Missense variants in SPG4: small differences, big consequences

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Abstract *(in English, max 250 words)*

Background: SPG4 patients show marked phenotypic heterogeneity, with symptom onset ranging from early childhood to over 70 years. Missense variants are generally associated with earlier onset than truncating variants, despite a residual variability. We explored whether the nature of the missense variant may influence clinical expression in SPG4 patients. We notably focused on the main functional domain of Spastin (AAA) composed of three conserved key motifs, the Walker A, Walker B and Arginin finger - having key function in the regulation of the ATP hydrolysis activity.

Aim of the study: We aimed to decipher a genotype-phenotype correlation between patients carrying a variant in one of these motifs compared with other variants within the AAA domain.

Methods: Using a combination of biochemical analyses in patient cells, molecular predictions, and clinical data, we characterized ten missense variants observed in SPG4 patients and searched for genotype-phenotype correlations.

Results: Clinical data showed an early onset in patients carrying missense variants in Walker A, Walker B and Arginine finger motifs, often associated with intellectual deficiency or epilepsy, suggesting a phenotype with a developmental component. When quantifying the levels of Spastin we evidenced that most of them lead to protein expression in patients. Co-expression experiments of the wild-type form and *SPAST* variants suggest that the one linked with an early onset also present a dominant-negative effect.

Conclusion: We identified a subgroup of variants with a dominant negative effect, associated with early onset and cognitive impairment. This work highlights the importance of the research/personalized medicine tandem to enable a more accurate interpretation of the effects of genetic variants, with a direct clinical impact.

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Comprehensive and personalized gait training is feasible and effective in adults with hereditary spastic paraplegia: an interim analysis of the FRAME trial

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Abstract

Background: There is a paucity of interventional trials investigating neuromotor rehabilitation in patients with hereditary spastic paraplegia (HSP). Most studies consider monothematic therapeutic strategies, lacking personalization of treatment according to patient's needs.

Aim of the study: To investigate the feasibility and effectiveness of FRAME (Flexibility, Resistance, Aerobic, Motor Execution), a comprehensive training to improve gait competence in HSP patients.

Methods: Patients performed 10 to 16 sessions of training, 60' to 120' of duration for each session, once or twice weekly. Outcomes collected at baseline and at post-intervention were considered for the present interim analysis, including 6-minute walking test (endurance), 10-metre walking test (speed), SPRS (disease severity), HSP-SNAP (subjective impact of motor symptoms), Functional Reach Test (standing balance), 5-Times Sit-To-Stand (functional lower limb muscle strength), passive range of motion (flexibility), and isometric muscle strength (dynamometer).

Results: Five males and seven females (median age=53.5, IQR=26) completed the protocol. Diagnoses were SPG4 (4), SPG7 (4), SPG31 (2), and SPG72 (2)**.** The intervention was feasible in terms of safety, adherence (median=100%, IQR=9.38), and patients' satisfaction. Significant improvements were found for endurance (Z=-2.98, p=.003), disease severity (Z=2.80, p=.005), lower limb functional strength (Z=2.49, p=.01), and flexibility (Z=-2.04, p=.04). Among the eight outcomes considered, every patient improved in at least three outcomes and 75% of patients improved in at least six outcomes.

Conclusion: Comprehensive and personalized gait training is feasible and effective for adults with HSP. Results need to be confirmed by study completion and put in perspective with long-term maintenance of therapeutic gains.

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Title: Unravelling the Complexity of ATL1-Linked Hereditary Spastic Paraplegia

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Mutations in Atlastin-1 (ATL1) are a frequent cause of autosomal dominant Hereditary Spastic Paraplegia (HSP), yet patients display a wide range of clinical severity, from slowly progressive “Pure” HSP to severe, early-onset “Complicated” forms. The cellular mechanisms underlying these differences are not well defined. This study investigates the molecular basis of these clinically distinct forms of HSP using *Drosophila* models genetically engineered to express orthologous disease-causing ATL1 mutations.

Both Pure and Complicated *Drosophila* HSP models exhibit locomotor deficits, but the Complicated HSP model demonstrates significantly worse movement and survival outcomes, mirroring disease severity in patients with these ATL-1 mutations. Immunofluorescence of motor neurons and glia reveal divergent cellular pathologies: Pure HSP models show lipid droplet (LD) accumulation within axons, a phenotype alleviated by the lipid-regulating drug LXR-623. In contrast, the Complicated HSP model lacks LD accumulation. Significantly, LXR-623 treatment improves locomotor performance only in Pure HSP models, suggesting that lipid dysregulation is a key driver in Pure but not Complicated HSP. These results indicate that distinct Atlastin mutations differentially impact cellular function, with lipid homeostasis implicated in Pure HSP.

Overall, this study reveals mutation-specific pathological mechanisms in Atlastin-linked HSP and underscores the necessity of tailored therapeutic strategies that address the underlying molecular pathology in each disease subtype.

Clinical and molecular characterization of SPG7 patients carrying the p.Ala510Val (A510V) variant

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Abstract *(in English, max 250 words)*

Background: The p.Ala510Val (A510V) is the most frequent pathogenic variant identified in SPG7 patients.

Aim of the study: Exploring SPG7 patients carrying the p.Ala510Val (A510V) variant.

Methods: We analysed 95 patients from SPATAX/BIOMOV cohorts (Paris Brain Institute) with biallelic SPG7 variants including at least one A510V variant. Proteomics and functional studies were performed.

Results: We identified 29 homozygous A510V and 66 compound heterozygous patients, including 18 with a second missense variant and 48 with a loss-of-function (LoF) variant. Age at onset differed significantly across group (p=0.02), being earliest in A510V/missense patients (mean 34.5 years), intermediate in A510V/LoF patients (38 years), and latest in A510V homozygotes (42 years). Signs at onset were similar across groups with ataxia more frequent (82%) than spasticity (26%). Ataxia severity was moderate in all groups (mean SARA score 12/40). Pyramidal syndrome was common (>85%), with spasticity more frequent in the LoF group (74%), followed by the homozygous (52%) and missense (33%) groups. Cerebellar atrophy was systematic in A510V/missense patients and observed in 60–70% of the other groups. Functional studies in fibroblasts from one A510V homozygous and four A510V heterozygous patients showed no detectable alteration in mitochondrial respiratory chain levels, consistent with

preliminary quantitative proteomic analysis (12 controls, 6 homozygous A510V, 7 A510V compound heterozygous), revealing no genotype-dependent differences in protein expression.

Conclusion: Homozygous A510V patients and compound heterozygous SPG7 patients showed a consistent clinical spectrum, with earlier onset in A510V/missense carriers. Mitochondrial function appeared preserved, and preliminary proteomic data did not identify genotype-dependent molecular differences.

From Mutation to Degeneration: Generating CRISPR Models to examine Warburg Micro Syndrome Pathophysiology

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Warburg Micro Syndrome (WARBM) is a rare autosomal recessive genetic disorder predominantly characterised by ocular, neurodevelopmental and endocrine abnormalities. Through early childhood affected individuals experience progressive spasticity beginning in the lower limbs, which then often ascends to the upper limbs resulting in spastic quadriplegia around five years of age, reflecting a clinical overlap with hereditary spastic paraplegia (HSP). WARBM is genetically heterogeneous with pathogenic loss-of-function mutations in one of *RAB18*, *RAB3GAP1*, *RAB3GAP2* or *TBC1D20* genes known to be causative. Mutations in *RAB3GAP2* are associated with Autosomal Recessive Spastic Paraplegia Type 69, highlighting a genetic overlap with HSP.

The aim of this project is to generate *in vivo* models of WARBM and to identify the mechanisms by which neurons degenerate in WARBM. To date I have used CRISPR/Cas9 gene editing to introduce loss-of-function mutations into *Rab3GAP1* and *CG17883,* the *Drosophila melanogaster* (fly) homologs of *RAB3GAP1* and *TBC1D20*. I have validated that my models have disrupted Rab3GAP1 / CG17883 sequence by Sanger sequencing and gene expression by quantitative PCR. Examination of disease relevant behavioural phenotypes including development, locomotion and survival revealed a progressive locomotor and survival deficit in *Rab3GAP1* and *CG17883* mutant *Drosophila*. Ongoing work focuses on examining the cellular events underpinning neurodegeneration associated with Rab3GAP1 and CG17883 deficiency in *Drosophila* with a particular focus on lipid droplet homeostasis, autophagy and endoplasmic reticulum organisation.

Overall, these models will enable more precise study of the underlying pathogenic effects of WARBM-causing mutations *in vivo.*

Hereditary Spastic Paraplegia type 78: Deepening into Cell Mechanisms

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Abstract *(in English, max 250 words)*

Background: SPG78 is a form of hereditary spastic paraplegia (HSP) with biallelic loss-of-function variants in *ATP13A2* associated with a spectrum of neurological manifestations. Within the STOP-HSP.net[1] project, whole-exome sequencing identified a homozygous c.1144C>T/p.(Gln382*) in *ATP13A2* [NM_022089.4], associated with markedly divergent phenotypes in two brothers.

Aim of the study: To integrate clinical phenotyping with advanced neuroimaging and the identification of a potential cellular biomarker in patient-derived fibroblasts.

Methods: Disease-specific clinical scales were applied together with 7T brain MRI to assess *nigrosome* integrity, DaT-SCAN imaging, and BODIPY 493/503 staining in skin fibroblasts. Data from the two affected brothers were compared with cultured skin fibroblasts from two healthy controls and a CLN5 patient, as a model of lipid-associated neurodegeneration.

