Outcome Measures and Biomarkers for Hereditary Spastic Paraplegia

A thesis submitted to the University of Sydney, Faculty of Medicine and Health in fulfilment of the requirements for the degree of Doctor of Philosophy

Sue Faye Siow

BMedSci, MBBS, FRACP

Faculty of Medicine and Health

Northern Clinical School

Kolling Institute of Medical Research, Royal North Shore Hospital

The University of Sydney

2024

Declaration of originality

This is to certify that the content of this thesis is the result of my own investigation, and all references to ideas and work of other researchers have been specifically acknowledged. The work embodied in this thesis has not been submitted in candidature for any other degree.

I hereby certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

Signature:

Name: Sue Faye Siow

Date: 26 August 2024

Authorship attribution

This thesis contains work carried out by the author under the supervision of Dr Kishore Kumar, Dr Gautam Wali, and Professor Carolyn Sue.

Scoping review in Chapter 1 is published as "Siow SF, Yeow D, Rudaks LI, Jia F, Wali G, Sue CM, Kumar KR. Outcome Measures and Biomarkers for Clinical Trials in Hereditary Spastic Paraplegia: A Scoping Review. Genes (Basel). 2023 Sep 3;14(9):1756. doi: 10.3390/genes14091756. PMID: 37761896". I was responsible for study conception, study design, data collection, data analysis, writing the draft manuscript, journal submission, and revisions.

Systematic review in Chapter 2 is published as "Siow SF, Cameron Smail R, Ng K, Kumar KR, Sue CM. Motor Evoked Potentials in Hereditary Spastic Paraplegia-A Systematic Review. Front Neurol. 2019 Sep 18;10:967. doi: 10.3389/fneur.2019.00967. PMID: 31620065". I was responsible for study conception, study design, data collection, data analysis, writing the draft manuscript, journal submission, and revisions.

Cross-sectional cohort study in Chapter 2 is published as "Siow SF, Waters A, Coward S, Wali G, Ng K, Sue CM, Kumar KR. Outcome measures for hereditary spastic paraplegia clinical trials: Learnings from an Australian HSP centre. J Neurol Sci. 2024 Jul 15;462:123100. doi: 10.1016/j.jns.2024.123100. PMID: 38914513". KRK, CMS, and I were responsible for study conception. I was responsible for study design, patient recruitment, data collection, data analysis, writing the draft manuscript, journal submission and revisions.

The results from Chapter 3 are published as "Wali G*, Siow SF*, Liyanage E, Kumar KR, Mackay-Sim A, Sue CM. Reduced acetylated α-tubulin in *SPAST* hereditary spastic paraplegia patient PBMCs. Front Neurosci. 2023 Mar 28;17:1073516. doi: 10.3389/fnins.2023.1073516. PMID: 37144097". (* equal first author) GW and I were responsible for study conception and design, writing the draft manuscript and revisions. I was

responsible for patient recruitment, and flow cytometry experiments. GW was responsible for animal studies, HTRF experiments, and journal submission.

Mixed methods study from Chapter 4 is available on the medRxiv preprint repository as "Siow SF, Fleming J, Barlow-Stewart K, Kumar KR, Sue CM. Designing and validating a Hereditary Spastic Paraplegia-specific Quality of Life Rating Scale (HSPQoL). medRxiv 2024.07.23.24310834; doi: https://doi.org/10.1101/2024.07.23.24310834" and is under review. JF, KBS, and I were responsible for study design. I was responsible for study conception, patient recruitment, data collection, data analysis, writing the draft manuscript, and journal submission.

In addition to the statements above, in cases where I am not the corresponding author of a published article, permission to include the published material has been granted by the corresponding author.

Signature:

Name: Sue Faye Siow

Date: 26 August 2024

Attesting authorship statement

As supervisors for the candidature upon which this thesis is based, I can confirm that the authorship attribution statements above are correct.

Signature:

Name: Dr Kishore Raj Kumar, Principal Supervisor

Date: 26 August 2024

Signature:

Name: Dr Gautam Wali, Co-Supervisor

Date: 26 August 2024

Acknowledgements

Thank you first and foremost to my supervisors, Dr Kishore Kumar, Dr Gautam Wali, and Prof Carolyn Sue. Your guidance and support have been instrumental in my research and clinical career. I am most grateful for the opportunities you have provided. A special thank you to Erandhi Liyanage for guiding me through various laboratory techniques and for your friendship in the lab. Thank you to the Neurogenetics Lab team for your support.

Thank you to Associate Professor Karl Ng, Dr Jane Fleming, and Associate Professor Kristine Barlow-Stewart. Your patience and guidance in teaching me has been invaluable. Without your guidance, my research outputs would not have been possible.

To my collaborators and co-authors, thank you for your insights and hard work. I have thoroughly enjoyed working with you and learning from you. I hope for more opportunities to work together in the future.

Thank you to all the patients and their families who have generously participated in this research. Thank you to Frank McKeown and the HSP research foundation for your support and ongoing advocacy for people with HSP.

Most importantly, thank you to my husband, Peter. This thesis wouldn’t exist without your unwavering support and belief in me, and unlimited proofreading. To my children, Luka and Nico, thank you for inspiring me to always do my best.

List of Publications and Presentations

This is a “Thesis with Publications”. For all chapters that have been published or submitted, the author made substantial contributions to the study design, assembly, analysis, and interpretation of data. The author contributed to drafting manuscripts, coordinating submission and revision of manuscripts after peer review, and compiled this thesis.

