, we profiled a total of 400 unique proteins. The most promising candidate biomarker that was consistently elevated across independent cohorts of CMT1A samples relative to controls, was the TMPRSS5/Transmembrane protease serine 5 gene (also known as spinesin). The average upregulation was 2.05 fold (p = < 0.0001). Expression databases show that TMPRSS5 is most highly expressed in human peripheral nerve and central nervous system, and mouse studies show high levels in purified Schwann cells and oligodendrocytes.

In parallel, we found elevated levels of NEFL in the same samples, using the Olink detection system. These results confirmed the previously reported upregulation of NEFL in CMT1A patient samples, across multiple disease and normal cohorts (average of 1.58 fold, p < 0.0001); and also a positive correlation to disease clinical score. The levels of TMPRSS5 and NEFL were tested for correlation with CMT1A disease clinical scores and conduction velocities. These data provide the first identification of a Schwann cell specific protein that is elevated in plasma of CMT1A patients, and may provide a sensitive treatment-responsive biomarker, with good disease specificity, for clinical trials.


Keywords: CMTR, Schwann Cell

Grant Support:
Charcot-Marie-Tooth Association

NINDS/NCATS-ORD U54NS065712
Preclinical Gene Therapy Studies for FIG4/CMT4J and GARS/CMT2D.

Robert Burgess¹, Kathryn Morelli², Maximiliano Presa³, Laurent Bogdanik³, Rachel Bailey⁴, Steven Gray⁴, Nettie Pyne⁵, Lindsay Wallace⁵, Allison Fowler⁵, Scott Harper⁵, Cathleen Lutz³

¹The Jackson Laboratory, Bar Harbor, ME, USA, ²The Jackson Laboratory, The University of Maine, Bar Harbor, USA, ³The Jackson Laboratory, Bar Harbor, USA, ⁴University of Texas Southwestern, Dallas, USA, ⁵The Research Institute at Nationwide Children’s Hospital, Columbus, USA

Inherited peripheral neuropathies are candidates for gene therapy approaches, although the diversity of mutations will require a variety of strategies. Here, we report the results of two preclinical studies for Charcot-Marie-Tooth types 4J and 2D. In both studies, mouse models were dosed with adeno-associated virus 9 (AAV9) to deliver the gene therapy payload to peripheral neurons. For CMT4J, recessive loss-of-function mutations in mouse Fig4 were rescued by a codon-optimized human FIG4 gene. Treatment by intracerebroventricular (ICV) injection at postnatal day (PND) one or four resulted in survival up to a year, compared to a median lifespan of ~35 days in untreated mice. Treatment by intrathecal delivery at PND seven or eleven also increased life span (median ~100 days in PND7 treated mice). Motor neurons showed improved conduction velocity, decreased vacuolization in the ventral horn, and decreased axon loss in the femoral motor nerve. Treated mice showed grip strength and motor performance that closely approximated wild type littermates. Thus, this treatment is effective within the limited treatment window provided by the aggressive Fig4 mouse model.

To treat dominant mutations in GARS underlying CMT2D, we used AAV9-delivered RNAi specifically targeting mutant GARS transcripts. This induce allele-specific knockdown was tested in two mouse models of CMT2D, one of which is an engineered human disease allele. When delivered ICV at birth, this approach was able to almost completely prevent the neuropathy, which otherwise develops by three weeks-of-age. Body weight, grip strength, sciatic nerve conduction velocity and femoral nerve axon number were all indistinguishable from wild type littermates. However, treating after the onset of symptoms did not promote regeneration, and had limited benefit that went down quickly with age. Thus, our studies show that allele-specific knockdown is technically feasible and is efficacious for dominant mutation in GARS, but early treatment is needed for maximal benefit.

References: None.

Keywords: Pre-clinical Studies, Axonal Biology

Grant Support: Muscular Dystrophy Association, National Institutes of Health U54 OD020351, R21 NS105116, Talia Duff Foundation
Monday, June 24, 2019 - 17:45 - 18:00

Treatment of Arg98Cys MPZ Mice In Vitro and In Vivo with IFB088

Michael Shy¹, Yunhong Bai¹, Mason LaMarche¹, David Wang¹, Rosa Mastrangelo², Caroline Treins³, Philippe Guedat³, Maurizio D'Antonio², Michael Shy¹

¹University of Iowa, Carver College of Medicine, Iowa City, IA, USA, ²San Raffaele Scientific Institute, Milano, Italy, ³InFlectis BioScience, Nantes, France

**Objective:** To determine whether IFB088 provides therapeutic benefit for a second model of Myelin Protein Zero (MPZ) induced Charcot Marie Tooth 1B (CMT1B) in which the unfolded protein response (UPR) contributes to the neuropathy.

**Background:** Patients with Arg98Cys mutations in myelin protein zero (MPZ) develop a severe infantile onset CMT1B. Arg98Cys MPZ mice recapitulate the disease and have demonstrated that activation of the UPR contributes to the pathogenesis of the neuropathy. IFB088, a specific inhibitor of the Gadd34/Ppp1r15a phosphatase, successfully treated Ser63del MPZ mice by prolonging the P-eIF2a-mediated attenuation of protein synthesis. We hypothesized that similar treatment may prove effective with Arg98Cys MPZ animals.

**Methods:** Arg98CysMPZ/+ DRGs were treated with 50, 75, 100 and 125nM of IFB-088 and assessed for myelination. IFB088 or vehicle was fed by gavage BID to Arg98Cys MPZ or WT mice from P30 through P180 and evaluated clinically, physiologically and morphologically. Western blot and immunohistochemistry (IHC) were used to analyze expression of relevant proteins including transcription factors that regulate PNS myelination.

**Results:** Most concentrations IFB-088 increased myelination in MPZArg98Cys DRGs and at 100 and 125 nM, the number of myelinating internodes approached wild-type levels. Treated Arg98Cys/+ mice increased holding time on the accelerating rotarod, increased their grip strength and increased both motor and sensory conduction velocity. Morphological and molecular studies are ongoing.

**Conclusions:** These data demonstrate that IFB088 improved the phenotype of Arg98Cys MPZ CMT1B in vitro and in vivo. This suggests that IFB088 may prove beneficial in other cases of CMT1B involving UPR activation.

**References:** None.

**Keywords:** CMTR, Schwann Cell

**Grant Support:** Support from InFlectis BioScience
Charcot-Marie-Tooth disease (CMT) is a group of rare inherited peripheral neuropathies with no therapeutic cure to date. The most common type of CMT, CMT1A, is due to a large 1.4 Mb duplication on chromosome 17p11.2 encompassing the peripheral myelin protein 22 gene (PMP22) gene. It is widely accepted that the increased dosage of PMP22, an integral component of the Schwann cell myelin, is the main cause of CMT1A. PMP22 protein folds with low efficiency under normal conditions. Nearly 80% of newly synthesized PMP22 is rapidly degraded by the proteasome, and only a small proportion of PMP22 is fully matured and reaches the myelin sheath. The maintenance of correct protein homeostasis is tightly controlled by protein quality control mechanisms. When they fail, stress response pathways are activated leading to phosphorylation of the alpha subunit of eukaryotic translation initiation factor 2 (eIF2a) causing a reduction of global protein synthesis while allowing the translation of selected genes supporting stress recovery. By inhibiting eIF2a dephosphorylation, IFB-088 prolongs protein translation attenuation in response to stress to allow the cells to restore cellular homeostasis. We previously demonstrated that IFB-088 rescues phenotype in CMT1B mice with activated unfolded protein response. We hypothesized that in CMT1A the overproduction of PMP22 may overload the degradative system causing a failure of protein homeostasis. Here, we show that the P-eIF2a pathway is activated in the C3-PMP22 mice, validated animal model of CMT1A. IFB-088 treatment ameliorates motor, neurophysiological and morphological parameters of C3-PMP22 mice, confirming previous data generated in the CMT1A rat model. Through its mode of action, IFB-088, currently in phase I clinical trial, represents a new therapeutic option for CMT1A and has the unique potential to be effective in the treatment of different CMT subtypes.

References: None.

Keywords: Pre-clinical Studies, CMTR, Schwann Cell, Human Genetics

Grant Support: None.
TNC Oral Abstracts
Monday, June 24, 2019 - 15:30 - 17:30
Monday, June 24, 2019 - 15:30 - 15:40
Outcome Measures in the Assessment of Chemotherapy Induced Peripheral Neuropathy - Which Tools are Most Responsive?

Tiffany Li1, Hannah Timmins1, Michelle Harrison2, Lisa Horvath3, Michael Friedlander4, Siobhan O’Neill 4, Terry Trinh5, James McCravy6, David Goldstein6, Matthew Kiernan1

1University of Sydney, Sydney, Australia, 2Chris O’Brien Lifehouse, University of Sydney, Sydney, Australia, 3Prince of Wales Hospital, Sydney, Australia, 4Prince of Wales Clinical School, Sydney, Australia, 5Prince of Wales Hospital, University of New South Wales, Sydney, Australia

Introduction

Chemotherapy-induced peripheral neuropathy (CIPN) is a major dose-limiting side effect of many cancer treatments, often leading to dose reduction or treatment cessation. Although multiple outcome measures are utilised to quantify CIPN, there is disagreement on whether quantitative sensory outcomes or patient reported outcomes (PRO) are most sensitive at detecting change in CIPN symptoms. This study compared the responsiveness of quantitative sensory outcomes (Von Frey Monofilament (VF), Grating Orientation Task (GOT) and 2-Point Discrimination (2PD)) and PROs (FACT/GOG-NTX13 and CIPN20) to the extensively validated composite neurological grading scale, Total Neuropathy Score (TNSc).

Methods

237 patients (73 males; 54.6±12.8 years) receiving neurotoxic chemotherapies (taxanes, platinums, vinka-alkaloids, bortezomib, thalidomide) were prospectively assessed prior to receiving, and upon completion of treatment. Sensitivity to change for each outcome (TNSc, GOT, VF, 2PD, FACT/GOG-NTX13 and CIPN20) was calculated using Cohen’s d effect sizes (95% CI), with 0.2 representing minimal clinically important difference. Pearson’s correlation coefficients were used to compare outcomes to the TNSc.

Results

From baseline to follow-up assessment, participants exhibited increased neuropathy symptoms, evident by larger TNSc scores (mean difference=2.7±0.18, P<0.005), PRO report and declining performance on quantitative outcomes. Effect sizes for all outcomes were clinically significant, however PROs demonstrated the most responsiveness (FACT/GOG 1.23(1.01-1.44); CIPN20 1.24(1.03-1.46)), compared to quantitative outcomes (VF 0.21(0.02-0.41); 2PD 0.52(0.30-0.73); GOT 0.56(0.36-0.76)) and neurological grading scale (TNSc 1.18(0.96-1.40)). PROs were more strongly correlated to TNSc than quantitative outcomes (VF r=0.15; 2PD r=0.14; GOT r=0.21; FACT/GOG r=0.40; CIPN20 r=0.37), suggesting concurrent validity.

Discussion

These results demonstrate that PROs are valid and responsive measures of CIPN. Although quantitative sensory outcomes provide objective assessment of sensory dysfunction, use of PROs allow for a more global and sensitive measure of neuropathy. Further investigation in comparing psychometric properties of PROs will provide insight into the optimal assessment of CIPN.

References: None.

Keywords: Other

Grant Support: None.
Bortezomib Neuropathy: Clinical and Electrophysiological Features and Its Predictive Factor

Nagaaki Katoh, Akihiro Ueno, Nobuhiko Ohashi, Minori Kodaira, Yoshiki Sekijima
Shinshu University, Matsumoto, Japan

【Purpose】Bortezomib is one of the proteasome inhibitors which are now considered as a major treatment option for systemic light chain (AL) amyloidosis. However, its neurotoxic side effect has emerged as a problem because some bortezomib-treated patients develop subsequent peripheral polyneuropathy. The aim of this study is to clarify the clinical and electrophysiological features of bortezomib-induced neuropathy and to investigate the possible predictive factor of this side effect.

【Methods】All AL patients, except for those with amyloid neuropathy, who were treated with bortezomib-dexamethasone (BD) between Oct. 2012 and Apr. 2018 were enrolled. Clinical and electrophysiological findings of the patients with bortezomib-neuropathy were retrospectively investigated.

【Results】Among 40 enrolled patients, ten patients (25.0%) were found to develop bortezomib-neuropathy. Mean cumulative dose of bortezomib at neuropathy onset was 15.8 mg/m². Clinical presentation was length-dependent symmetrical sensory neuropathy without any motor-nerve symptom. Electrophysiological examination revealed sensory-nerve axonal involvement. Although this abnormal electrophysiological findings recovered within 6 to 12 months after treatment, clinical symptom remained in all patients (the number of patients with complete recover: 0, partial improvement: 7, no improvement: 3). Many clinical parameters were compared between patients who developed bortezomib-neuropathy and who did not, but no significant predictive factor was detected. Neuropathy development did not affect hematological response rate or overall survival.

【Conclusion】25% of bortezomib-treated AL patients developed sensory-nerve axonal neuropathy with mean cumulative dose of bortezomib 15.8 mg/m². There was no significant relationship between neuropathy development and treatment outcomes. It is important to pay attention to cumulative dose of bortezomib because neuropathy onset was unpredictable but clinical symptom was likely to remain.

References: None.

Keywords: Amyloidosis, Other

Grant Support: None.
Incidence and characteristics of neurological adverse events secondary to immunotherapy with checkpoint inhibitors

Roser Velasco¹, Jordi Bruna², Andreas Argyriou³, Roser Velasco⁴, Garifallia Anastopoulou⁵, Montse Alemany¹, Marta Simó¹, Josep Piulats⁶, Ernest Nadal⁶, Haralabos Kalofonos⁷

¹Unit of Neuro-Oncology, Hospital Universitari de Bellvitge-ICO L’Hospitalet-IDIBELL, Barcelona, Spain,
²Hospital Universitari de Bellvitge-ICO Hospitalet; Department of Cell Biology, Physiology and Immunology, UAB, Barcelona, Spain,
³Neurological Department, Saint Andrew’s General Hospital of Patras, Patras, Greece,
⁴Unit of Neuro-Oncology, Hospital Universitari de Bellvitge-ICO L’Hospitalet, Barcelona, Spain,
⁵Department of Medicine-Oncology Unit, Saint Andrew’s General Hospital of Patras, Patras, Greece,
⁶Department of Medical-Oncology, ICO L’Hospitalet, Barcelona, Spain,
⁷Department of Medicine, Division of Oncology, Medical School, University of Patras, Patras, Patras, Greece

Objective: To explore the incidence and clinical phenotype of serious neurological adverse events (NAEs) in cancer patients who received immunotherapy with checkpoint inhibitors (ICIs). Patients and methods: We reviewed files of cancer patients who were treated with ICIs from 2010 to 2018 and then searched for ICIs-related NAEs. Results: We identified 1185 ICIs-treated patients. Males and females were 63.7% and 36.3%, respectively, with a mean age of 63.4±7.3 years-old. Nivolumab was given to 536 (45.2%) patients, Pembrolizumab to 301 (25.4%) patients, Atezolizumab to 135 (11.4%), Ipilimumab to 104 (8.8%), Durvalumab-Tremelimumab to 77 (6.5%), and other ICIs to 32 (2.8%). Of those patients, 24 (2%) developed an ICI-related NAE. No differences were identified in age, sex, tumor type and class of ICIs between patients who developed neurotoxicity compared to those without neurologic adverse events. The distribution of NAE by agent was: Nivolumab (2.1%), Pembrolizumab (2%), Atezolizumab (1.5%), Ipilimumab (1%), Durvalumab-Tremelimumab (3.9%), and others (2.8%). The median number of cycles received before NAEs onset were 4.5 (1-10), and the median time was 110 days. PNS involvement was evident in 14 patients (58.3%) and CNS involvement in 41.7%, while 2 patients with aseptic meningitis also presented polyradicular involvement. Among PNS complications, there were 5 cases (20.8%) with axonal sensory neuropathies, 4 (16.7%) with Guillain-Barre-like syndromes, 4 (16.7%) with muscle involvement (myositis and myasthenias), and one with other syndromes. Seven patients (50%) with PNS-related NAEs were treated with steroids, 2 (14.3%) with IVIG, and 5 (35.7%) remained untreated. In general the outcome was good after ICIs discontinuation, with about half of patients improving or completely resolving NAEs. Conclusion: ICIs-related NAE although rare overall, might be severe, and are mostly represented by neuromuscular complications. Early discontinuation of ICIs and possibly treatment with immune-modulating therapies should early be commenced to achieve a favourable neurological outcome.

References: None.

Keywords: Other

Grant Support: None
Elevated Neurofilament Light Chain (NF-L) Levels in Pancreatic Cancer Patients with CIPN Receiving Abraxane
Catherine Stehman-Breen1, Thomas Engber1, Christine Alewine2
1Disarm Therapeutics, Cambridge, MA, USA, 2National Institute of Health; National Cancer Institute, Bethesda, MD, USA

Neurofilaments have gained significant attention as a biomarker of axonal injury. They are abundant structural scaffolding proteins expressed only in neurons and are critical for radial growth and the stability of axons, enabling effective nerve conduction. Mutations in genes that encode neurofilament proteins are associated with Charcot-Marie-Tooth disease type 2E or 1F, amyotrophic lateral sclerosis, and familial Parkinson disease.