Results: The 30yo elder brother developed progressive spastic paraparesis since age 22 years and required a walking aid by age 29 (SPRS 22 at latest examination). The 20yo younger brother showed mild learning and attention difficulties and mild lower-limb spasticity (SPRS 5). Conventional 3T-brain-MRI was unremarkable, and motor evoked potentials were mildly abnormal in both patients. Cultured skin fibroblasts from the elder brother showed increased lipid droplet size, but not number, alike other lysosomal storage disorders (e.g., CLN5). The mildly affected brother showed no cellular alterations. Ultra-high-field 7T-MRI revealed bilateral *nigrosome* atrophy whereas DaT-SCAN confirmed bilateral dopaminergic degeneration consistent with *nigrosome* involvement in the elder brother.

Conclusion: This study identifies a novel cellular phenotype potentially correlating with clinical severity in SPG78. Brain 7T-MRI may represent a sensitive tool for detecting preclinical dopaminergic neurodegeneration.

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iAXON-Brazil-HSP Network: Building a Trial-Ready National Cohort for Hereditary Spastic Paraplegias in the Global South

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Background: although the therapeutic landscape for hereditary spastic paraplegia (HSP) has advanced in recent years, a key limitation remains the absence of validated surrogate biomarkers or clinical outcome assessments (COAs) that can serve as endpoints in clinical trials. To address this challenge, the Inherited AXONopathies Translational Research Network – Hereditary Spastic Paraplegias Brazil (iAXON-HSP Brazil) was created as a multicenter collaborative initiative to characterize the natural history of HSP and establish adequate endpoint measures. **Objectives:** to present the general organizational principles and initial achievements of the iAXON-HSP Brazil network. **Methods:** iAXON-HSP Brazil was established after strategic planning, leading to the development of standardized operating procedures (SOP) and training of participating researchers. Subjects are recruited across all participating centers and undergo standardized evaluations including COAs, biosample collection, neuroimaging, and gait analysis with digital health technologies. Biosamples are stored in the coordinating center's repository, and all data are centralized in a shared database. **Results:** iAXON-HSP Brazil currently includes 7 participating centers from 4 of the 5 regions of Brazil. The detailed SOPs, as well as the strategic planning and operational implementation of the network, are presented. Between the start of the network's activities in April 2024 and December 2025, 155 patients and 29 controls were enrolled. **Conclusion:** The initial results of iAXON-HSP Brazil demonstrate the network's potential to advance the development and validation of disease-modifying therapies for HSP. As a multicenter initiative based in the Global South, it highlights the feasibility and importance of generating high-quality translational research in underrepresented regions, providing a model for collaborative studies in other rare diseases.

Keywords: Hereditary Spastic Paraplegia; Translational Research Network; Clinical Outcome Assessments (COAs); Biomarkers; Global South

Title: Autosomal recessive spastic paraparesis due to homozygous mutations in *PNPT1* gene: the expansion of a phenotype.

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Abstract (in English, max 250 words)

Background: Hereditary Spastic Paraplegias and Ataxias (HSPs) represent significant groups of inherited neurological disorders that are associated with different clinical presentations, genetic inheritance patterns, and systemic and neurological complications.

Aim of the study: We present a case of a 64-years-old woman, with consanguineous parents, presented with slowly progressive infantile-onset spasticity in the lower limbs and cerebellar ataxia, associated with optic atrophy. Neuroimaging studies showed cerebellar atrophy and thinning of corpus callosum.

M**ethods:** Next-generation sequencing (NGS)-based multigene panel testing (including 300 genes related to inherited cerebellar ataxias, HSP, nuclear mitochondrial diseases, peroxisomal, and lysosomal storage diseases) was performed.

Results: Genetic testing disclosed the homozygous pathogenic variant c.866+4A>G in *PNPT1* gene, which potentially affects acceptor splice site, according to prediction bioinformatic tools (HSF, SpliceAI, SPiP). PNPT1 (polynucleotide phosphorylase) is a mitochondrial protein involved in RNA processing where it has a role in the import of small RNAs into mitochondria.

Conclusion: Biallelic variants are associated with Combined Oxidative Phosphorylation Deficiency Type 13 (MIM #614932), Autosomal Recessive Deafness Type 70 with or without adult-onset neurodegeneration (MIM #614934), Autosomal recessive nonsyndromic hearing impairment. Monoallelic variants in *PNPT1* gene are associated to SCA type 25 (OMIM# 608703).

Interpretation: We identified a novel homozygous variant in the *PNPT1* gene, which causes a mitochondrial disease of variable severity. Further studies on protein function and segregation of other affected and unaffected members of the family are ongoing to expand the clinical spectrum of *PNTP1*-related disorders.

References:

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An integrative approach for mapping molecular targets in Hereditary Spastic Paraplegia

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Abstract *(in English, max 250 words)*

Background:

Hereditary Spastic Paraplegia (HSP) comprises a clinically and genetically heterogeneous group of rare neurodegenerative disorders lacking shared molecular targets for diagnostic, prognostic, or therapeutic intervention.

Aim of the study:

We aim to define the mechanistic basis and identify convergent molecular targets in a subgroup of endolysosome- and autophagosome-associated HSP (SPG11, SPG15 and SPG48). Specifically, we investigate how loss of these related genes alter the cellular stress response and impacts regulation of the autophagosome-lysosome pathway.

Methods:

Using validated murine models, we performed transcriptomic profiling of immortalized embryonic fibroblasts (iMEFs) under basal and stress cell conditions as well as pre- and postsymptomatic brain tissue. Genotype-stress interactions and shared versus gene-specific transcriptional programs were defined by clustering, fold-change correlation, and pathway enrichment analyses.

Results:

Comparative transcriptomic analyses revealed distinct gene expression signatures across models; yet convergence at the level of affected subcellular pathways. Neurodegeneration-related gene sets (including Alzheimer's, Huntington's, Parkinson's, and ALS pathways) were significantly altered in both iMEF and CNS models, indicating shared molecular vulnerability networks. In contrast, MAPK signaling alterations were restricted to CNS tissue, suggesting tissue-specific regulatory mechanisms.

Conclusion:

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Our integrative approach identifies convergent molecular targets and pathway-level signatures, providing mechanistic insight into HSP pathogenesis and a framework for future diagnostic and therapeutic strategies.

Personalized ASO therapy for ALS associated with KIF5A mutations around exon 27: a multi-strategy approach

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Abstract *(in English, max 250 words)*

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that leads to paralysis and death within 3-5 years after diagnosis. For the majority of ALS-cases, no effective disease-modifying treatments exist. Several genes have been identified as causative, including KIF5A (kinesin heavy chain 5A). ALS-associated variants in KIF5A mostly cluster near exon 27. They cause frameshifts in exon 27/28 or skipping of exon 27, eventually leading to translation of an altered C-terminus with toxic gain-of-function.

Here, we aim to use splice-modulating antisense oligonucleotides (ASOs) to restore wild-type splicing or alternatively prevent the translation of the altered C-terminus. To achieve this goal, we designed four exon 28 skipping ASOs that truncate the C-terminus. We transfected four candidate ASOs into undifferentiated wild-type SH-SY5Y cells, leading to approximately 60 percent of exon 28 skipping of the most efficient ASO on mRNA level. In an independent approach, we sought to identify splice regulatory elements around exon 27 that might prevent mutation-induced exon 27 skipping and thus restore expression of wild-type protein. Detailed analysis of the locus using a series of minigene constructs led to the identification of regulatory elements promoting exon 27 retention. These will be targeted by splice-modulating ASOs, and the efficacy of the ASOs will be assessed in induced cortical neurons (iCNs) carrying different human pathogenic variants leading to exon 27 skipping. Additionally, the functional consequences of exon 28 skipping will be investigated in iCNs.

By advancing personalized ASO strategies against KIF5A mutations, this work aims to lay the foundation for future therapeutic approaches

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Assessing knockdown oligonucleotides in *Drosophila* as a therapeutic strategy for the treatment of severe ATL1-HSP

Lawless K, O'Sullivan NC

Atlastin-1 associated HSP is the second most common form of autosomal dominant HSP and is caused by mutations in the Atlastin-1 gene, a membrane-bound GTPase involved in maintaining the morphology of the endoplasmic reticulum. Recently, heterozygous *de novo* mutations in Atlastin-1 have been identified that result in a more severe disease presentation than is classically associated with ATL-1 associated HSP (Alecu *et al.,* 2023). My project aims to assess the viability of knockdown oligonucleotides targeting the disease-causing ATL-1 allele as a therapeutic strategy, using *Drosophila* models of disease.

To identify candidate allele-specific siRNAs, I have carried out an *in vitro* genetic screen comprising a dual luciferase assay of *Drosophila* S2 cells co-transfected with a luciferase reporter plasmid and an siRNA targeting the $Atl^{K382del}$ mutant allele. I have generated a psiCHECK2 luciferase reporter plasmid containing a mutant Atlastin fragment cloned into the 3'UTR of the Firefly luciferase reporter gene and a wild-type Atlastin fragment cloned into the 3'UTR of the *Renilla* luciferase reporter gene. I have validated expression of both of my engineered psiCHECK2 plasmids and used my dual luciferase assay to identify five promising siRNA sequences designed to specifically target the $Atl^{K382del}$ mutation.

I have begun assessing the effect of these candidate siRNAs on protein levels using Western blots. Plasmids were designed to contain either the tagged full-length wild-type or mutant Atlastin gene. A FLAG tag is common to both, while the wild-type plasmid specifically has a HA tag and the mutant plasmid has a 6xHis tag. I have confirmed that the S2 cells express the engineered plasmids well without significant toxicity and identified α-tubulin as a suitable loading control to permit quantification and validation of knockdown by candidate siRNAs identified in my *in vitro* genetic screen.