Journal Publications included in this thesis (* equal first authors)

1. Siow SF, Yeow D, Rudaks LI, Jia F, Wali G, Sue CM, Kumar KR. Outcome Measures and Biomarkers for Clinical Trials in Hereditary Spastic Paraplegia: A Scoping Review. Genes (Basel). 2023 Sep 3;14(9):1756. doi: 10.3390/genes14091756.
2. Siow SF, Cameron Smail R, Ng K, Kumar KR, Sue CM. Motor Evoked Potentials in Hereditary Spastic Paraplegia-A Systematic Review. Front Neurol. 2019 Sep 18;10:967. doi: 10.3389/fneur.2019.00967.
3. Siow SF, Waters A, Coward S, Wali G, Ng K, Sue CM, Kumar KR. Outcome measures for hereditary spastic paraplegia clinical trials: Learnings from an Australian HSP centre. J Neurol Sci. 2024 Jul 15;462:123100. doi: 10.1016/j.jns.2024.123100.
4. Wali G*, Siow SF*, Liyanage E, Kumar KR, Mackay-Sim A, Sue CM. Reduced acetylated α-tubulin in *SPAST* hereditary spastic paraplegia patient PBMCs. Front Neurosci. 2023 Mar 28;17:1073516. doi: 10.3389/fnins.2023.1073516.
5. Siow SF, Fleming J, Barlow-Stewart K, Kumar KR, Sue CM. Designing and validating a Hereditary Spastic Paraplegia-specific Quality of Life Rating Scale (HSPQoL).
   medRxiv 2024.07.23.24310834; doi: https://doi.org/10.1101/2024.07.23.24310834

Conference Oral Presentations

1. Human Genetics Society of Australia (HGSA) Annual Scientific Meeting, Gold Coast, Platform Presentation, “Designing and validating a Hereditary Spastic Paraplegia specific quality of life rating scale (HSPQoL)”, 12 August 2024.
2. University of Sydney Faculty of Medicine Higher Degree by Research Conference, Charles Perkin Centre, Sydney, Oral presentation, “Designing and validating a

Hereditary Spastic Paraplegia specific quality of life rating scale (HSPQoL)", 25 July 2024. Winner of best oral presentation in the Mental Health and Wellbeing category.

3. Human Genetics Society of Australia Special Interest Group Meeting, Melbourne, Platform Presentation, "Reduced acetylated α-tubulin in HSP-*SPAST* peripheral blood mononuclear cells", 18 November 2023
4. Australian and New Zealand Association of Neurologists (ANZAN) Annual Scientific Meeting, Hobart, Platform Presentation, "Reduced acetylated α-tubulin in peripheral blood mononuclear cells from patients with HSP-*SPAST*", 17 May 2023.

Conference Poster Presentations

1. Siow SF, Fleming J, Barlow-Stewart K, Sue C. "Refining a Hereditary Spastic Paraplegia quality of life rating scale using consumer consultation." Presented at ANZAN Annual Scientific Meeting 2023, Hobart, TAS
2. Siow SF, Fleming J, Barlow-Stewart K, Sue C. "Refining a Hereditary Spastic Paraplegia quality of life rating scale using consumer consultation." Presented at HGSA Special Interest Group Meeting 2023, Melbourne, VIC.
3. Wali G, Siow SF, Liyanage E, Kumar KR, Mackay-Sim A, Sue CM. "Reduced acetylated alpha-tubulin in *SPAST* hereditary spastic paraplegia patient blood." Presented at International Congress of Genetics 2023, Melbourne, VIC.
4. Wali G, Siow SF, Liyanage E, Kumar KR, Mackay-Sim A, Sue CM. "Reduced acetylated α-tubulin in *SPAST* hereditary spastic paraplegia patient blood." Presented at ANZAN Annual Scientific Meeting 2023, Hobart, TAS.
5. Siow SF, Fleming J, Barlow-Stewart K, Sue C. "Developing a quality of life measure for patients with Hereditary Spastic Paraplegia." Presented at HGSA Virtual Scientific Meeting 2020.
6. Siow SF, Kumar K, Wali G, Sue C. "A critical review of biomarkers for Hereditary Spastic Paraplegia." Presented at ANZAN Annual Scientific Meeting 2019, Sydney, NSW.
7. Siow SF, Sue C, Coward S, Waters A, Kumar K, Ng K. "Investigating motor evoked potentials as a biomarker for disease severity in hereditary spastic paraplegia." Presented at ANZAN Annual Scientific Meeting 2018, Darwin, NT.

This research is supported by:

1. Australian Government Research Training Program Fee Offset
2. National Health and Medical Research Council Postgraduate Scholarship
3. Australian and New Zealand Association of Neurologists Education and Research Fund Scholarship

Thesis Abstract

Hereditary Spastic Paraplegia (HSP) is an inherited neurodegenerative condition leading to impaired mobility and reduced quality of life. There is no curative treatment and therefore an urgent need for standardised outcome measures to demonstrate therapeutic efficacy of novel treatment options. The aim of this thesis is to identify outcome measures and biomarkers for HSP clinical trials.

In Chapter 1, I performed a scoping review of pre-existing outcome measures and biomarkers in HSP. There was heterogeneity and inconsistency of outcome measures and biomarkers used and limited evidence on validity and reliability in HSP. Patient reported outcome measures (PROM) were absent in the majority of published HSP clinical trials. Based on the findings, I proposed a core outcome set should include a clinician rated outcome measure (CROM), PROM, and a biomarker.