Abnormal levels of NF-L in the CSF and blood reflect axonal damage in a variety of neurodegenerative, inflammatory, vascular and traumatic diseases. Because neurofilaments are specific for axonal injury, they offer significant advantages over other biomarkers. Ultra-sensitive assay technology (SIMOA) has allowed for the reliable quantification of NF-L levels in blood. Data in acquired neuropathies suggest that NF-L levels are higher than in controls. However, there are no data assessing NF-L levels in CIPN. Since axonal damage and loss is observed in CIPN, detecting and monitoring CIPN with a serum biomarker would be advantageous in identifying those at risk and monitoring and evaluating treatment.

We measured serum NF-L levels in 17 samples from 6 patients with previously treated metastatic and/or locally advanced pancreatic ductal adenocarcinoma and mesothelin expressing solid tumors who participated in an NCI sponsored trial where they received LMB-100, a mesothelin-targeted immunotoxin in combination with nab-paclitaxel. Patients received 125 mg/m2 abraxane chemotherapy on D1 and D8 of the 21 day cycle. One patient had received prior abraxane and all received oxaliplatin. Three subjects had CIPN at baseline and an additional subject had a prior history of CIPN. These 4 patients exhibited CIPN during treatment and had substantially greater increases in NF-L (115.2 pg/mL) than the 2 patients that did not develop CIPN (13.2 mg/mL). This is the first description of changes in NF-L in patients receiving neurotoxic chemotherapy. Additional studies should be conducted to confirm these findings.

References: None.

Keywords: Axonal Biology

Grant Support: NIH Center for Cancer Research Intramural Program
Monday, June 24, 2019 - 16:10 - 16:20
Asymmetry in Chemotherapy-induced Peripheral Neuropathy: Differences in patient report and objective assessment

Hannah Timmins¹, Tiffany Li¹, Matthew Kiernan¹, Sally Baron-Hay², Gavin Marx³, Josie Rutovitz³, David Goldstein⁴, Susanna Park¹

¹University of Sydney, Sydney, Australia, ²Royal North Shore Hospital, Department of Oncology, Sydney, Australia, ³Sydney Adventist Hospital, SAN Integrated Cancer Centre, University of Sydney, Sydney, Australia, ⁴Prince of Wales Clinical School, Sydney, Australia

Chemotherapy-induced peripheral neuropathy (CIPN) is a dose-limiting side-effect of cancer treatment, typically producing symmetrical, length-dependent sensory symptoms and functional impairment. However, patients may report asymmetric-symptoms and the degree of concurrence with objective neurological assessment remains ill-defined. This study aimed to compare reported symptoms and objective assessments.

45 taxane-treated patients (F=43, 66±1.5years, 24.3±2.4months post-treatment) completed bilateral neurological assessments via the Total Neuropathy Score clinical version (TNSc) and bilateral sensory nerve conduction studies (NCS; sural: lateral malleolus; median: digit2). Patients reported symptom severity via FACT/GOG-Ntx13 questionnaires and were asked to report symptom symmetry. The laterality index (LI) was calculated as a ratio of smaller to larger side-to-side differences in CSAP amplitudes and TNSc scores indicating asymmetry.

Symptoms of neuropathy were reported by 88% of the cohort. On clinical examination, 83% had ≥2 abnormalities, with 35% having sural amplitudes below normative range. 35% indicated side-to-side symptom asymmetry, with 53% reporting discrepancy in upper limbs and 66% in lower limbs. Patients reporting asymmetry reported greater symptom burden (mean FACT-score=39.8±1.3) than those with symmetrical distribution (41.5±1.4, p<.05), as well as greater asymmetry on neurological examination (TNSc LI Asym=.72±.08, Sym=.85±.03, p<.05). However, there was no significant asymmetry in TNSc scores once patient report items were removed (LI Asym=.60±.10, Sym=.76±.05, NS) and no difference in side-to-side NCS (Median LI:Asym=.69±.06, Sym=.81±.04, NS; Sural LI: Asym=.80±.04, Sym=.81±.04, NS).

Discrepancies in symptom severity between hands and feet were reported by 31% of the cohort, with more severe symptoms typically in the feet. Of those reporting discrepancy, 54% demonstrated increased objective severity of neuropathy on clinical examination, in-line with patient report.

Discrepancies may exist between the patient experience of CIPN and objective assessments. Understanding these discrepancies may help to elucidate underlying mechanisms and inform treatment strategies. Accurate and early assessment of neuropathy, including identifying symptom asymmetry, may also assist to identify patients at-risk of severe neuropathy.

References: None.

Keywords: Other

Grant Support: None.
Mitochondrial vacuolation occurs independent of axon degeneration in paclitaxel-induced peripheral neuropathy

Anthony Cirrincione, Sandra Rieger

University of Miami Department of Biology, Coral Gables, FL, USA

Treatment with the chemotherapeutic agent paclitaxel causes peripheral neuropathy in the majority of patients. The mechanisms underlying this process have remained elusive. Evidence suggests that paclitaxel damages mitochondria in the axons, which is consistent with observations in other neuropathy models. Whether mitochondrial dysfunction underlies axon degeneration has however not been explored in detail. To re-examine this, we used our larval zebrafish paclitaxel model in combination with transmission electron and confocal microscopy to study mitochondrial function. Quantitative image analyses in transgenic zebrafish expressing the hydrogen-peroxide sensor, HyPer, shows a stronger mitochondrial oxidation in keratinocytes upon paclitaxel treatment compared with controls, suggesting a defect in keratinocyte-specific mitochondria. Nevertheless, transmission electron microscopy analyses shows that similar to mammalian studies, also axonal mitochondria show signs of damage. Axonal mitochondria form vacuoles within 3 hours of treatment and these persist up to 4 days of treatment with paclitaxel, when axon degeneration has initiated. We previously demonstrated that paclitaxel treatment upregulates MMP-13 in the epidermis and that pharmacological inhibition of MMP-13 prevents paclitaxel neurotoxicity. We therefore tested whether axonal mitochondrial vacuolation can be prevented in the presence of the MMP-13 inhibitor, DB04760. We found that this is not the case. These results suggest that vacuolation may not play a role in axon degeneration given that 1) axonal mitochondrial vacuoles form rapidly, long before axon degeneration occurs, and they do not differ over time, 2) we previously established that keratinocyte damage due to MMP-13 activity is the primary cause of axon degeneration, however vacuoles do not form in this cell type, and 3) MMP-13 inhibition prevents axon degeneration but not vacuolation of axonal mitochondria.

References: None.

Keywords: Axonal Biology, Axonal Regeneration, Pre-clinical Studies, Pain, Small Fibers

Grant Support: 7R01CA215973-02
Molsidomine provides neuroprotection against vincristine-induced peripheral neurotoxicity

Francesco Lotti¹, Irina Utkina-Sosunova¹, Alessia Chiorazzi², Valentina Carozzi³, Annalisa Canta², Laura Monza², Paola Alberti², Giulia Fumagalli², Serge Przedborski³, Guido Cavaletti²

¹Department of Pathology & Cell Biology, Columbia University Medical Center, New York, NY, USA, ²Experimental Neurology Unit, School of Medicine and Surgery, University Milano-Bicocca, Monza, Italy, ³Experimental Neurology Unit, School of Medicine and Surgery, University Milano-Bicocca, Monza, Italy, ⁴Departments of Neurology, Pathology & Cell Biology, Columbia University Medical Center, New York, NY, USA

Peripheral neuropathy is the principal dose-limiting adverse reaction of the major frontline chemotherapeutic agents. Neuropathy can be so disabling that many patients will drop out of potentially curative therapy, negatively impacting cancer prognosis. Chemotherapy-induced peripheral neuropathy (CIPN) is refractory to treatment and persists in about 50% of cancer survivors limiting their quality of life.

Vincristine is highly active, but its use is limited by the severe neurotoxicity leading to autonomic, sensory and motor impairment. The hallmark of vincristine peripheral neurotoxicity is axonopathy. Yet the underpinning mechanisms of vincristine-induced axonopathy remains uncertain. We hypothesize that agents preventing vincristine-induced axonopathy will effectively mitigate CIPN symptoms, hence improving cancer treatment outcomes and survivors’ quality of life. Based on this premise, we developed a comprehensive drug discovery pipeline to identify small molecules with neuroprotective activity against vincristine-induced axon degeneration. Among the hits identified, SIN-1 – an active metabolite of molsidomine (Covaryl) – prevents VCR-induced axon loss in both motor and sensory neurons, and it does so without compromising vincristine anti-cancer potency.

To determine the neuroprotective effect of molsidomine, we used an extensively validated rat model of vincristine-induced peripheral neuropathy. Vincristine (0.2mg/kg/week) was administered via the tail vein for 4 weeks and molsidomine (10mg/kg/day or 20mg/kg/day) was administered orally for 4 weeks, starting the first day of vincristine treatment. As expected, the selected schedule of vincristine administration induced severe sensory and motor nerve damage. Importantly, vincristine-treated rats showed a significant decrease in the sensory threshold determined by the dynamic test that was prevented by molsidomine administration in a dose-dependent manner. A similar protective effect of molsidomine was evident on digital nerve amplitude and velocity.

This study provides preliminary evidence of the neuroprotective properties of molsidomine and opens the way to further investigations of this drug as a therapeutic agent to prevent vincristine-induced peripheral neuropathy.

References: None.

Keywords: Axonal Biology, Pre-clinical Studies, Pain, Axonal Regeneration

Grant Support:

This work is supported by the Thompson Family Foundation Initiative at Columbia University (TFFI) Innovation Award.
Effects of candesartan on mouse models of vincristine- and oxaliplatine-induced neuropathy

Hichem Bouchenaki¹, Flavien Bessaguet¹, Laurent Magy², Laurence Richard¹, Franck Sturtz¹, Alexis Desmoulière¹, Aurore Danigo¹, Claire Demiot¹

¹EA 6309 - Myelin Maintenance & Peripheral Neuropathy, Faculties of Medicine and Pharmacy, University of Limoges, Limoges, France, ²Department of Neurology, Reference Center for Rare Peripheral Neuropathies, University Hospital of Limoges, Limoges, France

Background: Neuropathic pain is the major dose-limiting effect of frequently-used chemotherapeutic agents such as vincristine (VCR) or oxaliplatine (OXP). We recently demonstrated that candesartan, an angiotensin II type 1 receptor antagonist, was neuroprotective against resiniferatoxin-induced sensory neuropathy and that this effect is mediated by stimulation of the angiotensin II type 2 receptor (AT2R). Thus, we chose to evaluate the effect of a preventive treatment by candesartan on mouse models of sensory neuropathy induced by VCR or by OXP.

Methods: VCR (100 µg/kg, intraperitoneally (i.p.)) was administered once per day for 7 days and OXP (15mg/kg, i.p.) was administered in male Swiss mice. Treatments with candesartan (0.5 mg/kg, i.p.) were started at day 1 before administration of the chemotherapeutic agent, then until day 7. Development of VCR/OXP-induced peripheral neuropathy and effect of treatments were evaluated by functional tests.

Results: Mice treated with VCR showed high mechanical allodynia but no modifications of motor performance or mechanical/thermal nociception. Mice treated with OXP showed mechanical allodynia and cold allodynia/hyperalgesia but no modifications of motor performance. While candesartan totally restored tactile sensitivity in VCR mice, it showed no effect on mechanical allodynia in OXP mice. Cold allodynia and cold hyperalgesia were partially prevented by candesartan in OXP mice during the first days following OXP injection.

Conclusion: Candesartan prevents mechanical allodynia induced by VCR but not mechanical allodynia induced by OXP. Moreover, candesartan seems to partially prevent cold allodynia and cold hyperalgesia induced by OXP. Our finding encourages evaluation of candesartan’s therapeutic potential in neuropathies induced by chemotherapeutic agents. Further investigations of differential mechanisms of chemotherapy-induced neurotoxicity is required for the discovery of new targets to tackle chemotherapy-induced neuropathic pain.

References: None.

Keywords: Pain, Pre-clinical Studies, Other

Grant Support: Hichem Bouchenaki was financially supported by Pharnext SA.
INC Oral Abstracts
Monday, June 24, 2019 - 16:00 - 16:30
Monday, June 24, 2019 -17:30 - 18:30
Outcomes after single-cycle rituximab in patients with anti-MAG polyneuropathy: an average eleven years follow-up analysis

Martina Garnero¹, Diego Franciotta², Chiara Brianì³, Chiara Demichelis⁴, Marina Grandis⁴, Valeria Prada⁴, Angelo Schenone⁴, Luana Benedetti⁴

¹Neurology Department, Ospedale Sanremo, ASL 1, Sanremo, Italy, ²Neuroimmunology Laboratory, IRCCS Mondino Foundation, Pavia, Pavia, Italy, ³Department of Neurosciences, University of Padova, Padova, Padova, Italy, ⁴DiNOGMI, University of Genova, IRCCS, Policlinico San Martino, Genova, Genova, Italy

Introduction. Several studies make rituximab the most efficacious drug in anti-myelin-associated glycoprotein (MAG) polyneuropathy.

Open questions still remain about the duration of the benefit of a single treatment course and how to repeat therapy in responsive patients.

We studied twenty-one patients (mean age, 74.7 years) with anti-MAG polyneuropathy, who improved after rituximab (375 mg/m², 4 consecutive weekly intravenous infusions), prospectively followed-up for an average of 11.2 years to assess the duration of clinical benefit and the best timing for retreatment.

Patients and Methods. We empirically decided not to perform maintenance therapies, but to treat the patients upon relapse, defined as a worsening by one point in at least two of the three scales (INCAT, MRC, ISS).

Duration of clinical benefit was related with the following parameters: age, sex, disease duration, scores of the clinical scales, anti-MAG antibody titres, and serum B-cell-activating factor (BAFF) levels pre-therapy.

Results. Clinical improvement after the first rituximab cycles lasted on average 6 years (range 2-12) and significantly correlated with pre-therapy lower scores of both the INCAT and ISS scales, lower serum anti-MAG antibody and BAFF levels. When the cohort of patients was split between those who relapsed after 2-3 years (1017.0 ± 117.2 pg/mL) vs those at 4 year, or later (627.9 ± 70.5 pg/mL) ROC curve analysis showed an AUC of 0.937 (95% CI, 0.810-1.065; p = 0.01), and a cut-off of 860 pg/mL (likelihood ratio, 12.0).

In conclusion our data indicate that, after an initial rituximab cycle, clinical improvements can last for on average 6 years. One of the most interesting pre-therapy predictors of clinical response is lower serum BAFF level; the cut-off value of 860 pg/mL might be useful to identify patients with poor response to treatment. Further studies on larger series of patients are needed to validate our findings and cut-off.

References: None.

Keywords: Inflammatory

Grant Support: None.
One year closer to clinical trials with a new antigen specific treatment for anti-MAG neuropathy

Pascal Häneggi¹, Butrint Aliu¹, Lijuan Pang¹, Delphine Demeestere¹, Andreas Steck², Beat Ernst¹, Ruben Herrendorff³

¹ University of Basel, Basel, Switzerland, ² University Hospital Basel, Basel, Switzerland, ³ Polyneuron Pharmaceuticals AG, Basel, Switzerland

Anti-myelin-associated glycoprotein (MAG) neuropathy is a rare and disabling autoimmune disorder affecting the peripheral nervous system. The pathogenicity of anti-MAG IgM autoantibodies that target the HNK-1 glycoepitope is well established. Patients suffer from severe symptoms including sensorimotor deficits, ataxia, paraesthesia and tremor. Nowadays, there are only off-label treatments with limited efficacy available. We have previously shown that the new antigen-specific drug candidate PPSGG efficiently depletes pathogenic anti-MAG IgM in vivo and abrogates the reactivity of patients’ antibodies to MAG on sciatic nerves ex vivo.

We recently performed a more in-depth assessment of PPSGG addressing the selectivity of anti-MAG IgM binding, the characteristics of the drug-antibody interaction, as well as the pharmacokinetic and metabolic properties. These studies enabled first dosing assumptions for clinical trials. Moving forward to its clinical evaluation, we performed safety pharmacology studies in rats and dogs with PPSGG.

Consistent with the data from a mouse model, we showed that PPSGG and the anti-MAG IgM:PPSGG complex was taken up actively into human macrophages (THP-1 cell line) and other cell types of the human mononuclear phagocyte system by phagocytosis. We demonstrated a 1:1 or 1:2 stoichiometry of the PPSGG:anti-MAG IgM binding by analytical ultra-centrifugation and size exclusion chromatography. In the dose assumption study, we determined the dose of PPSGG that is required to deplete specific anti-MAG IgM levels. The extrapolation indicates that a single dose of 5 mg/kg PPSGG is expected to remove a significant proportion of anti-MAG IgM in patients. In a dose range finding toxicity study, PPSGG showed a favorable safety profile, even at elevated dose levels. In dogs, multiple dosing of 200 mg/kg did not cause any drug related side effects.

Taken together, we are convinced that PPSGG is a very promising drug candidate and will be further developed for clinical trials in anti-MAG neuropathy.


Keywords: Inflammatory, Clinical Trials, Other

Grant Support: None.
POEMS syndrome: characterization of neuropathy and post-treatment outcome in 36 patients

Nathalie DESCHAMPS¹, Adeline NOT², Cécile CAUQUIL², Guillemette BEAUDONNET², Andoni ECHANIZ-LAGUNA², David ADAMS²

¹CHU Bicêtre, CHU Pointe à Pitre, Kremlin Bicetre, France; ²CHU Bicêtre, Kremlin Bicetre, France

Introduction

Polyneuropathy in POEMS syndrome (Polyneuropathy, organomegaly, endocrinopathy, monoclonal protein and skin change) has been rarely described in detail. Here, we describe the characteristics of neuropathy in a large series of patients, and try to identify prognostic factors.