Loss of HPDL drives bioenergetic failure and CoQ10 deficit: clinical and molecular characterization enhanced by a multi-omic studies on SPG83 pathogenesis

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Abstract

Background: HPDL is a recently identified enzyme that converts 4-hydroxymandelate (4-HMA) into 4-hydroxybenzoate (4-HB), a crucial precursor for the CoQ10 headgroup. Bi-allelic *HPDL* variants cause a broad neurodevelopmental spectrum—from early-onset encephalopathy to adolescent-onset SPG83—but the underlying mechanisms remain largely unexplored.

Aim of the study: This study aims to characterize new HPDL cases and investigate its biological role using *in vitro* and *in vivo* models. Furthermore, we sought to identify novel proteomic and lipidic biomarkers through integrated omic profiling to define the disease signature.

Methods: Exome sequencing was performed on cohorts of congenital HSP and diplegic cerebral palsy. We conducted functional studies in patient-derived fibroblasts and *hpdl*$^{-/-}$ zebrafish larvae using qPCR, western blot, micro-oxygraphy, and behavioral locomotion studies. Integrated proteomic and lipidomic analyses were employed to identify metabolic disruptions.

Results: Patient fibroblasts showed reduced HPDL expression, impaired respiration, and increased reactive oxygen species. Clinical samples revealed elevated plasma GFAP and decreased 4-hydroxybenzeneacetic acid. In *hpdl*$^{-/-}$ zebrafish, integrated proteomic and lipidomic data confirmed severe mitochondrial dysfunction and significant bioenergetic impairment, validated by a marked decrease in CoQ10 levels. These molecular signatures correlated with neurodevelopmental abnormalities and epilepsy-like behavior in the larvae. Notably, bypass therapy with 4-HMA, 4-HB, and MitoTempo partially rescued the zebrafish phenotype.

Conclusion: We expanded the clinical landscape of SPG83, demonstrating that HPDL deficiency impairs oxidative metabolism across species. Our findings highlight that *hpdl*$^{-/-}$ zebrafish proteo-lipidomic

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signatures provide essential biomarkers for diagnosis and therapeutic monitoring. This model offers a robust platform for screening potential bypass therapies to target the identified bioenergetic failure.

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High-throughput phenotyping of fibroblast-derived induced neurons (FiNs) in SPG4-HSP

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Abstract *(in English, max 250 words)*

Background:

Hereditary spastic paraplegia type 4 (SPG4), caused by mutations in the *SPAST* gene, is characterized by progressive neurodegeneration, particularly affecting corticospinal motor neurons. The disease typically presents in early adulthood with spasticity and weakness of the lower limbs, progressing over time.

Aim of the study:

Here, we aim to identify phenotypic abnormalities in a fibroblast-derived induced neuron (FiN) model of SPG4 that could serve as measurable readouts for future drug testing. This approach is particularly compelling as age- and disease-related epigenetic signatures of the donor fibroblasts are preserved in FiNs.

Methods:

We established a direct transdifferentiation protocol to generate FiNs from SPG4 patient and age- and sex-matched control fibroblasts. This method bypasses the induced pluripotent stem cell (iPSC) stage to retain the epigenetic and age-related characteristics of the donor cells.

Results:

Using imaging-based high-throughput phenotypic screens, we identified significant deficits in SPG4 FiNs, including reduced neurite lengths and elevated neurite disintegration indices compared to controls. These findings indicate neurite damage in SPG4 FiNs.

Conclusion:

This platform establishes a robust disease model to investigate SPG4 pathophysiology and provides a basis for high-throughput drug screening to identify compounds that restore neurite integrity and length in SPG4-affected neurons.

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CSF1R-related leukodystrophy: development of an HEK293-based model for a screening platform.

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Abstract:

CSF1R-related leukodystrophy is a devastating neurodegenerative disease characterized by rapidly progressive cognitive and motor decline, leading to death within a few years after onset. The disease-causing gene *CSF1R* encodes a transmembrane tyrosine kinase receptor activated by CSF1 or IL-34, leading to autophosphorylation within CSF1R and multiple downstream signaling activations. *CSF1R* is crucial for the maturation and survival of microglia, which play an important role in maintaining brain homeostasis. Patients with mutations in *CSF1R* exhibit reduced amounts of microglia in the brain, which shows the importance of microglia in this disease. Over 170 pathogenic mutations have been identified within the *CSF1R* gene, yet the mutational mechanism remains unclear. Here, using cell and molecular biology methods, we present the process of developing HEK293-based models of mutations found in *CSF1R* as a platform for fast recognition of the molecular outcomes of those mutations and testing promising therapies, such as Antisense Oligonucleotides (ASO). Several cell lines stably expressing CSF1R were established using Sleeping Beauty. Cells were then analysed on mRNA and protein levels for the presence of CSF1R. Then, using Western blot, the activity of downstream signaling kinases such as JNK was assessed. Our preliminary results suggest that the *CSF1R* (c.1859-119G>A) variant has a reduced ability to activate JNK, which is important for cell survival and proliferation, but further analysis and optimization of the cell lines establishment are needed.

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Preliminary videoculographic (VOG) data in patients with genetically confirmed pure versus complicated HSP

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Background: Hereditary spastic paraplegias (HSPs) are a group of genetically heterogenous neurodegenerative disorders characterized by progressive lower limb spasticity and weakness. While lower limb motor dysfunction is the hallmark of HSP, other symptoms such as oculomotor abnormalities remain understudied and undercharacterizied.

Aim of the study: To evaluate the frequency and types of oculomotor abnormalities in patients with HSP and to compare their occurrence between patients with pure (pHSP) and complex forms (cHSP).

Methods: In a cohort of 20 patients with genetically confirmed HSP divided into the pHSP and cHSP group VOG examinations were performed using the EyeSeeCam system. The eye movements were evaluated for the presence of spontaneous and gaze-evoked nystagmus, optokinetic nystagmus, quality of smooth pursuit eye movements, horizontal and vertical saccades.

Results: Oculomotor abnormalities were observed in both pHSP and cHSP. Spontaneous nystagmus was present in 33% of pHSP and 45% of cHSP patients, while gaze-evoked nystagmus occurred in 11% and 27%. Horizontal smooth pursuit abnormalities were noted in 89% of pHSP and 67% of cHSP patients, and vertical smooth pursuit abnormalities in 67% of both groups. Optokinetic nystagmus was not elicited in 11% of pHSP compared with 56% of cHSP patients. Prevalence of oculomotor abnormalities did not differ between pHSP and cHSP.

Conclusion: Oculomotor abnormalities are present in both pHSP and cHSP suggesting that oculomotor involvement might be a general feature of HSP, not limited to complex forms.

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Title: **Assessing therapeutic strategies to modify neurotoxicity caused by complex HSP SPG3A mutants**

List of authors :O'Leary AC, O'Sullivan NC

Complex Hereditary Spastic Paraplegia (HSP) SPG3A is associated with autosomal dominant mutations in the Atlastin-1 (*ATL1*) gene, such as $ATL1^{K407del}$ and $ATL1^{M408T}$, resulting in a severe neurodegenerative disease affecting young children. Current treatments for HSP try to manage the physical symptoms only but fail to slow or reduce the disease progression. My project aims identify treatments that can modify neurotoxicity caused by severe-disease causing mutations in *ATL1* using biological and small molecule approaches.

In the first approach, I want to identify whether anti-sense oligonucleotides (ASO) designed to target allele-specific gene silencing of disease-causing SPG3A mutations, offer potential as a novel therapeutic strategy. I have developed a dual luciferase assay to screen ASOs. Briefly, I generated plasmids expressing fusions of *Renilla* – $ATL1^{WT}$ sequence and *Firefly-*$ATL1^{Mut}$ in human U2-OS cells. Using these, I was able to identify ASO sequences that reduced A$TL1^{Mut}$ mRNA expression without disrupting $ATL1^{WT}$ expression. Next, I generated plasmids expressing full length ATL1 ($ATL1^{WT}$ and $ATL1^{Mut}$) tagged with FLAG. Western blot analysis confirmed that my top candidate ASO significantly reduces $ATL1^{Mut}$ but not $ATL1^{WT}$ expression. Future work will investigate whether treatment with my $ATL1^{Mut}$ ASO modifies disease-relevant phenotypes in a neuronal iPSC model of disease.

My second approach uses an *in vivo* model, *Drosophila melanogaster,* to conduct a small molecule screen of approved drugs that have shown benefits in models of different neurodegenerative diseases. The screen uses Atlastin mutant *Drosophila*, that have previously been shown to have locomotor and survival deficits, which are chronically treated with a range of drugs are administered via food. To date, the majority of drugs screened have not shown lessen disease-relevant phenotypes. However, treatment with a mitochondrial modulating drug has resulted in a small but significant improvement of locomotion ability in two independent experiments. Further work is in progress to validate this and to investigate the molecular mechanisms by which this drug might be mediating its effects.

Axonal endoplasmic reticulum architecture and its links to axon function and degeneration

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Abstract *(in English, max 250 words)*

Background: Axon ER comprises a tubular network, shaped by membrane proteins including REEPs and reticulons, mutations in which cause HSP. It is continuous over large distances, with unusually narrow tubules.

Aim of the study: How does ER architecture influence axon function and dysfunction?

Methods: Live microscopy, calcium imaging, electron microscopy of *Drosophila* axons and synapses.

Results:

ER levels control STIM signaling and presynaptic Ca^{2+} signaling. *Drosophila* presynaptic motor ER comprises cisternae connected by tubules. Loss of reticulon Rtnl1 (SPG12) reduces tubular ER, but not cisternae, lumen volume or calcium stores; evoked calcium fluxes between compartments, and synaptic strength are generally lowered, as is the ER Ca^{2+} store sensor, STIM. STIM overexpression rescues the presynaptic Ca^{2+} phenotype.