In Chapter 2, a systematic review of motor evoked potentials (MEP) in HSP found that although MEPs could be useful for diagnosis of HSP, there was insufficient evidence to support its use for measuring disease severity. I evaluated a set of outcome measures - Scale for Assessment and Rating of Ataxia (SARA), Spastic Paraplegia Rating Scale (SPRS), Short Form-36, and MEP in a cohort of patients with HSP. My findings support SARA as a supplement to the SPRS in patients with HSP and prominent ataxia symptoms. SF-36, a generic PROM, did not capture HSP-specific quality of life issues, and MEP findings did not correlate with clinically relevant outcome measures. This study highlighted the need for an HSP-specific PROM and biomarker.

In Chapter 3, I investigated acetylated α-tubulin levels in peripheral blood mononuclear cells (PBMCs) as a potential biomarker for HSP-*SPAST* using flow cytometry. I demonstrated reduced levels of acetylated α-tubulin in HSP-*SPAST* patient cells compared to controls. My findings support further investigation of acetylated α-tubulin as a potential biomarker for HSP-*SPAST* and measure of treatment response to tubulin-binding drugs, such as noscapine.

In Chapter 4, I developed and validated an HSP-specific quality of life survey (HSPQoL). HSP-specific items were developed by consulting patients with HSP, carers, and HSP clinicians using a modified Delphi process and cognitive interviews. The HSPQoL was validated in a cohort of 61 patients with HSP demonstrating validity and reliability of the scale.

This thesis provides crucial evidence to inform choice of outcome measures and biomarkers in HSP clinical trials and discusses avenues for future research. The findings are an essential step in the search for a cure for HSP.

List of Figures and Tables

Chapter 3

Chapter 4

Table of Contents

Chapter 1. Introduction and Literature Review

1.1 Preamble

Hereditary Spastic Paraplegia (HSP) refers to a group of inherited neurodegenerative disorders characterised by lower limb spasticity. Symptoms are due to length-dependent degeneration of corticospinal neurons though there is significant variability in terms of age of onset, rate of progression, and phenotypic spectrum. [1] Despite the significant impact of HSP symptoms on quality of life, a disease-modifying treatment remains elusive with barriers to research including variability and rarity of the condition. [2, 3] Novel research over the past decade has identified promising therapeutic agents that require testing in clinical trials. [4, 5] However, there is a lack of consensus on outcome measures to assess treatment efficacy. [3, 6] Therefore, there is an urgent need for standardised validated outcome measures for HSP to translate findings from laboratory-based research to clinical studies. The overarching aim of my thesis is to investigate outcome measures and biomarkers for HSP.

1.2 Hereditary Spastic Paraplegia: A literature review

1.2.1 Introduction

HSP was first recognised as hereditary by Adolf von Strumpell who published the first report of a family with HSP in 1880. [7] A century later, Anita Harding described the various inheritance patterns associated with HSP in a report of 22 families with HSP. [8] In 1983, she proposed classification of HSP based on clinical symptoms – pure HSP to describe individuals with lower limb pyramidal signs, vestibular impairment, and bladder symptoms; and complicated HSP to describe individuals with features additional to lower limb pyramidal signs. [9] More recently, the advent of next generation sequencing has led to rapid discovery of genes associated with HSP with over 80 associated genes reported in the literature. [10-12] In keeping with the heterogeneity of underlying genetic causes, HSP is associated with various inheritance patterns and has vast phenotypic heterogeneity. In this section, I will introduce the complex nature of HSP and the consequent challenges in identifying standardised outcome measures and biomarkers for this condition.

1.2.2 Prevalence and impact of HSP

HSP remains a rare condition despite the large number of HSP-associated genes. Reported prevalence varies between 0.3 to 5.5 per 100,000 people depending on country and genotype. [13-16] The wide range of prevalence across different countries can be attributed to differences in access to genetic testing, incidence of consanguinity, and study methodology used to determine case numbers. [16] An epidemiological model estimated the global prevalence of all HSP subtypes as 3.6 per 100,000 people with the most common genotypes being HSP-*SPAST*, HSP-*SPG7*, and HSP-*SPG11*. [16] Although the life expectancies for the most common HSP subtypes are similar to or slightly lower than the general population, HSP is clearly associated with worse quality of life indices, and a multitude of healthcare needs. [2, 16-18]

1.2.3 Clinical Features of HSP

HSP is characterised by pyramidal signs including limb spasticity, weakness, increased reflexes, bladder and/or bowel dysfunction, and dorsal column sensory loss. [19] In addition to characteristic pyramidal dysfunction seen in HSP, additional clinical features are described including neurological and non-neurological features (Table 1). As illustrated in Table 1, the phenotype of HSP is broad and therefore, several classification systems have been reported and used in the literature.

Table 1. Examples of additional neurological and non-neurological features seen in HSP (adapted from Meyyazhagan et al 2022, Murala et al 2021). [11, 19]

Neurological Features	Non-neurological features
Cerebellar dysfunction	Other ophthalmological features (cataracts, macular degeneration, retinitis pigmentosa)
Cognitive impairment	Dysmorphic features (skeletal or facial differences)
Epilepsy	Orthopaedic changes (scoliosis)
Peripheral neuropathy	
Myopathy	
Psychiatric disturbances	

Extrapyramidal features (chorea, parkinsonism, dystonia)

1.2.4 Classification of HSP

1.2.4.1 HSP classification by phenotype

One of the earliest proposed classification systems for HSP was by phenotypic presentation. Pure HSP was defined as presentations limited to lower limb pyramidal signs, dorsal column sensory dysfunction, and bladder/bowel dysfunction. [9] Complicated or complex HSP was used to describe HSP with extra features in addition to the characteristic lower limb pyramidal signs, such as the features described in Table 1.