Methods

In this monocentric retrospective study, we analyzed the clinical and neurophysiological characteristics (at diagnosis, 6 months and 1 year follow-up) of patients with POEMS syndrome diagnosed in our University Hospital between 1980 and 2017. The primary end-point was the Modified Rankin Scale (mRS) score.

Results

36 patients (mean age at diagnosis: 54 years; 58% male) with POEMS syndrome were analyzed. Hematologic diagnosis was monoclonal gammapathy of unknown significance (MGUS) in 28% of cases, solitary plasmocytoma (25%), myeloma (19%), and multiple plasmocytoma (14%). Diagnosis was delayed up to 12 months after symptom onset in 75% of cases. The mean mRS at diagnosis was 2.61 +/- 1.13. The neuropathy was clinically and electrically sensorimotor, length-dependent and severe (56% of patients had mRS ≥ 3). Nerve conduction studies showed a mixed axonal and demyelinating pattern (44% of cases), a pure axonal pattern (25%), or pure demyelinating features (28%). 39% (n=14) of patients were treated with autologous stem-cell transplantation (ASCT) and 33% had radiotherapy. At 1 year follow-up, 33% of patients had improved, 36% were stabilized, and 14% worsened. Age over 65 years and weight loss were of poor prognosis (p=0.0483 and p=0.0383). Patients treated with ASCT improved in 54% of cases whereas patients treated with radiotherapy improved in 33% of cases. ASCT was associated with good prognosis (OR=7, IC95 1.14-42.97, p=0.0253).

Conclusion

POEMS syndrome polyneuropathy is severe, rapidly progressing, length-dependent, with both axonal and demyelinating electrophysiological features in a majority of cases. Age over 65, weight loss are of poor prognosis whereas ASCT is associated with a good outcome.
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Keywords: Axonal Regeneration, Schwann Cell, Pain

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**Misdiagnosis and Diagnostic Pitfalls of CIDP**

Merel Broers¹, Carina Bunschoten¹, Tiago Beck¹, Hester Lingsma², Jeffrey Allen³, Richard Lewis⁴, Esther Brusse¹, Judith Drenthen⁵, Pieter van Doorn¹, Bart Jacobs⁶

¹Department of Neurology, Erasmus MC, University Medical Center Rotterdam, The Netherlands, ²Department of Public Health, Erasmus MC, University Medical Center Rotterdam, The Netherlands, ³Department of Neurology, University of Minnesota, Minneapolis, Minneapolis, MN, USA, ⁴Department of Neurology, Cedars-Sinai Medical Center, Los Angeles, Los Angeles, CA, USA, ⁵Department of Clinical Neuropathology, Erasmus MC, University Medical Center Rotterdam, The Netherlands, Rotterdam, Netherlands, ⁶Department of Neurology and Immunology, Erasmus MC, University Medical Center Rotterdam, The Netherlands, Rotterdam, Netherlands

**Purpose:** The diagnosis of chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is challenged by the disease rarity, heterogeneity and differential diagnosis. Misdiagnosis of CIDP is a common problem in the United States of America. We aim to determine the extent of misdiagnosis, including over- and underdiagnosis, and to identify pitfalls in the diagnosis of CIDP in the Netherlands.

**Methods:** We retrospectively included all tertiary patient referrals to our university hospital between April 2011 and March 2017 with CIDP, or another initial diagnosis that we revised into CIDP. Final diagnosis in all patients was made after an extensive discussion in consensus meetings. Overdiagnosis was defined as initial incorrectly diagnosed with CIDP. Underdiagnosis was defined as initial missed CIDP diagnosis.

**Results:** Of 297 patients screened, 113 patients met the inclusion criteria. Preliminary results are based on the first 38 patients, including 33 patients initially diagnosed with CIDP and 5 patients with another initial diagnosis revised into CIDP. Overdiagnosis was observed in 10/33 (30%) patients, of whom 5 received immunomodulatory treatment before referral. The diagnosis was changed to axonal polyneuropathy (n=4), IgM anti-MAG neuropathy (n=1), CANOMAD (n=1), anti-Hu related polyneuropathy (n=1), hereditary polyneuropathy (n=1), sensory neuropathy (n=1) or polyneuropathy not demonstrated (n=1). Identified pitfalls were absence of proximal weakness, cytoalbuminologic dissociation, presence of serum antibodies associated with polyneuropathy, and NCS classified as axonal polyneuropathy. Underdiagnosed patients (5/28) were referred with possible entrapment neuropathy (n=1), Guillain-Barré syndrome (n=1), spinal muscular atrophy (n=1), paraproteinemic polyneuropathy (n=1) or polyneuropathy not further specified (n=1). The diagnostic pitfall in the underdiagnosis group was an atypical clinical presentation.

**Conclusion:** Based on these preliminary results, misdiagnosis of CIDP is also common in the Netherlands and includes both over- and underdiagnosis. The results of the full cohort (n=113), will be

**References:** None.

**Keywords:** Inflammatory

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Immunomodulatory effects of bortezomib in experimental autoimmune neuritis in lewis rats.

Rafael Klimas¹, Melissa Sgodzai¹, Nuwin Mohamad¹, Xiomara Pedreiturria¹, Jeremias Motte¹, Min-Suk Yoon², Ralf Gold¹, Kalliopi Pitarokoili¹

¹Ruhr-University Bochum, St. Joseph University Hospital Bochum, Bochum, Germany, ²Ruhr-University Bochum, Ev. Augusta Hospital Hattingen, Hattingen, Germany

Introduction: In a case series of ten treatment refractory chronic inflammatory demyelinating polyradiculopathy (CIDP) patients Bortezomib (BTZ) was able to stop disease progression when administered subcutaneously 1.3 mg/m2 KOF at day 1, 4, 7 and 10. The exact mechanisms of action remain unknown while the dose-limiting side-effect of Bortezomib is an axonal polyneuropathy. Methods: To determine the minimum concentration inducing polyneuropathy we treated female Lewis rats with BTZ (0.05, 0.2 mg/kg) intraperitoneally (i.p.) and the control group with 5% DMSO. Subsequently, EAN was induced in 6 weeks old rats by P2-peptide (n=12/group). One group was treated with 0.05mg/kg BTZ i.p, another group was treated with 0.1mg/kg BTZ i.p. A control group received 5% DMSO at day 9, 13, 17 and 21. Von Frey hair-(vFH) and hot plate test were performed at day 10, 14, 18 and 22. Clinical score and weight were assessed daily. Electrophysiological analyses of sciatic nerve were performed at day 0 and 23. At day 23, we performed flow cytometric analyses of the immune cells in spleen, lymph nodes, blood, and bone marrow. Subsequently, sciatic nerve and bone marrow tissue were analyzed by immunofluorescence stainings. Analyses of mRNA expression were performed on sciatic nerve. Results: Treatment with 0.05 mg/kg BTZ i.p. improved EAN significantly, reduced inflammatory infiltrates and demyelination in the sciatic nerves and ameliorated electrophysiological neuritis signs without inducing axonal neuropathy. Furthermore, BTZ improved vFH and hot plate test. Flow cytometric analyses showed a reduction of CD4+, CD11b+, IgK+ cell populations in several lymphoid organs. Conclusions: We revealed for the first time the immunomodulatory effects of bortezomib in EAN. It improves clinical EAN score, electrophysiological signs of large myelinated axons and small-fiber function in vFH test, without causing neuropathy as a side-effect. The proposed relevant immunological mechanism is the modification of dendritic and B-cell populations.

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Keywords:Inflammatory, Axonal Regeneration, Small Fibers

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Sensory neuronopathies (SNN) are rare diseases of the peripheral nerve system. Although being often classified as idiopathic, many cases are associated with immune-mediated diseases, suggesting an active involvement of the immune system. Indeed, we have reported that an antibody reacting with the intracellular domain of the Fibroblast Growth Factor Receptor 3 (FGFR3) identifies a subgroup of patients with SNN. Here we report the results of a prospective, multicentric and international study in which 42 anti-FGFR3-positive SNN patients were identified in one Brazilian center and in 35 European centers. The incidence of the anti-FGFR3 antibodies was higher in SNN Brazilian patients than in European ones (15/42 (35.7%) vs 27/211 (12.8%), p = 0.0003). When we compared the clinical and paraclinical patterns of the anti-FGFR3-positive SNN cases from Brazil and Europe, we found that Brazilian cases were younger at the onset of the neuropathy (median age 44 (39-50) vs. 61 (50-68), p=0.0006) and were more frequently associated with other autoimmune diseases than in the European cases, particularly regarding the frequency of Sjögren syndrome (46.7% vs. 3.8%, p=0.001). The distribution of sensory symptoms among the Brazilian anti-FGFR3-positive patients was more frequently asymmetrical (93% vs. 24%, p<0.0001; odd ratio 169, 3.4-8424 95% confidence interval, p=0.01), proximal (73% vs. 26%, p=0.003), and affecting the upper limbs (100% vs. 52%; p=0.001). After several years of evolution, paresthesia (100% vs. 67%, p=0.01), ataxia in upper limbs (73% vs. 30%, p=0.0001), global areflexia (80% vs. 26%, p=0.001) and dysautonomia (67% vs. 9%, p=0.0002) were more frequent in Brazilians than in Europeans. Overall, our prospective and international study identified a striking difference in the prevalence of anti-FGFR3 antibodies and in the clinical and paraclinical pattern of anti-FGFR3-positive SNN patients from Brazil and Europe which suggests that environmental or genetic factors may lead to an autoimmune reaction against FGFR3.

References:


Keywords: Inflammatory, Clinical Trials, Other, Other

Grant Support: None.
Antibodies directed against peripheral neurons, Schwann cells and myelin are frequently found in Zika-exposed subjects

Simon Rinaldi¹, Alexander Davies¹, Cinta Lleixà², Ana Siles³, Carolina Ramírez Santana³, Juan-Manuel Anaya³, Claudia Romero-Vivas⁴, Andrew Falconar⁵, Lyda Osorio⁶, Beatriz Parra⁶, Carlos Pardo⁷, Susan Halstead⁸, Hugh Willison⁸, Luis Querol²

¹University of Oxford, Oxford, United Kingdom of Great Britain and Northern Ireland, ²Hospital de la Santa Creu i Sant Pau, Barcelona, Spain, ³Universidad del Rosario, Bogotá, Colombia, ⁴clromero@uninorte.edu.co, Barranquilla, Colombia, ⁵London School of Health and Tropical Medicine, London, United Kingdom of Great Britain and Northern Ireland, ⁶Universidad del Valle, Cali, Colombia, ⁷Johns Hopkins, Baltimore, MD, USA, ⁸University of Glasgow, Glasgow, United Kingdom of Great Britain and Northern Ireland

Introduction: Following the Zika outbreak in French Polynesia, a spike in Guillain-Barré syndrome (GBS) diagnoses was observed in the region. By the time the epidemic reached South America, it had been established that 1 excess GBS case occurred for every 4000 people infected. Whether Zika-GBS results from autoimmunity or direct viral toxicity remains unclear. This is important to establish, however, not only to aid diagnosis and direct appropriate treatment of this complication, but also for the development of safe Zika virus vaccines. Methods: Serum samples were obtained within Colombia from 53 patients who developed a neurological syndrome following Zika infection (43 GBS, 10 other), 74 subjects with uncomplicated Zika, and 38 non-Zika exposed controls. Some samples were collected several months after the acute event. Sera were tested for IgG and IgM reactivity against human-induced-pluripotent-stem-cell derived peripheral myelinating co-cultures, primary Schwann cells and dorsal root ganglion (DRG) neurons. The antigen target(s) of sera with distinct binding patterns were then sought using immunoprecipitation and mass spectrometry, with potential candidates further evaluated by ELISA and/or transfected-cell-based assays. Co-cultures were also used to assess the ability of selected sera to induce demyelination. Results: Overall, 16 /43 (37.2%) patients with Zika-GBS reacted moderately or strongly against primary DRG or Schwann cells, whereas only 2 (2.7%) uncomplicated Zika subjects and 4 (10.5%) controls reacted moderately. These differences were statistically significant. IgM binding to myelinating co-cultures was frequently detected with both Zika-GBS and infectious control sera. IgG reactivity was infrequent, but significantly more common with Zika-GBS patients’ sera compared to controls. Reactivity against the abaxonal membrane of myelinating Schwann cells was particularly notable. Serum from one uncomplicated Zika subject with this pattern of reactivity induced complement-dependent demyelination on exposure to the co-culture. Conclusion: Antibodies targeting peripheral nerve structures are common after Zika infection and can induce demyelination.

References: None.

Keywords: Inflammatory, Schwann Cell, Other

Grant Support: None.
Chronic Inflammatory Demyelinating Polyneuropathy (CIDP) Associated With Sarcoidosis Or Connective Tissue Disease.
Clément Vialatte de Pémille¹, Nicolas Noël², David Adams³, Cécile Cauquil³

¹Service de neurologie CHU de Bicêtre Paris, Service de neurologie Hôpital Saint Joseph Paris, Paris, France, ²Service de médecine interne CHU de Bicêtre Paris, Paris, France, ³Service de neurologie CHU de Bicêtre Paris, Centre de référence des neuropathies amyloïdes familiales CHU de Bicêtre, Paris, France
Introduction:

CIDP are heterogeneous pathologies. Diagnosis can be challenging because of atypical presentations (CIDP-chameleons) and differential diagnoses (CIDP-mimicking) to exclude. Neurosarcoïdosis (NS) and connective tissue diseases (CTD) are rarely associated with CIDP. We analysed the presentations of CIDP associated with these diseases and highlight the helpful “red flags”.

Methods:

We performed a retrospective study by analysing patients with NS (group S) and CTD (group C) fulfilling EFNS-PNS CIDP diagnosis criteria and compared them with patients with idiopathic CIDP (group I). Zajicek criteria were used for NS diagnosis and ACR criteria for CTD.

Results:

Forty-four patients were included (median age 60 yo, 23 women): 17 in group I, 16 in group S and 11 in group C. NS diagnosis, according to Zajicek criteria, was definite in 9 cases (granuloma within peripheral nerve tissue), probable in 6 cases and possible in 1 case. Among CTD diagnosis there were 7 Gougerot-Sjogren syndrome (GSS), 1 systemic lupus erythematos (SLE), 2 SLE with GSS and 2 mixed connective tissue diseases. CIDP diagnosis was definite in 57.1% in group S and 54.5% in group C. General status was significantly (p<0.05) altered in both groups, C and S, when compared to group I. Subacute onset (78.6% vs 5.9%) and unresponsiveness to treatment by Intravenous Immunoglobulins (IVIg) (14.3% vs 82.4%) were significantly observed in group S when compared to group I. Interestingly, there were more motor conduction blocs in group S compared to group I (46.6% and 83.3% respectively, p=0.12). CSF and histological analyses showed no differences except for the presence of granulomas.

Discussion:

Our results confirm and enhance the red flags of CIDP diagnosis. Altered general status, subacute onset or unresponsiveness to IVIG are observed in patients with CIDP and NS or CTD. Motor conduction blocs can be found in peripheral NS mimicking CIDP.

References: None.
Keywords: Inflammatory

Grant Support: None.
Holistic Characterization of the Repertoire of Targeted Autoantigens of Chronic Inflammatory Demyelinating Polyneuropathy (CIDP)

Christian Moritz¹, Oda Stoevesandt², Yannick Tholance³, Carole Rosier⁴, Karine Ferraud⁴, Jean-Philippe Camdessanché³, Jean-Christophe Antoine³

¹Institut NeuroMyoGene INSERM U1217/CNRS UMR 5310 at the University of Lyon, Saint-Étienne, France, ²Cambridge Protein Arrays Ltd., Cambridge, United Kingdom of Great Britain and Northern Ireland, ³University Hospital of Saint-Étienne, Institut NeuroMyoGene INSERM U1217/CNRS UMR of the University of Lyon, Saint-Étienne, France, ⁴Institut NeuroMyoGene INSERM U1217/CNRS UMR of the University of Lyon, Saint-Étienne, France

Chronic inflammatory demyelinating polyneuropathy (CIDP) is a rare disease of the peripheral nervous system. Patients suffer from progressively increasing limb weakness, ataxia, absent or diminished tendon reflexes, and paresthesia. The disease is considered as having an autoimmune background, hence the identification of autoantibodies has been a focus of research in recent years. Identifying corresponding autoantigens would improve the diagnosis, treatment, and understanding of the disease, but, recent studies failed to identify an antigen for the majority of classical CIDP patients. We screened sera from 50 CIDP patients with an improved immunoblotting-based technique.[1] Based on the resulting immunological fingerprints, the sera from 22 classical CIDP patients were selected and their set of targeted autoantigens profiled via protein arrays, containing about 16,000 different bait proteins. As controls, 12 other neurological diseases and 9 healthy controls were profiled in parallel. A patient with Sjögren syndrome manifesting the known autoantibodies anti-Ro/SSA/Trove2 and anti-SS-B, as well as an autoimmune hepatitis patient manifesting anti-SLA/SEPSECS, served successfully as positive controls. Rather than addressing single antigens, we have developed a novel idea of studying the total repertoire of antigens in a more holistic way. The size of the antigen repertoire appears to depend on the clinical situation; e.g. CIDP patients responsive to intravenous immunoglobulin therapy targeted three times more autoantigens than non-responders. Bioinformatic analyses of the repertoires as a whole revealed that a significant part of the autoantibody set specifically targets neuronal disease-related antigens as well as proteins involved in glycoprotein metabolic processes. Our project helps to understand the role of autoantibodies in CIDP patients by addressing the set of autoantigens holistically.