ER tubule diameter constrains molecular movement. After photobleaching luminal GFP, wider mutant ER tubules recover faster than narrow wildtype ones, implying that narrow tubules limit luminal diffusion. We suggest that narrow axonal ER tubules constrain spatial dynamics of presynaptic Ca^{2+} signaling and plasticity.

Does ER continuity explain differential HSP sensitivity? Some *Drosophila* HSP mutants show sporadic gaps in axonal ER, and this could be a plausible hypothesis for HSP length dependence. This predicts that narrower axons, with less ER, would be more susceptible than wider ones. We explore these predictions in non-human primates.

Conclusion: Genetically manipulating ER architecture in *Drosophila* can test its links with normal and impaired axon/presynaptic function. Our data suggest synaptic strength as a HSP symptom, and STIM signaling as a candidate pathway for therapy.

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Very late-onset Krabbe disease with concomitant dementia: case description and a critical review of the literature.

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Abstract

Background: Krabbe disease (KD) is a rare autosomal recessive lysosomal storage disorder caused by pathogenic variants in *GALC*. Despite accounting only for 5% of forms, reports of adult-onset KD cases are increasingly recognized.

Aim of the study: To describe a patient manifesting KD after the age of 60 years, and to review the scientific literature of KD case with onset > 10 years to refine the spectrum of later-onset KD manifestations.

Methods: A female patient manifesting with pure spastic paraplegia with onset after 60 years developed dementia a few years later. Lumbar puncture degeneration biomarkers and neuropsychological assessment supported diagnosis of concomitant Alzheimer's disease. A Next-generation sequencing dementia panel documented the *GALC* c.857G>A homozygous variant. KD diagnosis was confirmed by reduced GALC activity in leukocytes.

Results: Including ours, we identified 84 KD adolescent/adult-onset patients (mean age at onset 28.7±14.2 years). Most patients had limb spasticity as main characterizing neurological feature (58/84, 70.2%), followed by polyneuropathy (11/ 84, 13.1%), both upper and lower motor neuron signs (2/84, 2.4%), and epilepsy (2/84, 2.4%). Five out of 84 patients (6.0%) were asymptomatic. Most patients had cortico-spinal tracts involvement at brain MRI. The most common pathogenic *GALC* variants were the c.1901T>C, the c.857G>A, and the c.1161+6532_polyA+9kbdel.

Conclusion: Complicated spastic paraplegia is the most common manifestation in later-onset KD, rarely with normal brain MRI. KD should be always considered also in cases with very late-onset spastic paraplegia.

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Spastin level regulation and biomarker development in SPG4-HSP

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Abstract

Background: Hereditary Spastic Paraplegia (HSP) is a motor neuron disease with no effective treatments. The most common cause is heterozygous loss-of-function mutations in the SPG4 gene, which encodes spastin, a microtubule-severing ATPase. Increasing spastin levels has emerged as a potential therapeutic strategy, particularly for truncating mutations. Reliable biomarkers are needed to monitor disease progression and evaluate new therapies. Recently, a cell imaging–based method was validated to quantify microtubule organization defects in patient-derived cells, showing potential as a biomarker in SPG4-HSP.

Aim of the study: To identify and manipulate the pathways controlling spastin stability for restoring functional protein levels in SPG4-HSP, including missense mutations. In parallel, to test the possibility of integrating cell imaging data with other biomarkers (molecular or fluid) to identify a disease signature.

Methods: We are dissecting factors that mediate selective spastin degradation and testing allele-specific silencing to normalize spastin dosage in cells carrying missense mutations. We are also analysing cells derived from the blood of SPG4 patients using a cell imaging-based method, integrated with spastin protein quantification and plasma neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP).

Results: We identified Ambra1 as a pro-degradation regulator of spastin; its targeting increases spastin levels. Allele-specific silencing approaches are under evaluation in missense contexts. Ongoing analyses integrate cell-imaging readouts with molecular biomarkers.

Conclusion: Modulating spastin degradation and carrying out allele-specific interventions are promising therapeutic strategies for SPG4-HSP. When integrated with complementary biomarkers, cell imaging–based method represents a promising tool for prognostic and predictive evaluation in SPG4-HSP, potentially guiding personalised therapeutic strategies and future clinical trials.

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Assessment of Autonomic Nervous System Involvement in Hereditary Spastic Paraplegias Using SUDOSCAN

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Abstract

Background: Hereditary spastic paraplegias (HSPs) are a heterogeneous group of disorders characterized by progressive degeneration of the corticospinal tract. Involvement of the autonomic nervous system (ANS) has been poorly investigated in HSPs.

Aim of the study: To assess autonomic nervous system involvement in patients with HSP using a non-invasive, multimodal approach in the frame of the STOP-HSP.net registry.

Methods: Thirty patients with genetically heterogeneous forms of HSP were evaluated using clinical disease severity scales (SPRS, SARA, FARS-ADL), plasma fluid biomarkers (NfL, GFAP, BD-Tau), and patient-reported autonomic symptoms assess

ed with the Composite Autonomic Symptom Score 31 (COMPASS-31). Patients with HSP were evaluated every six months using SUDOSCAN, a portable, non-invasive device suited for outpatient settings.

Results: The study cohort included 14 males and 16 females with a marked genetic heterogeneity, with SPG4 as the most frequent genotype. Sudomotor dysfunction, assessed by SUDOSCAN, was detected in 8 patients (27%), with a predominant involvement of the lower limbs. ANS involvement appeared to be correlated with selected disease-related parameters, including disease duration and genotype. According to COMPASS-31 data, gastrointestinal, bladder, and orthostatic domains were the most frequently involved.

Conclusion: SUDOSCAN is a feasible, non-invasive tool to screen for longitudinal monitoring of autonomic dysfunction in HSP and other motor-neuronopathies

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Refinement of the natural history, clinical and molecular spectrum of SPG11 and SPG15

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Abstract *(in English, max 250 words)*

Background: Despite being one of the most frequent forms of recessive HSP, very little is known about the natural history and clinical spectrum of SPG11 and SPG15, hindering potential clinical trials.

Aim of the study: We gathered a large, international cohort of SPG11 and SPG15 individuals to describe their natural history.

Methods: Calls for collaboration were launched to gather individuals diagnosed with SPG11 or SPG15. Age at onset, disability and clinical scales, neuropsychological evaluations, radiological and molecular data were collected in a standardized online Redcap form. Biosamples were available for 135 individuals, including plasma for 25.

Results: A total of 157 individuals were included (SPG11 n= 134, SPG15 n= 23). Mean age at onset of first motor symptoms was 16,2 years in SPG11 and 13,04 years in SPG15. Loss of running ability occurred at a mean age of 22 years in both diseases, and wheelchair dependence at 29 in SPG11 and 27 in SPG15. Intellectual development was delayed for 44% of SPG11 and 61% of SPG15 individuals. When available, brain MRI was abnormal in 90% and 82% of individuals, respectively, with corpus callosum atrophy being the most frequent feature (81% in SPG11 and 55% in SPG15). Genotype-phenotype correlations showed a later onset in SPG11 individuals with homozygous splice variants or compound heterozygous splice/loss-of-function variants compared with homozygous loss-of-function variants.

Conclusion: We describe the largest reported cohort of individuals with SPG11 and SPG15, providing relevant data on the natural history and clinical spectrum of both diseases.

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Missense variants in SPG4: small differences, big consequences

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Abstract *(in English, max 250 words)*

Background: SPG4 patients show marked phenotypic heterogeneity, with symptom onset ranging from early childhood to over 70 years. Missense variants are generally associated with earlier onset than truncating variants, despite a residual variability. We explored whether the nature of the missense variant may influence clinical expression in SPG4 patients. We notably focused on the main functional domain of Spastin (AAA) composed of three conserved key motifs, the Walker A, Walker B and Arginin finger - having key function in the regulation of the ATP hydrolysis activity.

Aim of the study: We aimed to decipher a genotype-phenotype correlation between patients carrying a variant in one of these motifs compared with other variants within the AAA domain.

Methods: Using a combination of biochemical analyses in patient cells, molecular predictions, and clinical data, we characterized ten missense variants observed in SPG4 patients and searched for genotype-phenotype correlations.

Results: Clinical data showed an early onset in patients carrying missense variants in Walker A, Walker B and Arginine finger motifs, often associated with intellectual deficiency or epilepsy, suggesting a phenotype with a developmental component. When quantifying the levels of Spastin we evidenced that most of them lead to protein expression in patients. Co-expression experiments of the wild-type form and *SPAST* variants suggest that the one linked with an early onset also present a dominant-negative effect.

Conclusion: We identified a subgroup of variants with a dominant negative effect, associated with early onset and cognitive impairment. This work highlights the importance of the research/personalized medicine tandem to enable a more accurate interpretation of the effects of genetic variants, with a direct clinical impact.

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Comprehensive and personalized gait training is feasible and effective in adults with hereditary spastic paraplegia: an interim analysis of the FRAME trial

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Abstract

Background: There is a paucity of interventional trials investigating neuromotor rehabilitation in patients with hereditary spastic paraplegia (HSP). Most studies consider monothematic therapeutic strategies, lacking personalization of treatment according to patient's needs.

Aim of the study: To investigate the feasibility and effectiveness of FRAME (Flexibility, Resistance, Aerobic, Motor Execution), a comprehensive training to improve gait competence in HSP patients.

Methods: Patients performed 10 to 16 sessions of training, 60' to 120' of duration for each session, once or twice weekly. Outcomes collected at baseline and at post-intervention were considered for the present interim analysis, including 6-minute walking test (endurance), 10-metre walking test (speed), SPRS (disease severity), HSP-SNAP (subjective impact of motor symptoms), Functional Reach Test (standing balance), 5-Times Sit-To-Stand (functional lower limb muscle strength), passive range of motion (flexibility), and isometric muscle strength (dynamometer).

