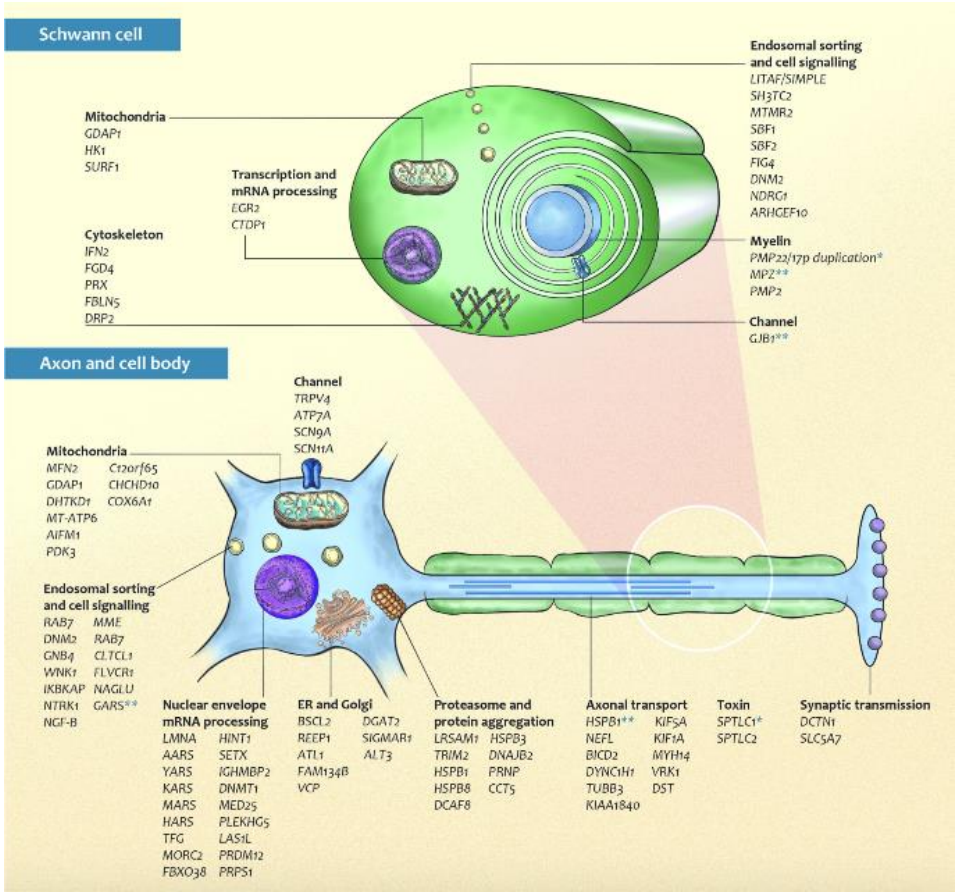
EDUCATIONAL COURSES

Mechanisms in inherited neuropathies (V. Timmerman)

- 94 genes, 1600 mutations *(Timmerman Strickland & Zuchner GENES (2014))*



Rossor et al. Curr Opin Neurol 2016

Mechanisms in inherited neuropathies (V. Timmerman)

- Inherited Neuropathy Variant Browser (Zuchner) : http://hihg.med.miami.edu/code/http/cmt/public_html/index.html#/
- Association of a mir-149 polymoprhism with onset age and severity in CMT1A *(Nam et al, Neuromuscular Disorder, 2018)*
- Second mutation of HSPB3 mutation in axonal CMT family
- Mutations in ATP1A1 cause dominant CMT2 *(Lassuthova et al. AIHG 2018)*
- COA7 mutations cause spinocerebellar ataxia with axonal neuropathy (+ cerebellar trophy ±leucopathy) *(Higuchi et al Brain 2018)*
  - Mildly elevated CPK levels
- Early onset axonal CMT due to SACS mutation
  - Pure sensorimotor axonal neuropathy without cerebellar ataxia, spastic paraplegia. Neuroimaging are similar as other sacsonopathies

Mechanisms in inherited neuropathies (V. Timmerman)

- tRNA synthetases : TARS
- HDAC6 inhibition : HDAC6 participates in neurodegeneration
- Atl3 mutations causes aberrant ER membrane tethering
- Mitochondria-associated-membranes as hubs for neurodegeneration
  - Lipid metabolism
  - Mitochondrial dynamism (MFN2)
  - Calcium metabolism (TRPV4)
  - Autophagy
- Autophagy as an emerging common pathomechanism in inherited peripheral neuropathies
  - Chaperon-Assistant-Selected-Autosystem (CASA) : BAG3...
  - Small Heat Shock Protein HSPB1

