2nd European CMT Specialist Conference

Abstract book

Selected Oral Presentations

Plenary Session 1 - Basic sciences and the many faces of CMT

- **PL1-01: Bieke Bekaert** (University of Antwerp, Belgium)
  Fully human iPSC-derived neuromuscular assembloids to model myelination and neuromuscular features in CMT
- **PL1-02: Francesc Palau** (Sant Joan de Déu Research Institute, Spain)
  Biallelic variants in the DARS2 gene as a novel cause of axonal Charcot-Marie-Tooth disease
- **PL1-03: Koen Kuipers** (Hasselt University, Belgium)
  ABCA1 inhibition improves Schwann cell maturation and cholesterol deficiency in Charcot-Marie-tooth disease type 1A
- **PL1-04: Barbara Tedesco** (Università degli Studi di Milano, Italy)
  Unveiling novel players in HSPB8 pathology caused by frameshift mutations
- **PL1-05: Stijn in 't Groen** (University of Antwerp, Belgium)
  Bioengineering the neuromuscular junction to investigate CMT2-pathophysiology using hiPSC-derived cell models
- **PL1-06: Ilaria Quartesan** (UCL Queen Square Institute of Neurology, UK)
  Long-read sequencing reveals SORD/SORD2P inversions as a common cause of SORD- CMT missed by short-read sequencing

Plenary Sesson 2: Methods: diagnostics / genetics of CMT neuropathies

- **PL2-01: Gorka Fernandez-Eulate** (Pitié-Salpêtrière University Hospital, France)
  Biallelic PIGB variants are a cause of childhood-onset, motor-predominant neuropathy with conduction blocks and neuromyotonia
- **PL2-02: Ayse Candayan** (VIB Center for Molecular Neurology, Belgium)
  Advancing genetic diagnostics in Charcot-Marie-Tooth disease: Lessons learned from long-read sequencing
- **PL2-03: Pedro José Tomaselli** (University of São Paulo, Brazil)
  Gradient-boosted discrimination of inflammatory neuropathies from hereditary Charcot-Marie-Tooth disease using motor nerve conduction metrics
- **PL02-04: Liedewei Van de Vondel** (University of Miami, USA)
  A CCG expansion in TBC1D7 defines a novel neuromuscular disorder – lessons for the next wave of gene discovery

Plenary Session 3: Therapeutic approaches on CMT neuropathies

- **PL3-01: Nathalie Bernard-Marissal** (Aix-Marseille University, France)
  Rescue of CMT2A pathology by two therapeutic approaches aimed at restoring defective organelle contacts and associated pathways
- **PL3-02: Nikolay Zhukovsky** (Ageronix, Switserland)
  Alpha-1 Antitrypsin demonstrates therapeutic efficacy in a mouse model of Charcot-Marie-Tooth Disease Type 1A
- **PL3-03: Lara Cantarero** (Institut de Recerca Sant Joan de Déu, Spain)
  A new selective HDAC6 inhibitor ameliorates disease phenotype in a Gdap1^{-}/$^{-}$ mouse model
- **PL3-04: Alberto Raoss** (University of Trento, Italy)
  Restoration of the physiological levels of PMP22 in CMT1A patient cells via base editing of the Kozak sequence
- **PL3-05: Hyeongseop Kim** (ENCell Corp, Republic of Korea)
  Therapeutic Potential and Safety of allogeneic mesenchymal stromal cell (EN001) in CMT1A and CMT1E: First-in-Human Evidence from South Korea
- **PL3-06: Natalia Dominik** (Queen Square UCL, UK)
  Loss of ARHGAP19 function disrupts RhoA regulation in Charcot-Marie-Tooth disease: mechanisms and therapeutic targets
- **PL3-07: Antonella Vitale** (Università Cattolica del Sacro Cuore – Roma, Italy)
  Ultrasound Evaluation of the Plantar Fascia in CMT Patients: Clinical-Functional Correlations and Rehabilitative Implications

Plenary Session 4: Clinical trials, data sharing and outcome measurement

- **PL4-01: Connor Maltby** (Ulysses Neuroscience Ltd., Ireland)
  Acetylated α-Tubulin as a clinical plasma biomarker of disease severity in CMT1A
- **PL4-02: Michael Shy** (University of Iowa, USA)
  Charcot-Marie-Tooth disease type 1E: Clinical Natural History and Molecular Impact of PMP22 Variants
- **PL4-03: Marion Masingue** (Pitié-Salpêtrière University Hospital, France, France)
  A COA8 homozygous mutation presenting as a demyelinating CMT with leukopathy
- **PL4-04: Alessandro Bertini** (Istituto Neurologico C Besta, Italy)
  Genotype-phenotype correlation in a large cohort with HSPB1-related neuropathy

Presentation PL1-01

Fully human iPSC-derived neuromuscular assembloids to model myelination and neuromuscular features in CMT

B. Bekaert (1), T. van Wermeskerken (1), N. Donies (1), S. in't Groen (1), T. Vangansewinkel (1), Y. Lambrechts (2-4), K. Libberecht (2-5), E. Wolfs (2), V. Timmerman (1)

(1) Peripheral Neuropathy Research Group, Department of Biomedical Sciences, University of Antwerp, Belgium
(2) Functional Imaging & Research on Stem Cells (FIERCE), BIOMED Research Institute, Department of Cardio and Organ Systems, Hasselt University, Belgium
(3) Lab of Neurobiology, VIB-KU Leuven Center for Brain & Disease Research, Department of Neurosciences, KU Leuven, Belgium
(4) Experimental Neurology and Leuven Brain Institute (LBI), Department of Neurosciences, KU Leuven, Belgium
(5) Laboratory for Histology and Regeneration (HISTOREGEN), Department of Cardio and organ systems, Hasselt University, Belgium

Animal models for CMT often fail to translate into therapies, emphasizing the need for human-derived systems. We developed neuromuscular assembloids to study myelination and neuron-muscle interactions. Initially, we created hybrid models by fusing induced pluripotent stem cells (iPSC)-derived motor neuron spheres with immortalized muscle spheres. We are now advancing to complete iPSC-based models by fusing motor neuron spheres with skeletal muscle spheres, to which Schwann cell precursors can be added, all derived from iPSCs.

We assess myelination and neuromuscular characteristics through immunostaining (whole-mount and cryosections), Western blot, and RT-qPCR, with a focus on cellular maturation. Functional analyses using calcium imaging and multielectrode array assays are underway. We are currently introducing endogenously marked cell types to facilitate downstream analyses. Additionally, we are implementing optogenetics to create a more controllable experimental environment.

In response to the limited Schwann cell marker S100B signal observed in hybrid assembloids, Schwann cell precursors were incorporated into fully iPSC-derived assembloids. In these preliminary experiments, S100B-positive cells were initially detected in the 3D structure but declined over time, likely due to suboptimal culture conditions that are currently being optimized. While Myosin Heavy Chain (MyHC) signals were also detected in the hybrid assembloids, the fully iPSC-derived assembloids exhibited more widespread MyHC expression, suggesting enhanced muscle maturation. Further refinement of the muscle compartment is currently underway. Additionally, α-bungarotoxin signals were detected.

Overall, fully iPSC-derived neuromuscular assembloids offer a more suitable platform to study myelination and neuromuscular dynamics. We plan to extend this model to include CMT1A-derived cells in future studies.

Presentation PL1-02

Biallelic variants in the DARS2 gene as a novel cause of axonal Charcot-Marie-Tooth disease

B. Estévez-Arias (1,2,3*), S. Sarv (4*), N. Bonello-Palot (5,6), L. Carrera-García (1,7), C. Ortez (1,7), J. Expósito-Escudero (1,7), D. Yubero (8,9), J. Muchart (10), E. Delmont (11), E. Õiglane-Shlik (4,12), T. Meren (13), S. Puusepp (14), Ü. Murumets (13), G. S. Salomons (15), B. Udd (16,17,18), L. Väli (4,19), L. Cantarero (2,8), C. G. Bönnemann (20), A. Nascimento (1,7,8), S. Ramón-Maiques (8,21,22), K. Õunap (4,13), J. Hoenicka (2,8#), D. Natera-de Benito (1,7,8#), **F. Palau** (2,8,23,24#)

(1) Neuromuscular Unit, Department of Neurology, Hospital Sant Joan de Déu, Barcelona, Spain
(2) Laboratory of Neurogenetics and Molecular Medicine, Center for Genomic Sciences in Medicine, Institut de Recerca Sant Joan de Déu, Barcelona, Spain
(3) Universitat de Barcelona (UB), Barcelona, Spain
(4) Institute of Clinical Medicine, University of Tartu, Tartu, Estonia
(5) Marseille Medical Genetics, Aix-Marseille University–Inserm UMR 1251, Marseille, France
(6) Genetics Department, Timone Hospital, APHM, Marseille, France
(7) Applied Research in Neuromuscular Diseases, Institut de Recerca Sant Joan de Déu, Barcelona, Spain
(8) Center for Biomedical Research Network on Rare Diseases (CIBERER), ISCIII, Spain
(9) Department of Genetics, Hospital Sant Joan de Déu and Institut de Recerca Sant Joan de Déu, Barcelona, Spain
(10) Department of Radiology, Hospital Sant Joan de Déu, Barcelona, Spain
(11) Reference Center for Neuromuscular Disorders and ALS, APHM, CHU La Timone, Filnemus, ERN Neuro-NMD, Marseille, France
(12) Children`s Clinic, Tartu University Hospital, Tartu, Estonia
(13) Genetics and Personalized Medicine Clinic, Tartu University Hospital, Tartu, Estonia
(14) Department of Pathology, Tartu University Hospital, Tartu, Estonia
(15) Department Laboratory Medicine, Laboratory Genetic Metabolic Diseases, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands
(16) Folkhälsan Research Center, Helsinki, Finland
(17) Medicum, University of Helsinki, Helsinki, Finland
(18) Tampere Neuromuscular Center, Tampere University Hospital, Tampere, Finland
(19) Neurology Clinic, Tartu University Hospital, Tartu, Estonia
(20) Neuromuscular and Neurogenetic Disorders of Childhood Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, United States
(21) Structure of Macromolecular Targets Unit, Instituto de Biomedicina de Valencia (IBV), CSIC, Valencia, Spain
(22) Centro de Investigación Príncipe Felipe (CIPF), Associated Unit to IBV-CSIC, Valencia, Spain
(23) Hospital Sant Joan de Déu, Barcelona, Spain
(24) Division of Pediatrics, Faculty of Medicine and Health Sciences, Universitat de Barcelona, Barcelona, Spain

Aminoacyl-tRNA synthetases are associated with Charcot-Marie-Tooth (CMT) disease genetics and pathophysiology. DARS2, which encodes the mitochondrial aspartyl-tRNA synthetase, has been linked to leukoencephalopathy with brainstem and spinal cord involvement and elevated lactate levels (LBSL). This study aims to explore the connection between biallelic DARS2 variants and axonal CMT.

We examined five individuals from three unrelated families with axonal CMT and biallelic DARS2 variants. Functional studies in fibroblasts assessed effects on DARS2 expression, localization, and mitochondrial function. Enzymatic activity of recombinant proteins was tested in HEK293 cells.

The five individuals, including four adults, showed childhood-onset progressive axonal CMT. None had leukoencephalopathy, but one exhibited central nervous system involvement, with intellectual disability and

epilepsy. Genetic analysis identified compound heterozygous DARS2 variants: Family A, p.Ser238Phe and p.Arg336Cys; Family B, p.Ser238Phe and p.Ile25Thrfs*38; Family C, c.492+2T>C and p.Pro503Leu. Functional studies in Family A revealed reduced DARS2 protein levels, abnormalities in the mitochondrial network, and impaired mitochondrial respiratory chain activity. The p.Pro503Leu variant showed 25% residual aminoacylation activity.

Our findings expand the spectrum of DARS2-related diseases by identifying a new link to axonal CMT. We propose that p.Ser238Phe is a hypomorphic variant that, when combined with more damaging variants, causes isolated axonal CMT. More severe combinations—even though less harmful than those in LBSL—result in axonal CMT with central nervous system involvement but without leukoencephalopathy. These results suggest that DARS2-associated diseases may exist on a continuum rather than representing strictly separate disorders of the central or peripheral nervous system.

Estévez-Arias et al. Ann Neurol 2025 (in press)

Funding: Spanish Research Agency-AEI, PID2020-114655RB-I00

Presentation PL1-03

ABCA1 inhibition improves Schwann cell maturation and cholesterol deficiency in Charcot-Marie-tooth disease type 1A

K. Kuipers (1,2), Y. Lambrechts (1,3), T. Vangansewinkel (1,3,4), H. Jeurissen (1), K. Libberecht (1,3), N. Dirkx (1), F. Hermans (1), J. Dehair (5), JV. Swinnen (5), JFJ. Bogie (2), E. Wolfs (1)

(1) UHasselt, Department of Cardio and Organ Systems, BIOMED, Diepenbeek, Belgium
(2) UHasselt, Department of Immunology, BIOMED, Diepenbeek, Belgium
(3) VIB, Center for Brain & Disease Research, Laboratory of Neurobiology, Leuven, Belgium
(4) University of Antwerp, Peripheral Neuropathy Research Group, Department of Biomedical Sciences, Antwerp, Belgium
(5) KU Leuven, Department of Oncology, LKI - Leuven Cancer Institute, Laboratory of Lipid Metabolism and Cancer, Leuven, Belgium

Charcot-Marie-Tooth disease type 1A (CMT1A) is the most common inherited peripheral neuropathy, caused by a duplication of the PMP22 gene. This leads to impaired Schwann cell function and myelin sheath formation, causing motor and sensory dysfunction in patients. Increasing evidence suggests a link between PMP22 and ATP-binding cassette transporter 1 (ABCA1), a membrane co-transporter involved in cholesterol and phospholipid efflux. Notably, Schwann cells in CMT1A exhibit reduced cholesterol levels, identifying ABCA1 as a potential therapeutic target.

In this study, we investigated the role of ABCA1 in CMT1A pathology and assessed the effect of its inhibition using PSC-833 in human and murine disease models. In the C3-PMP22 mouse model, ABCA1 gene and protein expression were significantly upregulated in sciatic nerves from 4 weeks to 1 year of age. In addition, myelin displayed reduced cholesterol levels, as demonstrated by lower lipid polarity values using the cholesterol-sensitive dye Di-4-ANEPPDHQ, as well as a general decrease in lipid content.

Similarly, increased ABCA1 gene expression was observed in Schwann cells derived from CMT1A patient iPSC (iPSC-SC) and dental pulp stem cells (DPSC-SC), as well as in C3-PMP22 primary Schwann cells. ABCA1 inhibition with PSC-833 improves Schwann cell phenotype, as indicated by increased MPZ, and decreased CJUN, PMP22, and ABCA1 expression in healthy and CMT1A DPSC-SC. Furthermore, PSC-833 increases membrane cholesterol content in giant plasma membrane vesicles from both healthy and CMT1A Schwann cells.

Finally, in vivo treatment of C3-PMP22 mice using PSC-833 improved motor function and restoration of the myelin integrity.

Together, this study underlines a consistent lipid and cholesterol deficiency in CMT1A Schwann cells, and provides evidence that ABCA1 contributes to this starvation. Inhibiting ABCA1 restores key Schwann cell functions and represents a promising therapeutic avenue for functional recovery in CMT1A.

Presentation PL1-04

Unveiling novel players in HSPB8 pathology caused by frameshift mutations

B. Tedesco (1), V. Marchesi (1), V. Ferrari (1), M. Cozzi (1), P. Pramaggiore (1), R. Cristofani (1), V. Crippa (1), P. Rusmini (1), M. Chierichetti (1), L. Cornaggia (1), A. Mohamed (1), M. Galbiati (1), M. Piccolella (1), L. Weiss (2), V. Kimonis (2), V. Timmerman (3), A. Poletti (1)

(1) Laboratory of Experimental Biology, Dipartimento di Scienze Farmacologiche e Biomolecolari "Rodolfo Paoletti", Dipartimento di Eccellenza 2018-2027, Università degli Studi di Milano, Milano, Italy
(2) Department of Pediatrics, University of California Irvine, Irvine, CA, United States
(3) Peripheral Neuropathy Research Group, Department of Biomedical Sciences and Institute Born Bunge, University of Antwerp, Antwerpen, Belgium

Autosomal dominant mutations in heat shock protein B8 (HSPB8) cause a spectrum of diseases, including neuropathy, myopathy and cardiomyopathy. HSPB8 plays a pivotal role in striatal muscles and neurons, by facilitating the removal of damaged and aggregating-prone proteins in response to cellular stress. This function is mediated by its involvement in chaperone-assisted selective autophagy (CASA), alongside the BAG cochaperone 3 (BAG3), HSPA family members, and the E3-ubiquitin ligase STUB1. Recently, we defined a molecular mechanism through which HSPB8 frameshift mutations (fs) in the last exon of HSPB8 lead to this spectrum of diseases. These fs mutations cause a +1 or +2 nucleotide shift in the open reading frame, resulting in the expression of elongated HSPB8 proteins. In overexpressing cell models, both fs mutants exhibit similar pathological behaviors, including HSPB8 aggregation, sequestration of wild-type HSPB8, recruitment of CASA components, and proteostasis impairment. Intriguingly, analyses on patient-derived fibroblasts carrying an HSPB8 fs+1 mutation (c.515dupC) revealed the absence of the elongated HSPB8 protein and downregulation of the HSPB8 mRNA from the mutated allele. This suggests the activation of quality control (QC) mechanisms at the translational level. To investigate this further, we examined components of the ribosome-associated and protein QC systems, focusing on NEMF, the ubiquitin ligase LTN1, and valosin-containing protein (VCP). Using an HSPB8 fs+1 mutant as a reference in cell models, we observed upregulation of core RQC components NEMF and LTN1 at the transcript level, although their protein levels remained unchanged. In contrast, VCP was sequestered by HSPB8 mutants, and its silencing exacerbated protein aggregation, likely contributing to HSPB8-pathology. In summary, our findings define shared pathogenic mechanisms among various HSPB8 fs mutations and highlight novel molecular players involved in HSPB8-associated disease pathology.

Presentation PL1-05

Bioengineering the neuromuscular junction to investigate CMT2-pathophysiology using hiPSC-derived cell models

S.L.M. in 't Groen (1), B. Bekaert (1), P. Pramaggiore (1,2), A. Poletti (2), V. Timmerman (1)

(1) Peripheral Neuropathy Research Group, Department Biomedical Sciences, University of Antwerp, Belgium
(2) Laboratory of Experimental Biology, Dipartimento di Scienze Farmacologiche e Biomolecolari "Rodolfo Paoletti" (DiSFeB), Università degli studi di Milano, Milan, Italy

Charcot-Marie-Tooth disease (CMT), like most peripheral neuropathies, currently lacks effective therapeutic strategies. A major barrier to therapeutic development is the absence of suitable preclinical models that recapitulate the complex neuromuscular phenotypes observed in patients. Although animal models have significantly advanced our understanding of disease pathophysiology, they often fall short in translational relevance. Likewise, conventional 2D in vitro systems often lack the architecture and cell–cell interactions that influence disease pathology.

To address these challenges, we aim to develop in vitro platforms to investigate the human neuromuscular system using iPSC-derived motor neurons and skeletal muscle cells. First, we have utilized microfluidic devices to establish a 2D model containing the neuromuscular junction (NMJ), compatible with high-resolution microscopy and live-cell imaging. Second, we are optimizing a 3D-Innervated-Skeletal-Muscle-on-a-chip (3D-iSM) platform that allows for contractile force measurements and NMJ-mediated action potential transmission. Finally, we aim to establish a functional NMJ-on-a-microelectrode array (MEA) platform for electrophysiological assessment of neuromuscular activity.