Results: Five males and seven females (median age=53.5, IQR=26) completed the protocol. Diagnoses were SPG4 (4), SPG7 (4), SPG31 (2), and SPG72 (2)**.** The intervention was feasible in terms of safety, adherence (median=100%, IQR=9.38), and patients' satisfaction. Significant improvements were found for endurance (Z=-2.98, p=.003), disease severity (Z=2.80, p=.005), lower limb functional strength (Z=2.49, p=.01), and flexibility (Z=-2.04, p=.04). Among the eight outcomes considered, every patient improved in at least three outcomes and 75% of patients improved in at least six outcomes.

Conclusion: Comprehensive and personalized gait training is feasible and effective for adults with HSP. Results need to be confirmed by study completion and put in perspective with long-term maintenance of therapeutic gains.

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Abstract TWS conference

Title: Unravelling the Complexity of ATL1-Linked Hereditary Spastic Paraplegia

Authors: Emma Cadoria, Associate Prof. Niamh O'Sullivan

Affiliation: UCD School of Biomolecular and Biomedical Sciences, UCD Conway Institute,

University College Dublin, Dublin 4, Ireland.

Mutations in Atlastin-1 (ATL1) are a frequent cause of autosomal dominant Hereditary Spastic Paraplegia (HSP), yet patients display a wide range of clinical severity, from slowly progressive “Pure” HSP to severe, early-onset “Complicated” forms. The cellular mechanisms underlying these differences are not well defined. This study investigates the molecular basis of these clinically distinct forms of HSP using *Drosophila* models genetically engineered to express orthologous disease-causing ATL1 mutations.

Both Pure and Complicated *Drosophila* HSP models exhibit locomotor deficits, but the Complicated HSP model demonstrates significantly worse movement and survival outcomes, mirroring disease severity in patients with these ATL-1 mutations. Immunofluorescence of motor neurons and glia reveal divergent cellular pathologies: Pure HSP models show lipid droplet (LD) accumulation within axons, a phenotype alleviated by the lipid-regulating drug LXR-623. In contrast, the Complicated HSP model lacks LD accumulation. Significantly, LXR-623 treatment improves locomotor performance only in Pure HSP models, suggesting that lipid dysregulation is a key driver in Pure but not Complicated HSP. These results indicate that distinct Atlastin mutations differentially impact cellular function, with lipid homeostasis implicated in Pure HSP.

Overall, this study reveals mutation-specific pathological mechanisms in Atlastin-linked HSP and underscores the necessity of tailored therapeutic strategies that address the underlying molecular pathology in each disease subtype.

Clinical and molecular characterization of SPG7 patients carrying the p.Ala510Val (A510V) variant

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*Oral/poster presenter

Abstract *(in English, max 250 words)*

Background: The p.Ala510Val (A510V) is the most frequent pathogenic variant identified in SPG7 patients.

Aim of the study: Exploring SPG7 patients carrying the p.Ala510Val (A510V) variant.

Methods: We analysed 95 patients from SPATAX/BIOMOV cohorts (Paris Brain Institute) with biallelic SPG7 variants including at least one A510V variant. Proteomics and functional studies were performed.

Results: We identified 29 homozygous A510V and 66 compound heterozygous patients, including 18 with a second missense variant and 48 with a loss-of-function (LoF) variant. Age at onset differed significantly across group (p=0.02), being earliest in A510V/missense patients (mean 34.5 years), intermediate in A510V/LoF patients (38 years), and latest in A510V homozygotes (42 years). Signs at onset were similar across groups with ataxia more frequent (82%) than spasticity (26%). Ataxia severity was moderate in all groups (mean SARA score 12/40). Pyramidal syndrome was common (>85%), with spasticity more frequent in the LoF group (74%), followed by the homozygous (52%) and missense (33%) groups. Cerebellar atrophy was systematic in A510V/missense patients and observed in 60–70% of the other groups. Functional studies in fibroblasts from one A510V homozygous and four A510V heterozygous patients showed no detectable alteration in mitochondrial respiratory chain levels, consistent with

preliminary quantitative proteomic analysis (12 controls, 6 homozygous A510V, 7 A510V compound heterozygous), revealing no genotype-dependent differences in protein expression.

Conclusion: Homozygous A510V patients and compound heterozygous SPG7 patients showed a consistent clinical spectrum, with earlier onset in A510V/missense carriers. Mitochondrial function appeared preserved, and preliminary proteomic data did not identify genotype-dependent molecular differences.

From Mutation to Degeneration: Generating CRISPR Models to examine Warburg Micro Syndrome Pathophysiology

Authors: Ailbhe Conway, Niamh O'Sullivan

Affiliations: University College Dublin School of Biomolecular and Biomedical Science, Conway Institute University College Dublin

Warburg Micro Syndrome (WARBM) is a rare autosomal recessive genetic disorder predominantly characterised by ocular, neurodevelopmental and endocrine abnormalities. Through early childhood affected individuals experience progressive spasticity beginning in the lower limbs, which then often ascends to the upper limbs resulting in spastic quadriplegia around five years of age, reflecting a clinical overlap with hereditary spastic paraplegia (HSP). WARBM is genetically heterogeneous with pathogenic loss-of-function mutations in one of *RAB18*, *RAB3GAP1*, *RAB3GAP2* or *TBC1D20* genes known to be causative. Mutations in *RAB3GAP2* are associated with Autosomal Recessive Spastic Paraplegia Type 69, highlighting a genetic overlap with HSP.

The aim of this project is to generate *in vivo* models of WARBM and to identify the mechanisms by which neurons degenerate in WARBM. To date I have used CRISPR/Cas9 gene editing to introduce loss-of-function mutations into *Rab3GAP1* and *CG17883,* the *Drosophila melanogaster* (fly) homologs of *RAB3GAP1* and *TBC1D20*. I have validated that my models have disrupted Rab3GAP1 / CG17883 sequence by Sanger sequencing and gene expression by quantitative PCR. Examination of disease relevant behavioural phenotypes including development, locomotion and survival revealed a progressive locomotor and survival deficit in *Rab3GAP1* and *CG17883* mutant *Drosophila*. Ongoing work focuses on examining the cellular events underpinning neurodegeneration associated with Rab3GAP1 and CG17883 deficiency in *Drosophila* with a particular focus on lipid droplet homeostasis, autophagy and endoplasmic reticulum organisation.

Overall, these models will enable more precise study of the underlying pathogenic effects of WARBM-causing mutations *in vivo.*

Hereditary Spastic Paraplegia type 78: Deepening into Cell Mechanisms

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Abstract *(in English, max 250 words)*

Background: SPG78 is a form of hereditary spastic paraplegia (HSP) with biallelic loss-of-function variants in *ATP13A2* associated with a spectrum of neurological manifestations. Within the STOP-HSP.net[1] project, whole-exome sequencing identified a homozygous c.1144C>T/p.(Gln382*) in *ATP13A2* [NM_022089.4], associated with markedly divergent phenotypes in two brothers.

Aim of the study: To integrate clinical phenotyping with advanced neuroimaging and the identification of a potential cellular biomarker in patient-derived fibroblasts.

Methods: Disease-specific clinical scales were applied together with 7T brain MRI to assess *nigrosome* integrity, DaT-SCAN imaging, and BODIPY 493/503 staining in skin fibroblasts. Data from the two affected brothers were compared with cultured skin fibroblasts from two healthy controls and a CLN5 patient, as a model of lipid-associated neurodegeneration.

Results: The 30yo elder brother developed progressive spastic paraparesis since age 22 years and required a walking aid by age 29 (SPRS 22 at latest examination). The 20yo younger brother showed mild learning and attention difficulties and mild lower-limb spasticity (SPRS 5). Conventional 3T-brain-MRI was unremarkable, and motor evoked potentials were mildly abnormal in both patients. Cultured skin fibroblasts from the elder brother showed increased lipid droplet size, but not number, alike other lysosomal storage disorders (e.g., CLN5). The mildly affected brother showed no cellular alterations. Ultra-high-field 7T-MRI revealed bilateral *nigrosome* atrophy whereas DaT-SCAN confirmed bilateral dopaminergic degeneration consistent with *nigrosome* involvement in the elder brother.

Conclusion: This study identifies a novel cellular phenotype potentially correlating with clinical severity in SPG78. Brain 7T-MRI may represent a sensitive tool for detecting preclinical dopaminergic neurodegeneration.

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iAXON-Brazil-HSP Network: Building a Trial-Ready National Cohort for Hereditary Spastic Paraplegias in the Global South

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Background: although the therapeutic landscape for hereditary spastic paraplegia (HSP) has advanced in recent years, a key limitation remains the absence of validated surrogate biomarkers or clinical outcome assessments (COAs) that can serve as endpoints in clinical trials. To address this challenge, the Inherited AXONopathies Translational Research Network – Hereditary Spastic Paraplegias Brazil (iAXON-HSP Brazil) was created as a multicenter collaborative initiative to characterize the natural history of HSP and establish adequate endpoint measures. **Objectives:** to present the general organizational principles and initial achievements of the iAXON-HSP Brazil network. **Methods:** iAXON-HSP Brazil was established after strategic planning, leading to the development of standardized operating procedures (SOP) and training of participating researchers. Subjects are recruited across all participating centers and undergo standardized evaluations including COAs, biosample collection, neuroimaging, and gait analysis with digital health technologies. Biosamples are stored in the coordinating center's repository, and all data are centralized in a shared database. **Results:** iAXON-HSP Brazil currently includes 7 participating centers from 4 of the 5 regions of Brazil. The detailed SOPs, as well as the strategic planning and operational implementation of the network, are presented. Between the start of the network's activities in April 2024 and December 2025, 155 patients and 29 controls were enrolled. **Conclusion:** The initial results of iAXON-HSP Brazil demonstrate the network's potential to advance the development and validation of disease-modifying therapies for HSP. As a multicenter initiative based in the Global South, it highlights the feasibility and importance of generating high-quality translational research in underrepresented regions, providing a model for collaborative studies in other rare diseases.