Keywords: Inflammatory, Pain

Grant Support: German Research Foundation (DFG; MO 3240/1-1:1), CSL Behring (Fonds de dotation)
IDNC Oral Poster
Monday, June 24, 2019 - 17:00 - 17:25
Tale of two states: A comparison study of baseline health data of neuropathy patients

Mamatha Pasnoor¹, Gordon Smith², Patricia Kluding¹, Laura Herbelin¹, Robin Marcus³, Cathy Revere³, Alexis Hawks¹, Mazen Dimachkie¹, Richard Barohn¹, Robinson Singleton³

¹The University of Kansas Medical Center, Kansas City, KS, USA, ²Virginia Commonwealth University, Richmond, VA, USA, ³The University of UTAH Health, Salt Lake City, UT, USA

Introduction: Utah ranked 5th in the United Health Foundation 2018 America's health rankings whereas Kansas is ranked 27th. We compared the baseline data on the diabetic peripheral neuropathy (DPN) patients in the Activity for Diabetic Polyneuropathy (ADAPT) study.

Objective: Compare the baseline health data of DPN patients between the two states.

Methods: Data collected included demographic, weight, body mass index (BMI), glycosylated hemoglobin (HgbA1C), lipid profile, activity information (number of steps), Norfolk Quality of Life - Diabetic Neuropathy (N-QOL), cholesterol, skin biopsy, Utah Early Neuropathy Scale (UENS), nerve conduction studies and 6-minute walk distance.

Results: Total number of subjects screened at Kansas and Utah was 95 and 67 respectively. There was no significant difference in the mean age 62.6 ± 7.5 years and 62.4 ±10.5 years (p=0.89). Mean body mass index (BMI) was not different 34.9± 8.8 and 33.4±5.9 (p=0.22). Significant difference was seen in HgbA1C (p=0.005) and high-density lipoprotein- cholesterol (HDL-C) (p=0.002) with higher mean HgbA1C and lower HDL-C in the Kansas DPN patients. There was no significant difference in LDL (p=0.83) and triglycerides (TG) (p=0.88). The Utah Early Neuropathy Scale (UENS) and distal thigh IENF density did not show significant difference (p=0.63; p=0.67). Mean N-QOL was significantly different (11.0±10.9 vs 16.8±11.7; p=0.001). Sural amplitude, peroneal distal latency (DL) and conduction velocity (CV) showed significant difference (p<0.0001, p=0.038, 0.017). The activity level and fitness measured by step count and 6-minute walk total distance was higher in Utah compared to Kansas (p=0.02, p<0.0001). VO2max was higher in Utah than Kansas (mean 17.4± 5.5 vs. 15.8±5.0, p=0.05).

Conclusions: Baseline significant differences were seen in hgbA1C, HDL, sural amplitude, peroneal CV, total step count and VO2max indicating Utah has a healthier population compared to Kansas.

References: None.

Keywords: Diabetes, Clinical Trials, Pain

Grant Support: NIH/ NIDDK: R01 DK064814
Monday, June 24, 2019 - 17:05 - 17:10
Expression of GAP-43 in type 2 diabetes and IGT: a longitudinal study
Xin Pan¹, Baohan Pan¹, Yanning Shou¹, Krish Chandrasekaran², Lindsay Zilliox², Neda Ilieva², James Russell², Michael Polydefkis¹

¹Johns Hopkins School of Medicine, Baltimore, MD, USA. ²University of Maryland, Baltimore, MD, USA

Diabetes is a common cause of peripheral neuropathy and loss of intraepidermal nerve fiber density (IENFD) is described in impaired glucose tolerance (IGT) and DM2. Here, we examined GAP-43 expression and its relation to IENFD among newly-diagnosed, well-controlled DM2 (N=22), IGT (N=15) and age/gender matched controls (N=30) in a 12 month longitudinal study. 3mm skin biopsies were obtained at the distal leg (DL) and proximal thigh (PT) at baseline and after 12 months. Immunohistochemistry (IHC) for GAP-43 and PGP9.5 were performed. Double IHC assessed co-localization. Subepidermal nerve fiber density (SENFD) of GAP-43+ fibers was assessed using a validated, unbiased stereology protocol (CE<0.1). IENFD was determined with PGP and GAP-43.

GAP-43 and PGP were co-expressed in most nerve fibers. DM2 and IGT subjects had similar age, gender and MNSI scores. Both had excellent A1c values (6.6±0.2% vs. 5.8±0.1%, p<0.05); TG levels were elevated in DM2 vs. IGT (124±13 vs. 85±13 mg/dl, p<0.05). IENFD-PGP and GAP43-IENFD were similar in IGT and DM subjects and significantly reduced compared to controls at both sites.

At 12 month, DM2 (N=11) and IGT (N=6) showed similar patterns. Combining the groups, IENFD-PGP remained reduced at DL and increased (0.85±0.38 fibers/mm, p=0.04) at PT. Proximal thigh SENFD-GAP43 (p=0.01) and IENFD-GAP43 (p=0.10) levels increased at 12-months vs. baseline, while DL SENFD-GAP43 (p<0.01) values increased but DL IENFD-GAP43 did not.

Cutaneous nerve GAP43 expression is dynamic in IGT/early well-controlled DM2. Surprisingly, GAP43 expression increased at 12-months to a similar degree in both IGT and DM2 subjects, approaching control subject levels. This was associated with a small but significant increase in IENFD at the proximal thigh. These results suggest that increased GAP43 expression can be associated with IENFD increases in the setting of well controlled early-onset diabetes.

References: None.

Keywords: Diabetes, Small Fibers, Axonal Regeneration, Axonal Biology, Metabolic

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Genome-wide DNA Methylation Profiling Identifies Epigenetic Clues into Human Peripheral Neuropathy in Type 2 Diabetes

Stephanie Eid¹, Kai Guo², Sarah ElZinga¹, Claudia Figueroa-Romero¹, Lucy Hinder¹, Crystal Pacut¹, Junguk Hur³, Eva Feldman¹

¹University of Michigan, Ann Arbor, MI, USA, ²University of North Dakota, Grand Forks, ND, USA, ³University of North Dakota, Grand Forks, MI, USA

Diabetic peripheral neuropathy (DPN) is one of the most common complications of diabetes worldwide, affecting up to 60% of subjects with diabetes. Emerging evidence suggests that aberrant DNA methylation is implicated in the pathogenesis of diabetes and diabetic complications, but its role in DPN is not well understood. In this study, we aimed to examine the association between DNA methylation and DPN progression in type 2 diabetes.

Using reduced-representation bisulfite sequencing, we performed genome-wide DNA methylation profiling of 12 human sural nerve samples from subjects with type 2 diabetes and DPN. These subjects were divided into two groups based on changes in sural nerve myelinated fiber density over a 52-week period: subjects with significant nerve regeneration (regenerators) and subjects with significant nerve degeneration (degenerators).

Between the two groups, we identified 3,460 differentially methylated CpG dinucleotides and 246 differentially methylated regions. The genes associated with differentially methylated CpGs were highly enriched in biological processes such as nervous system development, neuron development, and axon guidance, as well as glycerophospholipid metabolism and mitogen-activated protein kinase (MAPK) signaling.

To our knowledge, this is the first study to identify changes in sural nerve methylome associated with myelinated nerve fiber regeneration and degeneration in type 2 diabetes. Our results suggesting that pathophysiological pathways known to be implicated in DPN pathogenesis may be under epigenetic control will facilitate the development of new mechanism-based therapies.

References: None.

Keywords: Diabetes, Axonal Regeneration

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Regeneration of cutaneous unmyelinated axons has been shown to be reduced in subjects with diabetes compared to healthy controls. However, it is not clear if the regeneration of sensory distal axons ever fully recovers in diabetic subjects if given sufficient time, or if there are regeneration differences between type I and type II diabetes.

Here we measured the rate of axonal regeneration 6 months after chemical denervation using a capsaicin model in 47 diabetic patients (N=11 T1, N=36 T2) without neuropathy. Punch skin biopsies (thigh) were obtained at baseline, 48hrs after applying capsaicin, and then at 30, 90, 150 and 180 days. Blood glucose level and HgbA1C were serially measured. Comparisons between groups were made using analysis of variance and linear regression models. The effect of other covariates (height, weight, BMI, HbA1C, and cholesterol) was assessed through multiple linear regression and the rate of regeneration with time was estimated using a linear mixed-effects model.

Sural responses, blood glucose level, hypercholesterolemia status, and HbA1C were similar between patients with type 1 and type 2 diabetes at baseline and subsequent measurements. After six months, iENFD levels in both type 1 and type 2 diabetic patients remained significantly below baseline levels, with type 1 patients 5.2 [95% CI, 2.1-8.3] fibers/mm below baseline levels and type 2 patients 8.6 [95% CI, 6.8-10.4] fibers/mm below. Patients with type 1 attained a significantly higher percentage of baseline levels (80% versus 60%, \( p=0.01 \)) than type 2 subjects.

While we found significant differences between study groups in age, weight, height, and BMI, we found no significant effect for any of these covariates on absolute regeneration amount, percent of baseline regeneration, or rate of regeneration, when controlling for diabetes type. These results suggest that the long-term outcome of intraepidermal nerve fiber regeneration is affected by the type of diabetes.

References: None.

Keywords: Axonal Regeneration, Diabetes, Small Fibers

Grant Support: None.
Epidermal Axon Changes in Patients with Prediabetes: The PACMAN Study

Dan Elliott, Michelle Vitztum, Janelle Ryals, Patricia Kluding, Mamatha Pasnoor, Doug Wright
University of Kansas Medical Center, Kansas City, KS, USA

Prediabetic and diabetic patients often complain of debilitating neuropathic pain. However, not everyone with prediabetes or diabetes develops pain and the predisposing risk factors to painful neuropathy remain elusive. The epidermis receives dual innervation via peptidergic and nonpeptidergic axons and our previous studies in mice show that a high fat diet induces mechanical allodynia and increases peptidergic epidermal axons. We hypothesize that patients with prediabetes and painful neuropathy have an abnormal proportion of epidermal peptidergic axons. In our ongoing clinical trial, patients are recruited into 3 groups, dependent on their metabolic status: 1) normal (n=6), 2) prediabetes (n=7), or 3) prediabetes with neuropathic symptoms (n=5). Metabolic status (healthy vs. prediabetic) is determined using ranges established by the 2016 American Diabetes Association (ADA) Guidelines. Assignment to prediabetic category requires lab values within each of the following three ranges: A1c (5.7-6.4%), fasting glucose (100-125 mg/dl), or oral glucose tolerance (140-199 mg/dl). Data (vitals, demographic, social/lifestyle, medical history) is collected, and fasting patients undergo a blood draw (insulin, lipid panel, hematocrit, hemoglobin, and HBA1c) and a glucose tolerance test and to identify their metabolic status. In a second visit, patients undergo a clinical evaluation of their sensory status (Brief Pain Inventory for Diabetic Neuropathy (BPI-DPN), Michigan Neuropathy Screening Instrument, Utah Early Neuropathy Scale) and an ankle skin biopsy is obtained to quantify intraepidermal nerve fiber density (IENFD). PGP 9.5 is used to identify all axons, and TrkA+ fibers are used to identify peptidergic axons. Our analysis will compare differences in peptidergic fiber measures to neuropathy symptoms among the 3 groups. Our goal is to identify risk factors that modify peripheral innervation and increase the risk of pain associated with neuropathy. Discussion of this ongoing clinical study will be presented at the meeting.

References: None.

Keywords: Diabetes, Metabolic, Small Fibers, Pain, Clinical Trials

Grant Support: None.
Monday, June 24, 2019 - 17:30 - 17:45

Gut Microbiome and its Potential Role in Obesity-Induced Allodynia

Raiza Bonomo¹, Tyler Cook¹, Chaitanya Gavini¹, Laurent Gautron², Brian Layden³, Virginie Aubert¹

¹Loyola University Chicago, Chicago, IL, USA, ²University of Texas Southwestern Medical Center, Dallas, USA, ³University of Illinois Chicago, Chicago, USA

Obesity is considered a 21st century epidemic, affecting over 2 billion people worldwide. This condition is accompanied by complications, including peripheral neuropathy (PN): a prevalent disease with no available treatment. The initial stages of PN are clinically presented as peripheral allodynia and hyperalgesia, that later may progress to loss of sensation. Despite the high prevalence, the molecular mechanisms underlying disease onset are poorly understood and affected patients have no other choice than to use pain killers. In addition, an increasing body of evidence has now linked metabolic disorders to alterations of gut microbiome composition and their secreted metabolites, such as short-chain fatty acids (SCFAs – acetate, butyrate, propionate). Notably, we observed a decreased abundance of gut butyrate-producing bacteria in hypersensitive, Western-diet (WD)-fed mice. This change in bacteria composition was associated with changes in plasma SCFAs’ concentration. We hypothesize that there is a relationship between gut microbiome and PN onset. To test our hypothesis, we subjected WD-fed mice to fecal microbiome transplantation (FMT) or to butyrate treatment. We evaluated changes in i) mechanical and thermal sensitivities, ii) glucose homeostasis, iii) gut bacterial composition, iv) plasma SCFA levels, v) gene expression and protein acetylation in the dorsal root ganglia (DRG) and in the sciatic nerve (SN). We observed that FMT protects from obesity-induced allodynia, indicating that gut microbiota may play a role in PN onset in obese mice. Our results also suggest that gut bacteria may change sensory neurons function via modulating gene expression and histone deacetylases activity in the DRGs and SN of neuropathic mice. The gut microbiome and its metabolites could be novel valuable targets to delay or cure pain associated with obesity. More studies need to be done in humans and mice models to better understand the cellular and molecular neurobiology underlying these findings to identify specific targets.

References: None.

Keywords: Pain, Small Fibers

Grant Support: Loyola Cardiovascular Research Institute Collaborative Grant
A Keratinocyte-Derived Mechanism of Nicotinamide Riboside to Prevent and Reverse Diabetic Neuropathy

Cheng-Ying Ho 1, Krish Chandrasekera 2, James Russell 2

1 University of Maryland School of Medicine, Baltimore, MD, USA, 2 University of Maryland School of Medicine, Baltimore VA Medical Center, Baltimore, MD, USA

Skin keratinocytes produce neurotrophins to provide trophic support to cutaneous sensory axons. Early studies have suggested deficient neurotrophin signaling as a potential pathogenic mechanism of diabetic neuropathy (DN). Attempts to treat DN by exogenous neurotrophin administration, however, have failed due to intolerable injection site pain. We hereby presented a novel mechanism of nicotinamide riboside (NR) to ameliorate DN by physiologically inducing the expression of keratinocyte-derived neurotrophins.

For the reversal study, C57BL6 mice were fed either a control diet (CD) or high-fat diet (HFD) for 5 months. The mice subsequently received a daily oral dose of 300 mg/kg NR or saline for 4 months. For the prevention study, the diet modification and NR administration started simultaneously. Neuropathy was determined by mechanical allodynia thresholds (MAT) and intraepidermal nerve fiber density (IENFD). HFD-fed mice in both the prevention and reversal group demonstrated increased MAT and decreased IENFD. These findings of DN were ameliorated in mice treated with NR for two months. Of note, BDNF expression levels were decreased in the paw skin of HFD-fed mice. Since BDNF is a known target of SIRT1, a protein deacetylase activated by NR, a potential neuroprotective mechanism of NR is to enhance expression of keratinocyte-derived BDNF. In primary human keratinocyte cultures, we demonstrated that NR treatment increased NAD+ levels and induced BDNF expression over time. In addition to BDNF, transcriptome analysis by RNA-seq showed a substantial increase of other neurotrophins including FGF18 and ciliary neurotrophic factor (CNTF) in keratinocytes treated with NR. We are currently using transgenic mouse models and sensory neuronal cultures to evaluate the effect of keratinocyte-derived neurotrophins on sensory nerve protection and neurite outgrowth.

Our findings not only provide a novel neuroprotective mechanism for DN, but also highlight induced expression of keratinocyte-derived neurotrophins by pharmacological compounds as a potential treatment strategy for DN.

References: None.

Keywords: Diabetes, Small Fibers

Grant Support:

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NIH NIDDK R01DK107007 (JR)
Mrgprd as a Potential Therapeutic Target for Painful Diabetic Neuropathy

Dale George¹, Nirupa Jayaraj¹, Dongjun Ren¹, Abdelhak Belmadani², Sandra Hackelberg², Richard Miller¹, Daniela Menichella²

¹Northwestern, Chicago, IL, USA, ²Northwestern, Chicago, USA

Diabetes is a major global health problem and about 25% of these patients develop painful diabetic neuropathy (PDN), a debilitating complication of diabetes. In patients with PDN, nociceptors within the dorsal root ganglion (DRG) become hyperexcitable and eventually degenerate. Despite the prevalence of the disease, the pathogenesis of the disease is unclear. Our overall aim is to identify changes in the gene expression profile in PDN pathology for the discovery of novel druggable targets. To specifically study changes to the nociceptive neurons in PDN, we used the Na₁.8-Cre; Ai9 mice fed a regular or a high-fat diet for 10 weeks. The Na₁.8+ nociceptors from the DRG were sorted and followed by deep RNA sequencing. We identified 58 overexpressed and 360 underexpressed genes and observed overexpression of several GPCRs, including the Mas-related G protein-coupled receptor D (Mrgprd), a gene implicated in neuropathic pain. Mrgprd+ neurons are a subset of the Na₁.8 population and interestingly, we discovered several of the candidate genes clustered to the Mrgprd subpopulation, indicating a functional role of Mrgprd and associated genes in the pathogenesis of PDN. Mrgprd neurons are unmyelinated axons and do not express the neurofilament-200 (NF200), a marker of myelination. Interestingly, we observed Mrgprd expression within a population of NF200+ neurons in diabetic mice. Additionally, we examined the hairy skin of Mrgprd-EGFP reporter mice and saw a significant reduction of the Mrgprd intra-epidermal nerve fiber innervation in diabetic mice. Currently, efforts are directed towards the transcriptomic analysis of Mrgprd neurons and examining the effects of reducing the expression of Mrgprd receptors using CRISPR based approaches. Overall, we propose Mrgprd as a viable target for the development of disease-modifying therapeutics for PDN.