Thus far, we have visualized and quantified axonal transport of mitochondrial and lysosomal cargo in healthy iPSC-derived motor neurons using the microfluidic system. We are currently investigating the impact of HSPB1 and HSPB8 mutations (CMT2F and CMT2L, respectively). In parallel, we have engineered healthy 3D-iSM tissues displaying aligned myofibers, myonuclear chains, and acetylcholine receptor clusters, representing motor endplates and potential NMJ sites.

Next, we will evaluate bioengineering strategies to promote guided axonal extension and muscle innervation. We also plan to adapt the 3D-iSM model for MEA-based assessment of neuromuscular activity and further investigation into CMT pathophysiology.

Presentation PL1-06

Long-read sequencing reveals SORD/SORD2P inversions as a common cause of SORD-CMT missed by short-read sequencing

I. Quartesan (1), S. Facchini (1,2), A. Manini (3), R. Parolin Schnekenberg (1), C. Pisciotta (4), S. Magri (5), S. Negri (6), C. Gaetano (6), A. Rebelo (7), J. Schatzman Raposo (7), R. Mazanec (8), R. Curro (1), N. Dominik (1), S. Efthymiou (1), M. Laurà (1), T. Grider (9), S. M.E. Feely (10), V. Fridman (11), A. Bertini (1,4), G. M. Alves (1,12), L. Ferullo (1,13), A. Ghia (2), C. Caccia (5), F. Balistreri (5), P. Saveri (4), L. Crivellari (4), I. Moroni (14), F. R. Danti (14), T. Mongini (15), F. Taroni (5), M. Auer-Grumbach (16), E. Bugiardini (1), J. N. Sleigh (1,17), A. Tucci (18,19), H. Houlden (1), P. Laššuthová (20), P. Seeman (20), A. Basile (21), E. Giorgio (21,22), M. E. Shy (23), S. Zuchner (7), M. M. Reilly (1), D. Pareyson (4), A. Cortese (1)

(1) Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, WC1N 3BG, UK
(2) Department of Brain and Behavioural Sciences, University of Pavia, 27100 Pavia, Italy
(3) Department of Pathophysiology and Transplantation, "Dino Ferrari" Centre, Università degli Studi di Milano, Milan, Italy
(4) Unit of Rare Neurological Diseases, Department of Clinical Neurosciences, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, 20133, Italy
(5) Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
(6) Laboratorio di Epigenetica, Dipartimento Medicina Riabilitativa NeuroMotoria - MeRiNM, Istituti Clinici Scientifici Maugeri IRCCS, Pavia, 27100, Italy
(7) Dr. John T. Macdonald Foundation, John P. Hussman Institute for Human Genomics, University of Miami, Miami, FL 33136, USA
(8) Neurological Department of the Motol University Hospital, Prague, Czech Republic
(9) Neurology, The University of Iowa Roy J and Lucille A Carver College of Medicine, Iowa City, IA, USA
(10) Division of Pediatric Neurology, Seattle Children's Hospital, University of Washington School of Medicine, Seattle, WA, USA.
(11) Department of Neurology, University of Colorado Denver School of Medicine, Aurora, CO 80045, USA
(12) Department of Neurosciences and Behaviour Sciences, Neuromuscular Disorders, University of São Paulo, Ribeirao Preto 14040-900, Brazil
(13) Department of Clinical and Experimental Sciences, University of Brescia, 25121 Brescia, Italy
(14) Department of Pediatric Neurosciences, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
(15) Department of Neurosciences Rita Levi Montalcini, University of Turin, Turin, Italy
(16) Department of Orthopaedics and Trauma Surgery, Medical University of Vienna, Vienna, Austria
(17) UK Dementia Research Institute, University College London, London, UK
(18) William Harvey Research Institute, Queen Mary University of London, London EC1M 6BQ, UK
(19) Department of Neurodegenerative Disease, UCL Queen Square Institute of Neurology, University College London, London, UK.
(20) Department of Paediatric Neurology, 2nd Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic
(21) Department of Molecular Medicine, Medical Genetics Unit, University of Pavia, 27100 Pavia, Italy
(22) IRCCS Mondino Foundation, Neurogenetics Research Centre, 27100 Pavia, Italy
(23) Department of Neurology, Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA.

Background

Pseudogenes are non-functional gene copies arising from duplication or retrotransposition. Although traditionally viewed as genomic relics, they can cause human disease via recombination with their parental genes. Despite the broad adoption of short-read whole-genome sequencing (srWGS), over half of Mendelian

disease cases remain undiagnosed, highlighting intrinsic limitations of short-read approaches in detecting structural variants (SVs), especially within repetitive or homologous genomic regions.

Methods

We first performed a genome-wide analysis of 1,019 long-read sequencing (LRS) samples from the 1000 Genomes Project to identify recurrent gene–pseudogene inversions present in healthy individuals. To assess whether these inversions might contribute to genetic disorders, we next applied LRS and optical genome mapping (OGM) to a cohort of unsolved axonal neuropathy cases.

Results

We found that recurrent gene–pseudogene inversions occur in at least 3.6% of healthy individuals yet remain systematically undetected by srWGS and mostly absent from gnomAD SVs database. The SORD/SORD2P locus exhibited the highest inversion frequency. We therefore studied patients clinically suspected of having SORD-related Charcot–Marie–Tooth disease (SORD-CMT) but with only a single pathogenic SORD variant identified by srWGS. Combining LRS and OGM, we demonstrated that recurrent inversions between SORD and SORD2P represent the third most common pathogenic allele for SORD-CMT. Crucially, these inversions explained 75% of patients with only one previously identified SORD variant, enabling enrolment for an ongoing therapeutic trial of the aldose-reductase inhibitor govorestat (NCT05397665).

Conclusions

These findings suggest that gene–pseudogene inversions may represent largely overlooked pathogenic SVs in Mendelian disease. Their systematic detection through long-read technologies has the potential to improve diagnostic yield and inform future clinical decision-making.

Presentation PL2-01

Biallelic PIGB variants are a cause of childhood-onset, motor-predominant neuropathy with conduction blocks and neuromyotonia

G. Fernández-Eulate (1), R. Duval (2), M. Konyukh (3,4), A. Bertini (5), A. Candayan (6,7), A. Jordanova (6,7,8), T. Brunet (9), W. Müller-Felber (10), B. Isidor (11), B. Cogné (11), Y. Péréon (12), A. Cortese (13), Y. Parman (14), A. Cakar (15), D. Pareyson (5), S. Azouzi (2), T. Stojkovic (1)

(1) Nord/Est/Ile-de-France Neuromuscular Diseases Reference Center, Institut de Myologie, Pitié-Salpêtrière Hospital, APHP, 75013 Paris, France
(2) Université Paris Cité, UMRS 1134, Biologie Intégrée du Globule Rouge (BIGR), INSERM, Paris, France
(3) Department of Medical Genetics, Henri Mondor Hospital, APHP, Créteil, France
(4) Laboratoire de biologie médicale multisites SeqOIA - PFMG2025, Paris, France
(5) Unit of Rare Neurological Diseases, Department of Clinical Neurosciences, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20133 Milan, Italy
(6) Molecular Neurogenomics Group, VIB Center for Molecular Neurology, Antwerp, Belgium
(7) Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium
(8) Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Medical University-Sofia, Sofia, Bulgaria
(9) Institute of Human Genetics, Klinikum rechts der Isar, School of Medicine, Technical University of Munich, 81675 Munich, Germany
(10) LMU Munich, University Hospital, Hauner Children's Hospital, Pediatric Neurology and Developmental Medicine, Munich, Germany
(11) Service of Medical Genetics, CHU Nantes, Université de Nantes, CNRS, INSERM, L'Institut du Thorax, Nantes, France
(12) Neuromuscular Diseases Reference Center AOC, Laboratoire d'Explorations Fonctionnelles, CHU de Nantes, FILNEMUS, Euro-NMD, Nantes, France
(13) Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, WC1N 3BG, UK
(14) Neuromuscular Unit, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey

Glycosylphosphatidylinositol (GPI)-anchored proteins (GPI-AP) play crucial roles in the nervous system and biallelic variants in phosphatidylinositol glycan (PIG) genes cause severe neurodevelopmental disorders in infants. We report the first adult patients carrying mostly novel PIGB variants, presenting with childhood-onset, motor-predominant neuropathy with conduction blocks and neuromyotonia.

Genotyping was performed via exome/genome sequencing. Clinical data were collected retrospectively. GPI-AP expression was analyzed in blood cells by flow cytometry. Variant validation using a PIGB KO/KI K562 model is ongoing.

One known [p.(Arg232His)] and seven novel [p.(Leu112Pro), p.(Trp182Leu), p.(Val295Met), p.(Val414Ile), p.(Met436Ile), p.(Met454Thr), de novo inversion inducing a PIGB(E1–E9):Rab7(E7) fusion] PIGB variants were identified in 6 adults from 5 families (3 females, median age 35.5 years). All had pes cavus and distal lower-limb weakness from childhood, and walked independently on last visit. ENMG showed motor-predominant neuropathy with conduction blocks in five patients and one had demyelinating sensorimotor neuropathy. Four showed electrical neuromyotonia and muscle hypertrophy, 2 only muscle hypertrophy. One had episodic proximal weakness with diffuse decrements in repetitive nerve stimulation responsive to pyridostigmine. All but one patient had mild psychomotor delay or behavioral problems and two had transit epilepsy during childhood. One had mild dysmorphic features and five had elevated alkaline phosphatase. GPI-AP expression in blood cells was mildly to moderately reduced in all patients. The p.(Leu112Pro) and p.(Met436Ile) have been validated in vitro.

PIGB is a novel cause of slowly progressive, motor-predominant neuropathy with conduction blocks. Neuromyotonia, more common in acquired neuropathies, was frequent, reminiscent of HINT1-associated CMT. PIGB should be screened in unexplained neuropathies with conduction block and neuromyotonia.

Presentation PL2-02

Advancing genetic diagnostics in Charcot-Marie-Tooth disease: Lessons learned from long-read sequencing

A. Candayan (1,2), L. L. P. Ramos (1,2), E. De Vriendt (1,2), T. De Pooter (3,4), P. De Rijk (3,4), M. Strazisar (3,4), A. Jordanova (1,2,5)

(1) Molecular Neurogenomics Research Group, Center for Molecular Neurology, VIB, University of Antwerp, Antwerpen, Belgium
(2) Molecular Neurogenomics Research Group, Department of Biomedical Sciences, University of Antwerp, Antwerpen, Belgium
(3) Neuromics Support Facility, Center for Molecular Neurology, VIB, University of Antwerp, Antwerpen, Belgium
(4) Neuromics Support Facility, Department of Biomedical Sciences, University of Antwerp, Antwerpen, Belgium
(5) Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Faculty of Medicine, Medical University-Sofia, Sofia, Bulgaria

Charcot-Marie-Tooth (CMT) disease is marked by striking clinical and genetic heterogeneity, leaving over 35% of patients without a definitive genetic diagnosis despite extensive testing. The causal gene discovery rate has significantly decelerated over the past years, partly due to technical limitations of short-read sequencing. In contrast, long-read sequencing (LRS) is emerging as a powerful tool to uncover elusive variants and previously unrecognized mutational mechanisms that contribute to the heritability gap in CMT disease.

In this study, we applied nanopore whole-genome sequencing using the PromethION platform to 35 individuals from 14 families presenting with CMT, all of whom had remained genetically undiagnosed after in-depth analyses with short-read technologies. This cohort provided a unique opportunity to explore the diagnostic potential of LRS. We achieved a 28% diagnostic uplift through a combination of targeted and gene-agnostic approaches, providing long-awaited answers for families who had previously reached a diagnostic dead end.

While LRS for rare disease research revealed substantial promise, it also uncovered significant practical challenges. Key issues included variable data quality from archived samples, limited availability of large, ancestry-matched structural variant databases, demanding bioinformatic workflows, and difficulty in orthogonal validation of candidate structural variants.

Our findings highlight the ability of LRS in improving diagnostic yield and demonstrate that this technology holds great promise as a first-line diagnostic tool. Moreover, our experience offers practical insights for other researchers navigating this rapidly evolving field. As long-read technologies continue to shape the next paradigm shift in human genetics, openly sharing experiences and knowledge among early adopters of the technology will be essential to accelerate progress, ultimately benefiting both patients and the research community.

Presentation PL2-03

Gradient-boosted discrimination of inflammatory neuropathies from hereditary Charcot-Marie-Tooth disease using motor nerve conduction metrics

T. Pardini Fagundes (1), L. Gondim Briand Vieira (1), M. Guerra de Albuquerque Bueno (1), R. Siqueira Soares Frezatti (1), G. Maximiano Alves (1), C. Ferreira da Rosa Sobreira(1), W. Marques Júnior(1), **P. J. Tomaselli** (1)

(1) Department of Neurosciences, Division of Neurology, Ribeirão Preto Medical School, University of São Paulo, Ribeirão Preto, SP, Brazil

Gradient-boosted discrimination of CIDP/POEMS from hereditary CMT using routine motor NCS metrics Introduction We developed a compact machine-learning classifier to distinguish CIDP and POEMS syndrome from hereditary CMT (CMT1A/B, CMTX1, HNPP) using only routine motor nerve-conduction study parameters. Methods In a retrospective cohort of 70 genetically or clinically confirmed cases (39 hereditary, 23 CIDP, 8 POEMS; 2014–2024), 18 features—including patient age, 13 left-median/ulnar metrics (distal latency, MNCV, CMAP amplitude, F-wave latency), two derived indices (NCV uniformity, proximal/distal amplitude ratio) and two F-wave-absence flags—were mean-imputed and class-balanced with SMOTE-Tomek. A 300-tree, depth-3 HistGradientBoostingClassifier (seed 42) was trained and evaluated by 5-fold stratified cross-validation and an independent 20 % hold-out set, with permutation-based feature importance. Results Cross-validation yielded a macro F1 = 0.78 (95 % CI 0.64–0.89) and AUROC = 0.89 (95 % CI 0.79–0.97). On the hold-out set (n = 14), accuracy = 0.64, balanced accuracy = 0.63, macro F1 = 0.63 and AUROC = 0.77 at a 0.50 threshold. A precision-recall analysis raised CIDP/POEMS sensitivity from 0.50 to 0.83 at a 0.95 cut-off, pending external validation. Permutation importance identified age, NCV uniformity and ulnar distal latency as the strongest discriminators. Calibration was acceptable (Brier = 0.22; ECE = 0.08). Conclusions This lightweight gradient-boosting model achieves robust performance (cross-validated macro F1 ≈ 0.8) for differentiating treatable inflammatory/paraneoplastic neuropathies from hereditary CMT using only motor NCS metrics. It integrates seamlessly into EMG workflows without specialized hardware. Prospective multicentre validation (> 150 exams) is underway, and the final model will be released in ONNX format for EMG workstations.

Presentation PL2-04

A CCG expansion in TBC1D7 defines a novel neuromuscular disorder – lessons for the next wave of gene discovery

L. Van de Vondel (1,2), R. Curro (3), S. Facchini (3), I. Xu (1), J. De Winter (2,4), I. Quartesan (3), A. Monticelli (2), A. Alonso-Jimenez (4), W. De Ridder (4), S. Zuchner (1), J. Baets (2,4), A. Cortese (3)

(1) Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL, USA
(2) Translational Neurosciences, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium
(3) Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, UK
(4) Neuromuscular Reference Centre, Department of Neurology, Antwerp University Hospital, Belgium

75 tandem repeat (TR) loci are established to be disease-causing, of which several play a causal role in rare neuromuscular diseases. TR loci are complex and technically challenging to characterise, a hurdle that can be overcome by employing novel long-read (LR) sequencing and analysis techniques.

In this study, we identify a 5' UTR CCG expansion in TBC1D7 as a novel cause of oculopharyngodistal myopathy (OPDM), analogous to previously identified 5' UTR CCG expansions (OPDM1-6). Combining several LR technologies, including PacBio HiFi, Oxford Nanopore Technology and Optical Genome Mapping, we delineate the TR locus in patient tissues and large control datasets. We characterise the locus to be particularly variable in the control population, a recently identified hallmark of pathogenicity. We furthermore establish somatic length variability as well as epigenetic silencing of the repeat locus, leading to non-penetrance. We find increased transcript levels in patient-derived fibroblasts and observe intranuclear inclusions on muscle biopsy, rendering it likely that this particular CCG expansion results in repeat-associated non-AUG translation and polyalanine-mediated aggregation.

Our findings establish the 5' UTR of TBC1D7 as a novel disease locus, and add to the disease-specificity of 5' UTR CCG expansions causing OPDM. We currently identified three families, of which one through a family-based LR analysis and the other two through a cohort-based analysis of patients with similar phenotypes. As the two converging analyses were performed independently, both avenues are valid options when analysing datasets of patients with unsolved Charcot-Marie-Tooth disease or related neuropathies. As increasing evidence points to genotype-phenotype relations for TRs to be dependent on TR motif and location, and less so specific gene function, we will use the identification of TBC1D7 as a guideline for the identification of novel pathogenic motifs causative of neuropathies.

Presentation PL3-01

Rescue of CMT2A pathology by two therapeutic approaches aimed at restoring defective organelle contacts and associated pathways

M. Tessier (1)*, Z. Hamze (1)*, N. Bonello (1), N. Roeckel-Trévisiol (1), K. Bertaux (2), M. Bartoli (1), E. Abgueguen (4), P. Miniou (4), V. Delague* (1), B. Schneider* (2), **N. Bernard-Marissal** (1)

(1) Aix Marseille Univ, INSERM, MMG, U 1251, Marseille, France
(2) Centre de Ressources Biologiques, Assistance Publique Hôpitaux de Marseille, CRB APHM, Hôpital Timone Adulte, Biogénopôle, Marseille, France
(3) Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne (EPFL), Station 19, 1015 Lausanne, Switzerland
(4) Inflectis Bioscience, Nantes, France

Charcot-Marie-Tooth disease type 2A (CMT2A) is the most common axonal form of CMT and results from mutations in the MFN2 gene, which encodes mitofusin-2 (MFN2), a critical regulator of mitochondrial fusion and ER-mitochondria contact sites. Our previous work first demonstrated that disruption of ER-mitochondria contacts in CMT2A leads to altered calcium signaling and ER stress, underlying the disease pathology. The large number of MFN2 mutations distributed across all domains of the protein presents a significant challenge for broad therapeutic approaches.

In this study, we directly addressed these pathomechanisms by developing and testing two novel therapeutic strategies. Our gene therapy approach involved neuron-specific overexpression of wild-type MFN2, as well as a modified MFN variant designed to compensate for mutated MFN2. In parallel, we tested a pharmacological approach using IFB-088, an enhancer of the integrated stress response, in collaboration with Inflectis Bioscience.

Utilizing iPSC-derived motor neurons from CMT2A patients, we showed that both gene therapy and IFB-088 effectively restore ER-mitochondria interactions and improve mitochondrial function, resulting in reduced axonal degeneration. Furthermore, in a preclinical mouse model expressing the MFN2Arg94Gln mutation, our intrathecal delivery of AAV9 carrying therapeutic MFN2 transgenes led to significant improvements in locomotor function and reduction of axonal pathology, which was associated with restored mitochondrial and ER-mitochondrial contacts in the spinal cord and nerves. Importantly, our recent data also reveal that IFB-088 alone can prevent locomotor deficits in these mice.