Keywords: Hereditary Spastic Paraplegia; Translational Research Network; Clinical Outcome Assessments (COAs); Biomarkers; Global South

Title: Autosomal recessive spastic paraparesis due to homozygous mutations in *PNPT1* gene: the expansion of a phenotype.

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Abstract (in English, max 250 words)

Background: Hereditary Spastic Paraplegias and Ataxias (HSPs) represent significant groups of inherited neurological disorders that are associated with different clinical presentations, genetic inheritance patterns, and systemic and neurological complications.

Aim of the study: We present a case of a 64-years-old woman, with consanguineous parents, presented with slowly progressive infantile-onset spasticity in the lower limbs and cerebellar ataxia, associated with optic atrophy. Neuroimaging studies showed cerebellar atrophy and thinning of corpus callosum.

M**ethods:** Next-generation sequencing (NGS)-based multigene panel testing (including 300 genes related to inherited cerebellar ataxias, HSP, nuclear mitochondrial diseases, peroxisomal, and lysosomal storage diseases) was performed.

Results: Genetic testing disclosed the homozygous pathogenic variant c.866+4A>G in *PNPT1* gene, which potentially affects acceptor splice site, according to prediction bioinformatic tools (HSF, SpliceAI, SPiP). PNPT1 (polynucleotide phosphorylase) is a mitochondrial protein involved in RNA processing where it has a role in the import of small RNAs into mitochondria.

Conclusion: Biallelic variants are associated with Combined Oxidative Phosphorylation Deficiency Type 13 (MIM #614932), Autosomal Recessive Deafness Type 70 with or without adult-onset neurodegeneration (MIM #614934), Autosomal recessive nonsyndromic hearing impairment. Monoallelic variants in *PNPT1* gene are associated to SCA type 25 (OMIM# 608703).

Interpretation: We identified a novel homozygous variant in the *PNPT1* gene, which causes a mitochondrial disease of variable severity. Further studies on protein function and segregation of other affected and unaffected members of the family are ongoing to expand the clinical spectrum of *PNTP1*-related disorders.

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An integrative approach for mapping molecular targets in Hereditary Spastic Paraplegia

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Abstract *(in English, max 250 words)*

Background:

Hereditary Spastic Paraplegia (HSP) comprises a clinically and genetically heterogeneous group of rare neurodegenerative disorders lacking shared molecular targets for diagnostic, prognostic, or therapeutic intervention.

Aim of the study:

We aim to define the mechanistic basis and identify convergent molecular targets in a subgroup of endolysosome- and autophagosome-associated HSP (SPG11, SPG15 and SPG48). Specifically, we investigate how loss of these related genes alter the cellular stress response and impacts regulation of the autophagosome-lysosome pathway.

Methods:

Using validated murine models, we performed transcriptomic profiling of immortalized embryonic fibroblasts (iMEFs) under basal and stress cell conditions as well as pre- and postsymptomatic brain tissue. Genotype-stress interactions and shared versus gene-specific transcriptional programs were defined by clustering, fold-change correlation, and pathway enrichment analyses.

Results:

Comparative transcriptomic analyses revealed distinct gene expression signatures across models; yet convergence at the level of affected subcellular pathways. Neurodegeneration-related gene sets (including Alzheimer's, Huntington's, Parkinson's, and ALS pathways) were significantly altered in both iMEF and CNS models, indicating shared molecular vulnerability networks. In contrast, MAPK signaling alterations were restricted to CNS tissue, suggesting tissue-specific regulatory mechanisms.

Conclusion:

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Our integrative approach identifies convergent molecular targets and pathway-level signatures, providing mechanistic insight into HSP pathogenesis and a framework for future diagnostic and therapeutic strategies.

Personalized ASO therapy for ALS associated with KIF5A mutations around exon 27: a multi-strategy approach

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Abstract *(in English, max 250 words)*

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that leads to paralysis and death within 3-5 years after diagnosis. For the majority of ALS-cases, no effective disease-modifying treatments exist. Several genes have been identified as causative, including KIF5A (kinesin heavy chain 5A). ALS-associated variants in KIF5A mostly cluster near exon 27. They cause frameshifts in exon 27/28 or skipping of exon 27, eventually leading to translation of an altered C-terminus with toxic gain-of-function.

Here, we aim to use splice-modulating antisense oligonucleotides (ASOs) to restore wild-type splicing or alternatively prevent the translation of the altered C-terminus. To achieve this goal, we designed four exon 28 skipping ASOs that truncate the C-terminus. We transfected four candidate ASOs into undifferentiated wild-type SH-SY5Y cells, leading to approximately 60 percent of exon 28 skipping of the most efficient ASO on mRNA level. In an independent approach, we sought to identify splice regulatory elements around exon 27 that might prevent mutation-induced exon 27 skipping and thus restore expression of wild-type protein. Detailed analysis of the locus using a series of minigene constructs led to the identification of regulatory elements promoting exon 27 retention. These will be targeted by splice-modulating ASOs, and the efficacy of the ASOs will be assessed in induced cortical neurons (iCNs) carrying different human pathogenic variants leading to exon 27 skipping. Additionally, the functional consequences of exon 28 skipping will be investigated in iCNs.

By advancing personalized ASO strategies against KIF5A mutations, this work aims to lay the foundation for future therapeutic approaches

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Assessing knockdown oligonucleotides in *Drosophila* as a therapeutic strategy for the treatment of severe ATL1-HSP

Lawless K, O'Sullivan NC

Atlastin-1 associated HSP is the second most common form of autosomal dominant HSP and is caused by mutations in the Atlastin-1 gene, a membrane-bound GTPase involved in maintaining the morphology of the endoplasmic reticulum. Recently, heterozygous *de novo* mutations in Atlastin-1 have been identified that result in a more severe disease presentation than is classically associated with ATL-1 associated HSP (Alecu *et al.,* 2023). My project aims to assess the viability of knockdown oligonucleotides targeting the disease-causing ATL-1 allele as a therapeutic strategy, using *Drosophila* models of disease.

To identify candidate allele-specific siRNAs, I have carried out an *in vitro* genetic screen comprising a dual luciferase assay of *Drosophila* S2 cells co-transfected with a luciferase reporter plasmid and an siRNA targeting the $Atl^{K382del}$ mutant allele. I have generated a psiCHECK2 luciferase reporter plasmid containing a mutant Atlastin fragment cloned into the 3'UTR of the Firefly luciferase reporter gene and a wild-type Atlastin fragment cloned into the 3'UTR of the *Renilla* luciferase reporter gene. I have validated expression of both of my engineered psiCHECK2 plasmids and used my dual luciferase assay to identify five promising siRNA sequences designed to specifically target the $Atl^{K382del}$ mutation.

I have begun assessing the effect of these candidate siRNAs on protein levels using Western blots. Plasmids were designed to contain either the tagged full-length wild-type or mutant Atlastin gene. A FLAG tag is common to both, while the wild-type plasmid specifically has a HA tag and the mutant plasmid has a 6xHis tag. I have confirmed that the S2 cells express the engineered plasmids well without significant toxicity and identified α-tubulin as a suitable loading control to permit quantification and validation of knockdown by candidate siRNAs identified in my *in vitro* genetic screen.

Loss of HPDL drives bioenergetic failure and CoQ10 deficit: clinical and molecular characterization enhanced by a multi-omic studies on SPG83 pathogenesis

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Abstract

Background: HPDL is a recently identified enzyme that converts 4-hydroxymandelate (4-HMA) into 4-hydroxybenzoate (4-HB), a crucial precursor for the CoQ10 headgroup. Bi-allelic *HPDL* variants cause a broad neurodevelopmental spectrum—from early-onset encephalopathy to adolescent-onset SPG83—but the underlying mechanisms remain largely unexplored.

Aim of the study: This study aims to characterize new HPDL cases and investigate its biological role using *in vitro* and *in vivo* models. Furthermore, we sought to identify novel proteomic and lipidic biomarkers through integrated omic profiling to define the disease signature.

Methods: Exome sequencing was performed on cohorts of congenital HSP and diplegic cerebral palsy. We conducted functional studies in patient-derived fibroblasts and *hpdl*$^{-/-}$ zebrafish larvae using qPCR, western blot, micro-oxygraphy, and behavioral locomotion studies. Integrated proteomic and lipidomic analyses were employed to identify metabolic disruptions.

Results: Patient fibroblasts showed reduced HPDL expression, impaired respiration, and increased reactive oxygen species. Clinical samples revealed elevated plasma GFAP and decreased 4-hydroxybenzeneacetic acid. In *hpdl*$^{-/-}$ zebrafish, integrated proteomic and lipidomic data confirmed severe mitochondrial dysfunction and significant bioenergetic impairment, validated by a marked decrease in CoQ10 levels. These molecular signatures correlated with neurodevelopmental abnormalities and epilepsy-like behavior in the larvae. Notably, bypass therapy with 4-HMA, 4-HB, and MitoTempo partially rescued the zebrafish phenotype.

Conclusion: We expanded the clinical landscape of SPG83, demonstrating that HPDL deficiency impairs oxidative metabolism across species. Our findings highlight that *hpdl*$^{-/-}$ zebrafish proteo-lipidomic

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signatures provide essential biomarkers for diagnosis and therapeutic monitoring. This model offers a robust platform for screening potential bypass therapies to target the identified bioenergetic failure.