Mechanisms in inherited neuropathies (V. Timmerman)

- Potential biomarkers
  - Expression of transcripts in skin biopsy
  - Elevated plasma NFL conencentration
  - Proteomics revealed PFN2 and GAMT as commonly downregulated proteins in different CMT2 genotypes
    PFN2 decrease xith age in patients whereas it increases in healthy paptients.
    Profilins (PFN2) are actin dynamics regulatros essentials for actin polymerization
    GAMT belongs to creatine biosynthetic pathway

Genetic Therapies in CMT
(John Svaren)

- Vector-mediated gene therapy
  - AAV
  - lentiviral : adaptaded retrovirus
- (Non viral gene delivery)

- Axonal CMT : target neurons (+ Schwann celles for neuroprotection)
  Demyelinating CMT : target Schwann cells
- Type of mutation : gain or loss of function mutation/ point-mutation/copy number variants...

- Therapeutic options in CMT:
  - Disease genere replacement : recessive/X-linked
  - Disease Gene Silencing : reducing overexpression or allele specific
  - Modifier-gene /genome editing: NT3, neuregulin, NGF, CNTF, GDNF...

Genetic Therapies in CMT
(John Svaren)

- **CMTX** : GJB1/Connexin 32 (>400 mutations)

  Lentivirus uses MPZ regulatory elements to drive Schwann cells specific expression of GJB1

  Intrathecal delivery

- **ATTR** : reduce TTR production in order to minimize aggregation

  RNA interference: siRNA and shRNA binduce sequence-directed mRNA degradation by the RISC complex (cf plus loin)

  Antisense oliconucléotides targeting specfic sequence

- **SMA** : mutated SMN1 and non-entirely functional SMN2

  Manipulate splicing in SMN2 increases the level of production of the protein

  Spinraza: oligonucleotide antisense increases SMN2

  AAV mediated gene repoacement intravenously

  Epigenetic regulation : epigenetic repression of expression of SMN1 and 2 by a long non coding area. Using an ASO you can block interaction of this noncodant ARN and increase SMN2 expression

PLENARY SESSIONS

PRESENTATIONS

Patisiran in hATTR : APOLLO trial
(Senda Ajroud-driss)

- Patisiran : rNA interfence targeting hepatic production of TTR. I/3 weeks
- Placebo controlled study
- Outcome : neuropathic score
- Adverse events : peripheral oedema, injection reaction
- Patisiran : improved health status, scales

Lysosomal and mitochondrial disorder in CMT2B (Yvette Wong)

- Mitochondria and lysosomes form dynamic membrane contact sites
- Rab7 GTP hydrolysis promotes mitochondria-lysosme contact untethering
- Rab7 GTP hydrolysis regulates mitochondrial fission marked by mitochondria-lysosome contact sites

- ➔ new pathway for mitochondria-lysosome contact involved in CMT2B, regulated by Rab7 GTP hydrolysis

PMP22 antisense oligonucleotides in rodent models of CMT1A duplication
(John Svaren)

- ASO can target exonic, intronic UTR sites and can increase or decrease expression of genes
- (weekly sc in 5 weeks rodents during 8 weeks)
- PMP22 ASO treatment reverses motor deficits in the C22 moiuse modelPMP22 ASO reverses slow conducion velocities
- PMP22 ASO restores myelination in C22 mouse model
- Only a subset of deregulated gens are normalized by ASO
  Those genes may be the most sensitive biomarkers in clinical trial
  - Pou3f1 (transcription factor which expression inhibits myelination)
  - Sox4 (delays myelination)
  - C-Jun, Id2
- Schwann cell profiling in Human skin biopsies : measure PMP22 and other Schwann cell-specific genes

CMT

αII-spectrin haploinsufficiency due to *SPTAN1* nonsense mutations causes juvenile onset dominant hereditary motor neuropathy

Danique Beijer[1,2], Tine Deconinck[1,2], Jan De Bleecker[3], Alessandro Malandrini[4], Miren Zulaica Ijurco[5], J. Andoni Urtizberea[5], Peter De Jonghe[1,2,6], Jonathan Baets[1,2,6]