Overall, our work establishes proof of concept for both gene and pharmacological therapies that specifically target the organelles dysfunctions we previously identified in CMT2A. These promising results open avenues for future clinical development with the potential to benefit CMT patients.

Presentation PL3-02

Alpha-1 Antitrypsin demonstrates therapeutic efficacy in a mouse model of Charcot-Marie-Tooth Disease Type 1A

N. Zhukovsky (1), C. Faria (1), M. Silvano (1), T. Filloux (1), K-H. Krause (1)

(1) Ageronix SA, 64 Avenue de la Roseraie, 1205 Geneva, Switzerland

Peripheral neuropathies that are characterized by compromised myelin sheath integrity and innate Schwann cell dysfunction, such as Charcot-Marie-Tooth disease type 1A (CMT1A), lead to progressive motor and sensory deficits with debilitating consequences. Myelin sheath defects are often associated with chronic immune activation, suggesting a potential therapeutic role for anti-inflammatory and immunomodulatory agents like Alpha-1 Antitrypsin (AAT).

In this study, we conducted a detailed comparative analysis of AAT's therapeutic potential in a C3-PMP22 CMT1A mouse model. Mice (n=8) received bi-daily dose of 90mg/kg subcutaneous AAT (human plasma-derived), and outcomes were evaluated through behavioral and electrophysiological testing, nerve histology, and plasma cytokine profiling at defined time points.

AAT administration over a two week period significantly improved the nerve conduction velocity (NCV) and compound muscle action potential (CMAP) and enhanced both the axonal diameter and myelin sheath thickness (g-ratio) in CMT1A mice. Functionally, AAT-treated mice exhibited marked improvements in neuromuscular strength and coordination compared to untreated controls.

These findings reveal previously unrecognized neuroprotective and restorative effects of AAT in an animal model of an inherited demyelinating neuropathy. Our results support further investigation of AAT as a promising therapeutic strategy for CMT1A and potentially other inherited and acquired neuropathies.

Presentation PL3-03

A new selective HDAC6 inhibitor ameliorates disease phenotype in a Gdap1$^{-/-}$ mouse model.

M. Porras (1), Y. Vara (2), E. Aldaba (2), J. Hoenicka (1,3), F. Palau (1,3,4,5), **L. Cantarero** (1,3)

(1) Laboratory of Neurogenetics and Molecular Medicine, Center for Genomic Sciences in Medicine, Institut de Recerca Sant Joan de Déu, Barcelona, Spain
(2) Quimatryx S.L., San Sebastián, Spain
(3) Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain
(4) Hospital Sant Joan de Déu, Barcelona, Spain
(5) Division of Pediatrics, Faculty of Medicine and Health Sciences, University of Barcelona, Barcelona, Spain

GDAP1-related Charcot-Marie-Tooth (CMT) disease can be expressed as an axonal or demyelinating neuropathy, either recessive or dominant. In Spain, GDAP1 mutations are the leading cause of axonal CMT, accounting for approximately 11.5% of clinically diagnosed CMT cases. In recent years, histone deacetylase (HDAC) inhibitors have emerged as promising epigenetic therapeutics, with five currently approved by the FDA. Among these, considerable efforts have focused on developing HDAC6-selective inhibitors, as HDAC6 plays a deacetylase activity–dependent inhibitory role in axonal transport and regeneration, both of which are critical processes affected in axonal CMT. The specific involvement of HDAC6 in axonal dysfunction, along with its selective druggability, makes it an attractive therapeutic target.

Based on the structural properties of the HDAC6 enzyme and Quimatryx S.L. expertise in designing and synthesizing HDAC6-selective inhibitors, a new family of HDAC6 inhibitors that cross the blood-brain barrier was designed. In this sense, a different group from the hydroxamic was chosen as a zinc-chelating group, together with groups that reduce lipophilicity in the rest of the molecule structure.

In this study, we evaluated the therapeutic potential of QTX153, a novel selective HDAC6 inhibitor, in the Gdap1-/- mouse model. Preclinical trials were conducted using a 60 mg/kg dose in both male and female Gdap1-/- mice under two treatment paradigms: a presymptomatic regimen and an intervention initiated after the onset of phenotype. In both approaches, QTX153 administration led to significant improvements in motor function, measured by hind limb angle, severity score, hanging test, and maximum distance on rotarod.

These promising preliminary results demonstrate that QTX153 exerts therapeutic effects even in symptomatic animals, aligning more closely with real-world clinical scenarios and supporting further development of HDAC6-targeted therapies for GDAP1-related CMT.

Presentation PL3-04

Restoration of the physiological levels of PMP22 in CMT1A patient cells via base editing of the Kozak sequence

A. Raoss (1), S. Sanniti (1), M.C. Diener (1), E. Destefanis (3), E. Rossi (1), G. Zappacosta (1), C. Ambrosini (2), V. Sidarovich (1), A. Quattrone (1)

(1) Dept CIBIO, University of Trento, Italy
(2) Fondazione Human Technopole, Milano, Italy
(3) Fralin Life Sciences Institute, Virginia Tech Cancer Research Center DC, Washington, D.C., United States

Hereditary peripheral neuropathies, including Charcot-Marie-Tooth (CMT) disease, are prevalent genetic disorders of the nervous system, affecting around 1 in 2500 people. CMT1A, the most common form of the disease, is caused by a duplication of the PMP22 gene on chromosome 17, which is sensitive to gene dosage. This leads to the overproduction of the PMP22 protein. Currently, there are no approved therapies that halt or reverse CMT1A at its root, so management remains symptomatic and supportive, focusing on preserving strength, mobility, and quality of life.

We applied advanced gene-editing technology — specifically, base editing — to control PMP22 protein levels precisely and moderately. Our approach was to modulate the translational efficiency of the PMP22 mRNA by introducing targeted point mutations into the Kozak sequence of the PMP22 gene to decrease its codon recognition ability. This original approach does not cause genomic damage and aims to fine-tune PMP22 protein levels while avoiding the adverse effects associated with the loss of an allele.

To identify the variants required to restore physiological protein levels, we screened PMP22 Kozak variants in a high-throughput manner to identify and validate those that reduce translational efficiency. We introduced the three best-performing variants into HEK293T cells overexpressing PMP22.

We achieved editing efficiencies above 30% for each variant. Western blot analysis revealed a 30% decrease in PMP22 protein levels in two of the edited variants. We will validate our approach further in patient-derived induced pluripotent stem cells (iPSCs) differentiated to Schwann cell precursors. This strategy has already been patented and, if successfully developed, could form the basis of an advanced clinical approach to CMT1A, overcoming the limitations of other proposed solutions and potentially paving the way for a 'one-shot' gene therapy treatment.

Presentation PL3-05

Therapeutic Potential and Safety of allogeneic mesenchymal stromal cell (EN001) in CMT1A and CMT1E: First-in-Human Evidence from South Korea

Y. Choi (1,2), **H. Kim** (1,2), M. Kim (1), J. Hyun Park (1), S. Eon Park (1,2), J. Wook Chang (2,3), B-O. Choi (2-4)

(1) New Drug Development Division, ENCell Corp., Seoul 05854, Republic of Korea
(2) Cell & Gene Therapy Institute, Samsung Medical Center, Seoul 06351, Republic of Korea
(3) Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Seoul 06351, Republic of Korea
(4) Department of Neurology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 06351, Republic of Korea

Charcot–Marie–Tooth disease (CMT) is the most common inherited peripheral neuropathy, affecting 1 in 2,500 individuals, with an estimated 300,000 patients in Europe. Over 140 causative gene variants have been identified, with duplication of PMP22 responsible for nearly 50 % of all cases. Despite being first described in 1886, no EMA-approved therapy for CMT exists. Regenerative medicine offers a novel approach for intractable neuromuscular diseases such as CMT. Mesenchymal stromal cells (MSCs) exert regenerative and immunomodulatory effects via homing and paracrine actions. MSCs can be administered allogeneically due to their immune-privileged nature, allowing for off-the-shelf use. MSC-derived factors support immune-modulation, anti-apoptosis of nerves and muscles, and remyelination. In our previous preclinical studies, Wharton's jelly-derived MSCs (WJ-MSCs) enhanced Schwann-cell proliferation and remyelination in C3, C22, and Tr-J CMT mouse models. EN001, an allogeneic WJ-MSC therapy developed by ENCell Corp., was evaluated in two single-dose first-in-human studies in South Korea: one in patients with CMT1A (NCT05333406) and another in patients with CMT1E (NCT06218134). Safety and tolerability were the primary endpoints; exploratory efficacy was assessed via the CMT Neuropathy Score version 2 (CMTNSv2), Functional Disability Scale, Overall Neuropathy Limitation Scale (ONLS), and nerve conduction studies (NCS), etc. EN001 was well tolerated, with no dose-limiting toxicities and serious adverse events. Only transient, mild adverse events unrelated to EN001 were observed. Both patients with CMT1A and CMT1E showed reductions in CMTNSv2 after a single administration of EN001, indicating clinical improvement. These findings demonstrate the safety and preliminary therapeutic potential of EN001 and suggest it could become a viable therapeutic option for CMT. Large, multi-ethnic randomized controlled trials are now required to confirm safety and efficacy of EN001.

Presentation PL3-06

Loss of ARHGAP19 function disrupts RhoA regulation in Charcot-Marie-Tooth disease: mechanisms and therapeutic targets

N. Dominik (1), S. Efthymiou (1), CJ. Record (1), X. Miao (2,51), R. Lin (1), JM. Parmar (3), A. Scardamaglia (1), R. Maroofian (1), S. Lowe (4), G. Aughey (4), AD. Wilson (4), R. Curro (1,5), RP. Schnekenberg (1), S. Alavi (1), L. Leclaire (2,51), Y. He (2,51), K. Zhelcheska (1), Y. Bellaiche (6), I. Gaugué (6), M. Skorupinska (1), L. Van de Vondel (7,8), SI. Da'as (9), V. Turchetti (1), S. Güngör (10), G. Monahan (3), Ehsan Ghayoor Karimiani (1,11), Y. Jamshidi (11), PJ. Lamont (12), CA. Ricaurte (13,14), H. Topaloglu (15), A. Jordanova (13,14,16), M. Zaman (17), SH. Banu (17), W Marques (18), PJ. Tomaselli (19), B. Aynekin (1), A. Cansu (20), H. Per (21), A. Güleç (21), JR. Alvi (22), T. Sultan (22), A. Khan (23,24), G. Zifarelli (25), S. Ibrahim (26), GMS. Mancini (27), MM Motazacker (28), E. Brusse (27), V. Lupo (29), Teresa Sevilla (30,31), AN. Başak (32), S. Tekgul (32), RJ. Palvadeau (32), J. Baets (7,8,33), Y. Parman (34), A. Çakar (34), R. Horvath (35,36), TB. Haack (37,38), JH Stahl (39,40), K. Grundmann-Hauser (37,38), J. Park (37,38), S. Züchner (41,42), NG. Laing (3), L. Wilson (1), AM. Rossor (43), J. Polke (44), F. Barbosa Figueiredo (45), AL. Pessoa (46), F. Kok (45), A. Rodrigues Coimbra-Neto (47), MC. França Jr (47), G. Ravenscroft (3), SAhmed Hamed (48), WK. Chung (49), AM. Pittman (50), DP. Osborn (50), M. Hanna (1), A. Cortese (1,5), MM. Reilly (1), JEC. Jepson (4), N. Lamarche-Vane (2,51), H. Houlden (1)

(1) Department of Neuromuscular Disease, UCL Queen Square Institute of Neurology, UK
(2) Department of Anatomy and Cell Biology, McGill University, Canada
(3) Harry Perkins Institute of Medical Research, Centre for Medical Research, University of Western Australia, Australia
(4) Department of Clinical and Experimental Epilepsy, UCL Queen Square Institute of Neurology, UK
(5) Department of Brain and Behavioral Sciences, University of Pavia, Italy
(6) Institut Curie, Université PSL, Sorbonne Université, CNRS UMR3215, INSERM U934, Genetics and Developmental Biology, France.
(7) Translational Neurosciences, Faculty of Medicine and Health Sciences, University of Antwerp, Belgium.
(8) Laboratory of Neuromuscular Pathology, Institute Born-Bunge, University of Antwerp, Belgium
(9) Department of Human Genetics, Sidra Medicine, Qatar
(10) Inonu University, Faculty of Medicine, Turgut Ozal Research Center, Department of Pediatric Neurology, Turkey
(11) Molecular and Clinical Sciences Institute, St. George's, UK
(12) Royal Perth Hospital, Australia
(13) Molecular Neurogenomics group, VIB Center for Molecular Neurology, Belgium
(14) Molecular Neurogenomics group, Department of Biomedical Sciences, University of Antwerp, Belgium
(15) Department of Pediatric Neurology, Hacettepe University, Turkey
(16) Department of Medical Chemistry and Biochemistry, Medical University-Sofia, Bulgaria
(17) Department of Pediatric Neurology, Dr. M.R. Khan Shishu (Children) Hospital and ICH, Bangladesh
(18) Department of Neurosciences, School of Medicine of Ribeirão Preto, University of São Paulo, Brazi
(19) Clinical Hospital of Ribeirão Preto, Department of Neurosciences and Behaviour Sciences, University of São Paulo, Brazil
(20) Department of Pediatric Neurology, Faculty of Medicine, Farabi Hospital, Karadeniz Technical University, Turkey
(21) Department of Pediatric Neurology, Erciyes University, Turkey
(22) Children's Hospital & the Institute of Child Health, Pakistan
(23) Pediatric Neurology, Neuropedia Hospital, ARE
(24) Pediatric Neurology, Mohammed Bin Rashid University of Medicine and Health Sciences, ARE
(25) CENTOGENE GmbH, Am Strande 7, Germany
(26) Department of Pediatrics and Child Health, Aga Khan University, Pakistan
(27) Department of Clinical Genetics, ErasmusMC University Medical Center, The Netherlands
(28) Laboratory of Genome Diagnostics, Department of Human Genetics, Amsterdam UMC, University of Amsterdam, the Netherlands
(29) Rare Neurodegenerative Diseases Laboratory, Centro de Investigación Príncipe Felipe, Spain

(30) Hospital Universitari i Politècnic La Fe & IIS La Fe, Neuromuscular Diseases Unit, Department of Neurology, Spain
(31) Universitat de València, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Spain
(32) Suna and İnan Kıraç Foundation, Neurodegeneration Research Laboratory (NDAL), Research Center for Translational Medicine (KUTTAM), Koç University School of Medicine, Turkey
(33) Neuromuscular Reference Centre, Department of Neurology, Belgium
(34) Department of Neurology, Istanbul Medical School, Istanbul University, Turkey
(35) Department of Clinical Neurosciences, School of Clinical Medicine, University of Cambridge, UK
(36) Department of Clinical Neurosciences, John Van Geest Centre for Brain Repair, School of Clinical Medicine, UK
(37) Center for Rare Disease, University of Tübingen, Germany
(38) Institute of Medical Genetics and Applied Genomics, University of Tübingen, Germany
(39) Department of Epileptology, Center of Neurology, University of Tübingen, Germany
(40) Hertie Institute for Clinical Brain Research, University of Tübingen, Germany
(41) John P. Hussman Institute for Human Genomics, University of Miami, USA
(42) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami, USA
(43) Centre for Neuromuscular Diseases, Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, UK
(44) Neurogenetics Laboratory, The National Hospital for Neurology and Neurosurgery and the North Thames Genomics Laboratory Hub, London, UK
(45) Mendelics Genomic Analysis, São Paulo, SP, Brazil
(46) Universidade Federal Do Ceara - UFC and Hospital Infantil Albert Sabin, Fortaleza, Brazil
(47) Department of Neurology, School of Medical Sciences, University of Campinas (UNICAMP), Campinas, SP, Brazil
(48) Department of Neurology and Psychiatry, Faculty of Medicine, Assiut University, Assiut, Egypt
(49) Department of Pediatrics, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA
(50) Cancer Research Program, Research Institute of the McGill University Health Centre, Montréal, QC H4A 3J1, Canada

Charcot-Marie-Tooth Disease is a clinically and genetically heterogeneous group of hereditary neuropathies. Using next-generation sequencing, we identified biallelic variants in ARHGAP19, encoding for a RhoA-specific GTPase-activating protein, in 25 individuals from 20 unrelated families presenting with motor-predominant neuropathy.

In-vitro biochemical and cellular assays revealed that patient variants impair the GTPase-activating protein (GAP) activity of ARHGAP19 and reduce ARHGAP19 protein levels. Parallel in-vivo studies using Drosophila and zebrafish models revealed a conserved role for ARHGAP19 orthologs in regulating locomotor function, motoneuron axon length and branching, and neuromuscular junction morphology.

Leveraging these models and to address the limited therapeutic options, we established an in vivo movement-based drug screen to identify novel therapies, using a Drosophila model of ARHGAP19 ortholog, RhoGAP54D, loss of function. RhoGAP54D knock out flies exhibit motor defects that recapitulate patient phenotypes. To date, we have acutely fed the flies 120 drugs from a library of FDA-approved compounds and screened for drugs that rescued locomotor dysfunction. Any candidate compounds will be validated in patient-derived tissues to assess their clinical potential. This screening algorithm is adaptable and may accelerate therapeutic discovery across diverse CMT subtypes.

Our findings reveal ARHGAP19 loss-of-function as a novel driver of inherited neuropathy and support integrated Drosophila and cell-based studies as a means to enable drug repurposing in CMT.

Presentation PL3-07

Ultrasound Evaluation of the Plantar Fascia in CMT Patients: Clinical-Functional Correlations and Rehabilitative Implications

A. Vitale (1), N. Vallario (1), A. Zeni (1), E. Martire (1), R. Calciano (1), E. Di Ciesco (1), A. Fusco (1,2), L. Padua (1,2)

(1) Department of Geriatrics and Orthopaedics, Università Cattolica del Sacro Cuore, Rome, Italy
(2) UOC Neuroriabilitazione ad Alta Intensità, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy

Background: Charcot-Marie-Tooth (CMT) is the most common inherited neuromuscular disorder, often leading to disabling foot deformities, impaired gait, and loss of balance. Despite its high prevalence, little is known about the structural involvement of the plantar fascia, a key element in foot biomechanics and stability.

Objective: This study aims to explore, for the first time, the clinical relevance of plantar fascia alterations in CMT through non-invasive ultrasound imaging and their correlation with functional outcomes, highlighting a potential new avenue for early, targeted rehabilitation.

Methods: Twenty-six subjects with CMT (14F, 12M) at the High-Intensity Neurorehabilitation Unit, A. Gemelli University Hospital (Rome), underwent bilateral ultrasound of the plantar fascia. Parameters analyzed included thickness, echogenicity, and fibrillar pattern, categorized into three severity levels. Clinical-functional assessments included CMT-ES, Tinetti, Walk12, SPPB, and 10MWT. Statistical correlations were performed between ultrasound findings and clinical variables.

Results: Significant associations were found between fascial alterations and age (p=0.024), disease severity (CMT-ES, p=0.014), and functional performance (10MWT p=0.017; SPPB p=0.039).

Conclusions: This study suggests that plantar fascia changes, detectable via fast, non-invasive ultrasound, may serve as early markers of functional decline in CMT. These findings support the implementation of personalized physiotherapy and orthotic strategies. A patient-centered, multidisciplinary approach could enhance long-term functional outcomes and inform future therapeutic models.