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High-throughput phenotyping of fibroblast-derived induced neurons (FiNs) in SPG4-HSP

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Abstract *(in English, max 250 words)*

Background:

Hereditary spastic paraplegia type 4 (SPG4), caused by mutations in the *SPAST* gene, is characterized by progressive neurodegeneration, particularly affecting corticospinal motor neurons. The disease typically presents in early adulthood with spasticity and weakness of the lower limbs, progressing over time.

Aim of the study:

Here, we aim to identify phenotypic abnormalities in a fibroblast-derived induced neuron (FiN) model of SPG4 that could serve as measurable readouts for future drug testing. This approach is particularly compelling as age- and disease-related epigenetic signatures of the donor fibroblasts are preserved in FiNs.

Methods:

We established a direct transdifferentiation protocol to generate FiNs from SPG4 patient and age- and sex-matched control fibroblasts. This method bypasses the induced pluripotent stem cell (iPSC) stage to retain the epigenetic and age-related characteristics of the donor cells.

Results:

Using imaging-based high-throughput phenotypic screens, we identified significant deficits in SPG4 FiNs, including reduced neurite lengths and elevated neurite disintegration indices compared to controls. These findings indicate neurite damage in SPG4 FiNs.

Conclusion:

This platform establishes a robust disease model to investigate SPG4 pathophysiology and provides a basis for high-throughput drug screening to identify compounds that restore neurite integrity and length in SPG4-affected neurons.

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CSF1R-related leukodystrophy: development of an HEK293-based model for a screening platform.

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Abstract:

CSF1R-related leukodystrophy is a devastating neurodegenerative disease characterized by rapidly progressive cognitive and motor decline, leading to death within a few years after onset. The disease-causing gene *CSF1R* encodes a transmembrane tyrosine kinase receptor activated by CSF1 or IL-34, leading to autophosphorylation within CSF1R and multiple downstream signaling activations. *CSF1R* is crucial for the maturation and survival of microglia, which play an important role in maintaining brain homeostasis. Patients with mutations in *CSF1R* exhibit reduced amounts of microglia in the brain, which shows the importance of microglia in this disease. Over 170 pathogenic mutations have been identified within the *CSF1R* gene, yet the mutational mechanism remains unclear. Here, using cell and molecular biology methods, we present the process of developing HEK293-based models of mutations found in *CSF1R* as a platform for fast recognition of the molecular outcomes of those mutations and testing promising therapies, such as Antisense Oligonucleotides (ASO). Several cell lines stably expressing CSF1R were established using Sleeping Beauty. Cells were then analysed on mRNA and protein levels for the presence of CSF1R. Then, using Western blot, the activity of downstream signaling kinases such as JNK was assessed. Our preliminary results suggest that the *CSF1R* (c.1859-119G>A) variant has a reduced ability to activate JNK, which is important for cell survival and proliferation, but further analysis and optimization of the cell lines establishment are needed.

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Preliminary videoculographic (VOG) data in patients with genetically confirmed pure versus complicated HSP

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Background: Hereditary spastic paraplegias (HSPs) are a group of genetically heterogenous neurodegenerative disorders characterized by progressive lower limb spasticity and weakness. While lower limb motor dysfunction is the hallmark of HSP, other symptoms such as oculomotor abnormalities remain understudied and undercharacterizied.

Aim of the study: To evaluate the frequency and types of oculomotor abnormalities in patients with HSP and to compare their occurrence between patients with pure (pHSP) and complex forms (cHSP).

Methods: In a cohort of 20 patients with genetically confirmed HSP divided into the pHSP and cHSP group VOG examinations were performed using the EyeSeeCam system. The eye movements were evaluated for the presence of spontaneous and gaze-evoked nystagmus, optokinetic nystagmus, quality of smooth pursuit eye movements, horizontal and vertical saccades.

Results: Oculomotor abnormalities were observed in both pHSP and cHSP. Spontaneous nystagmus was present in 33% of pHSP and 45% of cHSP patients, while gaze-evoked nystagmus occurred in 11% and 27%. Horizontal smooth pursuit abnormalities were noted in 89% of pHSP and 67% of cHSP patients, and vertical smooth pursuit abnormalities in 67% of both groups. Optokinetic nystagmus was not elicited in 11% of pHSP compared with 56% of cHSP patients. Prevalence of oculomotor abnormalities did not differ between pHSP and cHSP.

Conclusion: Oculomotor abnormalities are present in both pHSP and cHSP suggesting that oculomotor involvement might be a general feature of HSP, not limited to complex forms.

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Title: **Assessing therapeutic strategies to modify neurotoxicity caused by complex HSP SPG3A mutants**

List of authors :O'Leary AC, O'Sullivan NC

Complex Hereditary Spastic Paraplegia (HSP) SPG3A is associated with autosomal dominant mutations in the Atlastin-1 (*ATL1*) gene, such as $ATL1^{K407del}$ and $ATL1^{M408T}$, resulting in a severe neurodegenerative disease affecting young children. Current treatments for HSP try to manage the physical symptoms only but fail to slow or reduce the disease progression. My project aims identify treatments that can modify neurotoxicity caused by severe-disease causing mutations in *ATL1* using biological and small molecule approaches.

In the first approach, I want to identify whether anti-sense oligonucleotides (ASO) designed to target allele-specific gene silencing of disease-causing SPG3A mutations, offer potential as a novel therapeutic strategy. I have developed a dual luciferase assay to screen ASOs. Briefly, I generated plasmids expressing fusions of *Renilla* – $ATL1^{WT}$ sequence and *Firefly-*$ATL1^{Mut}$ in human U2-OS cells. Using these, I was able to identify ASO sequences that reduced A$TL1^{Mut}$ mRNA expression without disrupting $ATL1^{WT}$ expression. Next, I generated plasmids expressing full length ATL1 ($ATL1^{WT}$ and $ATL1^{Mut}$) tagged with FLAG. Western blot analysis confirmed that my top candidate ASO significantly reduces $ATL1^{Mut}$ but not $ATL1^{WT}$ expression. Future work will investigate whether treatment with my $ATL1^{Mut}$ ASO modifies disease-relevant phenotypes in a neuronal iPSC model of disease.

My second approach uses an *in vivo* model, *Drosophila melanogaster,* to conduct a small molecule screen of approved drugs that have shown benefits in models of different neurodegenerative diseases. The screen uses Atlastin mutant *Drosophila*, that have previously been shown to have locomotor and survival deficits, which are chronically treated with a range of drugs are administered via food. To date, the majority of drugs screened have not shown lessen disease-relevant phenotypes. However, treatment with a mitochondrial modulating drug has resulted in a small but significant improvement of locomotion ability in two independent experiments. Further work is in progress to validate this and to investigate the molecular mechanisms by which this drug might be mediating its effects.

Axonal endoplasmic reticulum architecture and its links to axon function and degeneration

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Abstract *(in English, max 250 words)*

Background: Axon ER comprises a tubular network, shaped by membrane proteins including REEPs and reticulons, mutations in which cause HSP. It is continuous over large distances, with unusually narrow tubules.

Aim of the study: How does ER architecture influence axon function and dysfunction?

Methods: Live microscopy, calcium imaging, electron microscopy of *Drosophila* axons and synapses.

Results:

ER levels control STIM signaling and presynaptic Ca^{2+} signaling. *Drosophila* presynaptic motor ER comprises cisternae connected by tubules. Loss of reticulon Rtnl1 (SPG12) reduces tubular ER, but not cisternae, lumen volume or calcium stores; evoked calcium fluxes between compartments, and synaptic strength are generally lowered, as is the ER Ca^{2+} store sensor, STIM. STIM overexpression rescues the presynaptic Ca^{2+} phenotype.

ER tubule diameter constrains molecular movement. After photobleaching luminal GFP, wider mutant ER tubules recover faster than narrow wildtype ones, implying that narrow tubules limit luminal diffusion. We suggest that narrow axonal ER tubules constrain spatial dynamics of presynaptic Ca^{2+} signaling and plasticity.

Does ER continuity explain differential HSP sensitivity? Some *Drosophila* HSP mutants show sporadic gaps in axonal ER, and this could be a plausible hypothesis for HSP length dependence. This predicts that narrower axons, with less ER, would be more susceptible than wider ones. We explore these predictions in non-human primates.

Conclusion: Genetically manipulating ER architecture in *Drosophila* can test its links with normal and impaired axon/presynaptic function. Our data suggest synaptic strength as a HSP symptom, and STIM signaling as a candidate pathway for therapy.

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Very late-onset Krabbe disease with concomitant dementia: case description and a critical review of the literature.

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Abstract

Background: Krabbe disease (KD) is a rare autosomal recessive lysosomal storage disorder caused by pathogenic variants in *GALC*. Despite accounting only for 5% of forms, reports of adult-onset KD cases are increasingly recognized.

Aim of the study: To describe a patient manifesting KD after the age of 60 years, and to review the scientific literature of KD case with onset > 10 years to refine the spectrum of later-onset KD manifestations.

Methods: A female patient manifesting with pure spastic paraplegia with onset after 60 years developed dementia a few years later. Lumbar puncture degeneration biomarkers and neuropsychological assessment supported diagnosis of concomitant Alzheimer's disease. A Next-generation sequencing dementia panel documented the *GALC* c.857G>A homozygous variant. KD diagnosis was confirmed by reduced GALC activity in leukocytes.