In keeping with the broad phenotypic spectrum of HSP, there is recognised phenotypic overlap with other neurodegenerative conditions, posing a challenge to classification and diagnosis of the condition (Figure 1). [20, 21] To address this, an international Task Force was convened to develop a nomenclature and classification system for movement disorders as described in section 1.2.4.4. [22]

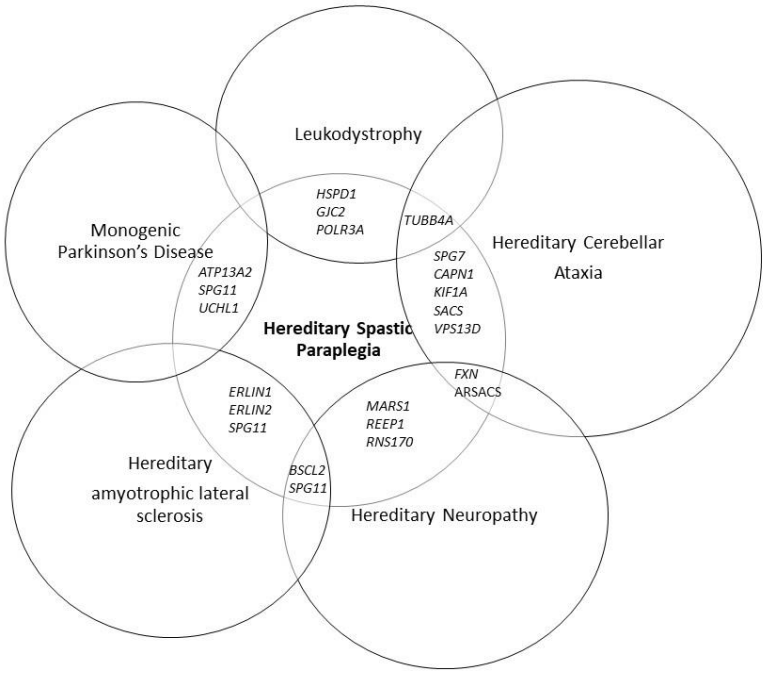


Figure 1. Clinical and genetic overlap of HSP with other neurodegenerative conditions. [10, 12, 21]

1.2.4.2 HSP classification by age of onset

Harding *et al* also proposed classification of HSP based on age of onset: early onset defined as age of onset before 35 years old, and later onset defined as age of onset after 35 years old. [8] This form of classification is no longer commonly used though the variable age of onset in HSP is a well-recognised feature of the condition.

1.2.4.3 HSP classification by inheritance pattern

Another form of classification, also first described by Harding *et al*., is by mode of inheritance. [8] HSP can be inherited in various manners depending on the underlying genetic cause, including autosomal dominant, autosomal recessive, X-linked, and mitochondrial. In addition, certain HSP subtypes can have both autosomal dominant and autosomal recessive patterns of inheritance. HSP-*KIF1A* and HSP-*REEP2* are associated with autosomal dominant and autosomal recessive forms of HSP. [23, 24] With the improved understanding of underlying genetic causes of HSP, this form of classification is now superseded by HSP classification by genotype as described below.

1.2.4.4 HSP classification by genotype

Prior to the identification of individual genes associated with neurogenetic conditions, locus symbols (e.g. DYT1) [25] were used to designate specific chromosomal regions linked to a familial disorder in order of discovery. As the underlying genetic basis of these conditions have been elucidated, clinicians and researchers continued to use the locus symbols to identify the conditions of interest (e.g. SPG4 to describe HSP caused by pathogenic *SPAST* variants) [26]. Figure 1 illustrates the use of the previous system. [21] This old system of naming and classifying neurogenetic conditions had several problems including that it does not account for genes that are associated with more than one movement disorder, HSP-associated genes that have not been assigned a locus symbol, and locus symbols assigned to chromosomal regions or genes that are not definitively associated with HSP. [27] In 2016, a new classification system was proposed, adapted to the latest knowledge, with a further update in 2022. [22, 28] The current system recommended describing movement disorders with a prefix denoting the predominant phenotypic feature with a suffix identifying the gene name associated with that disorder, e.g. HSP-*SPAST* to describe HSP associated with a pathogenic *SPAST* variant. [22] This new system accounts for phenotypic and genotypic heterogeneity of many movement disorders, clearly identifying the condition being described. I have adopted this latest form of nomenclature throughout my thesis and publications.

To date, over 80 HSP subtypes have been identified. A detailed description of each subtype is beyond the scope of this thesis and several publications have reviewed the various HSP subtypes. [11, 19] HSP-*SPAST* is the most common genotype in my patient cohort and I provide an overview of HSP-*SPAST* in Chapter 3.

1.2.5 Diagnosis of HSP

The diagnosis of HSP is usually made on clinical examination where the characteristic features of lower limb pyramidal signs are identified. [8] Family history can be helpful to identify the inherited nature of the condition though the absence of a family history does not preclude the diagnosis. Further investigations with magnetic resonance imaging (MRI) of the central nervous system; biochemical tests, such as vitamin B12 levels and serum copper levels; and infectious serology, such as HTLV1, HIV and syphilis if indicated, are recommended to exclude other causes of spasticity. [10] MRI of the spinal cord is usually normal though may show cervical cord thinning in HSP. MRI brain can sometimes show features suggestive of particular HSP subtypes, such as thinning of the corpus callosum in HSP-*SPG11* and cerebellar atrophy in HSP-*SPG7*. [29] Motor evoked potentials can be studied to evaluate central motor conduction time, which is commonly prolonged in HSP, though this finding is not universal and the absence of abnormal motor evoked potentials does not preclude a diagnosis of HSP. [30, 31] Metabolic investigations, such as plasma amino acids, very long chain fatty acids, and CSF glucose, are helpful to identify potentially treatable metabolic conditions that cause HSP. [32] Other investigations may be indicated for additional features such as nerve conduction studies, neuropsychological testing, ophthalmological review, and/or electroencephalography. Genetic testing to identify a pathogenic HSP-associated gene variant can confirm a genetic diagnosis of HSP.