1. Neurogenetics Group, Center for Molecular Neurology, VIB Antwerp, Belgium; 2. Laboratory of Neuromuscular Pathology, Institute Born-Bunge, University of Antwerp, Belgium; 3. Department of Neurology, University Hospital Ghent, Belgium; 4. Department of Medicine, Surgery and Neuroscience, University of Siena, Italy; 5. Neuromuscular Reference Center, Hôpital Marin, AP-HP, Hendaye, France; 6. Neuromuscular Reference Centre, Department of Neurology, Antwerp University Hospital, Belgium

INTRODUCTION

distal Hereditary Motor Neuropathies (dHMN)

- Most commonly features distal weakness of the extremities.
- Generally only slowly progressive towards more proximal involvement
- Genetically and clinically heterogeneous
  > 90 genes are associated with IPN
- ~60-80% of patients remain undiagnosed

HMN

IDENTIFICATION OF *SPTAN1* MUTATIONS

Cohort

- NGS data for patients from **~60 families with hereditary motor neuropathies (HMN)** is available. WES or WGS from the index patient and in some families additional affected or unaffected family members
- NGS data was generated as part of the NeurOmics consortium
- **3 separate nonsense mutations in *SPTAN1*** were identified in 3 separate multigenerational families

	Mutation	CADD	GERP	MutationTaster	Gnomad
Family A	c.415C>T (p.R139*)	38	5.26	Disease causing	Absent
Family B	c.4615C>T (p.Q1539*)	52	5.65	Disease causing	Absent
Family C	c.6385C>T (p.Q2149*)	47	5.53	Disease causing	Absent

CLINICAL FINDINGS

-/+ mutation carrier, -/- non-carrier, # clinically examined

Family A

- Childhood-onset gait difficulties
- Easily sprained ankles, frequent falls
- Bilateral toe-drop, heel walking impossible
- Slow progression, walks unaided at 30

Family B

- Childhood-onset gait difficulties
- LL weakness and a mildly shortened Achilles tendon
- Overall slow progression
- ↓ reflexes LL

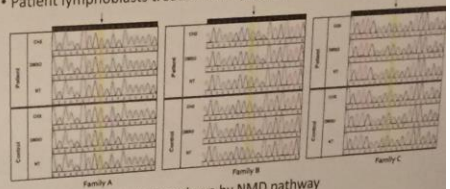
Family C

- Childhood-onset gait difficulties
- Inability to run and frequent falls
- Bilateral Achilles tendon shortening, distal leg hypotrophy
- Slow progression, walking at age 59 with cane
- ↓ LL and ↓ in UL

HAPLOINSUFFICIENCY MECHANISM

mRNA nonsense-mediated decay

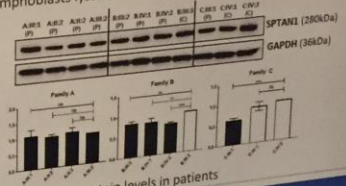
- Patient lymphoblasts treated with cycloheximide



→ Mutant transcripts broken down by NMD pathway

Assessment of protein levels

- Patient lymphoblasts lysates on western blot



→ Reduced αII-spectrin protein levels in patients

SPTAN1 IN dHMN VS EPILEPSY

***SPTAN1* causal gene for epilepsy**

- 22 mutations: 11 missense, 10 small duplication/deletions, 1 LoF
- Presumed dominant-negative mechanism

Staining of αII-spectrin and βII-spectrin

- Epilepsy patient fibroblasts showed spectrin aggregates
- Performed staining of dHMN patient fibroblasts

→ No αII-spectrin aggregates in dHMN patients as opposed to epilepsy patients in literature[1]

Future Perspectives

Frequency of *SPTAN1* mutations in dHMN

- Panel sequencing in a larger cohort of dHMN patients is planned

Mouse model of heterozygous knockout

- In literature: "Heterozygous animals (Spna2+/–) display no phenotype by 2 years of age"[2]
- Plans to assess possible mild neuromuscular phenotype

Contact: danique.beijer@uantwerpen.vib.be

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SPTAN1 nonsense mutation causes a spectrum of juvenile onset hereditary motor neuropathy
(Danique Beijer)

- NGS identified 3 different nonsense mutations in 3 separate families
- Childhood onset but slow progression
  Reflexes may be present
  Axonal motor neuropathy
- SPTAN1 encodes alpha-II spectrin : essential for AIS and nodes of Ranvier assembly in myelinated neurons and prevents axon degradation in larger diameter axons.
- Involved in epilepsy (dominant negative mutations)
- dHMN : haploinsufficiency mechanism