Presentation PL4-01

Acetylated α-Tubulin as a clinical plasma biomarker of disease severity in CMT1A

CJ. Maltby (1); C. Laurini (2,3,4), J. Pinto (1), B. Sorrenti (2,3,4), M. Patelli (4), K. Ootake (5), SC. Previtali (2,3,4), M. Bianchi (1)

(1) Ulysses Neuroscience Ltd, Pioneer Life Sciences, Building 10 Cherrywood Business Park, Loughlinstown, Co. Dublin, Republic of Ireland
(2) Neurology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy
(3) Neuromuscular Repair Unit, Institute of Experimental Neurology (INSPE) and Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy
(4) Vita-Salute San Raffaele University, Milan, Italy
(5) Neuroscience Drug Discovery Unit, Takeda Pharmaceutical Company Limited, 26-1, Muraoka-Higashi 2-chome, Fujisawa 251-8555, Japan

Charcot-Marie-Tooth disease type 1A (CMT1A) is the most common inherited peripheral neuropathy and is caused by a 1.5Mb duplication on chromosome 17p11.2 encompassing the PMP22 gene. This results in PMP22 overexpression, progressive demyelination, and peripheral nerve dysfunction. Clinically, CMT1A presents with distal muscle weakness, sensory deficits, foot deformities, and gait disturbances, typically beginning in childhood. Although several clinical and electrophysiological scales exist to monitor disease progression, they are limited by subjectivity and insensitivity to slow progression. Neurofilament light chain (Nf-L), a biomarker of axonal injury, has shown limited correlation with CMT1A severity or progression, restricting its utility in clinical trials.

To address this, we evaluated acetylated α-tubulin (Acet-Tub)—a microtubule-stabilising post-translational modification—as a candidate blood-based biomarker in the plasma of 45 genetically confirmed CMT1A patients and age- and sex-matched healthy controls. Acetylation at lysine 40 (K40) of α-tubulin, catalyzed by αTAT1 and removed by HDAC6, supports microtubule stability and axonal transport, key for neuronal health. Dysregulated α-tubulin acetylation is implicated in neurodegeneration, suggesting that reduced acetylation may reflect axonal dysfunction in CMT1A.

We confirmed that plasma Nf-L is elevated in CMT1A but does not correlate with clinical severity. In contrast, Acet-Tub levels were significantly reduced in CMT1A patients and showed a strong inverse correlation with the Overall Neuropathy Limitations Scale (ONLS) and the Charcot-Marie-Tooth Examination Score (CMTES).

These findings indicate that impaired axonal integrity is coupled with a decrease in microtubule stability. Acetylated α-tubulin is a downstream target of HDAC6 currently under investigation for the treatment of CMT, and thus serves as a novel and disease-relevant biomarker reflecting molecular pathology and clinical severity in CMT1A.

Presentation PL4-02

Charcot-Marie-Tooth disease type 1E: Clinical Natural History and Molecular Impact of PMP22 Variants

K. S. Ward (1), C. P. Ptak (2), N. Pashkova (3), T. Grider (1), T. A. Peterson (3), D. Pareyson (4), C. Pisciotta (4), P. Saveri (4), I. Moroni (5), M. Laura (6), J. Burns (7), M. P. Menezes (8), K. Cornett (8), R. Finkel (9), B. Mukherjee-Clavin (10), C. J. Sumner (10), M. Greene (11), O. Abdul Hamid (12), D. Herrmann (13), R. Sadjadi (14), D. Walk (15), S. Züchner (16, 17), M. M. Reilly (6), S. S. Scherer (18), Inherited Neuropathy Consortium, R. C. Piper (3), **M. E. Shy** (1)

(1) Department of Neurology, University of Iowa Health Care Medical Center, Iowa City, IA 52242, USA
(2) Biomolecular Nuclear Magnetic Resonance Facility, University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA
(3) Department of Molecular Physiology and Biophysics, University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA
(4) Department of Clinical Neurosciences, Fondazione IRCCS Instituto Neurologico Carlo Besta, 20133 Milan, Italy
(5) Department of Pediatric Neurosciences, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20133 Milan, Italy
(6) Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, WC1N 3BG, UK
(7) Department of Epidemiology and Cancer Control, St. Jude Children's Research Hospital, Memphis, TN, USA
(8) University of Sydney School of Health Sciences, Faculty of Medicine and Health; Paediatric Gait Analysis Service of New South Wales, Sydney Children's Hospital Network, Sydney, 2145 Australia
(9) Center for Experimental Neurotherapies, St. Jude Children's Research Hospital, Memphis, TN USA
(10) Department of Neurology, John Hopkins University School of Medicine, Baltimore, MD 21205
(11) Department of Neurology, Stanford University, Stanford, CA 94304, USA
(12) Department of Neurology, Nemours Children's Hospital, Orland, FL 32827, USA
(13) Department of Neurology, University of Rochester, Rochester, NY 14618, USA
(14) Department of Neurology, Massachusetts General Hospital, Boston, MA 02114, USA
(15) Department of Neurology, University of Minnesota, Minneapolis, MN 55455, USA
(16) Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL, 33136, USA
(17) John P. Hussman Institutue for Human Genetics, University of Miami Miller School of Medicine, Miami, FL 33136, USA
(18) Department of Neurology, University of Pennsylvania, Philadelphia, PA 19104, USA

Charcot-Marie-Tooth disease type 1E (CMT1E) is a rare, autosomal dominant peripheral neuropathy caused by missense variants, deletions, and truncations within the peripheral myelin protein-22 (PMP22) gene. CMT1E phenotypes vary depending on the specific variant, ranging from mild to severe, and there is little natural history and phenotypic progression data on individuals with CMT1E. Patients with CMT1E were evaluated during initial and follow-up visits at sites within the Inherited Neuropathy Consortium. Clinical characteristics were obtained from history, neurological exams, and nerve conduction studies. Clinical outcome measures were used to quantify baseline and longitudinal changes. The trafficking of PMP22 variants in transfected cells was correlated to disease severity. Twenty-four presumed disease-causing PMP22 variants were identified in 50 individuals from 35 families, including 19 missense variants, three in-frame deletions, and two truncations. Twenty-nine patients presented with delayed walking during childhood. At their baseline evaluation, the mean CMTESv2-R in 46 patients was 16 ± 7.72 (out of 32), and the mean CMTPedS from 17 patients was 28 ± 6.35 (out of 44). Six individuals presented with hearing loss, eleven with scoliosis, three with hip dysplasia, and one with both scoliosis and hip dysplasia. Twenty variants were localized within in transmembrane domains; 31 of 35 individuals with these variants had moderate to severe phenotypes. Three variants were found in the

extracellular domain and were associated with milder phenotypes. Reduced expression of PMP22 at the cell surface, and the location of missense variants within in the transmembrane domain correlated with disease severity. Pathogenic PMP22 variants located within the transmembrane regions usually cause a moderate to severe clinical phenotype, beginning in early childhood, and have impaired trafficking to the plasma membrane.

Presentation PL4-03

A COA8 homozygous mutation presenting as a demyelinating CMT with leukopathy

A. Dupic (1), Dias-Evangelista T. (2), M. Konyukh (3,4), **M. Masingue** (1)

(1) Reference Center for Neuromuscular Disorders, Nord/Est/Ile de France, Institut de Myologie, CHU La Pitié-Salpêtrière, APHP, Paris, France
(2) Neuromuscular Morphology Unit, Myology Institute, Pitié-Salpêtrière Hospital, 75013 Paris, France
(3) Département de Génétique Médicale, Hôpital Henri Mondor, Assistance Publique des Hôpitaux de Paris, Créteil, France
(4) Laboratoire de biologie médicale multisites SeqOIA - PFMG2025, Paris, France

Patients with cytochrome c oxidase (COX) deficiency exhibit clinical heterogeneity, with the onset of symptoms ranging from infancy to adulthood. COA8-related disorders typically present in childhood with acute symptoms and cavitating posterior leukoencephalopathy, though milder, muscle-predominant forms have recently been reported.

We describe a 54-year-old woman with a pauci-symptomatic demyelinating sensorimotor neuropathy (25-30 m/s in the lower limbs, 30-40 m/s in the upper limbs). Neurological examination showed short stature, preserved motor function, hammer toes, mildly high-arched feet, mild dysmetria without lateralization, and distal hypoesthesia with intact proprioception and vibration sense. Further investigations revealed a history of migraines, hearing loss, early menopause, and symmetrical posterior-predominant leukoencephalopathy.

Targeted NGS for hereditary neuropathies was unremarkable. The neurometabolic workup was negative, and there was no evidence to support a diagnosis of MNGIE.

Whole genome sequencing identified a homozygous COA8 mutation (c.476+1G>A), confirmed by diffuse COX deficiency and significantly reduced complex IV activity on muscle biopsy.

Historically, the only mitochondrial gene associated with both demyelinating neuropathy and leukoencephalopathy was TYMP (MNGIE). Later, CMT4K (SURF1) was described, as well as rare cases involving mutations in POLG, RRM2B, and DARS2 (which more commonly cause axonal neuropathies), as well as COA8.

Brain MRI can be useful in the genetic diagnosis of a demyelinating neuropathy, and the mitochondrial hypothesis should be considered when leukoencephalopathy is also present, especially in the context of other suggestive symptoms (in this case: migraine, deafness, short stature, early menopause).

Presentation PL4-04

Genotype-phenotype correlation in a large cohort with HSPB1-related neuropathy

A. Bertini (1), A. Rossor (2), M. Russo (3), S. Tozza (4), M. Skorupinska (2), P. Saveri (1), T. Cavallaro (5), M. Tagliapietra (5), L. Gentile (3), S. Massucco (6), Y. Matteo Falzone (7), F. Taioli (5), F. D'arma (3), E. Cavalca (1), L. Crivellari (1), C. Laurini (7), C. Briani (8), T. Rosso (9), M. Ferrarini (5), E. Bellone (6), A. Geroldi (6), S. Magri (10), M. Laurà (2), M. Grandis (6), G. Maria Fabrizi (5), S. Carlo Previtali (7), A. Schenone (6), F. Manganelli (4), A. Mazzeo (3), M. M Reilly (2), D. Pareyson (1), C. Pisciotta (1)

(1) Unità di Malattie Neurologiche Rare, Dipartimento di Neuroscienze Cliniche, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
(2) Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London
(3) Unità di Neurologia e Malattie Neuromuscolari, Dipartimento di Medicina Clinica e Sperimentale, Università di Messina, Messina, Italy
(4) Dipartimento di Neuroscienze, Scienze Riproduttive ed Odontostomatologiche, Università Federico II di Napoli, Naples, Italy
(5) Dipartimento di Neuroscienze, Biomedicina e Movimento, Università di Verona, Verona, Italy
(6) Dipartimento di Neuroscienze, Riabilitazione, Oftalmologia, Genetica e Scienze materno-infantili, Università di Genova, Genoa, Italy
(7) INSPE and Division of Neuroscience, IRCCS Ospedale San Raffaele, Milan, Italy
(8) Department of Neurosciences, Neurology Unit, University of Padova, Padova, Italy
(9) UOC di Neurologia, Ospedale San Bassano, Vicenza, Italy
(10) Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

HSPB1 has three domains: N-terminal (1-87aa), alpha-crystallin (ACD) (88-168aa), and C-terminal (169-205aa). In vitro studies show that variants in N-terminal might be less severe than variants in ACD and C-terminal. We performed a genotype-phenotype correlation study in patients with CMT due to heterozygous pathogenic variants in HSPB1.

We retrospectively gathered a minimal dataset of information in patients with pathogenic variants in HSPB1 at baseline and follow-up visits, across seven Italian centres and UCL (UK), including type of variant, motor milestones occurrence, and CMTES.

We included 106 patients with HSPB1-related neuropathy followed up for 9.2±4.8 years (276 visits overall): 43 patients had pathogenic variants in N-terminal domain, 50 in ACD, and 13 in C-terminal.

Disease onset occurred earlier in patients with variants in C-terminal (33±11.4 years), followed by those with variants in ACD (39±9.5), and N-terminal (48±10.8) (p=0.023).

We found that variants in N-terminal were associated to milder disease (CMTES 4.5±2.5), compared to those in the ACD (6.4±4.1) and C-terminal domain (7.4±4.4) (p=0.008). Accordingly, 28% and 26% of patients with variants in ACD and C-terminal, respectively, used AFOs (vs 9% of those with variants in N-terminal, p=0.040), 32% and 17% used stick (vs 6% p<0.001), 8% and 8% were wheelchair-bound (vs 0%, p=0.040), 40% and 42% had difficulties with buttons (vs 15%, p=0.014), and 29% and 27% presented with proximal weakness (vs 9%, p=0.032).

Similarly, disease progression (ΔCMTES/year) showed a direct gradient of severity from N-terminal (0.33/year), to ACD (0.55/year), and C-terminal (0.70/year) (p=0.040). Mean time from disease onset to stick use was 9 years for variants in C-terminal, 15 years for those in the ACD, and 32 years in the N-terminal domain.

Variants in ACD and C-terminal domain are associated to earlier disease onset, more rapid progression and severe disease burden compared to variants in N-terminal.

Posters

Poster Session 1

- **P1: Paola Saveri** (Fondazione IRCCS Istituto Neurologico Carlo Besta, Italy)
  CMT1E Under the Reflex Hammer and the Microscope: A Clinical and Biological Analysis
- **P3: Karen Libberecht** (Hasselt University, Belgium)
  Lysosomal dysbalance and cargo release by Schwann cells in Charcot-Marie-Tooth disease type 1A
- **P5: Alexandra Ekshteyn** (VIB Center for Molecular Neurology, Belgium)
  HINT1 deficiency impairs actin cytoskeleton and calcium signaling
- **P7: Alessandro Bertini** (UCL, Italy)
  CGG repeat expansions in Charcot-Marie-Tooth disease: insights from the 100 000 Genomes Project
- **P9: Amelie Blum** (University Hospital RWTH Aachen, Germany)
  Motor neuropathy gene FAM169A interacts with other neuropathy-causing genes at the nuclear envelope
- **P11: Melanie Van Brussel** (University of Antwerp, Belgium)
  Unraveling the macrophage – Schwann cell crosstalk in Charcot-Marie-Tooth disease type 1A
- **P13: Michael Sereda** (Max Planck Institute of Multidisciplinary Sciences, Germany)
  Schmidt-Lanterman Incisure number supports Schwann cell function in Charcot-Marie-Tooth disease 1A (CMT1A)
- **P15: Danique Beijer** (Uniklinik RWTH Aachen , Germany)
  Novel heterozygous NARS1 variant in a family with axonal peripheral neuropathy
- **P17: Arman Cakar** (Istanbul University, Turkey)
  Genetic distribution of inherited peripheral neuropathies in Türkiye
- **P19: Alexia Kagiava** (The Cyprus Institute of Neurology and Genetics, Cyprus)
  Efficacy of PLGA nanoparticles for Schwann cell targeted gene delivery
- **P21: Mehrnaz Hamedani** (IRCCS Polyclinic Hospital San Martino, Italy)
  Static and Dynamic Stabilometric Evaluation and Rehabilitation in Peripheral Neuropathy
- **P23: Evan Bailey** (Applied Therapeutics , United States of America)
  Understanding the Time Course and Evolution of CMT-SORD (Sorbitol Dehydrogenase Deficiency)
- **P25: Valérie Delague** (INSERM/Aix Marseille Université , France)
  Gene replacement therapy efficiently restores normal phenotype in hiPSC-derived in vitro models of VRK1-related motor neuropathies
- **P27: Beschan Ahmad** (Max-Planck-Institute for multidisciplinary sciences Göttingen , Germany)
  Circulating transcriptional biomarkers serve as surrogates of disease progression in Charcot-Marie-Tooth 1A disease
- **P29: Gita Ramdharry** (Queen Square Centre for Neuromuscular Diseases, UCL Institute of Neurology, UK)
  Better Balance-CMT: Protocol for a randomised, controlled, efficacy and implementation trial of a home-based, balance training intervention for people with Charcot-Marie-Tooth Disease
- **P31: Filippo Genovese** (ACMT-Rete per la malattia di Charcot-Marie-Tooth OdV)
  ACMT-Rete: A Patient-Led Network Integrating Research, Awareness, and Community for Charcot-Marie-Tooth

Poster Session 2

- **P2: Nathan Donies** (University of Antwerp , Belgium)
  SCREEN4PN: Efficient evaluation of therapeutic compounds for Charcot-Marie-Tooth disease using patient-derived induced motor neurons and neuromuscular organoids
- **P4: Francesco Gentile** (San Raffaele Scientific Institute , Italy)
  Genetic and pharmacologic modulation of the ATF6 UPR-related pathway affect disease pathogenesis in CMT1B
- **P6: Nathalie Dirkx** (Hasselt University , Belgium)
  Phenotypic and functional impairments in human CMT1A Dental Pulp Stem Cell-derived Schwann cells
- **P8: Hanne Jeurissen** (Hasselt University , Belgium)
  Cellular stress responses induced by PMP22 overexpression in Charcot-Marie-Tooth disease type 1A
- **P10: Akram Khanghahi** (UAntwerpen - VIB Center for Molecular Neurology, Belgium)
  Modelling YARS-Related Charcot-Marie-Tooth Disease: Establishing a Link Between YARS1-related Neurodegeneration and Mitochondrial Dysfunction
- **P12: Yara Lambrechts (**BIOMED-UHasselt + VIB-KU Leuven , Belgium)
  The impact of Schwann cell differentiation on transgene expression in human stem cells
- **P14: Katherine Forsey** (Charcot-Marie-Tooth Association , USA / UK)
  An In Vitro Model for Motor Neurons Displays Axonal and Mitochondrial Deficiencies Associated With Charcot-Marie-Tooth Disease Type 2A
- **P16: Edouard Berling** (Paris-Saclay , France)
  Whole-Body muscle MRI in Non-5q Spinal Muscular Atrophy: Patterns, Genotype Prediction, and Diagnostic Implications
- **P18: Christopher J Record** (UCL Queen Square IoN , UK)
  A neuropathy-mitochondrial multidisciplinary team meeting: enhancing diagnosis
- **P20: Laurence Lee** (University College London , United Kingdom)
  “Getting the Balance Right”: Co-creating a Sensory-Integrated Balance Programme for People Living with Charcot-Marie-Tooth Disease
- **P22: Barbara Chaloupek** (CMT-Austria Patients Self-help Organisation , Austria)
  Patient survey: The lived experience with Charcot-Marie-Tooth in Austria: therapies actually used and medical care
- **P24: Eleonora Cavalca** (Fondazione IRCCS Istituto Neurologico Carlo Besta, Italy)
  Physiotherapy in Charcot-Marie-Tooth Disease: insights from the Italian CMT Registry
- **P26: Martina D'Agostino** (IRCCS Ca’ Granda Ospedale Maggiore Policlinico , Italy)
  AAV9 MFN1 gene therapy as proof of principle for the treatment of CMT2A
- **P28: Ana Claudia Mattiello-Sverzut** (Ribeirão Preto Medical School, University of São Paulo , Brazil)
  Responses to the cardiopulmonary exercice test for upper limbs in children and adolescents with Charcot-Marie-Tooth disease
- **P30: Filippo Genovese** (European CMT Federation (ECMTF), Belgium)
  The European CMT Federation (ECMTF): A Unified Voice to Accelerate Research and Raise Awareness for CMT
- **P32: Lydia Jestice (University of Sheffield, UK)**
  Investigating Axonal Transport In Charcot-Marie-Tooth Disease Type 2A Using A Pluripotent Stem Cell-based Model

Poster P1

CMT1E Under the Reflex Hammer and the Microscope: A Clinical and Biological Analysis

P. Saveri (1), C. Ferri (2), L. Crivellari (1), C. Pisciotta (1), R. Bonaccorso (3), E. Cavalca (1), C. Ciano (4), B. Casini (5), P. Fossa (5), F. Balistreri (6), S. Magri (6), F. Taroni (6), S.C. Previtali (3), D. Pareyson (1), M. D'Antonio (2)

(1) Unit of Rare Neurological Diseases, Department of Clinical Neurosciences, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
(2) Myelin Biology Unit, Division of Genetics and Cell Biology, IRCCS Ospedale San Raffaele, Milan, Italy
(3) INSPE Institute of Experimental Neurology and Division of Neuroscience, IRCCS San Raffaele Scientific Institute, Milan, Italy
(4) Unit of Neurophysiology, Department of Diagnostics and Applied Technology, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
(5) Department of Pharmacy, Section of Medicinal Chemistry, School of Medical and Pharmaceutical Sciences, University of Genoa, Genoa, Italy
(6) Unit of Medical Genetics and Neurogenetics, Department of Diagnostics and Applied Technology, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

PMP22 point mutations cause the rare dysmyelinating Charcot-Marie-Tooth type 1E neuropathy (CMT1E), with the more severe cases also classified as Dejerine-Sottas syndrome (DSS).