1.2.6 Genetics of HSP

There are over 80 genes reported to be associated with HSP in the literature. [11, 33, 34] Genetic testing approaches for HSP have evolved over time, starting with linkage studies in families to identify associated loci, followed by single gene testing, targeted gene panels, whole exome sequencing and whole genome sequencing. With the advancement of genetic testing technologies, the number of associated genes continues to grow. The diagnostic yield from

genetic testing varies with the type of genetic testing, and inclusion criteria for testing. [35] More recently, diagnostic yields of ~30% are reported for HSP cohorts that undergo genetic testing with HSP gene panels and/or whole exome sequencing. [34] In clinical practice, the choice of genetic test for individuals with HSP depends on several factors including clinical presentation (e.g. pure vs complex HSP, presence of family history, clinical features suggestive of particular subtypes), availability of test (e.g. cost, access to accredited laboratories), and patient factors (e.g. availability of parental samples, concern for incidental findings). [35] Genetic counselling is an important component in genetic testing, particularly for HSP where the genetics of the condition can be complex. A positive genetic diagnosis can have potential implications for genetic relatives, such as recurrence risk in children and/or siblings. On the other hand, an uninformative result may leave an individual with uncertainty with regards to their diagnosis and the implications for their health and family members. Individuals with a clinical diagnosis of HSP and an uninformative genetic test result may wish to consider research genetic testing as improving genetic knowledge and technology will continue to increase diagnostic yield. Identifying a genetic diagnosis may open up the possibility of targeted treatments in the future though successful targeted treatment of HSP has not yet been achieved. [3]

1.2.7 Pathophysiology of HSP

The cardinal symptoms of lower limb spasticity and weakness seen in HSP are due to length-dependent axonal degeneration of corticospinal tracts and sensory tracts in the spinal cord. [36] Discovery of HSP-associated genes has shed further light on the underlying mechanisms leading to this axonal degeneration. Figure 2 illustrates the various cellular pathways implicated in HSP based on underlying genotype. [37] Cellular pathways affected in HSP include impairment of endoplasmic reticulum function, impaired axon plotting, impaired lipid metabolism, dysmyelination, impaired axonal transport, abnormal endocytic trafficking, dysfunctional mitochondrial activity, and disrupted microtubule dynamics. [38] Improved understanding of the underlying pathophysiology of each HSP subtype is essential to developing targeted treatments for this progressive disease.

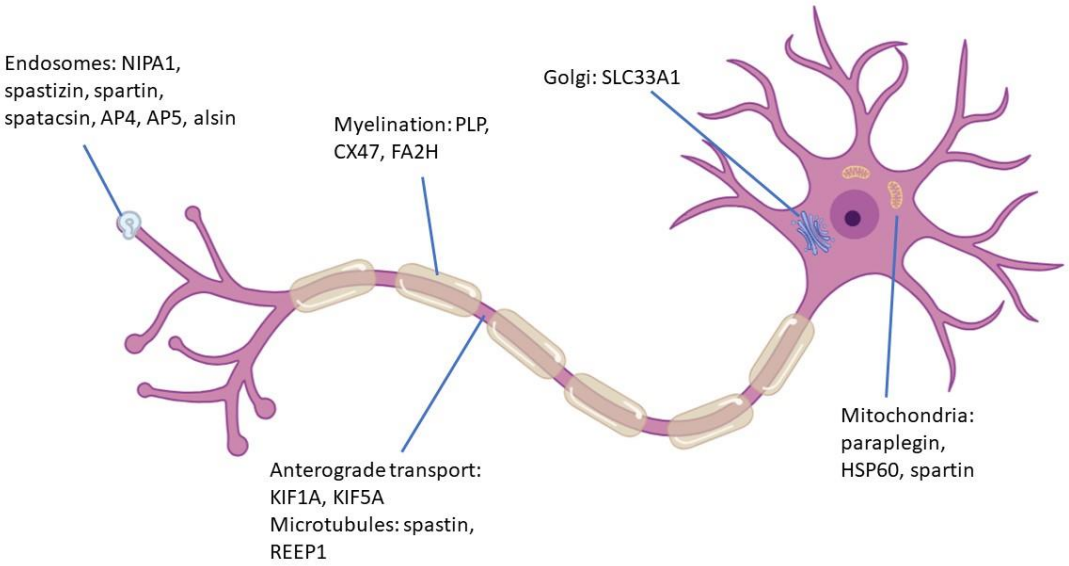


Figure 2. Cellular processes affected in various HSP subtypes. [37] Created with Biorender.com.

1.2.8 Natural history and prognosis of HSP

HSP is a progressive disease with rates of progression varying depending on the underlying genetic cause. Progression and clinical involvement of HSP is variable though identifying the genetic diagnosis may provide information on age of onset, rate of progression, and spectrum of clinical features. To complicate things further, many HSP subtypes demonstrate inter- and intra-familial variability despite identical genotypes. [1]

1.2.9 Management and Treatment options for HSP

Currently, there is no disease-modifying treatment for HSP. Treatment is symptomatic with variable efficacy. Overall, there have been limited clinical trials of treatment options for HSP, most with negative or inconclusive outcomes, and poor study quality. [3] Treatment options reported in the literature are listed in Table 2.