References: None.

Keywords: Small Fibers, Pain, Diabetes

Grant Support: NIH/NINDS R01 NS104295-01 (DMM)
Real Time Analysis of ATP Levels in DRG Neurons Derived from Normal or Diabetic Rats

Reza Aghanoori¹, Mohamad-Reza Aghanoori¹, Victoria Margulets¹, Lorrie Kirshenbaum¹, Daniel Gitler², Paul Fernyhough¹

¹University of Manitoba, Winnipeg, Canada, ²Ben-Gurion University of the Negev, Beer-Sheva, Israel

The distal dying-back of nerve fibers is a hallmark of many neurodegenerative disorders including diabetic sensorimotor polyneuropathy (DSPN). There is a high energy demand at the nerve ending and provision of energy in the form of ATP is challenging for neurons with long axons where the primary metabolite is glucose. We hypothesized that energy supplementation via glycolysis and/or mitochondrial oxidative phosphorylation is compromised in nerve endings thus contributing to axonal degeneration in diabetic conditions. DRG neuron cultures from age-matched control or streptozotocin (STZ)-induced type 1 diabetic rats were used for in vitro studies. Three plasmids containing ATP sensors of varying affinities (medium to low affinity, and one mutant; detectable by FRET technology and live cell confocal imaging) were transfected into neurons to study endogenous ATP levels in real time. FRET efficiency (YFP/CFP ratio) of the ATP sensors AT1.03 (low affinity) and AT1.03 YEMK (medium affinity) were significantly higher than the mutant (AT1.03 R122/6K) in DRG neurons in both cell bodies and neurites (P<0.0001). Using the AT1.03 YEMK construct, treatment with oligomycin (an ATP synthase inhibitor in mitochondria) decreased the ATP levels in neurites and cell bodies of DRG neurons (P<0.05). Blockade of glycolysis using 2-Deoxy-D-glucose (2-DG: a glucose analog) also lowered ATP levels (P<0.001). Both neurites and cell bodies of DRGs from diabetic rats showed a diminishment of ATP levels when compared to neurons from control rats (P<0.01). In conclusion, low ATP levels in cell bodies and distal axons may contribute to the energy deficit in nerve in diabetes and could trigger distal dying-back nerve degeneration.

References: None.

Keywords: Axonal Biology, Diabetes, Axonal Regeneration

Grant Support: None.
Novel biomarkers and therapeutic approaches in Charcot-Marie-Tooth Disease (CMT)

Michael Sereda

University Medical Centre Goettingen, Max-Planck-Institute of Experimental Medicine, Goettingen, Germany

Charcot-Marie-Tooth disease 1A (CMT1A) is the most common inherited neuropathy caused by a duplication of the gene encoding PMP22. CMT1A is characterized by slow disease progression and a high variability, even among twins. The cause of disease variability is unknown and epigenetic disease modifiers have been suggested. Previously, we could identify skin-derived disease and progression biomarkers in a rat model of CMT1A, which was translated to patients and was validated in a large European and US-based cohort. We now found that differentially methylated DNA regions and transcriptomic target genes correlate with disease severity in peripheral nerves in CMT1A rats. Clinically relevant, we also identified novel candidates from blood in both rats and CMT1A patients that may serve as easily accessible novel biomarkers. We are currently validating these candidates in blood of patients with CMT1A but also other CMT forms within the German CMT Disease Network (CMT-NET). Apart from the clinical projects we focus on the molecular understanding of failed myelin assembly in CMT in order to identify novel therapeutic approaches. I will present preclinical therapeutic approaches targeting different molecular mechanisms of mutant Schwann cells that contribute to failed myelination in CMT1A: dysdifferentiation, altered intracellular signaling and reduced lipid synthesis. I will also show how these therapeutic strategies may be relevant for other demyelinating neuropathies and how these findings may become relevant for therapeutic trials in patients.

References: None.

Keywords: Pre-clinical Studies, Schwann Cell, Clinical Trials

Grant Support: MWS was supported by the German Ministry of Education and Research (BMBF, CMT-BIO, FKZ: 01ES0812, CMT-NET, FKZ: 01GM1511C, CMT-NRG, ERA-NET 'ERARE3', FKZ: 01GM1605). MWS was awarded a DFG Heisenberg Professorship (SE 1944/1-1).
**Novel NDRG1 mutations causing CMT4D with milder phenotype**

Shawna Feely, Riccardo Zuccarino, Rosemary Shy, Michael Shy  
University of Iowa, Iowa City, IA, USA

*N-Myc Downstream-Regulated Gene 1 (NDRG1)* mutations have previously been reported to cause a demyelinating, recessive form of Charcot Marie Tooth (CMT) disease, specifically CMT4D. The phenotype is typically severe with onset in the first or second decade, motor and sensory impairment with hearing loss. We describe novel mutations in *NDRG1*, Cys289fs and Ala143Thr, which are likely causing CMT4D in our patient with a mild phenotype. Patient was a product of a normal pregnancy and delivery. Early milestones were on time and she kept up with peers. At 12 years her parents noticed that she was toe walking. She had muscle atrophy noted at ankles, could not get up on her heels, and structural changes noted in feet. She started to trip and fall, and had frequent ankle sprains. She was 15 years of age at her initial examination which showed weakness in her hands, as the FDI, APB, and ADM was 4/5 on the left and 4/5 on the right. She also had weakness in her lower extremities getting 4/5 bilaterally for her anterior tibialis, foot eversion, and great toe dorsi flexion. Sensory examination was normal for pinprick, vibration, and joint position sense. She had diffusely reduced reflexes. Overall CMT Exam Score version 2 (CMTESv2) was in the mild range at 3/28 and the CMT Pediatric Score (CMTPeds) was also mild with a score of 10/44. Nerve conduction studies were performed and revealed intermediate median NCV of 38 m/s with 3.5mV amplitude. Parents were each found to be carriers of the *NDRG1* mutations identified in the proband confirming phase. The other more common mutations identified in this gene lead to a truncated protein and loss of function. These mutations may allow for partial protein function thus leading to a milder phenotype and NCVs that are intermediate compared to significantly slowed.

**References:** None.

**Keywords:** CMTR, Human Genetics

**Grant Support:** None.
Monday, June 24, 2019 - 18:25 - 18:30
Patisiran, a silencing RNA in Hereditary Transthyretin Amyloid polyneuropathy: First experience in real life
Thierry Gendre1, Abir Wahab1, Farida Gorram1, Amandine Ladaïque2, Philippe Le Corvoisier3, Diane Bodez4, Jean-Pascal Lefaucheur5, Violaine Planté-Bordeneuve1
1Department of Neurology, Henri Mondor Hospital, East Paris University, Créteil, France, 2Department of Pharmacy, Henri Mondor Hospital, East Paris University, Créteil, France, 3Department VERDI, Inserm, CIC1430, Créteil, France, 4Department of Cardiology, Henri Mondor Hospital, East Paris University, Créteil, France, 5Department of Neuropsychology, Henri Mondor Hospital, East Paris University, Créteil, France

Background: Patisiran, a transthyretin silencing RNA, is a new effective therapeutic in hereditary transthyretin amyloid polyneuropathy (hATTR-PN) evaluated in the phase 3 Apollo trial.

Aim: To report our first experience on the safety and efficacy of patisiran in hATTR-PN patients previously treated by TTR stabilizers.

Methods: After premedication, patisiran 0.3mg/kg was administered intravenously every 3 weeks. An assessment was performed at baseline and every 6 months, including the Neuropathy Impairment Score (NIS), polyneuropathy disability (PND) score, Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN), and serum TTR level. Adverse events were recorded.

Results: Twenty-one hATTR-PN patients (17 males, 13 ATTR-Val30Met, mean age: 66 years [range: 45-86]) received patisiran. Four were enrolled in the Apollo open-label extension study, since April 2016, 17 others in the early access program since May 2018. Disease course averaged 7.2 years [range: 1.9-19.1]. Eleven patients had a PND score ≥IIIa. The mean NIS was 62 [range: 12-128] at baseline. Twenty patients received a prior TTR stabilizer. Under TTR stabilizer, the mean delta-NIS during the past 12 months was 9.6 (range: 0-58) and the PND score worsened in 10 patients. Under patisiran, the NIS was unchanged in all cases evaluated at 6 months (N1=8) and at 12 months (N2=2). The delta-NIS change was less than 5 at 24 months (N3=2). Under patisiran, PND score was stable in all patients but one. At last evaluation, the mean serum TTR level had decreased by 75% from baseline (18 cases). All patients but two had an improved Norfolk QOL-DN score (mean change: -13.6). Safety was generally good. Local erythema or flush occurred in 3 cases. Two patients presented a serious adverse event attributable to the premedication (dexamethasone) including hyperglycemia and a cardiac failure episode.

Conclusion: Patisiran appears an effective and well-tolerated treatment to stabilize hATTR-PN. Longer follow-up will be presented.

References: None.

Keywords: Amyloidosis

Grant Support:

Dr. Planté-Bordeneuve received supports for meeting, traveling fees and consulting from Alnylam, Pfizer, and Akcea.
Tuesday, 25 June

Oral Abstract Presentations
Tuesday, June 25, 2019 - 09:00:09:15

The impact of eculizumab on neurological improvement in Guillain–Barré syndrome: Subanalysis of JET-GBS study

Sonoko Misawa1, Satoshi Kuwabara1, Yukari Sekiguchi1, Hiroshi Amino1, Tomoki Suichi1, Susumu Kusunoki2

1Department of Neurology, Chiba University Graduate School of Medicine, Chiba, Japan, Chiba, Japan,
2Department of Neurology, Faculty of Medicine, Kindai University, Osaka-Sayama, Japan

Japanese eculizumab trial for Guillain–Barré syndrome (JET-GBS) study has shown that eculizumab could facilitate neurological improvement, and increase the probability to regain full muscle strength in patients with severe Guillain–Barré syndrome. This study aimed to determine impact of eculizumab on neurological improvement in GBS. We analyzed changes in manual muscle testing score (sum of the scores from 13 muscles, full score = 65) and calculated mEGOS score and probability unable to walk at week 24 in 34 GBS patients (23 in the eculizumab group and 11 in the placebo group) who participated in JET-GBS study. Most patients were severely affected and 87 % of the eculizumab group and 91% of the placebo group were graded as functional grade 4 or 5. Intravenous immunoglobulin plus either eculizumab (900 mg) or placebo were administrated for 4 weeks. The proportion of patients who achieved full recovery of manual muscle testing score was 60.9% in the eculizumab group and 27.3% in the placebo group (p=0.141). The mEGOS at hospital admission score was similar for the ecuizumab (median 4.5, range 1 – 9; at week 1, 5, 1 - 12) and placebo (5.5, 3 – 8; 5, 1 - 11) groups. However, eculizumab substantially shifted the curve in the predicted probability unable to walk at 24 weeks to the right (more favorable direction). These sub-analysis results suggest that eculizumab could substantially contribute to almost complete recovery of motor function in GBS and mEGOS can be useful to determine indication of eculizumab.

References: None.

Keywords: Clinical Trials, Inflammatory

Grant Support: None.
The plasticity of adult sensory neurons is relevant to their ability to mount a regenerative response following injury or disease. Outcomes following axonal damage to nerves remain unsatisfactory, rendering permanent disability. Manipulation of neurons downstream of growth factor receptors offers a newer approach to improve regenerative success. Here we summarize recent work on four, apparently independent pathways that impact adult sensory neuron plasticity.

PTEN (phosphatase and tensin homolog deleted on chromosome 10) a tumour suppressor, impairs downstream signaling of PI3K/pAkt. Its inhibition or knockdown (KD) enhances neurite outgrowth in vitro and improves early axon regeneration in vivo. PTEN is upregulated in sensory neurons from experimental diabetes mellitus that have a regenerative deficit. Rb1 (retinoblastoma 1) inhibits a divergent transcriptional growth signal E2F1 and like PTEN, its KD improves outgrowth in vitro and regeneration in vivo. Both PTEN and Rb1 KD have greater impacts on neurons already 'preconditioned', a unique instance of regrowth beyond that offered by preconditioning paradigms. BRCA1 (breast cancer 1) acts to repair damaged DNA, a previously unrecognized feature of both intact and regenerating sensory neurons. Unlike PTEN and Rb1, its KD inhibits growth, indicating a role in maintaining the integrity of regenerating adult neurons. The latest addition to this list is APC (adenomatous polyposis coli) that binds to and inhibits nuclear translocation of β-catenin, a widely acting transcription factor that supports growth. APC KD is associated with increases in neurite growth in vitro and improved regeneration in vivo. β-catenin localization to sensory neuron nuclei is enhanced. Both APC and PTEN have heightened expression in slower growing IB4 nonpeptidergic neurons. Overall, the commonalities among these intrinsic growth pathways requires additional investigation, but they each offer new approaches to enhance nerve regeneration.

References: None.

Keywords: Axonal Regeneration

Grant Support: Supported by the Canadian Institutes of Health Research
Long-Term Efficacy and Safety of Inotersen for Hereditary Transthyretin Amyloidosis: NEURO-TTR Open-Label Extension 2-Year Update

Thomas Brannagan1, Marcia Waddington Cruz2, Annabel Wang3, Michael Polydefkis4, Peter Dyck5, Sami Khella6, Violaine Plante-Bordeneuve7, John Berk8, Fabio Barroso9, Giampaolo Merlini10, Isabel Conceição11, Steven Hughes12, Jesse Kwok12, Shiangtung Jung12, Spencer Guthrie13, Michael Pollock13, Merrill Benson14, Morie Gertz5, Teresa Coelho15

1Columbia University Medical Center, New York, NY, USA, 2Federal University of Rio de Janeiro, University Hospital, Rio de Janeiro, Brazil, 3University of California, Irvine, Orange, CA, USA, 4Johns Hopkins University, Baltimore, MD, USA, 5Mayo Clinic, Rochester, MN, USA, 6University of Pennsylvania, Philadelphia, PA, USA, 7CHU Henri Mondor, Creteil, France, 8Boston University, Boston, MA, USA, 9FLENI, Buenos Aires, Argentina, 10Amyloidosis Center, IRCCS Policlinico San Matteo, University of Pavia, Pavia, Italy, 11CHLN—Hospital de Santa Maria, Lisbon, Portugal, 12Ionis Pharmaceuticals Inc, Carlsbad, CA, USA, 13Akcea Therapeutics, Cambridge, MA, USA, 14Indiana University School of Medicine, Indianapolis, IN, USA, 15Centro Hospitalar do Porto, Porto, Portugal

Hereditary transthyretin amyloidosis (hATTR) is a rare protein misfolding disorder that causes progressive and debilitating polyneuropathy. A randomized, placebo-controlled phase 3 trial (NEURO-TTR; NCT01737398) demonstrated efficacy and safety of inotersen in patients with hATTR polyneuropathy.1 Patients who completed NEURO-TTR were eligible to enroll in an ongoing open-label extension (OLE) study (NCT02175004). We report an update on the long-term efficacy and safety of inotersen in patients with hATTR polyneuropathy after 24 months in the OLE. Assessments included modified Neuropathy Impairment Score +7 neuropathic tests composite score (mNIS+7), Norfolk Quality of Life—Diabetic Neuropathy questionnaire total score (Norfolk QOL-DN), Short Form 36 Health Survey version 2 (SF-36v2) Physical Component Summary (PCS), and safety monitoring. Of 139 patients who completed NEURO-TTR, 135 (97.1%) enrolled in the OLE. As of 5/31/2018, some patients were ongoing but had not yet completed 24 months in the OLE, and the longest inotersen exposure in both studies was 5.2 years. Patients who switched from placebo to inotersen in the OLE demonstrated slowing of neurologic disease progression by mNIS+7 and Norfolk QOL-DN as early as 6 months after starting inotersen (mean change from OLE baseline to month 6/year 2: 6.22/5.08 in mNIS+7 and 0.54/2.26 in Norfolk QOL-DN). Patients who received inotersen for 39 months (15 months in NEURO-TTR + 24 months in OLE) continued to show benefit (mean change from OLE baseline to year 2: 11.18 in mNIS+7 and 5.22 in Norfolk QOL-DN). Patients who continued inotersen also showed stabilization of health-related quality of life as measured by SF-36v2 PCS. No evidence of increased risk of grade 4 thrombocytopenia or severe renal events has been observed with increased exposure duration; no new safety concerns have been identified. In the OLE, inotersen improved, halted, or slowed progression of hATTR polyneuropathy, with greater stabilization observed in patients who initiated inotersen earlier.