The underlying molecular mechanisms are still unknown but studies in different models suggested that mutant PMP22 protein mistrafficking and activation of the unfolded protein response (UPR) may play a role.

We investigated five index patients, carrying either the already described PMP22 mutations W28R, S72L, L80P or the novel variants A106V, A113P. All the subjects were clinically and neurophysiologically characterized: three cases were sporadic; for the remaining two, family members were also evaluated. We performed molecular modelling evaluation of mutant PMP22 proteins and in vitro studies; moreover, we collected serum and plasma for biomarkers' assessment and skin biopsies for immunohistochemical and fibroblasts analysis.

Four patients (W28R, S72L, L80P, A113P) showed early-onset and moderate-to-severe phenotype (CMTES range 9-20/28). Motor nerve conduction velocities (MCV) were in the DSS range (2.1-7.3 m/s). The youngest A106V subject was characterized by later onset (~14 years) and a very mild phenotype (CMTES=1, MCV=49.8 m/s), though 2 of 4 affected family members showed an aggressive demyelinating neuropathy. Molecular modelling suggested that all the mutations alter aminoacidic interactions, increasing protein rigidity, and negatively affecting plasticity and functionality. In in vitro studies, RT-4 Schwannoma cells were transfected with WT or mutant PMP22. Unlike PMP22-wt, which reached the membrane, mutants were intracellularly retained, co-localized with ER or Golgi markers, and triggered a UPR, as confirmed by CHOP staining in patient skin biopsies.

Our results strongly suggest a shared mechanism involving mistrafficking and ER stress for PMP22 mutations causing DSS/CMT1E. Ongoing studies on patient-derived cells will be crucial to confirm the UPR activation in humans.

Poster P2

SCREEN4PN: Efficient evaluation of therapeutic compounds for Charcot-Marie-Tooth disease using patient-derived induced motor neurons and neuromuscular organoids

N. Donies (1), T. van Wermeskerken (1), B. Bekaert (1), S. in ’t Groen (1), T. Vangansewinkel (1), V. Timmerman (1)

(1) Peripheral Neuropathy Research Group, Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium

Charcot-Marie-Tooth (CMT) disease is the most common peripheral neuropathy, affecting over 2.5 million people worldwide. CMT research is slow due to its genetic and phenotypic heterogeneity. CMT1 primarily involves myelin degeneration, whereas CMT2 is marked by axonal degeneration. Each category includes multiple subtypes with distinct genetic causes, further complicating therapeutic development.

To address this challenge, we developed SCREEN4PN, a service platform designed for efficient drug testing. It utilizes 2D motor neuron and 3D organoid cell models derived from induced pluripotent stem cells (iPSCs). We identified several pathological phenotypes shared across CMT genotypes, enabling SCREEN4PN to facilitate therapeutic screening despite disease heterogeneity. Treatment effects are evaluated using microscopy, qPCR, and protein biomarkers.

SCREEN4PN utilizes iPSCs from CMT patients with various genotypes, along with isogenic and healthy controls. These cells are differentiated into models tailored to specific subtypes. The 2D motor neuron model assesses axonal degeneration in CMT2, while the 3D organoid model enables myelin formation to study the CMT1 phenotype.

Additionally, our cell banks undergo rigorous quality control to ensure standardized, high-quality cells for research and therapeutic applications.

So far, we have completed two services using 2D motor neuron cultures. In the future, we aim to expand SCREEN4PN to other diseases, optimize existing models, and incorporate advanced systems such as assembloids and microfluidics.

By reducing the time, cost, and reliance on animal models compared to traditional approaches, SCREEN4PN offers a more ethical and efficient platform for pharmaceutical companies, clinical research organizations, and academic partners.

Poster P3

Lysosomal dysbalance and cargo release by Schwann cells in Charcot-Marie-Tooth disease type 1A

K. Libberecht (1-4), H. Jeurissen (1), N. Dirkx (1), S. Hasevoets (4,5), R. Prior (2,3), K. Kuipers (1,6), F. Hermans (4,5), K. Fluiter (7), F. Baas (7), I. Lambrichts (4), T. Vangansewinkel (1,2,8), L. Van Den Bosch (2,3), E. Wolfs (1)

(1) Lab for Functional imaging & research on stem cells, Hasselt University, Diepenbeek, Belgium
(2) KU Leuven, Department of Neurosciences, experimental Neurology and Leuven brain institute (LBI), Leuven, Belgium.
(3) VIB, Center for Brain & Disease Research, Laboratory of Neurobiology, Leuven, Belgium
(4) Laboratory for Histology and Regeneration, Hasselt University, Diepenbeek, Belgium
(5) Laboratory for research in ischemic stroke, stem cells & angiogenesis, Hasselt University, Diepenbeek, Belgium
(6) Department of Immunology and Infection, Hasselt University, Diepenbeek, Belgium
(7) Department of Clinical genetics, Leiden University Medical center, Leiden, The Netherlands
(8) Peripheral Neuropathy Research Group, Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium

Charcot-Marie-Tooth disease (CMT) is the most prevalent peripheral neuropathy, with CMT1A as the predominant subtype. CMT1A results from a duplication of the peripheral myelin protein 22 (PMP22) gene, mainly expressed in Schwann cells. While the precise mechanisms linking PMP22 overexpression to Schwann cell dysfunction remain unclear, PMP22 accumulation has been associated with lysosomes. However, its contribution to the CMT1A pathology remains unclear. We identify lysosomal abnormalities in the CMT1A C3 mouse model and validate our findings in CMT1A patient-derived Schwann cell precursors (SCP). Western blotting and immunohistochemistry demonstrated significantly increased levels of the lysosomal marker LAMP1 and enzymes Cathepsin B (CtB) and Cathepsin D (CtD) in sciatic nerves of C3 compared to wild-type (WT) mice. These results were confirmed in primary murine Schwann cells from C3 mice and human CMT1A SCP via immunocytochemistry. Transmission electron microscopy revealed increased lysosomal content and lysosomal membrane permeabilization in CMT1A Schwann cells. Additionally, we observed a significant upregulation of lysosomal exocytosis, characterized by regulated secretion of lysosomal enzymes into the extracellular milieu. Conditioned medium (CM) from CMT1A Schwann cells confirmed elevated CtB and CtD levels and activity. Functional degradation assays confirmed that the CM of CMT1A Schwann cells exhibits increased proteolytic activity, resulting in enhanced degradation of the extracellular matrix (ECM) protein collagen IV compared to healthy controls. Consistent with these findings, reduced ECM protein levels were detected in sciatic nerves of C3 mice compared to WT littermates. Our results underscore lysosomal upregulation, destabilization, and amplified extracellular cathepsin release in CMT1A Schwann cells, contributing to ECM breakdown. Hence, targeting extracellular cathepsin activity may represent a promising therapeutic strategy for CMT1A.

Poster P4

Genetic and pharmacologic modulation of the ATF6 UPR-related pathway affect disease pathogenesis in CMT1B

F. Gentile (1), F. A. Veneri (1), N. Sorgiati (1), L. Morelli (1), C. Ferri (1), S. Valenzano (2), U. Del Carro (2), R. J. Kaufman (3), R. Luke Wiseman (4), J. Svaren (5), M. D'Antonio (1)

(1) Biology of Myelin Unit, Division of Genetics and Cellular Biology, IRCCS Ospedale San Raffaele, Milan (Italy).
(2) Division of Neuroscience, INSPE, IRCCS Ospedale San Raffaele, Milano (Italy).
(3) Degenerative Diseases Program, SBP Medical Discovery Institute, La Jolla, CA, United States of America.
(4) Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA 92037, USA.
(5) Waisman Center, University of Wisconsin-Madison, Madison, WI, United States of America.

Background - Charcot-Marie-Tooth type 1B is a hereditary neuropathy caused by mutations in myelin protein zero (MPZ/P0). Many of these mutations trigger a canonical unfolded protein response (UPR). Here, we explore the effects of the modulation of the ATF6 pathway of the UPR in a mouse model of CMT1B.

Material and Methods - the ATF6 gene was systemically ablated in CMT1B mice (P0S63del/ATF6KO). Locomotor, neurophysiology and morphologic studies were performed, as well as transcriptomics in sciatic nerves. Selective pharmacologic activation of ATF6 through the experimental compound AA147 was assessed in explants of myelinating dorsal root ganglia (DRG). A pharmacodynamic study was also performed in mice by administering AA147 at 2, 4, and 8 mg/kg through intraperitoneal injection and verified by gene expression analysis.

Results - We observed a worsening of disease phenotype in P0S63del/ATF6KO mice, with reduced motor capacity and neurophysiology and thinner myelin observed in morphology. RNAseq analysis indicated that ablation of ATF6 leads to a strong suppression of genes related to ER protein folding, protein degradation, oxidative stress and inflammation.

In P0S63del DRG cultures, treatment with AA147 increased myelination. In WT mice, 8 mg/kg AA147 induced an increase in BiP expression 24 hours post-injection, accompanied by an increase also of XBP1s and CHOP gene expression. Interestingly, the same UPR genes showed a dose-dependent reduction in P0S63del nerves 24 hours post-injection. Instead, no significant difference in BiP expression was observed after 72 hours between placebo- and AA147- treated mice.

Conclusions - Genetic and/or pharmacologic modulation of the ATF6 pathway of the UPR may affect neuropathy severity and progression. The drug AA147 seems to exert a short-lasting but significant pharmacological activity. A pilot study of AA147 treatment at 8 mg/kg is currently ongoing to test if the compound activation could have disease-modifying effects.

Poster P5

HINT1 deficiency impairs actin cytoskeleton and calcium signaling

A. Ekshteyn (1,2), S. Amor Barris (1,2,3), M. Malcorps (1,2), R. Bilbao-Canalejas (1,2), L. Mateiu (4), S. Manzella (5), B. Asselbergh (5), R. La Rovere (3), K. Peeters (1,2), G. Bultynck (3), A. Jordanova (1,2)

(1) Molecular Neurogenomics Group, VIB Center for Molecular Neurology, VIB, Belgium
(2) Molecular Neurogenomics Group, Department of Biomedical Sciences, University of Antwerp, Belgium
(3) Laboratory of Molecular and Cellular Signaling, Department of Cellular and Molecular Medicine &Leuven Kanker Instituut, KULeuven, Belgium
(4) Cognitive Genetics (CONGET), Center for Medical Genetics (CMG), Department of Biomedical Sciences, University of Antwerp, Belgium
(5) Neuromics Support Facility, VIB Center for Molecular Neurology, VIB, Belgium

Loss of functional histidine triad nucleotide-binding protein 1 (HINT1) causes a rare form of inherited peripheral neuropathy with neuromyotonia (NMAN). Patients suffer from motor-greater-than-sensory polyneuropathy with an age of onset within the first decade of life. HINT1 is an ubiquitously expressed purine phosphoramidase that functions as an inhibitor of pro-oncogenic transcription factors, and acts as an adaptor protein of the endocannabinoid signaling pathway in the central nervous system (CNS). Yet, HINT1's role in the peripheral nerves remains uncharacterized.

Currently, 28 NMAN-causing variants have been described resulting in loss of HINT1 function, e.g. by impairing conformational stability and leading to protein degradation (R37P) or abolishing enzymatic activity without affecting protein stability (H112N). We genetically engineered HeLa cell lines deficient for HINT1 using the CRISPR/Cas9 editing technology and studied their transcriptome profile. Gene ontology and pathway analysis identified actin cytoskeleton remodeling and integrin signaling as affected pathways. Further validation of misregulated genes involved in intracellular signaling pathways showed downregulation of calcium-related proteins in particular. Using our HINT1-KO HeLa model and NMAN patient-derived fibroblasts homozygous for the R37P or H112N variants, we functionally validated the effect of HINT1 deficiency on the actin cytoskeleton dynamics using a wound healing assay. We found mobility and attachment impediments in the HINT1 deficient cells. Additionally, we showed HINT1-associated effect on IP3-mediated Ca2+ response that depends on HINT1 protein abundance and the nature of applied stimuli. Our findings identify and characterize two functionally related affected cellular pathways as a result of loss of HINT1 that might underlie the NMAN disease mechanism.

Poster P6

Phenotypic and functional impairments in human CMT1A Dental Pulp Stem Cell-derived Schwann cells

N. Dirkx (1), K. Libberecht (1,2,4), K. Kuipers (1), T. Vangansewinkel (1,3), L. van den Bosch (4), I. Lambrichts (2), E. Wolfs (1)

(1) Functional Imaging & Research on Stem Cells (FIERCE) lab, BIOMED Research Institute, Hasselt University, 3590 Diepenbeek, Belgium
(2) Laboratory for Histology and Regeneration (HISTOREGEN), BIOMED Research Institute, Hasselt University, 3590 Diepenbeek, Belgium
(3) Peripheral Neuropathy Research group, University of Antwerp, 2100 Wilrijk, Belgium
(4) Center for Brain & Disease Research, VIB-KULeuven, 3000 Leuven, Belgium

CMT1A is the most prevalent type of CMT, which is caused by the duplication of the peripheral myelin protein 22 (PMP22) gene, resulting in peripheral nerve demyelination. The failure of many candidate therapies reflects a shortage of physiologically relevant human Schwann cell models. iPSC-derived Schwann cell precursors are valuable, but immaturity and the need for reprogramming raise the need for alternatives.

Dental Pulp Stem Cells (DPSC) reside in adult human teeth and share a neural crest origin with Schwann cells. They can be efficiently differentiated into DPSC-derived Schwann cells (DPSC-SC). Hence, we suggest patient-derived DPSC-SC as a highly translatable in vitro model to research Schwann cell behavior and interactions in CMT1A. DPSC were isolated from third molars of four CMT1A donors and age-matched controls. Following DPSC-SC differentiation, Schwann cell phenotypes were evaluated using RNA sequencing, qPCR, ICC, and proliferation assays. Next, cells were co-cultured with human iPSC-derived motor neurons (iPSC-MN) in 2D microfluidic chambers and 3D collagen type I hydrogels.

RNA sequencing revealed downregulated differentiation, cytoskeletal, and motility pathways in CMT1A, but proliferative and ECM pathways were upregulated. Additionally, MPZ and laminin proteins decreased in CMT1A DPSC-SC, while levels of the immature marker P75NTR increased. CMT1A DPSC-SC showed higher proliferation rates but decreased migration to iPSC-MN in microfluidic co-cultures. Finally, in 3D hydrogels, CMT1A DPSC-SC showed lower contractile function and decreased pericellular density of collagen fibers, compared to controls.

To conclude, our results suggest that CMT1A DPSC-SC display a more immature, repair-like phenotype compared to control cells with disruptions in migration and collagen-linked contraction, which may underlie the pathology. These 2D and 3D patient-specific models provide a reproducible and translatable platform for mechanistic studies and drug screening.

Poster P7

CGG repeat expansions in Charcot-Marie-Tooth disease: insights from the 100 000 Genomes Project

A. Bertini (1,2), S. Facchini (1,3), I. Quartesan (1), R. Currò (1), R. Parolin Schnekenberg (1), N. Dominik (1), G. Alves (1,4), L. Ferullo (1,5), A. Tucci (6), H. Houlden (1), M. M Reilly (1), A. Cortese (7)

(1) Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, UK
(2) Unit of Rare Neurological Diseases, Department of Clinical Neurosciences, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy
(3) Department of Brain and Behavioral Sciences, University of Pavia, Pavia, Italy
(4) Clinical Hospital of Ribeirão Preto, Department of Neurosciences and Behaviour Sciences, University of São Paulo, Ribeirão Preto, Brazil
(5) Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
(6) William Harvey Research Institute, Faculty of Medicine and Dentistry, Queen Mary University of London, London, UK
(7) Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, UK

Background: CGG expansions in NOTCH2NLC and LRP12 were recently identified as a cause of Charcot-Marie-Tooth disease (CMT) in 1.2%-10.6% of genetically undiagnosed patients in China, Taiwan and Japan. However, their relevance in CMT patients of different ethnic origin is still unknown.

Methods: Here, we leveraged short-read whole genome sequencing data from the 100 000 Genomes Project to investigate the presence and frequency of CGG expansions in NOTCH2NLC, LRP12 and additional genes associated with oculopharyngodistal myopathy (OPDM), in 560 genetically unsolved patients diagnosed with CMT and 32 509 non-neurological controls.

Results: Repeat expansions in NOTCH2NLC, LRP12, RILPL1, NUTM2B-AS1 and ABCD3 were absent from 560 genetically unsolved patients with CMT, mostly of Northern European ancestry. One patient of African ancestry carried an expanded allele in GIPC1, below the reported pathogenic threshold. However, rare expansions in this gene, as well as in NOTCH2NLC, NUTM2B-AS1 and ABCD3, were also detected in controls (≤0.05%). The distribution of repeat size at these loci varied significantly across different ethnicities, with larger non-pathogenic intermediate alleles of NOTCH2NLC and LRP12 typically observed in East Asians.

Conclusions: CGG expansions in NOTCH2NLC, LRP12 and other OPDM-associated genes do not appear to be a relevant cause of CMT in the UK. The larger size of non-pathogenic intermediate alleles of NOTCH2NLC and LRP12 in East Asians could explain their ancestry-specific propensity to further expand into the full pathogenic range.

Poster P8

Cellular stress responses induced by PMP22 overexpression in Charcot-Marie-Tooth disease type 1A

H. Jeurissen (1), K. Libberecht (1,2,3,4), I. De Fays (1), K. Kuipers (1,5), N. Dirkx (1), L. Van Den Bosch (2,3), T. Vangansewinkel (1,2,6), E. Wolfs (1)

(1) Lab for Functional Imaging & Research on Stem Cells (FIERCE Lab), Hasselt University, Diepenbeek, Belgium
(2) Experimental Neurology and Leuven Brain Institute (LBI), Department of Neurosciences, KU Leuven, Leuven, Belgium
(3) Laboratory of Neurobiology, Center for Brain & Disease Research, VIB, Leuven, Belgium
(4) Laboratory for Histology and Regeneration, Hasselt University, Diepenbeek, Belgium
(5) Department of Immunology and Infection, Hasselt University, Diepenbeek, Belgium
(6) Peripheral Neuropathy Research Group, Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium

Charcot-Marie-Tooth (CMT) disease is the most common peripheral neuropathy, affecting 1 in 2500 people worldwide. The most common subtype, CMT1A, is predominantly demyelinating and is caused by a duplication of the peripheral myelin protein 22 (PMP22) gene, mainly expressed in Schwann cells (SC). However, the mechanisms by which PMP22 overexpression disrupts SC function remain unclear. We hypothesize that PMP22 overexpression induces endoplasmic reticulum (ER) stress through protein overload, which may exacerbate mitochondrial dysfunction, contributing to SC impairment. Given the role of mitochondria-associated membranes (MAMs) in ER-mitochondria communication, we further explored their contribution to ER and mitochondrial stress in CMT1A.