Results: Including ours, we identified 84 KD adolescent/adult-onset patients (mean age at onset 28.7±14.2 years). Most patients had limb spasticity as main characterizing neurological feature (58/84, 70.2%), followed by polyneuropathy (11/ 84, 13.1%), both upper and lower motor neuron signs (2/84, 2.4%), and epilepsy (2/84, 2.4%). Five out of 84 patients (6.0%) were asymptomatic. Most patients had cortico-spinal tracts involvement at brain MRI. The most common pathogenic *GALC* variants were the c.1901T>C, the c.857G>A, and the c.1161+6532_polyA+9kbdel.

Conclusion: Complicated spastic paraplegia is the most common manifestation in later-onset KD, rarely with normal brain MRI. KD should be always considered also in cases with very late-onset spastic paraplegia.

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Spastin level regulation and biomarker development in SPG4-HSP

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Abstract

Background: Hereditary Spastic Paraplegia (HSP) is a motor neuron disease with no effective treatments. The most common cause is heterozygous loss-of-function mutations in the SPG4 gene, which encodes spastin, a microtubule-severing ATPase. Increasing spastin levels has emerged as a potential therapeutic strategy, particularly for truncating mutations. Reliable biomarkers are needed to monitor disease progression and evaluate new therapies. Recently, a cell imaging–based method was validated to quantify microtubule organization defects in patient-derived cells, showing potential as a biomarker in SPG4-HSP.

Aim of the study: To identify and manipulate the pathways controlling spastin stability for restoring functional protein levels in SPG4-HSP, including missense mutations. In parallel, to test the possibility of integrating cell imaging data with other biomarkers (molecular or fluid) to identify a disease signature.

Methods: We are dissecting factors that mediate selective spastin degradation and testing allele-specific silencing to normalize spastin dosage in cells carrying missense mutations. We are also analysing cells derived from the blood of SPG4 patients using a cell imaging-based method, integrated with spastin protein quantification and plasma neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP).

Results: We identified Ambra1 as a pro-degradation regulator of spastin; its targeting increases spastin levels. Allele-specific silencing approaches are under evaluation in missense contexts. Ongoing analyses integrate cell-imaging readouts with molecular biomarkers.

Conclusion: Modulating spastin degradation and carrying out allele-specific interventions are promising therapeutic strategies for SPG4-HSP. When integrated with complementary biomarkers, cell imaging–based method represents a promising tool for prognostic and predictive evaluation in SPG4-HSP, potentially guiding personalised therapeutic strategies and future clinical trials.

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Assessment of Autonomic Nervous System Involvement in Hereditary Spastic Paraplegias Using SUDOSCAN

Nunzio Setola [1], Lorenzo Cipriano [1], Sara Satolli [2], Gianmarco Dalla Zanna [1], Flavio Dal Canto [1], Guja Astrea [1], Filippo Maria Santorelli [1]

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Abstract

Background: Hereditary spastic paraplegias (HSPs) are a heterogeneous group of disorders characterized by progressive degeneration of the corticospinal tract. Involvement of the autonomic nervous system (ANS) has been poorly investigated in HSPs.

Aim of the study: To assess autonomic nervous system involvement in patients with HSP using a non-invasive, multimodal approach in the frame of the STOP-HSP.net registry.

Methods: Thirty patients with genetically heterogeneous forms of HSP were evaluated using clinical disease severity scales (SPRS, SARA, FARS-ADL), plasma fluid biomarkers (NfL, GFAP, BD-Tau), and patient-reported autonomic symptoms assess

ed with the Composite Autonomic Symptom Score 31 (COMPASS-31). Patients with HSP were evaluated every six months using SUDOSCAN, a portable, non-invasive device suited for outpatient settings.

Results: The study cohort included 14 males and 16 females with a marked genetic heterogeneity, with SPG4 as the most frequent genotype. Sudomotor dysfunction, assessed by SUDOSCAN, was detected in 8 patients (27%), with a predominant involvement of the lower limbs. ANS involvement appeared to be correlated with selected disease-related parameters, including disease duration and genotype. According to COMPASS-31 data, gastrointestinal, bladder, and orthostatic domains were the most frequently involved.

Conclusion: SUDOSCAN is a feasible, non-invasive tool to screen for longitudinal monitoring of autonomic dysfunction in HSP and other motor-neuronopathies

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Refinement of the natural history, clinical and molecular spectrum of SPG11 and SPG15

Quentin THOMAS MD PhD[1,2], Pauline Lallemant-Dudek MD PhD[1,3], Giulia Coarelli MD PhD[1] , Anna Heinzmann MD[1], Claire Ewenczyk MD PhD[1], Mariana Atencio[1], Rania Hilab[1], Alexis Brice MD[1], Frederic Darios, PhD[1] , Alexandra Durr MD PhD[1]

1 Sorbonne Université, Paris Brain Institute, ICM Institut du Cerveau, AP-HP, INSERM, CNRS, University Hospital Pitié-Salpêtrière, Paris, France

2 Université Bourgogne Europe, CHU Dijon Bourgogne, Service de Neurologie, Centre de Référence Maladies Rares Neurogénétiques, Inserm UMR1231 équipe GAD, Dijon, France

3 Service de Médecine Physique et de réadaptation, CH Armand Trousseau, APHP, 75012, Paris

Abstract *(in English, max 250 words)*

Background: Despite being one of the most frequent forms of recessive HSP, very little is known about the natural history and clinical spectrum of SPG11 and SPG15, hindering potential clinical trials.

Aim of the study: We gathered a large, international cohort of SPG11 and SPG15 individuals to describe their natural history.

Methods: Calls for collaboration were launched to gather individuals diagnosed with SPG11 or SPG15. Age at onset, disability and clinical scales, neuropsychological evaluations, radiological and molecular data were collected in a standardized online Redcap form. Biosamples were available for 135 individuals, including plasma for 25.

Results: A total of 157 individuals were included (SPG11 n= 134, SPG15 n= 23). Mean age at onset of first motor symptoms was 16,2 years in SPG11 and 13,04 years in SPG15. Loss of running ability occurred at a mean age of 22 years in both diseases, and wheelchair dependence at 29 in SPG11 and 27 in SPG15. Intellectual development was delayed for 44% of SPG11 and 61% of SPG15 individuals. When available, brain MRI was abnormal in 90% and 82% of individuals, respectively, with corpus callosum atrophy being the most frequent feature (81% in SPG11 and 55% in SPG15). Genotype-phenotype correlations showed a later onset in SPG11 individuals with homozygous splice variants or compound heterozygous splice/loss-of-function variants compared with homozygous loss-of-function variants.

Conclusion: We describe the largest reported cohort of individuals with SPG11 and SPG15, providing relevant data on the natural history and clinical spectrum of both diseases.

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Dear Madame

Quentin Thomas` email for some more information:

My name is Quentin THOMAS, I am a French neurologist working on rare neurogenetic disorders in Dijon's teaching hospital. This year i am working in Pr Alexandra Durr's team at the Paris brain institute and together we are working on a large cohort of people with SPG11 and SP15. We have gathered a large cohort of nearly 160 individuals for whom we offer detailed and genuine information on their story, symptoms, evolution of disability and so on. I believe this data is of interest and falls within the scope of your symposium. Unfortunately the 250 words format does not really fit such a large work with many different informations, but i hope the abstract will give you a glimpse of the potential fo this work.

Thank you in advance for your interest in our work, i am looking forward to the symposium.

Best regards

Dr Quentin THOMAS, PHU

MD PhD

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From Omics to Precision Medicine: decoding the molecular signatures of IAHSP and SINO syndrome.

A. Vicidomini[1], E. E. Ng'umbi[1], S. Della Vecchia[2], M. Rossi Sebastiano[3], N. Y. A. Abdallah[1], E. Mazzolini[1], G. Caron[3], G. Ermondi[3], F. M. Santorelli[2], E. F. Fornasiero[1,4], F. Cesca[5].

[1] University of Trieste, Trieste, Italy ; [2] IRCCS Stella Maris, Pisa, Italy ; [3] University of Turin, Turin, Italy ; [4] University Medicine, Gottingen, Germany ; [5] University of Padua, Padua, Italy

Abstract

Hereditary Spastic Paraplegias (HSPs) are clinically and genetically heterogeneous incurable disorders. Understanding the disease progression and the underlying molecular mechanisms is crucial to develop targeted therapeutics and relieve the healthcare burden on patients and families.

Background:

All forms of HSP show a progressive impairment of the lower limbs with muscular spasticity and weakness. Existing treatments only address the symptoms rather than molecular causes. We examine two genetically distinct HSP forms. Pure infantile-onset autosomal recessive HSP (IAHSP) is caused by the loss of Alsin, a factor critical for endosomal trafficking and neurite outgrowth. Conversely, complex SINO syndrome is due to mutation of KIDINS220, a neurotrophin-receptor scaffold protein essential for neuronal survival and synaptic plasticity.

Aim of the study:

This study focuses on IAHSP and SINO, assessing genotype-phenotype correlations and identifying convergent pathogenic pathways through clinical history data and patient-derived fibroblasts.

Methods:

Natural history curves from 85 IAHSP patients were generated by stratifying mutation profiles. Transcriptomic and proteomic profiling on patient-derived fibroblasts harbouring mutations in KIDINS220 and ALS2 genes was compared to healthy controls.

Results:

Natural history analysis revealed distinct disease progression severity, correlating with specific mutation types and providing a predictive model for the quantification of patient populations needed for clinical trials. Multi-omic characterization revealed shared alterations in endomembrane trafficking, cytoskeletal dynamics and cellular adhesion.

Conclusion:

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Combining clinical natural history with molecular profiling provides prognostic tools for patient counselling, while quantification of eligible patient populations demonstrates feasibility for future clinical trials. The identification of shared dysregulated pathways across HSP subgroups supports common pathogenic mechanisms, opening avenues for developing pathway-based therapeutics for patients sharing coherent molecular signatures.

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