References:


Keywords: Amyloidosis, Clinical Trials, Clinical Trials, Human Genetics

Grant Support: Sponsored by Akcea Therapeutics.
Autoantibodies and Pain: The Role of Leucine-Rich Glioma Inactivated 1 in Primary Sensory Neurons

John Dawes, Gregory Weir, Paddy Waters, Sarosh Irani, David Bennett

University of Oxford, Oxford, United Kingdom of Great Britain and Northern Ireland

A number of patients with autoantibodies directed against the voltage-gated potassium channel complex (VGKCC) have neuropathic pain which can be reduced with immunotherapy (Klein et al., 2012). Autoantibodies are not directed against the Kv1 channels themselves, but instead to proteins with which they interact. Recent work has shown that autoantibodies directed against one of these neuronal targets, CASPR2, are causal to pain, not through inflammation or neuronal damage, but instead by directly impacting on dorsal root ganglion (DRG) neuron excitability via Kv1 channel disruption (Dawes et al., 2018). LGI1 (Leucine-rich glioma inactivated 1) is another component of the VGKCC and also targeted by autoantibodies in neuropathic pain patients. Studies have focused on the role of LGI1 in the CNS; LGI1-autoantibodies (-Abs) are commonly associated with limbic encephalitis and LGI1 is genetically linked to the development of epilepsy. In terms of pain, LGI1-Abs might also target DRG neurons (similar to CASPR2-Abs); however the role of LGI1 in the peripheral nervous system is unclear. Using in situ hybridisation and immunohistochemistry, we find that LGI1 mRNA is highly expressed by a variety of mouse DRG neurons including nociceptors. Serum from LGI1-Ab patients with neuropathic pain show IgG binding to live mouse DRG neurons in vitro. LGI1 is a secreted molecule and studies have shown exogenous application can regulate neuronal excitability (Seagar et al., 2017). We find that LGI1-EGFP targets DRG neurons in vitro and regulates their function. In summary, LGI1 is highly expressed at the level of the DRG and therefore primary sensory neurons represent a plausible site of action for LGI1-Abs in neuropathic pain patients. Furthermore, exogenous application of LGI1 reduces the excitability of DRG neurons, suggesting that this molecule is important in regulating sensory neuron function and this approach represents a possible avenue to reduce their excitability in pathological pain states.

References:


Keywords: Pain

Grant Support:

Wellcome trust

MRC
Gene Therapy For Peripheral Neuropathy CMT1A

Benoit Gautier¹, Hélène Hajjar¹, Jade Berthelot¹, Scarlette Abbou¹, Virginie François Le Razavet², Caroline Le Guiner Blanvillain², Ruth Stassart³, Robert Fledrich³, Nicolas Tricaud¹

¹INSERM U1051, Institut des Neurosciences de Montpellier, Montpellier, France, ²INSERM UMR 1089, Nantes, France, ³Leipzig University, Leipzig, Germany

CMT1A, the most common of Charcot-Marie-Tooth diseases, results from the duplication of the Peripheral Myelin Protein 22 (PMP22) gene. This gene encodes for a small protein of 22 kDa, PMP22, mainly produced by Schwann cells and the excess of PMP22 leads to demyelination. There is still no cure for this disease, but one approach for a treatment is gene therapy. A transgenic rat model exists for CMT1A, which possesses 3 copies of the mouse PMP22 gene. Our goal is to provide a proof of principle for gene therapy in peripheral nerves using this rat model of CMT1A. Our strategy is to reduce the overexpression of mouse PMP22 protein in rats Schwann cells using short hairpin RNAs (shRNAs). shRNAs are small non-coding RNAs that specifically bind to targeted mRNAs resulting in their degradation. Adeno-associated serotype 9 (AAV9) viral vector was used to deliver these molecular tools to cells affected by the disease. AAV9 was selected for its high transduction rate of myelinating Schwann cells for its good diffusion and low immunogenicity. We performed bilateral injections in the sciatic nerve of control and CMT1A rats. The efficiency of this gene therapy was high as muscle strength (grip test), mobility (Rotarod) and nerve conduction velocity of treated CMT1A rats were maintained to wildtype levels on a long range of time (at least 12 months, which represent the third of the life expectancy). AAV9 injection did not generate an immune response in most of the animals and the unwanted AAV9 off target infection was very limited as well. All together, these results highlight this strategy as a promising one to treat CMT1A disease. We are now looking for a preclinical validation of the molecular tools and injection methods on large animal model, such as non-human primates, before entering the clinical phase.

References: None.

Keywords: CMTR, Pre-clinical Studies, Schwann Cell

Grant Support: None.
Mutations in the GJB1 gene, encoding gap junction protein connexin32 (Cx32), cause X-linked Charcot-Marie-Tooth disease, one of the commonest forms of inherited demyelinating peripheral neuropathy. Our previous studies have shown that gene addition mediated by an intrathecally injected lentiviral vector carrying the GJB1/Cx32 gene under the myelin protein zero (Mpz) promoter, ameliorates the phenotype of the Cx32 knockout (KO) mouse model of the disease, through Schwann cell-targeted expression. In order to develop a more translatable approach, we cloned the Mpz.Egfp (mock vector) and Mpz.GJB1 (full vector) expression cassettes into the AAV transfer plasmid and used the AAV9 vector serotype with established safety in clinical trials for other disorders to target Schwann cells. Following lumbar intrathecal injection of the AAV9-Mpz.Egfp vector in 2-month old wild type (WT) mice, EGFP reporter gene expression was detected in the perinuclear compartment of Schwann cells in lumbar roots, sciatic and femoral nerves, at rates similar to those observed after lentiviral delivery. After delivery of the AAV9-Mpz.GJB1 therapeutic vector into 2-month old Cx32 KO mice, Cx32 expression was detected in the paranodal non-compact myelin areas of myelinated fibers. We then performed a post-onset treatment trial in which 6-month old Cx32 KO mice were randomized to receive either the mock or the full AAV9 vector. Outcome was assessed at 8 and 10 months of age by behavioral, electrophysiological and morphological analyses. We observed improved motor performance and sciatic nerve conduction velocities along with improved myelination and reduced inflammation in PNS tissues of treated mice. Blood neurofilament light levels, a clinically relevant biomarker, were also significantly ameliorated in treated compared to mock-treated mice. This study provides evidence that a more clinically translatable AAV9-mediated gene therapy approach targeting Schwann cells could be potentially used for the treatment of CMT1X, even after the onset of the disease.

References: None.

Keywords: CMTR, Schwann Cell

Grant Support:

Muscular Dystrophy Association and Charcot-Marie-Tooth Association (Grants MDA 480030 and MDA603003 to KAK). Dr. Kagiava is a recipient of a Young Research’s Award by the Cyprus Research Promotion Foundation (KOYATOYPA/BP-NE/0416/07).
PNS Oral Poster Session III
Tuesday, June 25, 2019 - 11:30 - 12:20
Bortezomib neurotoxicity is associated with altered MAP2 levels and distribution within human iPSC-derived sensory neurons

Nathan Staff1, Sybil Hrstka2, Soneela Ankam2, Jon Klein2, Busranur Agac2, Bhavya Narapureddy2, Ron Hrstka2

1Mayo Clinic, Rochester, MN, USA, 2Mayo Clinic, Rochester, USA

INTRODUCTION: The neurotoxic effects of the chemotherapeutic agent bortezomib are well documented, yet the mechanistic underpinnings that govern these cellular processes remain incompletely understood. In this study, system-wide proteomic changes were identified in human induced pluripotent stem cell (iPSC)-derived sensory neurons (iSN) exposed to a clinically relevant dose of bortezomib.

METHODS: Our CIPN-in-a-dish model involved the differentiation of iPSCs created from 3 healthy individuals into neural crest derivatives followed by neuronal subtype specification and maturation using an established, directed approach with typical iSN yields of 60-70%. At 21 days post-induction, iSN were treated with 100nM bortezomib, DMSO, or left untreated for 48h.

RESULTS: Bortezomib treatment of iSN led to altered axonal morphology, microtubule distribution and decreased mitochondrial motility. Label-free mass spectrometry facilitated the identification of approximately 2800 differentially expressed proteins in iSN exposed to bortezomib. Pathway analysis revealed microtubule dynamics, cytoskeletal and cytoplasmic organization, and molecular transport as significantly affected cellular processes. Microtubule Associated Protein-2 (MAP2) emerged as a topmost influential candidate, which was reduced in proteomics and confirmatory Western blot analyses. Furthermore, 24h bortezomib exposure was sufficient to lead to the accumulation of MAP2 proximal to the plasma membrane.

CONCLUSIONS: Studying neurotoxicity of bortezomib is challenged by its broad effects on multiple protein pathways. Unbiased proteomics followed by pathway analysis aided the discovery of putative pathomechanisms. One of these molecules, MAP2, was further discovered to be concentrated in the perisomatic region and through pathway analysis was associated with processes of axonogenesis, neurite extension and branching, and neurite morphology.

References: None.

Keywords: Axonal Biology, Other

Grant Support: National Institutes of Health: CA211887 (NPS)
Rate of progression of Utah Early Neuropathy Scale (UENS) score in diabetic neuropathy

J. Robinson Singleton¹, Peter Hauer¹, Cathy Revere¹, Stormy Foster-Palmer¹, Adrienne Aperghis¹, A. Gordon Smith²

¹University of Utah, Salt Lake City, UT, USA, ²Virginia Commonwealth University, Richmond, VA, USA

The Utah Early Neuropathy Scale (UENS) is a brief, validated exam scale focused on injury to small diameter nociceptive fibers. Twenty-four of 42 possible points are related to measurement of length dependent loss of pin sensation in legs and feet. While the UENS has been accepted as a standard clinical measure for diagnosis of small fiber neuropathy, its utility as a primary outcome measure in clinical trials has not been evaluated. Here we report natural history progression of the UENS. In an NIDDK DP3 single center study, 190 patients with diabetes were screened for neuropathy using Toronto criteria, then 83 found to have neuropathy were followed longitudinally at 9 month intervals for up to 27 months using a variety of clinical and ancillary measures, including the UENS. Among those with neuropathy, baseline UENS score significantly correlated with scores of symptom questionnaires including the Norfolk Quality of Life-Diabetes Neuropathy (NQoL-DN) and the NTSS-6; with nerve conduction study measures including sural sensory amplitude, peroneal amplitude and proximal conduction velocity; with intraepidermal nerve fiber density (IENFD) from distal thigh and distal leg 3m punch skin biopsies; but not with confocal corneal microscopy measures of nerve fiber length and density. Overall, 75 participants were followed for 9 months, and 56 for at least 18 months for a total of 149 nine-month segments. UENS worsened (increased) 1.08 (+/- StDev 3.51) points per 9 month segment overall. For those followed at least 18 months, there was a 2.2 (+/-4.1) point increase over this period. Change in UENS significantly correlated with change in NQoL-DN and IENFD, among other measures. The UENS is an objective, responsive, validated clinical instrument for which a linear natural progression slope can be measured, and would be an appropriate primary endpoint in small fiber neuropathy clinical trials.

References: None.

Keywords: Small Fibers, Diabetes, Metabolic

Grant Support: None.
Biomarker Profiling of Neuropathic Pain in Idiopathic Peripheral Neuropathy

Perry Van Doormaal, Simone Thomas, Senda Ajroud-Driss, Mazen Dimachkie, Roy Freeman, David Simpson, Robinson Singleton, Gordon Smith, Ahmet Höke

1Johns Hopkins University School of Medicine, Baltimore, MD, USA, 2Johns Hopkins University School of Medicine, Baltimore, USA, 3Northwestern University, Chicago, USA, 4Kansas University Medical Center, Kansas City, USA, 5Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA, 6Icahn School of Medicine at Mount Sinai, New York, USA, 7University of Utah, Salt Lake City, USA, 8Virginia Commonwealth University, Richmond, USA

Introduction: Neuropathic pain is a common symptom in idiopathic peripheral neuropathy that has a significant effect on quality of life. However, not all patients develop this symptom. The mechanisms responsible for the occurrence or absence of neuropathic pain in these patients have not been unraveled.

Methods: We collected blood samples from the Peripheral Neuropathy Research Registry (PNRR), a prospective multicenter cohort study from six tertiary neuromuscular referral centers in the United States, to construct a biomarker profile containing proteomics, lipidomics and metabolomics data. In total 60 idiopathic peripheral neuropathy patients were included; 30 patients with severe neuropathic pain and 30 patients without any pain symptoms or pain medication. Both groups were matched for age, gender and BMI. Biomarker profiling was performed at Johns Hopkins University School of Medicine, using liquid chromatography mass spectrometry.

Results: After multiple quality controls we included 476 proteomic variables, 618 lipidomic variables and 109 metabolomic variables (1203 variables in total) for analysis.

We will present the results from univariate logistic regression analysis, as well as more in-depth multivariable penalized regression modeling and co-expression analysis of these biomarker profiles in the two patient groups.

Conclusion: Using this extensive biomarker profiling approach, we aim to find new risk factors and pathways leading to neuropathic pain in patients with peripheral neuropathy. This information not only gives us more knowledge on the development of neuropathic pain, but also might provide us with new therapeutic targets to decrease the burden of neuropathic pain.

References: None.

Keywords: Pain, Other

Grant Support: None.
Influence of Body Mass Index on disability in Children with CMT

Gabrielle Donlevy1, Sarah Garnett1, Kayla Cornett2, Marnee McKay3, Jennifer Baldwin4, Joshua Burns (Joint Senior Author)1, Manoj Menezes (Joint Senior Author)1

1The University of Sydney, The Children’s Hospital at Westmead, Sydney, New South Wales, Australia, 2Sydney, Australia, 2The University of Sydney, New South Wales, Australia, Columbia University, Irving Medical Center, New York, NY, USA, Sydney, Australia, 3The University of Sydney, Sydney, New South Wales, Australia, Sydney, Australia, 4School of Clinical Sciences, Auckland University of Technology, Auckland, New Zealand, Auckland, New Zealand

Growth and body mass influence disability in many childhood neuromuscular disorders. In this study we examined the relationship between Body Mass Index (BMI) and disability in children with CMT. We conducted a cross sectional analysis of 477 patients with CMT aged 3-20 years from the Inherited Neuropathies Consortium, and 316 age-and-sex matched controls from the 1000 Norms Project[1]. BMI was categorised according to the International Obesity Task Force (IOTF)[2], and compared with scores on the CMT Pediatric Scale (CMTPedS) [3]. IOTF categories were collapsed into five age-and-sex equivalent BMI groups: severely underweight (BMI <17kg/m²); underweight (≥17kg/m² to <18.5kg/m²); healthy weight (≥18.5kg/m² to <25kg/m²), overweight (BMI ≥25 kg/m² to <30 kg/m²); obese (BMI ≥30 kg/m²). Compared to normative reference data, there was a significantly higher proportion of children with CMT categorised as severely underweight (5.6% vs 0.3%), underweight (10.4% vs 5.1%), and obese (7.6 vs 3.8%) (p<0.05). There was fewer children categorised as healthy weight (61.2% vs 74.4%) (p<0.05), and the distribution of overweight (15.1 % vs16.5%) between the two groups was comparable. Mean CMTPedS scores for each group were: severely underweight (27 ±9), underweight (20 ±8), healthy weight (17± 9), overweight (17± 9) and obese (22 ±10). Compared to healthy weight children with CMT, being severely underweight with CMT was significantly more disabling (p< 0.0001), as was being obese (p=0.015). There is a higher frequency of underweight and obese children with CMT compared to age-and-sex matched healthy children. Underweight and obese children with CMT are more disabled than children of healthy weight. A longitudinal study is required to determine the need for specific nutritional intervention to reduce the burden of CMT.

References:


Keywords: CMTR, Other

Grant Support: None.
A mitochondrial ATP6 mutation causing a slowly progressive myeloneuropathy

Tanya Bardakjian, Steven Scherer

University of Pennsylvania, Philadelphia, PA, USA

We report on a 53-year-old man with an insidiously progressive myeloneuropathy that progressed over 20 years to more typical picture of CMT. The proband has had high arches for as long as he can remember, and developed hammertoes in his teens. He presented to a neurologist at the age of 32, and was found to have hammer toes and high arches, brisk reflexes in the arms and knees (but absent at the ankles) and prominent extensor plantar reflexes. Nerve conduction studies and EMG showed a motor > sensory axonal neuropathy, with severe, chronic denervation in distal leg muscles. At age 52, he reported more difficult ambulating. His exam showed minimally worsened deficits, reflexes were less pronounced at the knees, vibration sense was absent at the toes, pinprick sensation was normal above the knees, and he had subtle extensor plantar responses. Nerve conductions and EMG showed little change from the prior study. His CMT neuropathy score was 17. His mother was examined at age 68. She had hammertoes, mild (4+) weakness in extensor hallicus longus and tibialis anterior. Vibration was absent in her toes, and she had bilateral extensor plantar responses. Her nerve conductions (showed reduced motor but not sensory amplitudes in the feet, and EMG showed moderate, chronic denervation in distal leg muscles. A hereditary neuropathy panel of the proband through GeneDx identified a VUS in the SCN9A. Reflex testing to whole exome sequencing with mitochondrial sequencing identified a homoplasmic pathogenic variant in MT-ATP6 - m.9176T>C/p.Leu217Pro in the proband and his mother. These findings add to the evidence that MT-ATP mutations can present as CMT or evolve into a CMT-like presentation. The presence of a mitochondrial disorder changes the genetic counseling and risk assessment for a patient. Therefore, mitochondrial sequencing should be a part of a complete CMT evaluation.