Sciatic nerve tissue and primary SC were isolated from the C3-PMP22 CMT1A mouse model and wild-type controls. Furthermore, we used Schwann cell precursors (SCP) and immature iPSC-derived SC (iPSC-SC), derived from CMT1A patient-induced pluripotent stem cells (iPSCs) and isogenic controls.

Immunocytochemistry indicated increased protein ubiquitination in CMT1A SCP and C3-SC, and increased ER chaperone calnexin intensity in C3-SC. ER stress markers revealed a non-significant increase in BiP/GRP78 in CMT1A SCP, and a non-significant decrease in XBP1s and p-eIF2α/eIF2 ratio in CMT1A SCP compared to controls. Live-cell mitochondrial imaging using MitoTracker Green revealed increased mitochondrial density in C3-SC and CMT1A SCP. Lastly, live-cell confocal imaging using MitoTracker Green and ER-Tracker Red demonstrated reduced ER-mitochondria contact sites in CMT1A SCP, further supported by decreased PE/PC ratios in CMT1A iPSC-SC, suggesting MAM dysfunction.

Our findings indicate activation of the integrated stress response, alongside ER and mitochondrial alterations and reduced mitochondria-ER contacts in CMT1A. Further research is needed to clarify how these changes affect SC myelination and contribute to CMT1A pathogenesis.

Poster P9

Motor neuropathy gene FAM169A interacts with other neuropathy-causing genes at the nuclear envelope

A. Blum (1,2), W. Antonin (1), H. Lue (1), M. Dohrn (2)

(1) Institute of Biochemistry and Molecular Cell Biology, RWTH Aachen University, Aachen, Germany
(2) Department of Neurology, Medical Faculty RWTH Aachen University, Aachen, Germany

Heterozygous loss-of-function mutations in FAM169A were identified in six unrelated families with autosomal dominant inheritance, manifesting as early adult-onset, progressive distally pronounced muscle atrophy, gait abnormalities, and upper motor neuron signs. These mutations are absent from the general population, suggesting FAM169A is prone to haploinsufficiency and likely acts as a disease gene.

FAM169A encodes the nuclear envelope protein SLAP75 (soluble lamin-associated protein, 75 kDa), whose biological function remains to be discovered.

Upon western blot, SLAP75 appeared to form a dimer of 150 kDa. Immunofluorescence in HEK293T cells and stable expression of EGFP-FAM169A in HeLa cells confirmed its nuclear envelope localization. siRNA-mediated knockdown of SLAP75 reduced its signal, whereas the nuclear shape still remained intact, suggesting FAM169A is not essential for nuclear envelope integrity.

Mass spectrometry-based pull-down analyses of tagged FAM169A revealed interactions between SLAP75 and several nuclear envelope components, notably Lamin-A/C—linked to neuropathy and myopathy—and NUP50, an ALS-associated nuclear pore protein.

LMNA (encoding Lamin-A/C) and NUP50 knockdown did not affect SLAP75 localization. Vice versa, knockdown of SLAP75 did not have an influence on the localization of LMNA and NUP50. However, FAM169A band size decreased in LMNA- and NUP50-depleted cells, requiring further quantification.

We are generating dTAG-based HEK293T cell lines for more rapid, reversible SLAP75 degradation, enabling detailed assessment of loss- and gain-of-function phenotypes. The dTAG and EGFP cell lines will allow live-cell imaging to directly observe FAM169A dynamics, interactions, and functional consequences of acute or reversible depletion.

By the time of the conference, we anticipate providing more insights into FAM169A function and dysfunction and discussing the importance of the nuclear envelope for future treatment developments.

Poster P10

Modelling YARS-Related Charcot-Marie-Tooth Disease: Establishing a Link Between YARS1-related Neurodegeneration and Mitochondrial Dysfunction

A. Khanghahi (1,2) , E. De Vriendt (1,2), A. Jordanova (1,2)

(1) Center for Molecular Neurology, VIB, University of Antwerp, Antwerpen, Belgium
(2) Department of Biomedical Sciences, University of Antwerp, Antwerpen, Belgium.

Dominant intermediate CMT type C (DI-CMTC) has been linked to mutations in the YARS1 gene, which encodes tyrosyl-tRNA synthetase. Emerging evidence suggests that YARS mutations cause neuropathy through a toxic gain-of-function mechanism affecting transcription, axonal transport, and actin dynamics. While previous studies showed altered mitochondrial morphology in YARS-mutant fibroblasts, a functional motor neuron-based model to assess mitochondrial involvement in DI-CMTC has been lacking. In this project, motor neurons were generated from iPSCs derived from four distantly related YARS E196K patients and corresponding isogenic controls. To examine axonal phenotypes, neurons were cultured in Xona Microfluidic Devices, enabling compartmentalisation of proximal and distal axons. Live-cell imaging was performed on day 28 post-differentiation to visualise organelles movement. Mitochondrial morphology was assessed using both immunofluorescent staining and live MitoTracker imaging in 2D and 3D cultures. To assess neuronal identity, Islet-1-positive nuclei were quantified across patient and control lines. Also, cellular stress was modelled by treating neurons with sodium arsenite. Motor neuron differentiation efficiency was comparable between YARS-mutant and control lines, confirming the mutation does not impair neural development. However, mitochondrial morphology analysis revealed an increase in fragmentation and decreased size in the distal but not the proximal compartments of YARS-mutant neurons. Live imaging demonstrated a selective reduction in mitochondrial transport velocity in distal axons. Additionally, stress granule analysis showed smaller G3BP1-positive granules in mutant neurons following oxidative stress, pointing to an altered stress response. Our findings describe how YARS mutations cause distal axonal degeneration that disrupts mitochondrial trafficking and morphology, and suggest mitochondrial dynamics as a potential therapeutic target.

Poster P11

Unraveling the macrophage – Schwann cell crosstalk in Charcot-Marie-Tooth disease type 1A

M. Van Brussel (1), B. Bekaert (1). S. in't Groen (1), M. Kleinewietfeld (2), L. Van Den Bosch (3,4), E. Wolfs (5), J. Bogie (6), R. Fledrich (7), T. Vangansewinkel (1,3,5), V. Timmerman (1)

(1) Peripheral Neuropathy Research Group, Department of Biomedical Sciences, University of Antwerp, Belgium
(2) Laboratory of Translational Immunomodulation, BIOMED Research Institute, Department of Immunology, University of Hasselt, Belgium
(3) Experimental Neurology, Department of Neurosciences, KU Leuven, Belgium
(4) Laboratory of Neurobiology, Center for Brain & Disease Research, VIB, Belgium
(5) Functional Imaging & Research on Stem Cells (FIERCE), BIOMED Research Institute, Department of Cardio and Organ Systems, University of Hasselt, Belgium
(6) Medical Biochemistry and Immunology Lab (MBIL), BIOMED Research Institute, Department of Immunology, University of Hasselt, Belgium
(7) Glial Biology in Neuromuscular Disorders (fledrichLAB), Institute of Anatomy, University of Leipzig, Germany.

Charcot-Marie-Tooth disease type 1A (CMT1A), the most common inherited peripheral neuropathy, is caused by a duplication of the PMP22 gene in myelinating Schwann cells. Growing evidence points to a significant involvement of the immune system in CMT1A pathogenesis, with macrophages increasingly being implicated in driving disease progression. Despite these associations, the functional role of macrophages and the specific mechanisms by which they interact with Schwann cells to influence disease progression remain poorly understood.

Here, we aimed to investigate macrophage biology and the complex crosstalk between Schwann cells and macrophages to better understand the underlying disease mechanisms of CMT1A. We stimulated macrophages derived from peripheral blood mononuclear cells with human myelin extracts to induce myelin uptake and activate their lipid metabolism. Compared to healthy controls, macrophages of a CMT1A patient showed a significant increase in lipid droplets, suggesting altered lipid handling. In parallel, we have been developing a 2D co-culture system to explore direct and indirect interactions between Schwann cells and macrophages in CMT1A. Co-cultures of Schwann cells and blood-derived macrophages from CMT1A patients and healthy controls were maintained for 24 to 72 hours to investigate intracellular communication. Various media compositions were tested, and a 50% mix of Schwann cell and macrophage media supported their viability and typical cell morphology. Moreover, a close interaction was observed between both cell types and myelin-related gene expression was upregulated in Schwann cells, suggesting an impact on differentiation and maturation.

In summary, our co-culture system offers a powerful platform to unravel the molecular mechanisms driving Schwann cell–macrophage interactions in CMT1A. These findings, coupled with a deeper understanding into macrophage biology, may uncover novel therapeutic strategies targeting immune responses in CMT1A.

Poster P12

The impact of Schwann cell differentiation on transgene expression in human stem cells.

Y. Lambrechts (1,2), K. Libberecht (1), L. Van Den Bosch (2*), E. Wolfs (1*).

(1) Laboratory for Functional Imaging & Research on Stem Cells, Biomedical Research Institute (BIOMED), Faculty of Medicine and Life Sciences, Hasselt University, Diepenbeek, Belgium

(2) Center for Brain & Disease Research, Laboratory of Neurobiology, Leuven, Belgium

Human stem cells, including induced pluripotent stem cells (iPSCs) and dental pulp-derived stem cells (DPSCs), are powerful tools for disease modeling and regenerative medicine. Their ability to differentiate into specialized cell types makes them ideal platforms for studying cellular behavior under both physiological and pathological conditions. To facilitate such studies, genetic modification with reporter genes such as green fluorescent protein (GFP) is commonly used for cell tracking and visualization. However, the stability of transgene expression during differentiation remains an important consideration.

In this study, we examined the effects of Schwann cell (SC) differentiation on transgene expression following either CRISPR/Cas9 gene editing or lentiviral transduction. iPSCs were genetically modified using CRISPR/Cas9 to create a GFP-positive master line with a hygromycin resistance cassette under the constitutive CAG promoter, enabling FLPe-mediated cassette exchange. Separately, DPSCs were transduced with a lentiviral vector encoding GFP, firefly luciferase (fLUC), and a puromycin resistance cassette, driven by the EF1alpha promoter.

Both iPSCs and DPSCs were differentiated into SCs. Differentiation was confirmed by the upregulation of SC markers, including neurotrophin receptor P75 (P75 NTR), laminin-211, and laminin-411. GFP expression was evaluated before and after differentiation using fluorescence microscopy, qPCR, and western blotting, while luciferase activity was assessed using fLUC assays.

Our results show that differentiation into SCs significantly reduces transgene expression in both iPSCs and DPSCs. These findings highlight the need for protocol optimization to maintain or enhance reporter gene expression following differentiation. Despite this reduction, modified cells remain useful for research involving the peripheral nervous system requiring SC visualization, including co-culture systems and transplantation models.

Poster P13

Schmidt-Lanterman Incisure number supports Schwann cell function in Charcot-Marie-Tooth disease 1A (CMT1A)

D. Stausberg* (1), D. Krauter* (1), P. Döbbe (1), T. Kungl (3), L. Coutelle (1), M. A. Eichel (1), D. Ewers (1,2), L. Linhoff (1), C. Stadelmann-Nessler (4), R. Fledrich (3), R. M. Stassart (5), K.-A. Nave (1), **M. W. Sereda** (1,2)

(1) Department of Neurogenetics, Max Planck Institute of Multidisciplinary Sciences, Göttingen, Germany
(2) Department of Neurology, University Medical Center Göttingen, Göttingen, Germany
(3) Institute of Anatomy and Department of Neuropathology, Leipzig University, Leipzig, Germany
(4) Department of Neuropathology, University Medical Center Göttingen, Göttingen, Germany
(5) Paul Flechsig Institute of Neuropathology, University Hospital Leipzig, Leipzig, Germany
**The authors contributed equally to this work.*

Schmidt-Lanterman Incisures (SLIs) are funnel-shaped cytoplasmic channels in the compact myelin internode of peripheral nerves. Although first described over 150 years ago, their functional role remains largely unknown. In demyelinating diseases such as Charcot-Marie-Tooth disease (CMT1A), caused by duplication of the PMP22 gene, increased numbers of SLIs are observed in both human patients and mouse models. A Schwann cell specific knockout of Vcl, which encodes the actin-binding protein vinculin, results in a reduced number of SLIs, while radial myelination, motor behavior or electrophysiological measurements are unaltered. Thus, vinculin conditional knockout mice (VclcKO) provide a useful model to study the relevance and function of SLI in physiological and pathological conditions. While a reduced number of SLIs has no effect on the phenotype of healthy Schwann cells, deletion of vinculin in the context of PMP22 overexpression deteriorates the CMT1A disease pathology, indicating a critical role for SLIs under demyelinating conditions.

Following peripheral nerve crush injury an increase in the number of SLIs can be observed. However, VclcKO mice fail to upregulate SLI formation following injury and show a delayed regeneration. Based on these findings we hypothesize that increased SLI numbers are beneficial during chronic and acute nerve injury.

Poster P14

An In Vitro Model for Motor Neurons Displays Axonal and Mitochondrial Deficiencies Associated With Charcot-Marie-Tooth Disease Type 2A

M. H. Magdesian (1), G. Viana (1), L. Kuytjes (1), R. V. DaSilva (1), K. Xu (2), A. Johnson (2), H. Rueth (2), M. Hendrickson (2), M. Scheideler (3), **K. Forsey** (3), S. S. Lin (3)

(1) Ananda Devices, 500 Bd Cartier O Suite 135, Laval, Quebec H7V 5B7, Canada
(2) BrainXell Inc., 455 Science Dr #210, Madison, WI 53711, USA
(3) Charcot-Marie-Tooth Association, PO Box 105 Glenolden, PA 19036, USA

CMT is the most common inherited neuropathy, affecting peripheral sensorimotor nerves, causing muscle weakness, atrophy and pain. CMT2A is the most frequent axonal subtype and an earlier onset form of CMT Type 2. It is associated with mutations in Mitofusin 2 (MFN2), a mitochondrial membrane anchored GTPase involved in equilibrium of mitochondrial fusion/fission. We investigated the impact of the MFN2 R364W mutation on aspects of neuronal cell morphology and mitochondria activity, using microfluidic plates containing compartments to accommodate neuronal elongation. iPSC-derived motor neurons were generated from a healthy human control line and a patient-derived line with the MFN2 R364W mutation and cultured on microfluidic plates to organize neurons into standard reproducible networks with cell bodies positioned in one compartment and axons elongated to a second compartment. Consistent orientation of axons enabled automated imaging/assessment using custom-created software of seven neuromorphological factors including cell size, axonal length, neurite connections, mitochondrial mobility and morphology.

Standardization of neuronal network cultures and analysis with the platform identified key differences between CMT2A and healthy controls, including statistically significant changes in cell number, size, and mitochondrial movement. Non-statistically significant trends were observed for other traits including axonal material, branching, number of branching junctions, axon breadth, and neurite straightness, as well as average anterograde mitochondrial velocity and aspect ratio of upper chamber, neurite channel, and lower chamber mitochondria.

Mouse models for CMT2A are limited. Our study highlights the advantage of using human-derived in vitro models for faster and more effective disease modeling, rapid drug screening, and a deeper understanding of the mechanisms underlying a devastating disease with a huge impact on children's development.

Poster P15

Novel heterozygous NARS1 variant in a family with axonal peripheral neuropathy

D. Beijer (1), S. M. Laureano-Figueroa (2,3), A. Antonellis (2,4), I. Kurth (1), M. Elbracht (1), K. Eggermann (1)

(1) Center for Human Genetics and Genomic Medicine, Medical Faculty, Uniklinik RWTH Aachen, Germany
(2) Department of Human Genetics, University of Michigan Medical School, Ann Arbor, MI, USA
(3) Medical Scientist Training Program, University of Michigan Medical School, Ann Arbor, MI, USA
(4) Department of Neurology, University of Michigan Medical School, Ann Arbor, MI, USA

Introduction: Aminoacyl-tRNA synthetases (ARSs) are enzymes that charge tRNA molecules with cognate amino acids in the process of protein synthesis. Dysfunction of these ARSs have been implicated in different neurological disorders. Particularly dominant mutations in ARSs are associated with peripheral neuropathies including Charcot-Marie-Tooth (CMT) disease. NARS1 mutations have been observed in dominant and recessive inheritance. While recessive NARS1 variants are primarily associated with complex central nervous system disease and dominant NARS1 variants with peripheral neuropathies, some overlap between the associated phenotypes has been observed.

Methods: Whole-genome sequencing was performed in a family with 5 individuals affected by axonal CMT (CMT2). This analysis revealed a heterozygous candidate variant in NARS1. Sanger sequencing of an unaffected family member was performed for additional segregation. Additional yeast experiments to study the functional effect of the identified variant on NARS function are ongoing.

Results: We identified a heterozygous NARS1 variant NM_004539.4:c.896C>A, p.(Ser299Tyr) in all 5 affected individuals in this family. The variant could not be detected in another related unaffected individual. The variant is absent from gnomAD v4.1 and in-silico prediction of the variant supports pathogenicity (Revel 0.913; CADD 27.1). Yeast-complementation assays are ongoing to assess the potential and expected loss-of-function effect, a common feature of neuropathy-associated ARS variants.

Conclusion: We identified another putative pathogenic variant in NARS1, segregating with disease in a family with axonal CMT. Pathogenicity is to be further supported by yeast complementation assays, allowing the expansion of known dominant pathogenic NARS1 variants associated with peripheral nervous system phenotypes.

Poster P16

Whole-Body muscle MRI in Non-5q Spinal Muscular Atrophy: Patterns, Genotype Prediction, and Diagnostic Implications

E. Berling (1,2), H. Reyngoudt (3), B. Marty (3), JY. Hogrel (3), Y. Fromes (3), A. Le Guillou(4), G. Fernandez-Eulate (5), M. Masingue (5), A. Behin (5), G. Bassez (5), A. Rouyer (1), C Lefeuvre (1), P. Laforêt (1,2), G. Nicolas (1,2), RY. Carlier (1,2), T. Stojkovic (5)

(1) APHP, Service de Neurologie, Hôpital Raymond Poincaré, Centre de référence Nord-Est-Ile-de-France Garches, France
(2) Université de Versailles Saint-Quentin-en-Yvelines, U 1179 INSERM, Paris-Saclay, France
(3) NMR Laboratory, Neuromuscular Investigation Center, Institute of Myology, Paris, France
(4) Department of Epidemiology, Emory University, Atlanta, Georgia
(5) APHP, Sorbonne Université, Service de Neuromyologie, Centre de référence Nord-Est-Ile-de-France, Institut de Myologie, Hôpital Pitié-Salpêtrière, Paris, France

Non-5q spinal muscular atrophies (SMA) are genetically heterogeneous hereditary motor neuropathies with progressive motor-predominant weakness and minimal sensory involvement. Over half remain unresolved, and muscle MRI may reveal distinctive fatty replacement patterns that support diagnosis.

We evaluated 25 adults (mean age 42.1 ± 13.5 y) meeting clinical and electrophysiological criteria for SMA, all negative for SMN1 deletions/mutations. Nineteen (76%) carried pathogenic variants in 13 genes, most often VRK1, DYNC1H1, and VWA1 (n=3 each). Whole-body MRI was graded with the Mercuri scale across 42 paired muscles and compared to published gene-specific signatures by counting discordant muscles.