Table 2. Treatment options for HSP.

Therapeutic options for HSP	**Evidence of efficacy**
Gabapentin	No benefit over placebo[39]
Dalfampridine (4-aminopyridine)	Improved mobility and spasticity scores though study quality rated low. [3, 40]
Botulinum toxin injection	No benefit over placebo. [41]
Botulinum toxin with physiotherapy	Improved tone, gait and quality of life. [42]
Botulinum toxin and stretching	Improved gait parameters. [43]
Cholesterol-lowering drugs	Biochemical response to statins in two clinical trials in HSP-*CYP7B1* though no clinical correlate demonstrated. [4, 44]
Robotic Gait Training	No improvement in spasticity but some improvement in gait, [45] but limitations in study quality. [3]
Hydrotherapy	Inconclusive results, limitations in study quality. [3, 46]
Intrathecal Baclofen	Reduced spasticity but limitations in study quality, [3, 47] improved spasticity and quality of life but small sample size. [48]
Dorsal rhizotomy	Improved lower limb spasticity in one study but limitations in study quality. [3, 49] Other study showed 14% improved mobility but 17% deteriorated mobility whilst 70% remained unchanged. [50]
Spinal direct current stimulation	Improved spasticity. [51]
Repetitive transcranial magnetic stimulation	No benefit over sham treatment, [52] improved spasticity compared to sham but small sample size. [53]

1.2.10 Challenges in identifying biomarkers and outcome measures

In this introduction section, I have presented the complexities of HSP – variable phenotype, variable genotype, variable pathophysiology, rarity, limited diagnostic yield from genetic

testing, and limited treatment options. These features of HSP have led to challenges in developing standardised and validated outcome measures for treatment. Phenotypic variability limits the use of a single outcome measure, such as the Modified Ashworth Score for spasticity, as it does not evaluate other clinical features of HSP. Variable genotype and pathophysiology limit the use of biomarkers targeted to cellular processes, such as markers of microtubule stability, as different forms of HSP have different underlying pathophysiological mechanisms. The rarity of HSP and limited diagnostic rate results in difficulty meeting sufficient sample sizes for studies of outcome measures and therapeutic agents. In the following scoping review, I explore outcome measures and biomarkers used in HSP studies, discuss potential strategies to address the identified challenges with developing these measures, and provide recommendations of outcome measures for HSP.

1.3 Scoping review of outcome measures and biomarkers for HSP

Siow SF, Yeow D, Rudaks LI, Jia F, Wali G, Sue CM, Kumar KR. Outcome Measures and Biomarkers for Clinical Trials in Hereditary Spastic Paraplegia: A Scoping Review. Genes (Basel). 2023 Sep 3;14(9):1756. doi: 10.3390/genes14091756. PMID: 37761896.

genes

MDPI

Review

Outcome Measures and Biomarkers for Clinical Trials in Hereditary Spastic Paraplegia: A Scoping Review

Sue-Faye Siow [1,2,*], Dennis Yeow [1,3,4,5,6], Laura I. Rudaks [1,2,4,5], Fangzhi Jia [1], Gautam Wali [1,3,†], Carolyn M. Sue [1,3,6,7,†] and Kishore R. Kumar [1,4,5,7,†]

1 Sydney Medical School, University of Sydney, Camperdown 2050, Australia; c.sue@neura.edu.au (C.M.S.)
2 Department of Clinical Genetics, Royal North Shore Hospital, St Leonards 2065, Australia
3 Neuroscience Research Australia, University of New South Wales, Randwick 2031, Australia
4 Rare Disease Program, Garvan Institute of Medical Research, Darlinghurst 2010, Australia
5 Translational Neurogenomics Group, Molecular Medicine Laboratory and Department of Neurology, Concord Hospital, Concord 2139, Australia
6 Neurodegenerative Service, Prince of Wales Hospital, Randwick 2031, Australia
7 School of Clinical Medicine, UNSW Medicine & Health, University of New South Wales, Kensington 2052, Australia
* Correspondence: suefaye.siow@health.nsw.gov.au
† These authors share senior authorship.

Abstract: Hereditary spastic paraplegia (HSP) is characterized by progressive lower limb spasticity. There is no disease-modifying treatment currently available. Therefore, standardized, validated outcome measures to facilitate clinical trials are urgently needed. We performed a scoping review of outcome measures and biomarkers for HSP to provide recommendations for future studies and identify areas for further research. We searched Embase, Medline, Scopus, Web of Science, and the Central Cochrane database. Seventy studies met the inclusion criteria, and eighty-three outcome measures were identified. The Spastic Paraplegia Rating Scale (SPRS) was the most widely used (27 studies), followed by the modified Ashworth Scale (18 studies) and magnetic resonance imaging (17 studies). Patient-reported outcome measures (PROMs) were infrequently used to assess treatment outcomes (28% of interventional studies). Diffusion tensor imaging, gait analysis and neurofilament light chain levels were the most promising biomarkers in terms of being able to differentiate patients from controls and correlate with clinical disease severity. Overall, we found variability and inconsistencies in use of outcome measures with a paucity of longitudinal data. We highlight the need for (1) a standardized set of core outcome measures, (2) validation of existing biomarkers, and (3) inclusion of PROMs in HSP clinical trials.