References:


Keywords: Human Genetics
Grant Support:

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Tuesday, June 25, 2019 - 11:55 - 12:00

Nusinersen in Adults with Spinal Muscular Atrophy, A Single Center Experience

Orly Moshe-Lilie, Chafic Karam, Chahin Nizar, Amy Visser, Diana Dimitrova
Oregon Health and Science University (OHSU), Portland, OR, USA

Objective: To report a single center’s experience of treating adult SMA patients with Nusinersen.

Background: Studies in adult SMA patients are lacking, especially of those treated with Nusinersen.

Design/Methods: We conducted a chart review of adult patients with genetically confirmed SMA types 2 or 3 seen between 2017-2019. Results: Twenty-three patients were included, 9 type 2 and 14 type 3, median age was 35 (range 20-71). Twenty-two were nonambulatory. Seventeen had severe scoliosis, 12 had undergone thoracolumbar fusion. Of those, 6 were referred for bone laminectomy to establish access for treatment and three underwent procedure. Ten had significant respiratory impairment necessitating ventilation and 2 underwent tracheostomy. Ten were treated with Nusinersen for median of 10 months (range 4-16). All treated patients exhibited stability in %MRC at 4-6 months. 5 were treated for 12 months. Of those, 2 exhibited stability, three demonstrated very modest improvement with %MRC change mean of 3.5% and 5% in upper and lower limbs respectively. Two patients were treated for 16 months, exam in one patient remained stable, the other had modest improvement with %MRC change mean of 7.5%. One patient died shortly after treatment initiation from respiratory failure. One stopped treatment due to recurrent pneumonias and lack of improvement at 12 months. All untreated patients’ %MRC remained stable. Four patients declined treatment due to comorbidities, lack of data in adults or advanced stage. Four patients awaiting treatment. Treatment side effects included post lumbar puncture headache in 5 patients, two of which necessitated blood patch, 1 developed bacterial meningitis requiring admission for long term antibiotics. Conclusions: The degree of impact on strength and function in adult SMA treated with Nusinersen remains unclear, and complications secondary to its intrathecal administration can be serious. More data is needed to better understand the role of Nusinersen in adult SMA patients.

References: None.

Keywords: Human Genetics, Other

Grant Support: None.
The overlapping spectrum of Chronic Inflammatory Demyelinating Polyradiculoneuropathy and anti-MAG neuropathy

Giuseppe Liberatore¹, Claudia Giannotta¹, Dario Cocito², Fiore Manganelli³, Raffaella Fazio⁴, Chiara Briani⁵, Massimiliano Filosto⁶, Luana Benedetti⁷, Anna Mazzeo⁸, Girolama Marlia⁹, Andrea Cortese¹⁰, Giuseppe Cosentino¹¹, Stefano Jann¹², Angelo Clerici¹³, Marinella Carpo¹⁴, Angelo Schenone¹⁵, Marco Luigetti¹⁶, Giuseppe Lauria¹⁷, Giovanni Antonini¹⁸, Tiziana Rosso¹⁹, Gabriele Siciliano²⁰, Guido Cavaletti²¹, Pietro Doneddu¹, Lucio Santoro³, Erdita Peci², Stefano Tronci², Marta Ruiz⁵, Stefano Cotti Piccinelli²², Antonio Toscano⁶, Giorgia Mataluni⁹, Luca Leonardi¹⁸, Mario Sabatelli²³, Eduardo Nobile Orazio¹

¹Neuromuscular and Neuroimmunology Service, Humanitas Clinical and Research Institute – IRCCS -, Rozzano, Milan, Italy,
²Presidio Sanitario Major, Istituti Clinici Scientifici Maugeri, Turin, Italy,
³Department of Neuroscience, Reproductive Sciences and Odontostomatology, University of Naples ‘Federico II’, Naples, Italy,
⁴Department of Neurology, Institute of Experimental Neurology (INSPE), San Raffaele Scientific Institute, Milan, Italy,
⁵Neurology Unit, Department of Neuroscience, University of Padova, Padova, Italy,
⁶Center for Neuromuscular Diseases and Neuropathies, ASST ‘Spedali Civili’, University of Brescia, Brescia, Italy,
⁷Neurology Unit, Sant’Andrea Hospital, La Spezia, Italy,
⁸Department of Clinical and Experimental Medicine, Unit of Neurology, University of Messina, Messina, Italy,
⁹Dysimmune Neuropathies Unit, Department of Systems Medicine, Tor Vergata University of Rome, Rome, Italy,
¹⁰IRCCS Foundation C. Mondino National Neurological Institute, Pavia, Italy,
¹¹Department of Experimental BioMedicine and Clinical Neurosciences (BioNeC), University of Palermo, Palermo, Italy,
¹²Department of Neurosciences, Niguarda Ca’ Granda Hospital, Milan, Italy,
¹³Neurology Unit, Circolo & Macchi Foundation Hospital, Insuibia University, DBS, Varese, Italy,
¹⁴Neurology Unit, ASST Bergamo Ovest-Ospedale Treviglio, Treviglio, Italy,
¹⁵Department of Neuroscience, Rehabilitation, University of Genoa and IRCCS AOU San Martino-IST, Genoa, Italy,
¹⁶Fondazione Policlinico Universitario A. Gemelli IRCCS, UOC Neurologia, Catholic University of Sacred Heart, Rome, Italy,
¹⁷Unit of Neuroalgology, IRCCS Foundation ‘Carlo Besta’ Neurological Institute, Milan, Italy,
¹⁸Unit of Neuromuscular Diseases, ‘Sapienza’ University of Rome, Sant’Andrea Hospital, Rome, Italy,
¹⁹ULSS2 Marca Trevigiana, UOC Neurologia-Castelfranco Veneto, Treviso, Italy,
²⁰Neurology Unit, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy,
²¹School of Medicine and Surgery and Experimental Neurology Unit, University of Milano-Bicocca, Monza, Italy,
²²Center for Neuromuscular Diseases and Neuropathies, ASST ‘Spedali Civili’, University of Brescia, Brescia, Italy,
²³NEuroMuscular Omnicentre (NEMO)- Pol. A. Gemelli, Catholic University of Sacred Heart, Rome, Italy
Objective: diagnosis of Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) can be challenging and one of the possible pitfalls is the possible presence of the neuropathy associated with anti-myelin-associated-glycoprotein (MAG) IgM monoclonal gammopathy.

Methods: We implemented a multicentric web-based database to collect data from patients with CIDP followed throughout Italy. In order to exclude anti-MAG neuropathy we tested our patients for anti-MAG protein on ELISA (Buhlmann method). We considered as positive a titer>7000 Buhlmann.

Results: By February 2019 we tested for anti-MAG protein 267 of the 530 included patients with a diagnosis of typical or atypical CIDP. Eighteen patients (6.7%) had high anti-MAG IgM antibodies (mean titer 45,500, range 9,300-125,000). Twelve patients had a diagnosis of typical CIDP (67%), 5 of DADS (28%) and one of sensory CIDP. Compared to patients without antibodies (249), patients with anti-MAG IgM had an older mean age of disease onset (61 vs 48.8 years) and a shorter disease course (5.5 vs 8.8 years). Patients with and without antibodies had a similar frequency of cramps, ataxia, fatigue at onset, relapsing course (50% in both groups) and increased CSF proteins (about 70%). On nerve conduction studies, patients with anti-MAG antibodies had a more frequent presence of sensory abnormalities compatible with demyelination (50% vs 30%) and of increased distal latency (50% vs 19%). Patients with anti-MAG antibodies had an overall similar response to therapy compared with CIDP patients, but a less frequent response to IV Ig (61% vs 72%) and a similar response to steroids (50% vs 51%).

Discussion: Some patients with the clinical features of CIDP may have anti-MAG antibodies with a similar clinical and electrophysiological spectrum and response to immunotherapy compared to patients without antibodies. Even if these patients are currently excluded from the diagnosis of CIDP they share the same clinical and therapeutic features of CIDP.

References: None.

Keywords: Inflammatory

Grant Support: None.
**Tuesday, June 25, 2019 - 12:05 - 12:10**

**International Validation of the modified Erasmus GBS Outcome Score (mEGOS) for Guillain-Barré Syndrome**

Alex Doets¹, Hester Lingsma¹, Christa Walgaard¹, Badrul Islam², Amy Davidson³, Yuko Yamagishi⁴, Susumu Kusunoki⁴, Mazen Dimachkie⁵, Kenneth Gorson⁶, Bart Jacobs¹, the IGOS Consortium⁷

¹Erasmus MC University Medical Center Rotterdam, Rotterdam, Netherlands, ²The International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), Dhaka, Bangladesh, ³University of Glasgow, Glasgow, United Kingdom of Great Britain and Northern Ireland, ⁴Kindai University Faculty of Medicine, Osaka-Sayama, Japan, ⁵University of Kansas Medical Center, Kansas City, USA, ⁶St. Elizabeth’s Medical Center, Tufts University School of Medicine, Boston, USA, ⁷n.a., n.a., Unknown or unspecified country

**Introduction.** The modified Erasmus GBS Outcome Score (mEGOS) estimates the probability of being unable to walk independently (GBS disability score >2) in individual patients with Guillain-Barré syndrome (GBS), based on age, preceding diarrhoea and limb strength. The mEGOS was based on Dutch patients and has been validated in a Japanese cohort. The aim of this study was to validate mEGOS in the International GBS Outcome Study (IGOS) cohort.

**Methods.** The study was based on data from patients included in the IGOS-1300 cohort that were aged over 6 years and were unable to walk. Patients from Bangladesh were excluded. Model performance was assessed regarding discrimination (area under the receiver operating characteristic curve, AUC) and calibration, in separate regions. Outcome was inability to walk at 4, 13 and 26 weeks. Missing values were imputed using single imputation.

**Results.** For validation of mEGOS at admission 606 patients were eligible (Europe n=394, North-America n=116, Asia n=52), and 517 for validation of mEGOS at week 1 (Europe n=353, North-America n=78, Asia n=49). For the full IGOS cohort and for patients from Europe, discriminative ability of mEGOS was good at all three time points, with AUC-values equal to or higher than 0.7. Model calibration was suboptimal in these subgroups, with observed percentages of poor prognosis exceeding predicted probabilities (e.g. mEGOS at admission, week 4, observed percentage poor prognosis/predicted probability poor prognosis: full cohort 63%/55%, Europe 65%/55%). Similar performance of mEGOS was found for patients from North-America and Asia, but 95% confidence intervals around AUC-values and calibration curves were wide.

**Conclusion.** The mEGOS is a promising tool for the prediction of poor prognosis in GBS worldwide. Recalibration is required to further improve model performance and enable region-specificity. Larger sample sizes are required for North-America and Asia to increase reliability and create a region-specific model.

**References:** None.

**Keywords:** Inflammatory, Other

**Grant Support:** This study is funded by GBS-CIDP Foundation International, gain, Erasmus MC University Medical Centre Rotterdam, University of Glasgow, CSL Behring, Grifols and Annexon.
Incidence of antibodies against the node of Ranvier in a prospective cohort of 1000 CIDP

Emilien Delmont¹, Alexandre Brodovitch¹, Shahram Attarian¹, Jose Boucraut²

¹Referral centre for neuromuscular diseases and ALS, APHM, Marseille, France
²Immunology department, APHM, Marseille, France

IgG4 antibodies against proteins of the node of Ranvier have been recently described in severe CIDP resistant to intravenous immunoglobulins treatment (IVIg). Their incidence varies from 4% to 16% in sera banks of CIDP patients analyzed retrospectively.

Our objective was to determine the incidence of IgG4 antibodies against proteins of the node of Ranvier in a multicenter, prospective cohort of 1000 consecutive CIDP.

IgG4 antibodies against neurofascin 155 (NF155), neurofascin 186 (NF186), contactin 1 (CNTN1), contactin associated protein 1 (Caspr1) were tested with a flow cytometry technique using HEK cells transfected with the plasmid of the proteins of interest.

IgG4 antibodies against proteins of the node of Ranvier were detected in 19 patients (incidence 1.9%): 10 anti-NF155, 8 anti-CNTN1 and 1 anti-Caspr1. These patients had subacute onset (29%), marked sensory ataxia (94%), postural tremor (59%) and were resistant to IVIg treatment (73%). Half of the patients with antibodies against CNTN1 also had membranous glomerulonephritis. The patient with antibodies against Caspr1 did not have painful paraesthesia.

Incidence of antibodies against the node of Ranvier was lower than previously described. It could be explained by several hypotheses. First, other studies have retrospectively analyzed sera banks of CIDP patients, while we have prospectively analyzed sera sent for routine detection of antibodies against nodal and paranodal proteins. Secondly, only IgG4 antibody isotype were considered as positive samples. We confirmed the clinical features previously described namely the resistance to IgIV treatment. Unlike the 2 previously reported cases, the patient with anti-Caspr1 antibodies did not express excessive pain. Renal involvement seems frequent in patients with anti-CNTN1 antibodies.

Antibodies against the proteins of the node of Ranvier are uncommon in CIDP patient but they are clinically relevant

References: None.

Keywords: Inflammatory, Node

Grant Support: None.
Introduction: Subcutaneous immunoglobulin (SCIG) IgPro20 (Hizentra®, CSL Behring) was efficacious in chronic inflammatory demyelinating polyneuropathy (CIDP) in the PATH study. A 48-week open-label extension study to the PATH study, explored long-term outcomes of IgPro20 (0.2 g/kg and 0.4 g/kg) in CIDP.

Methods: Subjects started with 0.4 g/kg weekly IgPro20 and switched to 0.2 g/kg weekly after 24 weeks. In case of CIDP relapse, 0.4 g/kg was re-initiated. After a study amendment, subjects started on 0.2 g/kg weekly with dose increase at relapse. The primary endpoint was relapse rate (≥1 point deterioration in total adjusted INCAT [Inflammatory Neuropathy Cause and Treatment] score versus baseline). Secondary endpoints included change in INCAT, I-RODS (Inflammatory Rasch-built Overall Disability) centile score, Medical Research Council (MRC) sum score, mean grip strength, and patient treatment preference.

Results: Eighty-two subjects were enrolled, most received both doses. Relapse rates were 10% (during treatment with 0.4 g/kg, n=72) and 48% (during treatment with 0.2 g/kg [n=73]; 89% of whom recovered within 4 weeks upon switching to 0.4 g/kg). At baseline, both dose groups had similar median scores on all secondary endpoints. All scores showed deterioration at relapse, both on 0.2 g/kg (change from baseline: INCAT, +1 point; I-RODS, -8.0 points; MRC, -3 points; grip strength, -63 kPa) and 0.4 g/kg (+1, -13, -8, -12, respectively) with improvement at recovery after dose increase in the 0.2 g/kg group. For non-relapsers, endpoints generally remained unchanged. Most patients (≥80%) kept preferring SCIG over previous treatment during this long-term follow-up study.

Discussion: Secondary endpoints confirm efficacy of IgPro20 in long-term maintenance treatment of CIDP. IgPro20 0.4 g/kg was more efficacious than 0.2 g/kg. However, many subjects didn’t relapse on 0.2 g/kg, and most who did deteriorate improved after dose increase. Patient preference for SCIG over previous treatment remained high during long-term follow-up.

References: None.

Keywords: Inflammatory

Grant Support: This study was supported by CSL Behring.
Tuesday Clinical Trials Session
Tuesday, June 25, 2019 - 14:30 - 16:30
Tuesday, June 25, 2019 - 14:30 - 14:43
Improving Neuropathy and Mobility in Diabetes: the INMED trial
Lindsay Zilliox, Neda Ilieva, Min Zhan, James Russell
University of Maryland, VA Maryland Health Care System, Baltimore, MD, USA

Introduction

Currently there are no effective disease modifying treatments for diabetic neuropathy (DN). Short term, uncontrolled trials in DN have been performed. However, data from randomized control trials is lacking. To test the effect of a lifestyle intervention on subjects with impaired glucose tolerance (IGT) or type 2 diabetes mellitus (T2DM), a blinded, randomized, intention-to-treat, parallel group intervention study was performed over 12 months.

Methods

The lifestyle intervention program consisted of a weight-loss dietary intervention that was tailored to the participants’ caloric expenditure and a graded, increasing, moderate intensity aerobic physical activity intervention that was tailored to the participants’ baseline physical activity (TDPA). The TDPA intervention group was compared to a standard care (SC) group receiving general dietary and exercise advice. The primary caregivers, those measuring endpoints, and the statistician were completely masked to the trial intervention. Masking to the trial intervention was achieved in 81% of participants. The primary efficacy measure was improvement in the 6 minute walk test (6MW) and the secondary efficacy measure was improvement in the intraepidermal nerve fiber density (IENFD).

Results

72 subjects were randomized into the study with equal numbers completing the study in each group. Baseline measurements in each group were not statistically different. At 6 months, the difference in the 6MW (change from baseline) was >25 meters (P=0.02) and >6 meters at 12 months in the TDPA compared to SC groups (P=0.03). At 12 months the change from baseline in the mean thigh IENFD was +1.29 in the TDPA and -0.39 fibers/mm in the SC group (P = 0.04).

Conclusions

In this randomized, blinded, intention-to-treat study of a lifestyle intervention in diabetic neuropathy, there is improvement in the IENFD, as a measure of neuropathy, and mobility in the tailored lifestyle intervention group. This offers a potential therapy for diabetic neuropathy.

References: None.