Motor weakness was mainly proximo-distal (80%) with a mean clinical diagnostic delay of 20.4 ± 14.9 y. MRI patterns were highly concordant for DYNC1H1 and BICD2 with only 3.3 ± 1.2 and 4 discordant muscles, respectively. Reproducible patterns of fatty replacement were seen in following genes: VRK1: severe gluteal, thigh, and leg involvement with iliopsoas sparing; DYNC1H1: selective adductor magnus and anterior thigh involvement, long head of biceps femoris affected with short head spared, severe triceps surae with tibialis posterior sparing, and consistent gluteus maximus sparing; VWA1: severe anterior and posterior thigh involvement with gracilis and sartorius sparing. Cohort-wide key patterns" included selective sartorius/gracilis sparing despite quadriceps involvement (n=5), sparing of the long head of biceps femoris (n=4), and tibialis posterior (n=12) or extensor digitorum longus (n=6) sparing in the leg.

WB-MRI in non-5q SMA identifies reproducible gene-associated pattern (DYNC1H1, VWA1 and BICD2) and distinctive patterns improving genotype prediction. WB-MRI may resemble that of dystrophies, as VWA1-mutated patients show typical gracilis and sartorius sparing, suggesting dual pathophysiology, giving rise to motor neuropathies as well as myopathic involvement.

Poster P17

Genetic distribution of inherited peripheral neuropathies in Türkiye

A. Cakar (1), A. Candayan (2, 3, 4), G. Bagırova (5), Erzurumluoğlu (6), H. Durmus (1), S. Ceylaner (7), H. Houlden (8), ZO Uyguner (5), E. Battaloglu (2), Y. Parman (1)

(1) Neuromuscular Unit, Istanbul University, Istanbul Faculty of Medicine, Istanbul, Türkiye
(2) Department of Molecular Biology and Genetics, Bogazici University, Istanbul, Türkiye
(3) Molecular Neurogenomics Group, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium
(4) Molecular Neurogenomics Group, Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium
(5) Department of Medical Genetics, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Türkiye
(6) Department of Medical Genetics, Faculty of Medicine, Eskişehir Osmangazi University, Eskişehir, Türkiye
(7) Medical Genetics, Intergen Genetics Laboratory, Ankara, Türkiye
(8) Department of Neuromuscular Disorders, UCL Queen Square Institute of Neurology, London, UK

Background: Inherited neuropathies encompass a spectrum of diseases, including subtypes of Charcot-Marie-Tooth disease (CMT), hereditary neuropathy with liability to pressure palsies (HNPP), hereditary sensory neuropathy (HSN), distal hereditary motor neuropathy (dHMN), and other rare and complex neuropathies. Expanding our understanding of the genetic landscape in these populations is essential for accurate diagnosis and improved patient care.

Methods: The distribution of genetically diagnosed patients with CMT, dHMN, and HSN, from 314 families (370 patients), is evaluated. Patients with HNPP or other complex neuropathies were excluded from the analysis.

Results: The most frequent subtype was CMT1 (135 families), followed by CMT4 (45 families), dHMN (33 families), CMT-I (31 families), CMT2 (27 families), AR-CMT2 (26 families), and HSN (11 families. Interestingly, biallelic variants were responsible for the disease in 110 families. Parental consanguinity was noted in 117 patients. Pathogenic variants were identified in 48 different genes. The most frequent variation was PMP22 duplication in all cohort, followed by the pathogenic variants in GJB1, MFN2, SH3TC2, GDAP1, HINT1, and SORD, respectively. Regarding subtypes, SORD-neuropathy was the leading cause in dHMN, whereas RETREG1 variants were the most frequent in HSN.

Conclusion: This study highlights the significant genetic heterogeneity of inherited neuropathies, emphasizing the predominance of biallelic variants in our cohort, which contrasts with findings from similar studies in European populations. These results underscore the importance of examining genetic variations across diverse populations to gain a deeper understanding of the underlying genetic architecture.

Poster P18

A neuropathy-mitochondrial multidisciplinary team meeting: enhancing diagnosis

C. J. Record (1), W. L. Macken (1), M. Skorupinska (1), S. Haddad (1), M. Pipis (1), C. Pizzamiglio (1), A.M. Rossor (1), M. Laura (1), R.D.S. Pitceathly (1), M. M. Reilly (1)

(1) Department of Neuromuscular Diseases, Centre for Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London UK

Introduction

Next-generation sequencing has highlighted the clinical and genetic overlap of genetic neurological disorders. However, geographic and organisational distance between departments can limit interdisciplinary working. We hypothesised that a multidisciplinary team meeting (MDT) bridging neuropathy and mitochondrial teams could facilitate enhanced diagnostics and management of our patients.

Methods

Patients were recruited at a specialist inherited neuropathy centre or the Highly Specialised Services for rare mitochondrial disorders. Genetically undiagnosed families were discussed over three, one-hour meetings.

Results

Eighteen families were discussed and action plans generated based on a mitochondrial variant (4/18) or phenotype (14/18). These included reanalysis of existing genomic data, further clinical phenotyping, fadvanced analysis of existing histological samples e.g. muscle biopsies, and additional genetic testing. Nine families (50%) have a confirmed genetic diagnosis, accounting for at least part of their neurological syndrome, and a further two have a promising genetic candidate. These include POLG-related neuropathy, intellectual disability and retinitis pigmentosa syndrome due to recessive SCAPER variants, NDUFAF2-related neuropathy, ataxia and optic atrophy syndrome and MT-ATP6 myeloneuropathy. Diagnoses were made with testing available via the clinical service in 44% (4/9) of solved cases, as directed by clinical expertise.

Conclusions

An MDT combining the approaches of two specialist services has resulted in a 50% diagnostic rate for previously unsolved patients. This highlights the benefit of interdisciplinary working where complex phenotypes bridge classical sub-specialities.

Poster P19

Efficacy of PLGA nanoparticles for Schwann cell targeted gene delivery

A. Kagiava (1), M.Xinari (1), S. Matsia (2), G.Lazopoulos (2), A. Salifoglou (2), K.A. Kleopa (1,3)

(1) Department of Neuroscience, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus
(2) Laboratory of Inorganic Chemistry and Advanced Materials, School of Chemical Engineering, Faculty of Engineering, Aristotle University of Thessaloniki, Greece
(3) Center for Neuromuscular Disorders, The Cyprus Institute of Neurology and Genetics, Nicosia, Cyprus

The unique properties of nanoparticles (NPs) and their ability to cross blood-brain-barrier (BBB) make them an attractive vehicle for the treatment of neurological diseases. In our previous studies we showed that gene replacement delivered by adeno-associated viral (AAVs) vectors including AAV9 and AAVrh10 can partially rescue the demyelinating neuropathy in different models of Charcot-Marie-Tooth (CMT) disease. Although AAVs proved to be efficient, certain properties may limit their potential for clinical translation. These issues motivated us to develop a potentially safer and more targeted gene delivery approach for Schwann cells through the development of targeted NPs for the treatment of peripheral demyelinating neuropathies.

We created conjugated and non-conjugated PLGA NPs encapsulating a plasmid expressing the reporter gene EGFP driven by the Schwann cell specific myelin protein zero (MPZ) promoter. Targeting was achieved by conjugation of the NP to a tripeptide that has the ability to bind on a protein located on the abaxonal outer membrane of Schwann cells, in order to facilitate binding of the NP to the cell of interest. NPs were delivered in adult mice by lumbar intrathecal injection at the dose of 60 mg/kg. We examined possible toxicity in peripheral organs and EGFP expression in Schwann cells in PNS tissues including lumbar roots and sciatic nerves, comparing targeted to non-targeted NPs.

We detected EGFP in perinuclear cytoplasm of a subset of Schwann cells in lumbar roots and sciatic nerves but at low expression rates. Neither the conjugated nor the non-conjugated NPs resulted in any inflammatory responses in the peripheral tissues examined including the liver, indicating that this approach is safe.

In conclusion, we have developed a safe and promising approach for Schwann cell targeting to deliver gene therapies for demyelinating CMT neuropathies. Further optimization is needed in order to achieve higher expression rates in the cells of interest.

This work was co-funded by CMTA-MDA (MDA 952763), awarded to AK

Poster P20

"Getting the Balance Right": Co-creating a Sensory-Integrated Balance Programme for People Living with Charcot-Marie-Tooth Disease

L.E. Lee (1), M.M. Dudziec (1), C.H. Ford (2), S. Naveed (3), G.M. Ramdharry (1)

(1) Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, UK
(2) Independent Researcher, and Public Contributor, UK
(3) School of Design, University of Greenwich, UK

Introduction:

People with Charcot-Marie-Tooth disease (CMT) experience progressive distal weakness and sensory loss, contributing to impaired postural control and increased fall risk. Balance interventions incorporating sensory integration targeting visual, vestibular, and somatosensory systems are effective in other neurological conditions, and early studies suggest potential benefit in CMT. Co-design methods can enhance the relevance, acceptability, and uptake of such interventions by incorporating lived experience.

Methods:

A series of co-creation workshops were conducted with six adults living with CMT, facilitated by physiotherapists, an expert patient, and an artist-designer. Experience-based co-design approaches were used to identify priorities for balance training, shape the content and presentation of both paper-based and digital resources, and develop strategies for engagement and dissemination within the global CMT community.

Results:

Participants emphasised the importance of individualisation, variety, and psychological safety in balance training. Their input informed the structure and delivery of a modular resource that accommodates varying abilities and preferences, while encouraging exploration of different sensory inputs.

Conclusion:

The co-designed resource offers a novel, user-informed approach to sensory-integrated balance training in CMT. While it is intended for immediate community use, insights from the co-design process will inform the refinement and evaluation of future clinical interventions and research in this area.

Poster P21

Static and Dynamic Stabilometric Evaluation and Rehabilitation in Peripheral Neuropathy

M. Hamedani (1), E. Roveta (1), S. Massucco (2), C. Gemelli (1), M. Grandis (1), C. Schenone (2), M. Leandri (2), A. Schenone (1)

(1) Department of Neurology, IRCCS Polyclinic Hospital San Martino, Genova, Italy
(2) Department of Neuroscience, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), University of Genoa, Genova, Italy

Objectives

This study aims to evaluate the effectiveness of the GeaMaster stabilometric platform (GMSP) in detecting balance impairments and supporting rehabilitation in individuals affected by Charcot-Marie-Tooth (CMT) disease.

Materials

The full study plans to enroll 40 patients with genetically confirmed CMT and 60 healthy controls (HC), aged between 18 and 80 years. At this stage, preliminary data are available from 15 patients and 25 HC. Eligibility criteria required the absence of significant comorbidities, recent orthopedic surgery, and a pain score below 3 on the Visual Analogue Scale (VAS<3).

Methods

All participants underwent balance assessments using the GMSP in static (Eyes Open [EO], Eyes Closed [EC]) and dynamic conditions (Eyes Open Toes Up Small Perturbation [EOTUS], Eyes Open Backward Stimulation [EOBWS]). Dynamic trials were paired with surface electromyography to assess activation in the tibialis anterior and soleus muscles. The CMT group also performed functional tests: Berg Balance Scale, Modified Barthel Index, Tinetti Scale, 10-Meter and 6-Minute Walking Tests. Patients were randomized to either traditional physiotherapy or GeaMaster-based rehabilitation, both delivered 30 daily 1-hour sessions.

Results

No significant test–retest differences were found in HCs, confirming platform reliability. In patients, significant post-treatment improvements were observed in Sway Area and Sway Path during static EO and EC trials ($p = 0.01$). Dynamic testing showed reduced muscle activation delay in the tibialis anterior during EOTUS ($p = 0.001$) and in the soleus during EOBWS ($p < 0.001$) from baseline (T0) to post-treatment (T1). Although functional scores improved, changes were not statistically significant ($p > 0.05$). Correlation analysis revealed significant associations between Sway Area and activation times of the tibialis anterior ($r = 0.65$, $p = 0.03$) and soleus ($r = 0.72$, $p = 0.01$). Due to the current sample size, no statistical comparison between platform-based and traditional rehabilitation has been performed. Results reflect the general rehabilitation effect in CMT patients, without group differentiation. Further analysis will follow upon full enrollment.

Discussion

The GMSP reliably detected functional deficits and post-rehabilitation changes. Static tests were more sensitive to balance alterations, while dynamic trials better captured muscular responses. The platform offers objective, individualized data that complement clinical scales and may detect subtle changes not otherwise observable.

Conclusions

The combination of traditional assessments with quantitative metrics enhances sensitivity to small functional variations. Since the study is still in progress, more comprehensive results and interpretations will follow upon completion of the study.

Funded by the Italian Ministry of Research(Fit4MedRob,Grant#PNC0000007)

Poster P22

Patient survey: The lived experience with Charcot-Marie-Tooth in Austria: therapies actually used and medical care

B. Chaloupek (1), K. Mannsberger (2)

(1) Chair Woman CMT-Austria, Eichgraben, Austria
(2) Secretary CMT-Austria, Wolfsberg, Austria

The aim was to comprehensively analyse the reality of life for people with Charcot-Marie-Tooth neuropathy (CMT) in Austria and to record the therapy plans actually used.

Therapies

Non-pharmacological therapies are the cornerstone of CMT management. Physiotherapy is considered indispensable by almost all respondents, followed by therapeutic massage and electrotherapy. (utilisation 30 % upwards). Exercise therapy is relatively new in Austria, so the utilisation rate of 16% is very high. It is also interesting to take a look at other therapies mentioned by the respondents themselves, such as energy work, music therapy, psychotherapy and speech therapy. They show that the disease is debilitating overall and that there is a need for systemic therapy.

60% of respondents stated that they had invested a block of time (minimum 4 weeks) in an inpatient rehabilitation stay in the last 5 years, 38% of them several times. There is great demand for these interprofessionally organised therapy weeks, as they include a gait analysis and enable individual atrophied muscles to be specifically addressed and developed.

Orthopaedic shoes are widely used (71%). Only 24% use orthoses.

Medical management

Risk in diagnostic pathway: The data highlights the significant diagnostic delay of a median of 7 years. This is time lost for therapy.

Risk in consultations: CMT requires continuous medical care from a variety of specialities. On average, the participants consulted 6 different specialists. CMT was not mentioned in 35% of the visits. This means that patients themselves must ensure that no contraindicated medication (e.g. neurotoxic substances) is prescribed and that the various treatments are coordinated.

Other topics covered in the survey: regional differences in care, fears at the moment of diagnosis and assistance from the wealth of experience of self-help in response to this, specific demands on the healthcare system.

Report (16 pages) see https://qrco.de/bqC7Sk

Poster P23

Understanding the Time Course and Evolution of CMT-SORD (Sorbitol Dehydrogenase Deficiency)

E. Bailey (1), J. Luth (1), S. Summers (1), J. Charpentier (1)

(1) Applied Therapeutics, New York, NY United States of America

CMT-SORD is a newly-discovered progressive hereditary peripheral neuropathy grouped in the broader rare diseases Charcot-Marie-Tooth Type 2 (CMT2) or distal hereditary motor neuropathies (dHMNs), conditions in which the majority of patients never receive a genetic diagnosis or know the biochemical cause of their disease.

Biallelic mutations in the gene encoding sorbitol dehydrogenase are the cause of CMT-SORD. Sorbitol dehydrogenase catalyzes the second step in the polyol pathway, an alternative pathway of glucose metabolism. Sorbitol dehydrogenase is necessary to catabolize the sugar alcohol sorbitol, a toxic metabolite at high concentration that cannot be easily excreted. Patients with CMT-SORD have grossly elevated levels of sorbitol in their blood, nerves, and other tissues, resulting in progressive peripheral neuropathy.

Evidence suggests that disease manifestations are related to patients' total sorbitol exposure (i.e., exposure-years). Lowering total sorbitol in the body and sustaining this effect is therefore likely to produce a clinical benefit by slowing or stopping disease progression. Therapeutic intervention would require lowering whole body sorbitol and sustaining the reduction for sufficient time as to allow nerve function to stabilize or improve. Sorbitol exposure have been shown to be directly correlated to CMT-Functional Outcome Measures. Reduction of whole body and tissue sorbitol also promotes the degradation of intracellular sorbitol. Maintenance or improvement of motor neuron signaling may slow the conversion of muscle to fat, thereby effecting improvements in functional measures. Any reduction in sorbitol is likely to benefit patients with CMT-SORD by reducing the overall disease burden.

Understanding the relationship between SORD deficiency, sorbitol accumulation in nerves, and long-term clinical manifestations can inform potential therapeutic targets and enable the development of a disease-modifying therapy for CMT-SORD.

Poster P24

Physiotherapy in Charcot-Marie-Tooth Disease: insights from the Italian CMT Registry

E. Cavalca (1), A. Bertini (1), M. Serio (1), F. Manganelli (2), G.M Fabrizi (3), A. Schenone (4), L. Santoro (2), T. Cavallaro (3), M. Grandis (4), S.C Previtali (5), Y.M Falzone (5), I. Allegri (6), L. Padua (7), C. Pazzaglia (7), P. Saveri (1), A. Quattrone (8), P. Valentino (8), G. Vita (9), S. Tozza (2), L. Gentile (9), M. Russo (9), A. Mazzeo (9), F. Ferraro (10), C. Pisciotta (1), D. Pareyson (1); Italian CMT Network

(1) Unità di Malattie Neurologiche Rare, Dipartimento di Neuroscienze Cliniche, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy
(2) Dipartimento di Neuroscienze, Scienze Riproduttive ed Odontostomatologiche, Università Federico II di Napoli, Napoli, Italy
(3) Dipartimento di Neuroscienze, Biomedicina e Movimento, Università di Verona, Verona, Italy
(4) Dipartimento di Neuroscienze, Riabilitazione, Oftalmologia, Genetica e Scienze materno-infantili, Università di Genova, Genova, Italy
(5) INSPE e Divisione di Neuroscienze, IRCCS Ospedale San Raffaele, Milano, Italy
(6) Dipartimento di Neurologia, Azienda Ospedaliera di Parma, Parma, Italy
(7) Università Cattolica del Sacro Cuore, Rome, Italy
(8) Centro di Ricerche Neuroscienze e Istituto di Neurologia, Università Magna Graecia, Catanzaro, Italy
(9) Unità di Neurologia e Malattie Neuromuscolari, Dipartimento di Medicina Clinica e Sperimentale, Università di Messina, Messina, Italy
(10) Struttura Complessa di Riabilitazione Neuromotoria ASST Mantova Presidio di Bozzolo, Mantova, Italy

CMT treatment is still symptomatic. Despite extensive use of physiotherapy, no specific guidelines exist for adults. Data from direct patients' experience concerning access, benefits and satisfaction are also lacking. We investigated physiotherapy use, satisfaction and access in a well-characterized CMT cohort.

Questionnaires were administered through the Italian CMT Registry (2015-2018) or directly at CMT clinic of Besta Institute (2024), with clinical and genetic data collected. Questionnaires: Foot Function Index (FFI); tailored Questionnaire developed with input from experts and patients' groups about physiotherapy use (frequency and type), benefit duration, satisfaction (VAS scale 0-10).