Keywords: hereditary spastic paraplegia; outcome measures; biomarkers; clinical trials; scoping review

Citation: Siow, S.-F.; Yeow, D.; Rudaks, L.I.; Jia, F.; Wali, G.; Sue, C.M.; Kumar, K.R. Outcome Measures and Biomarkers for Clinical Trials in Hereditary Spastic Paraplegia: A Scoping Review. *Genes* **2023**, *14*, 1756. https://doi.org/10.3390/genes14091756

Academic Editor: Claudia Ricci

Received: 9 August 2023
Revised: 30 August 2023
Accepted: 30 August 2023
Published: 3 September 2023

1. Introduction

Hereditary Spastic Paraplegia (HSP) refers to a group of inherited neurodegenerative conditions characterized by lower limb spasticity and weakness. HSP is rare, with a prevalence of 0.3 to 5.5 per 100,000 people, depending on country [1–4]. HSP is associated with significant disability and a negative impact on quality of life [5]. There are over 80 recognized HSP-associated genes with broad phenotypic variability [6–8]. Clinically, HSP can be categorized into pure HSP—symptoms limited to weakness, spasticity, impaired vibration sense in the lower limbs, and bladder dysfunction; and complex HSP—where there are additional neurological and non-neurological manifestations [9]. The phenotypic and genotypic heterogeneity of HSP and the rarity of the condition pose a challenge to the development of suitable outcome measures and biomarkers.

Advances in genetic testing have led to the rapid discovery of genes associated with HSP [10], accelerating the discovery of therapeutic targets. Patient-derived stem cell and

animal models have identified potential drug treatment candidates targeting underlying pathogenesis for specific HSP genotypes, such as noscapine for HSP-*SPAST* [11–15]. Currently, the rate of drug discovery is far outpaced by the rate of gene discovery for HSP and a shift in HSP research to developing treatment options is required [10,16,17]. Outcome measures and biomarkers that can measure the efficacy of therapeutic interventions in clinical trials are needed to facilitate this.

The choice of appropriate outcome measures in interventional trials is critical to demonstrating a meaningful treatment effect [18]. Initiatives such as COnsensus-based Standards for the selection of health Measurement INstruments (COSMIN) and Core Outcome Measures in Effectiveness Trials (COMET) aim to guide the selection of appropriate outcome measures [19]. There is currently a lack of standardized outcome measures used in HSP clinical trials, with recent reviews of interventional trials in HSP showing heterogeneity of outcome measures [17,20]. Inconsistency of outcome measures leads to an increased risk of reporting bias due to post hoc selection of outcomes based on results rather than the use of pre-specified primary outcomes [21]. Furthermore, use of different outcome measures limits comparison and meta-analysis of results from different studies [21].

There are no current consensus guidelines for outcome measures in HSP clinical trials. To address this, we performed a scoping review of outcome measures and biomarkers in HSP to identify suitable outcome measures, provide recommendations for future HSP clinical trials, and identify areas for further research.

2. Materials and Methods

2.1. Search Strategy

This study was conducted according to published guidelines for conducting a systematic scoping review [22,23]. Under the guidance of an academic librarian, we performed a search in Embase, Medline, Scopus, Web of Science and the Central Cochrane databases using the search terms “Hereditary Spastic Paraplegia”, “biomarker”, “outcome measure”, and “patient reported outcome measure” (Appendix A). Additional studies were identified by searching the references of the included articles and relevant review articles.

2.2. Selection Criteria

We kept the selection criteria broad to capture all possible outcome measures and biomarkers. The inclusion criteria included studies involving patients with HSP of any age and gender, and that included a description of the outcome measures or biomarkers for HSP. Abstracts were included if novel outcome measures or biomarkers were described.

We excluded review articles, single-case reports, trial protocols with no published results, abstracts with no novel outcome measures/biomarkers, and articles that did not involve humans or human samples, did not include outcome measures/biomarkers, or were not in English.

2.3. Screening of Search Results

The screening and data extraction process was conducted using Covidence, a web-based collaboration software platform that streamlines the production of systematic and other literature reviews [24]. The first author (S.F.S.) excluded all irrelevant results—studies unrelated to HSP or not in English and duplicate studies. Authors S.F.S. and D.Y. independently reviewed the abstracts according to the selection criteria for the first 50 results. Authors S.F.S. and D.Y. independently reviewed the remaining abstracts, applying the finalized exclusion criteria.

2.4. Data Extraction and Analysis

Authors S.F.S., D.Y., L.R., and F.J. reviewed two full texts each to test the data extraction template. The template was modified by discussion at a team meeting. The team then reviewed six full texts, each with each author reviewing the same three articles as two other authors (~30% overlap) and met to resolve any discrepancies and standardize the extraction

process. The rest of the studies were reviewed by one reviewer (D.Y., L.R., or F.J.) and verified by a second reviewer (S.F.S.). At each stage, discrepancies were resolved through a team meeting. The data were extracted using a standardized data extraction template, including information on study characteristics, aim of study, participant characteristics, characteristics of interventions, outcome measures, and ability of outcome measure to (i) distinguish patients versus controls, (ii) demonstrate change over time; (iii) show response to the intervention; and (iv) correlate with other measures. Study quality analysis was not performed, as is usual for scoping reviews [22].

Data were analyzed descriptively to provide an overview of study characteristics and outcome measures. Outcome measures were grouped according to the types of clinical outcome assessments as defined by the U.S. Food and Drug Administration [25]:

1. Clinician-reported outcome measures (CROM): measurement of clinical signs or findings performed by a health professional.
2. Performance outcome measures (PerfOM): measurement with a standardized task, either administered by a trained individual or undertaken by the patient without assistance.
3. Patient-reported outcome measures (PROM): measurement of patient-reported health status.