Keywords: Diabetes, Clinical Trials, Metabolic, Small Fibers, Other

Grant Support:

Supported in part by the Office of Research Development, Department of Veterans Affairs (Rehabilitation Research and Development, 101RX001030 and 1K2RX001651, Diabetes Action Research and Education Foundation, and the GRECC.)
Second IVIg Course in Guillain-Barré Syndrome Patients with Poor Prognosis (SID-GBS); Double-blind Randomized Controlled Trial.

Christa Walgaard¹, Bart Jacobs¹, Hester Lingsma¹, Ewout Steyerberg², David Cornblath³, Pieter van Doorn¹

¹Erasmus Medical Center, Rotterdam, Netherlands, ²Erasmus Medical Center, LUMC, Rotterdam, Leiden, Netherlands, ³Johns Hopkins University, Baltimore, MD, USA

Introduction

Guillain-Barré syndrome (GBS) has a variable clinical severity and outcome and the standard treatment with IVIg (0.4 g/kg for 5 days) is insufficient for patients with the severest forms. In this RCT the additional value of a second IVIg course is investigated in GBS patients with a predicted poor outcome according to the modified Erasmus GBS Outcome Score (mEGOS). The SID-GBS study (NTR 2224) currently is the only large RCT in GBS worldwide.

Methods

Included were patients with GBS (age ≥12 years) and an indication to start standard IVIg treatment. One week after start of IVIg, patients with a poor prognosis predicted by the mEGOS model (score 6-12) were randomized to receive a second IVIg course of 0.4 g/kg for 5 days or placebo (double-blind). Primary endpoint: GBS disability score (range 0-6) at 4 weeks is compared between the treatment groups using a proportional odds model and pre-specified covariate adjustment to correct for known prognostic factors at baseline. Secondary endpoints included GBS disability score at 8, 12 and 26 weeks, MRC sum score and Overall Neuropathy Limitations Scale at 4, 8, 12 and 26 weeks and safety. Also nerve conduction study data and serial serum IgG levels are available. In total 59 hospitals in The Netherlands participated in this trial.

Results

Of the 339 included patients, 93 patients with a predicted poor prognosis were randomized. Recently the half year follow-up of the last included patient was completed. The database will be locked in February 2019. The results of this RCT will be presented at the congress.

Conclusion

This RCT potentially will change the treatment and perspective of GBS patients with a poor prognosis. It hopefully opens the way for a more individualized and better treatment of this severe disease.

References: None.

Keywords: Clinical Trials, Inflammatory

Grant Support:

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Maintenance IVIg treatment regimens vary largely between CIDP patients and the best strategy to find the optimal dosage and interval is unknown. Guidelines on how to dose IVIg more effectively and efficiently are needed. High peak serum IgG levels may not be needed in IVIg maintenance treatment and these might be responsible for side effects. More frequent lower dosing of IVIg is likely to result in more stable and higher trough levels of IgG which might improve the efficacy. The DRIP study is a double-blind randomised placebo-controlled cross-over study. The main objective is to investigate whether more frequent low dosage IVIg treatment is more effective than less frequent high dosage IVIg treatment. Secondary objectives are to investigate whether more frequent lower dosing leads to fewer side effects. CIDP patients proven to be IVIg dependent receiving an individually established stable dosage and interval of IVIg treatment are included. The intervention group (A) was treated with half their normal dosage of IVIg at half their interval; the contrast group (B) with their normal dosage and interval of IVIg followed by a placebo infusion at half their normal interval (total IVIg dosage remained the same over time). After a wash-out phase patients cross-over. Hand grip strength (Martin Vigorimeter) was used as the primary outcome measurement. A difference of > 8 kPa in the mean of the four Vigorimeter changes from baseline in favor of the group treated with half the dosage and interval was considered a relevant improvement. Secondary outcome measures were changes in the R-ODS, R-FSS, and SF-36 and the occurrence of side effects. Twenty-five patients have been included, of which 22 patients completed both treatment periods. Three patients did not receive the second treatment due to clinical worsening. The trial is completed and data entry almost finished. Results will be presented.

References: None.

Keywords: Inflammatory, Clinical Trials, Other

Grant Support: Baxalta/Shire
Background: Intravenous immunoglobulin (IVIg) is an efficacious treatment for chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). However, CIDP runs different and unpredictable disease courses, which may include spontaneous remissions. Prognostic factors for individual disease courses and biomarkers for disease activity are lacking, making it difficult to assess the need for ongoing IVIg treatment. Methods: The primary objectives of the IOC-trial are to determine whether subjects with CIDP are overtreated with maintenance IVIg treatment and to reduce overtreatment-associated subjects’ burden and healthcare costs. The secondary objective is to identify possible predictive factors for ongoing need of IVIg treatment. To study these objectives, we designed a multicenter, randomized, double-blind, standard IVIg treatment-controlled non-inferiority trial. We included 60 adult subjects with clinically stable CIDP who were receiving maintenance IVIg treatment. Subjects were randomized to either IVIg withdrawal or continuation of IVIg treatment. Those randomized to IVIg withdrawal started with a tapering phase consisting of three infusions (75%, 50% and respectively 25% of the subjects’ pre-study IVIg dose and brand combined with placebo), followed by 100% placebo infusions. Those randomized for continuation of treatment received the same IVIg brand, dose and interval prior to the study. The primary outcome was the mean Inflammatory Rasch-Overall Disability Scale (I-RODS) change score between baseline and 24-week follow-up. Results: The first patient was included in April 2014, and last follow-up visit of last patient is scheduled in May 2019. We will present the first results of this trial at the PNS 2019.

References: None.

Keywords: Clinical Trials, Inflammatory

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Restabilization after intravenous immunoglobulins (IVIg) withdrawal in patients with chronic inflammatory demyelinating polyneuropathy (CIDP)

Ilse Lucke¹, Max Adrichem¹, Alexander Vrancken², Ivo van Schaik¹, Filip Eftimov¹

¹Amsterdam UMC - university of Amsterdam, Amsterdam, Netherlands, ²University Medical Center Utrecht, Utrecht, Netherlands

Background: As biomarkers for disease activity in CIDP are lacking, IVIg withdrawal attempts are advocated to assess whether the disease is active. Patients and physicians can be reluctant to stop treatment, as it might lead to an increase in disability. Our objective was to assess the effectiveness of IVIg restabilization in patients deteriorating after IVIg withdrawal.

Methods: Patients randomized into the withdrawal arm of the double-blind controlled non-inferiority IOC-trial (registration number: ISRCTN1363769) were eligible for this prospective follow-up study if they deteriorated. Patients who were randomized for IVIg continuation and remained stable during the trial, could enter a withdrawal attempt after the trial. All patients who deteriorated were included. The withdrawal protocol consisted of infusions of 75%, 50% and 25% of the individual maintenance dose, followed by placebo. The restabilization protocol consisted of a loading dose of 2g/kg followed by patient's individual maintenance dose. After the trial, the withdrawal and restabilization protocols were recommended, but final decision was at discretion of the physician.

Primary outcome was the proportion of patients who restabilized at 12 weeks. Restabilization was defined as no change on a 5-point patient global impression scale and/or a change less than the MCID on i-RODS compared to baseline. Disability and impairment were scored using i-RODS, MRC-sum score and grip strength.

Results: A total of 28 patients were included. All patients were considered restabilized at 12 weeks. IVIg was restarted according to the restabilization protocol in 19 patients (68%), of which 14 patients (74%) recovered within three weeks. Nine patients received an extra dose of their previous maintenance dose or were restarted on therapy without a loading dose. All nine patients recovered within 12 weeks.

Conclusion: All patients restabilized within 12 weeks after restart of IVIg. The 3-step withdrawal schedule did not lead to any long-term disability or impairment.

References: None.

Keywords: Clinical Trials, Inflammatory

Grant Support: None.
Although IVlg efficacy for the treatment of CIDP has been demonstrated in randomized controlled trials, the optimal treatment approach for patients on chronic therapy is unknown. Herein we update progress on the investigator-initiated, multi-center “GRIPPER” study that prospectively evaluates “wear-off” or other IVlg treatment-related fluctuations in patients with CIDP. The primary outcome measure is Jamar grip strength (GS), performed daily for 6 months. Home nursing visits also capture Rasch-built Overall Disability Score (R-ODS), Timed Up and Go Test (TUGs), Overall Neuropathy Limitations Scale (ONLS), Modified Fatigue Severity Scale (mFSS), and Visual Analog Pain Severity Scale (VAS) weekly for 6 months. The QOL Short Form Physical Component Summary (SF-36v2®) is collected at baseline, week 12, and week 24. Serum IgG levels are collected at 3 time-points surrounding IVlg infusions (peak, trough, and mid-cycle). Study “wear-off” frequency data is currently being analyzed by assessing the proportion of subjects with any given degree of GS and RODS intracycle fluctuation and the proportion of cycles in which GS and R-ODS fluctuation occurs. To determine the extent of “wear-off” the degree of difference between maximum and minimum GS, R-ODS, TUGs, ONLS, and VAS scores are being analyzed. Study enrollment (n=29) and data collection are now complete. Preliminary study results are forthcoming. By better understanding the frequency and extent of IVIG treatment-related fluctuations we expect that these results will help facilitate development of CIDP treatment optimization strategies.

References: None.

Keywords: Inflammatory, Clinical Trials, Other

Grant Support: None.
A Randomized, Single-Blinded, Non-Inferiority Cross-Over Trial of Facilitated Subcutaneous Immunoglobulin in Multifocal Motor Neuropathy

Ali Al-Zuhairy¹, Johannes Jakobsen², Henning Andersen³, Søren Sindrup⁴, Lars Markvardsen⁵

¹Department of Neurology, Neuroscience Center, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark, ²Department of Neurology, Copenhagen University Hospital (Rigshospitalet), Copenhagen, Denmark, ³Department of Neurology, Aarhus University Hospital, Aarhus, Denmark, ⁴Department of Neurology, Odense University Hospital, Odense, Denmark, ⁵Department of Neurology, Aarhus University Hospital, Aarhus, Denmark

Purpose: To optimize subcutaneous therapy with immunoglobulins we compared large volume infusion of IgG facilitated by pretreatment with hyaluronidase (fSCIG) to conventional infusion of multiple small dosages (cSCIG) in 20 patients with multifocal motor neuropathy (MMN).

Methods: A randomized, non-inferiority, cross-over and observer-blinded design was applied with a treatment period of 24 weeks at each therapy. The primary study parameter was isometric strength. Secondary study parameters were the scores of the following tests: The Overall Disability Sum Score, Medical Research Council, grip strength using a hand-held dynamometer, 9 Hole Peg Test, Six Spot Step Test and EQ-5D-5L Index Value and VAS.

Results: In 18 patients fSCIG was feasible, 2 patients leaving the study due to side-effects. The results for the primary and secondary parameters will be presented at the meeting. - Mild and short-lasting generalized side-effects were similar in the two groups, whereas the relative frequency of localized side-effects at the injection site was increased after fSCIG (0.63 (95% CI: 0.23 – 1.00) vs 0.09 (95% CI: 0.00 – 0.22), P =0.005). The preference of the patients favoured fSCIG for 2 out of 5 VAS-scores as well as the total mean score of all preferences (P =0.03).

Conclusion: fSCIG seems feasible and safe. In addition, it is preferred by patients but is accompanied by a higher frequency of short lasting localized side-effects.

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European Clinical Trials Database: 2015-003453-18

References: None.

Keywords: Clinical Trials, Inflammatory

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Tuesday, June 25, 2019 - 16:01 - 16:14

**RECIPE: a phase II randomized controlled trial of rituximab for refractory CIDP with IgG4 autoantibodies**

Masahiro Iijima¹, Shinobu Shimizu¹, Yuki Fukami², Ryoji Nishi³, Yuichi Kawagashira³, Haruki Koike³, Hidenori Ogata⁴, Jun-ichi Kira⁵, Ken-ichi Kaida⁶, Michiaki Koga⁷, Takashi Kanda⁸, Masahiro Mori⁹, Satoshi Kuwabara⁹, Masahisa Katsuno³

¹Department of Advanced Medicine, Nagoya University Hospital, Nagoya, Japan, ²Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan, ³Department of Neurology, Nagoya University Graduate School of Medicine, Nagoya, Japan, ⁴Department of Neurology, Kyoto University Hospital, Kyoto, Japan, ⁵Department of Neurology, Neurological Institute, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, ⁶Department of Neurology, National Defense Medical College, Tokorozawa, Japan, ⁷Department of Neurology and Clinical Neuroscience, Yamaguchi University Graduate School of Medicine, Ube, Japan, ⁸Department of Neurology and Clinical Neuroscience, Yamaguchi University Graduate School of Medicine, Ube, Japan, ⁹Department of Neurology, Graduate School of Medicine, Chiba University, Chiba, Japan

Introduction: Potential efficacy for rituximab is indicated for refractory CIDP, especially IgG4 autoantibodies positive cases.

Methods: To evaluate the efficacy and safety of rituximab intravenously administered to CIDP patients with positive or negative IgG4 autoantibody, we are going to perform a multicenter, placebo-controlled, randomized, partially blind, parallel-group, comparative study. A total of 15 CIDP patients with positive IgG4 autoantibody (contactin-1 or neurofascin-155) will be divided into 10 in the rituximab group and 5 in the placebo group. On the other hand, a total of 10 refractory CIDP patients with negative IgG4 autoantibody will be administered by rituximab. The primary endpoint is the adjusted INCAT Disability Scale, and the analysis will compare the score evaluated prior to treatment and at each timepoint after week 26, 38, and 52 to calculate the proportion and its 95% confidence interval of patients who achieve one or more points improvement from the baseline with positive IgG4 autoantibody in the rituximab group and the placebo group. Scores at each timepoint and differences between scores between prior to treatment and at each timepoint are summarized as mean, standard deviation, median, minimum and maximum values in patients with positive IgG4 autoantibody (rituximab and placebo groups) and those with negative IgG4 autoantibody. The secondary endpoints include grip strength, R-ODS, MRC sum score, indices in nerve conduction study, CSF protein, B cell counts, expression of human anti-chimeric antibodies to rituximab, serum rituximab level, serum titers of IgG4 antibodies (anti-contactin-1 and anti-neurofascin-155) and those IgG subclasses.

Results: Registration of the initial trial participant will be scheduled from 1Q to 2Q in 2019.

Conclusions: The RECIPE Trial aims to clarify the efficacy of rituximab from IgG4 autoantibodies positive CIDP and refractory patients who are resistant to conservative therapies.

References: None.

Keywords: Clinical Trials, Inflammatory, Node

Grant Support: Japan Agency for Medical Research and Development (AMED)
Efficacy and safety of PXT3003 in patients with CMT1A: International Pivotal Phase III trial.

Attarian Shahram\(^1\), Boutalbi Youcef\(^2\), Fitoussi Serge\(^3\), Rinaudo Philippe\(^2\), Bertrand Viviane\(^2\), Hajj Rodolphe\(^2\), Nabirotchkin Serguei\(^2\), Cohen Daniel\(^2\), Thomas Florian\(^4\)

\(^1\)AP-HM et Aix Marseille Université, Marseille, France, Marseille, France, \(^2\)Pharnext, Issy-les-Moulineaux, France, \(^3\)Pharnext, Issy-les-Moulineaux, France, \(^4\)Hackensack University Medical Center, Hackensack, NJ 07601, Hackensack, NJ, USA

CMT1A is a rare, inherited, chronic peripheral neuropathy affecting 1 patient out of 5000. Patients suffer from distal dominant muscle atrophy compromising gait and activities of daily living, stocking-glove sensory loss, and overall reduced quality of life. To date, no treatment is available to stabilize or reverse the disease. PXT3003 is a novel oral fixed-dose 3 drug combination: baclofen, naltrexone and D-sorbitol targeting multiple disease pathways. Methods: PLEO-CMT is an international, multi-center, randomized, double-blind, placebo (Pb)-controlled pivotal phase III trial, assessing the efficacy and safety of 2 doses of PXT3003 given twice daily for up to 15 months to mild-to-moderate severity, genetically confirmed, CMT1A patients aged 16 to 65, with Dose 1 (D1) (3 mg baclofen, 0.35 mg naltrexone and 105 mg sorbitol) and Dose 2 (D2) at twice D1. Primary Objective: To assess the effect of PXT3003 on disability measured by the mean change from baseline of Overall Neurology Limitations Scale (ONLS) score at month 12 and 15. The 10-meter Walk Test (10-mWT) constituted one of the secondary efficacy endpoints. Results: 323 patients were randomized 1:1:1 in the study (D1=109, D2=113, Pb=101). Characteristics of the three groups were comparable at baseline. PXT3003 D2 met the primary endpoint: a clinically meaningful reduction of 0.37-point ONLS (95% CI [0.1,0.64], p=0.008) was observed vs. Pb. In addition, in group D2 a trend for improvement in the ONLS score was observed vs. baseline -0.20 (95% CI [-0.447, -0.039], p=0.098). A reduction of 0.47 sec (95%CI [0.09,0.85], p=0.016) was observed on the 10-mWT with D2 vs. Pb. The rate of treatment-emergent adverse events leading to treatment withdrawal was low and similar between groups (D2=5.3%, D1=5.5%, Pb=5.6%). Conclusion: PXT3003 is the first treatment for CMT1A demonstrated to be effective, safe and well tolerated.

References: For the Pleo CMT Investigators

Keywords: Clinical Trials, Human Genetics, Schwann Cell

Grant Support: None.