313 patients completed the questionnaire: 255 (81.5%) online and 58 (18.5%) in presence. 210 (69.3%) patients received physiotherapy at least once; as compared to non-physiotherapy ones, they had higher CMTES (9.6±5.1 vs 6.4±4.5; $p<0.001$); FFI pain (20.8±17.9% vs 13.9±15.2%; $p=0.002$), FFI Disability scores (34.7±17.5% vs 22.1±18.2%; $p<0.001$), and more frequent use of orthoses (29.5% vs 5.8%; $p<0.0001$). Most of the patients received physiotherapy in cycles (62.3%) via the National Health System (76.2%). Commonly reported exercises included stretching (85.2%), muscle strengthening (85.7%), posture (80%) and balance exercises (83.4%), with 70% reporting at least three combined types. 62% of patients rated their satisfaction ≥6. Perceived benefit after physiotherapy lasted 1-3 months in 57%, 3-6 months in 17% and more than 6 months in 12% of the cases. 47% of patients reported difficulties accessing physiotherapy.

Almost 70% of patients underwent physiotherapy at least once in their lives, in most cases with combined exercises and good satisfaction. Duration of perceived benefit, however, is limited, not exceeding three months for most patients, suggesting the need for repeated or continuous treatment. Access remains a significant barrier for almost half of the patients.

Poster P25

Gene replacement therapy efficiently restores normal phenotype in hiPSC-derived in vitro models of VRK1-related motor neuropathies

Z. Hamzé (1), S. El Hachem (1), N. Roeckel-Trévisiol (1), C. Provost (1), K. Bertaux (2), A. Mégarbané (3,4), N. Broucqsault (1), O. Hadadeh (1), N. Bernard-Marissal (1), **V. Delague** (1)

(1) Aix Marseille Univ, INSERM, MMG, U 1251, Marseille, France
(2) Centre de Ressources Biologiques, Assistance Publique Hôpitaux de Marseille, CRB APHM, Hôpital Timone Adulte, Biogénopôle, Marseille, France
(3) Department of Human Genetics, Gilbert and Rose-Marie Chagoury School of Medicine, Lebanese American University, Lebanon
(4) Institut Jérome Lejeune, Paris, France

The distal hereditary motor neuropathies (dHMN) comprise a heterogeneous group of diseases characterized by length-dependent predominantly motor neuropathy. We have described VRK1 as the gene responsible for dHMN, associated to upper motor neuron signs[1]. We have demonstrated that dHMN due to VRK1 mutations lead to reduced levels of VRK1 in the nucleus, and that this depletion alters the dynamics of coilin, a phosphorylation target of VRK1. Patients' hiPSC-derived Motor Neurons (hiPSC-MN) display Cajal Bodies (CBs) disassembly, defects in neurite outgrowth and branching, altered Action Potential (AP) waveform and decreased Axonal Initial Segment (AIS). length[2].

Here, we want to demonstrate, in vitro, that we can rescue the effect of the loss-of-function mutations in VRK1 using AAV-based transfer of the therapeutic gene inpatiens' hiPSC-MNs.

We treated hiSPC-MNs from two patients from different families and different mutations, with AAV6 vectors expression a GFP tagged VRK1 protein under the control of a CMV promoter (AAV6-CMV.Vrk1.GFP). The patients are the one published in [1] and a patient compound heterozygous for the following variants: NP_003375: p.Arg389Hisfs*7;Lys357Valfs*11.

Using a dose of 5,5*10^3 viral genome/MN, at Day 23 of differentiation for 24 hours, we were able to efficient transduce more than 50% of the total hiPSC-MN. In treated motor neurons at final Day 30 of differentiation, we observed restored levels of VRK1, restoration of the CB size to values similar to the control, restoration of a normal AIS length, correlated to a restoration of normal AP amplitude and amelioration of the global electrical parameters in treated versus non treated patient's hiSPC-MNs (n=3 independent experiments).

In conclusion, we made the proof of concept that re-expression of the wild-type VRK1 protein in motor neurons from patients with dHMN-VRK1 rescues the disease phenotype in vitro.

[1] doi: 10.1093/hmg/ddz060

[2]doi: 10.1016/j.nbd.2021.105609

Poster P26

AAV9 MFN1 gene therapy as proof of principle for the treatment of CMT2A

M. D'Agostino (1), E. Pagliari(2), L. Quetti (1), L. Sali (1), L. Ottoboni (2), GP. Comi (1,2), S. Corti(2,3), F. Rizzo (1)

(1) Neurology Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
(2) Department of Pathophysiology and Transplantation, Dino Ferrari Center, Università degli Studi di Milano, Milan, Italy
(3) Neuromuscular and Rare Diseases Unit, Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

Mitofusin-2 (MFN2) is a mitochondrial outer-membrane protein involved in mitochondrial dynamics in most tissues; its mutations are directly responsible for Charcot-Marie-Tooth disease type 2A (CMT2A), a disabling and currently incurable motor and sensory neuropathy. Although MFN2 is ubiquitous in cells, its alterations disproportionately impact the nervous system, potentially due to unique energy demands and diminished levels of MFN1, an MFN2 isoform. This makes neurons more susceptible to disruption caused by mutant MFN2. Interestingly, overexpressing MFN1 can compensate for MFN2 loss, as shown in vitro and in vivo when expressed as a transgene, surpassing MFN2-focused strategies.

For the first time in this study, we evaluate the therapeutic efficiency of MFN1 modulation by systemic or local administration in presymptomatic and symptomatic CMT2A mice (Thy1.2-MFN2R94Q mice). Considering that AAV9 displays CNS tropism and crosses the blood–brain barrier (BBB) after systemic and intrathecal administration, and that AAV9–mediated gene therapies have been developed for neurological diseases, we selected AAV9 as in vivo shuttle for delivering the MFN1 gene.

The western blot analysis showed significant increase of human MFN1 protein levels in the brain and spinal cord of the AAV9::MFN1 treated animals compared to AAV9-null treated ones, confirming the molecular efficacy of our strategy. Phenotypic and neuropathological analyses of treated CMT2A mice are now on going. While additional studies are needed, these results offer a promising starting point for future clinical applications, paving the way toward the development of an effective treatment for CMT2A patients.

Poster P27

Circulating transcriptional biomarkers serve as surrogates of disease progression in Charcot-Marie-Tooth 1A disease

B. Ahmad (1,2)*, L. Linhoff (1,2)*, A. Leha (3)*, T. Linhoff (1,2), S. Wernick, K. Kummer, S. Fritzsch (1) , E. Akova-Öztürk (4), B. Dräger (4,5), B. Schlotter-Weigel (7), S. Thiele (7), N. Garcia-Angarita (7), E. Greckl (7), L. Reinecke (1), M. Rossner (6), M.C. Walter (7), P. Young (4,8) and M.W. Sereda (1,2)

(1) Department of Neurology, University Medical Center Göttingen, Robert-Koch-Straße-40, Göttingen, Germany
(2) Translational Neurogenetics, Max-Planck-Institute of Multidisciplinary Sciences, Hermann-Rein-Straße 3, Göttingen, Germany
(3) Department of Medical Bioinformatics, University Medical Center, Robert-Koch-Straße 40, Göttingen, Germany
(4) Department of Sleep Medicine and Neuromuscular Disorders, University of Münster, Münster, Germany
(5) Department of Neurology with Institute of Translational Neurology, University Hospital Münster, Münster, Germany
(6) Department of Psychiatry, Ludwig-Maximilians-University, Munich, Germany
(7) Friedrich-Baur-Institute, Department of Neurology, Ludwig-Maximilians-University, Munich, Germany
(8) Medical Park International Department, Medical Park Bad Feilnbach, Reithof 1, Bad Feilnbach, Germany
(9) Molecular and Behavioral Neurobiology, Department of Psychiatry, Ludwig Maximilian University of Munich, Munich, Germany

Charcot-Marie-Tooth disease type 1A (CMT1A), the most common inherited peripheral neuropathy, results from PMP22 gene duplication and presents with progressive motor and sensory deficits. Despite well-defined genetics, therapeutic development is hindered by the lack of sensitive clinical outcome assessments (CAO) given slow disease progression and variable severity. This study aimed to identify robust transcriptomic biomarkers from blood which may serve as surrogate outcome measures for monitoring disease progression.

A cohort of 139 genetically confirmed CMT1A patients was longitudinally assessed over 2 years using standardized clinical tests and blood RNA sequencing at 3 expert sites across Germany. Parallel analyses were conducted in a Pmp22-overexpressing rat model over 12 weeks. Differential expression analysis, cross-species comparisons, and integration with functional data were employed to identify candidate biomarkers associated with disease severity and progression.

Over the two-year follow-up, we confirm deterioration of the Charcot-Marie-Tooth Neuropathy Score (CMTNSv2) (0.32 (95%-CI [0.05; 0.59]) per year ($p = 0.019$)). Out of a pool of consistently upregulated disease markers in CMT1A patients and CMT rats (vs.controls) based on RNA Seq data sets at baseline, 9 regulated genes were validated via Real-Time Quantitative PCR (RT-PCR). Transcriptional expression of Actb, E2F2, MFAP, SAMD14, SEMA5A and SPI1 show a significant increase over time, whereas UQCRB declined over time. These 7 genes show properties fitting to diagnostic, prognostic and predictive biomarkers.

Transcriptomic profiling revealed several blood-derived mRNA candidates in CMT1A patients correlate with longitudinal changes in CAOs and overlap with findings from the CMT rat model. These markers may offer a minimally invasive, scalable tool for disease monitoring in future clinical trials.

Poster P28

Responses to the cardiopulmonary exercice test for upper limbs in children and adolescents with Charcot-Marie-Tooth Disease

A.C. Mattiello-Sverzut (1), B.A. Soares (2), R.S.O. Odake (3), J.R. Silva (3), J. Crescêncio (4), P.H. Manso (5).

(1) Department of Health Sciences, Ribeirão Preto Medical School (FMRP), University of São Paulo (USP), Brazil
(2) External research collaborator, Ft-Rara Laboratory, FMRP-USP, Brazil
(3) Postgraduate Programme in Rehabilitation and Functional Performance, FMRP-USP, Brazil
(4) Department of Internal Medicine, FMRP-USP, Brazil
(5) Department of Paediatrics and Childcare, FMRP-USP, Brazil

Introduction: Charcot-Marie-Tooth disease (CMT) is a progressive hereditary neuropathy that impairs motor and cardiorespiratory function. Assessing responses to physical exertion can help detect functional decline.

Objective: To compare the cardiorespiratory response to exercise in children and adolescents with CMT versus those with typical development.

Methods: This cross-sectional study (ethics no. 83382524.0.0000.5440) included 34 participants (17 with CMT and 17 matched controls; mean age: 12.5 ± 3.4 years). Participants performed a maximal incremental exercise test on an upper-limb cycle ergometer (Lode Angio) with a 5 W/min ramp protocol. Cardiorespiratory parameters were collected using a portable gas analyzer (Cosmed K5). Outcomes included peak oxygen consumption (VO_2), total test time, workload, distance, carbon dioxide production (VCO_2), minute ventilation (VE), respiratory exchange ratio (RER), and heart rate (HR) at rest, peak, and delta. Data were analysed using Student's t-test ($p < 0.05$).

Results: The CMT group showed significantly lower values than controls for relative VO_2 peak (19.0 vs. 24.7 mL/kg/min; $p = 0.005$), absolute VO_2 peak (954.5 vs. 1181.5 mL/min; $p = 0.027$), RER (1.04 vs. 1.10; $p = 0.037$), test time (8:30 vs. 11:06 min; $p = 0.006$), workload (35.5 vs. 51.3 W; $p = 0.001$), distance (560.3 vs. 1077.9 m; $p = 0.001$), HR at rest (95.3 vs. 83.8 bpm; $p = 0.047$), HR peak (142.7 vs. 161.0 bpm; $p = 0.014$), HR delta (49.1 vs. 77.1 bpm; $p < 0.001$), VCO_2 peak (980.2 vs. 1339.1 mL/min; $p = 0.006$), and VE peak (33.4 vs. 45.8 L/min; $p = 0.003$).

Conclusion: Children and adolescents with CMT have reduced cardiorespiratory capacity and physical performance compared to typically developing peers. These findings highlight the importance of promoting exercise interventions, including aerobic training, to support cardiopulmonary and functional health.

Acknowledgements: We acknowledge the financial support and research scholarships provided by the Coordination for the Improvement of Higher Education Personnel (CAPES) and the São Paulo Research Foundation (FAPESP).

Poster P29

Better Balance-CMT: Protocol for a randomised, controlled, efficacy and implementation trial of a home-based, balance training intervention for people with Charcot-Marie-Tooth Disease

G. Ramdharry (1,2), L. Lee (1,2), M. Dudziec (2), M. Reilly (1,2)

(1) Queen Square Centre for Neuromuscular Diseases, National Hospital for Neurology and Neurosurgery, University College London Hospitals NHS Foundation Trust, London, UK
(2) Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology, London, UK

Multi-sensory rehabilitation has shown promising effects in people with sensory loss, and resistance training can improve proximal lower limb muscle strength. Studies of multi-sensory rehabilitation with and resistance training can improve balance in people with CMT in specialist clinics. We developed a pragmatic, home-based balance rehabilitation program and a proof-of-concept study found it to be safe and acceptable for people with CMT with excellent engagement. This will now be tested at scale through the Better Balance-CMT (BB-CMT) trial.

Methods: We will partner with patients and stakeholder to co-produce web-based resources for the BB-CMT intervention. It will be delivered at home by trained physiotherapists, through 3 face-to-face sessions, using self-management principles, digital materials and remote support.

We will then conduct a randomised, single blinded, two arm trial of the BB-CMT intervention compared to treatment as usual. A hybrid-1 trial design is planned, with the primary aim of exploring efficacy of BB-CMT and the secondary aim of exploring potential barriers and facilitators to “real-world” implementation into practice. The program will last 12 weeks and compared to a 12-week control period. A 12-week open label is included to assess continued engagement and carry over.

The primary outcome measure is the Berg Balance Scale (BBS), calculating mean difference in BBS score between the BB-CMT and the control group using a linear regression, adjusted for baseline BBS score. A target sample of 84 participants is based previous studies of balance training in CMT, with a detectable standardised effect size of 0.66, at 80% power and 5% 1-sided alpha, allowing for a 10% drop out. Participants will be recruited from 6 NHS hospitals in England.

Conclusion: Funding for this work has been acquired through a National Institute for Health Research award. The co-production work will start in autumn 2025, with the BB-CMT trial to commence in 2026.

Poster P30

The European CMT Federation (ECMTF): A Unified Voice to Accelerate Research and Raise Awareness for CMT

F. Genovese (1), M. Van Der Linden (1), A. Hoyau (1), A. Leysen (1), S. Bull (1), I. Pernice (1)

(1) European CMT Federation (ECMTF), Belgium

Charcot-Marie-Tooth (CMT) presents significant challenges across Europe, with fragmented research and varying standards of care. The European CMT Federation (ECMTF) is the umbrella organization for national CMT patient associations, created to unite these groups, amplify the patient voice, and accelerate progress.

A cornerstone of our strategy is the European CMT Research Association (ECRA). This innovative collaboration between PAGs and all the leading CMT stakeholders establishes a unified European research agenda. By coordinating efforts and funding promising projects, ECRA aims to eliminate redundant work and fast-track the most viable therapeutic pathways toward a cure.

To combat obscurity and diagnostic delays, ECMTF launched a coordinated pan-European awareness campaign. Leveraging shared branding and messaging across member countries (UK, France, Italy, Spain, and others), the campaign educates the public and non-specialist healthcare professionals about CMT's symptoms and impact, fostering earlier diagnosis and greater understanding.

By strategically aligning research through ECRA and amplifying our message through a unified awareness campaign, ECMTF breaks down national silos. Our federated model is essential for driving impactful research, influencing policy, and ensuring no patient is left behind in the journey towards effective treatments.

Poster P31

ACMT-Rete: A Patient-Led Network Integrating Research, Awareness, and Community for Charcot-Marie-Tooth

F. Genovese (1), D. Esposito (1), M. Garcea (1), F. Del Vita (1), E. Conte (1), P. Grespi (1), R. Riccò (1), A. Arcuri (1), F. Tiberio (1)

(1) ACMT-Rete per la malattia di Charcot-Marie-Tooth OdV, Italy

ACMT-Rete is Italy's patient-driven association for Charcot-Marie-Tooth (CMT), a progressive inherited neuropathy. Our mission is to build a collaborative "Rete" (Network) that unites patients, clinicians, and researchers to accelerate progress and provide holistic support.

We fund patient-centric research, like the 2024 project led by Dr. Yuri Matteo Falzone (University of Milan). His work investigates the complex mechanisms of balance deficits in CMT, aiming to develop targeted, more effective rehabilitation protocols to improve stability and reduce fall risk.

To demystify stigma and raise public awareness, we support innovative outreach like "The Ballad of Human Mutations", a unique art installation that translates CMT hands and feet into pieces of artwork with an engaging narrative about the importance of diversity.

Our fundraising is rooted in community. The "Bottoni che Fatica" (Button Jewelry) project transforms donated buttons into handcrafted pieces, symbolizing how our community creates value to directly fuel research.

Recognizing the unique challenges faced by youth, we launched "Generazione Z: Crescere con la CMT" (Generation Z: Growing up with CMT). This vital project provides dedicated support and a peer network for adolescents and young adults navigating the transition to adulthood with a chronic condition.

By integrating targeted research, creative awareness, grassroots fundraising, and peer support, ACMT-Rete fosters a holistic ecosystem that empowers patients and drives meaningful change in the fight against CMT.

Poster P32

Investigating Axonal Transport In Charcot-Marie-Tooth Disease Type 2A Using A Pluripotent Stem Cell-based Model

L. Jestice (1,2), L. Butler (1,2), K. Adamson (2,3,4), A. Grierson (2,3,4), K. De Vos (2,3), I. Barbarić (1,2)

(1) School of Biosciences, The University of Sheffield, Sheffield, S10 2TN, United Kingdom
(2) Neuroscience Institute, The University of Sheffield, Sheffield, S10 2TN, United Kingdom
(3) Sheffield Institute for Translational Neuroscience, The University of Sheffield, S10 2HQ, Sheffield, United Kingdom
(4) Bateson Centre, The University of Sheffield, S10 2TN, Sheffield, United Kingdom

Charcot-Marie-Tooth (CMT) disease is one of the most common forms of inherited peripheral neuropathy and has many different subtypes. One such subtype is sensory and motor neuropathy CMT Type 2A (CMT2A), for which no treatments currently exist. CMT2A is caused by mutations in Mitofusin 2, and it is unknown how these mutations drive disease. Hence, we set out to create the first human embryonic stem cell (hESC) model of CMT2A to investigate the impact of a CMT2A-causing mutation in a disease-relevant cell type.

We generated a panel of hESC clones by introducing the disease-causing heterozygous R94Q mutation into Mitofusin 2 via CRISPR-Cas9 editing. The clone panel was subsequently differentiated into limb-innervating motor neurons and used for live trafficking assays. Limb-innervating motor neurons containing the disease-causing mutation displayed a mitochondrial trafficking defect that could be rescued via HDAC6 inhibition. We further tested the same HDAC6 inhibitor in a zebrafish model of CMT2A, where chronic dosing rescued motor deficits in treated zebrafish. Furthermore, to obtain a mechanistic insight into CMT2A pathogenesis we show that Mitofusin 2 containing the R94Q mutation interacts more strongly with the trafficking adapter Trak1, leading to Trak1 having a reduced interaction with the axonal motor protein kinesin.

We have successfully created hESCs containing a CMT2A-causing mutation that can be differentiated into limb-innervating neurons, providing a new in vitro platform for CMT2A research. Furthermore, results here contribute to evidence that axonal transport deficits are a common CMT2 hallmark. This work provides a new hypothesis for CMT2A pathophysiology and is a foundation for the further study of axonal transport machinery and its functionality in CMT2A.