Biomarkers were grouped according to the assessed modality:

1. Laboratory-based biomarkers;
2. Neuroimaging biomarkers;
3. Neurophysiology biomarkers;
4. Other biomarkers.

As other groups have previously published reviews of non-randomized interventional clinical trials [17,20], we chose to perform further analysis of randomized controlled trials (RCTs) to compare the outcome measures used, as RCTs are the study type with the highest level of evidence for treatment effectiveness [26].

3. Results

3.1. Search Results

A total of 1930 search results were identified, and 1437 abstracts were screened after duplicates were removed, of which 1222 were identified as irrelevant. The full texts of the remaining results (n = 215) were assessed according to inclusion criteria. Ten more studies were identified by searching the references of the included articles (Figure 1).

3.2. Study Characteristics

A total of 70 studies that met the inclusion criteria were identified. These studies were published between 1991 and 2022; date limits were not set to include as many studies as possible.

The majority (78.6%) of the included studies were observational studies, and only a quarter (25.7%) of studies were interventional studies. Most studies did not include longitudinal data (75.7%), and over half did not include a control group (51.4%). Participant genotype was predominantly mixed or unknown (65.7%), and sample sizes were small (mean of 36.99 participants) (Table 1). A list of all studies with relevant details is included as Supplementary Material S1.

We assessed each outcome measure for (1) the ability to differentiate patients versus controls if a control group was included, (2) the ability to demonstrate disease progression if longitudinal data were included, (3) the ability to demonstrate response to the intervention if an intervention was assessed, and (4) any correlation with other biomarkers or outcome measures. Although we report the number of studies that fulfilled the criteria for (1), (2) and (3) to illustrate the available evidence for each outcome measure, it is important to note that (1) in studies with multiple outcome measures, the control group was compared to the patient group for some but not all outcome measures, (2) not all longitudinal studies were designed to assess the ability of an outcome measure to measure disease progression, (3) an outcome measure may not demonstrate a response to the intervention for many reasons

including efficacy of intervention, duration of trial, and timing of the outcome measure relative to the intervention.

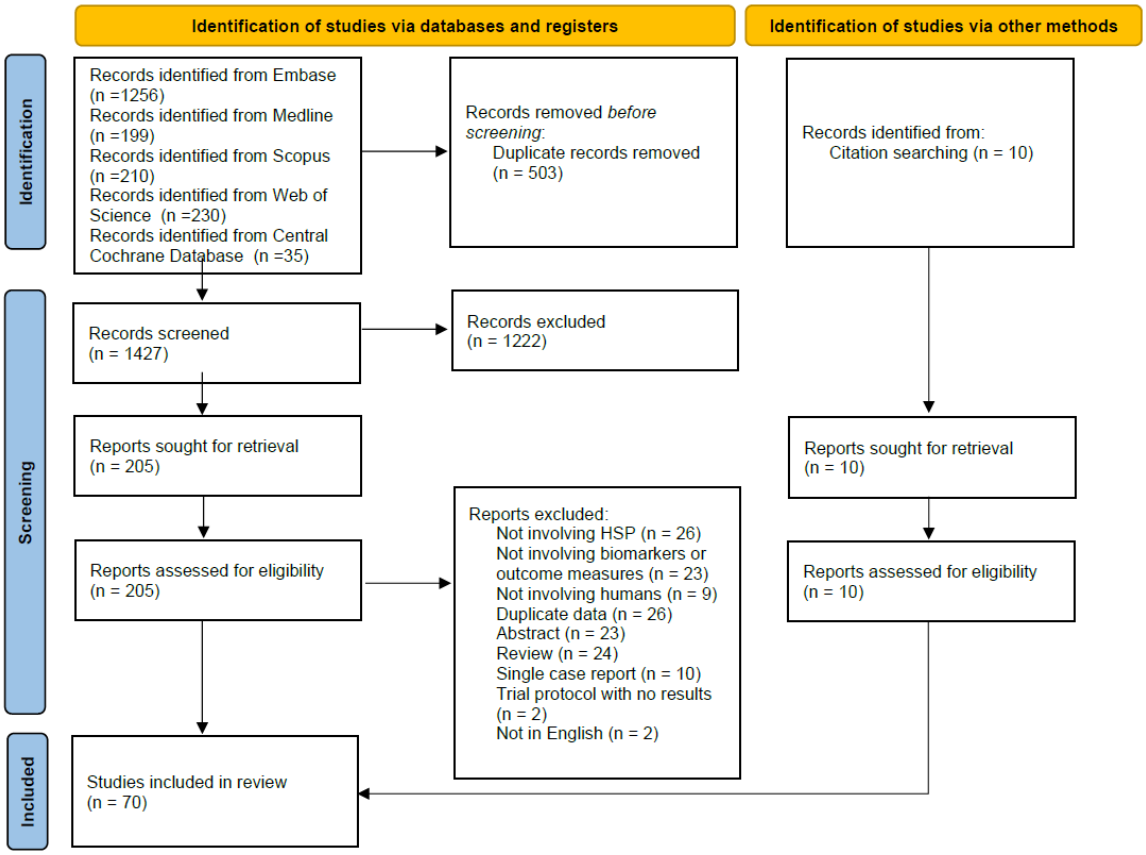


Figure 1. PRISMA flow chart [27].

Table 1. Descriptive findings.

Study Type	Number of Studies (%)
Observational (total)	55 (78.6)
• Case series	9
• Cohort	14
• Case–control	2
• Cross-sectional	30
Diagnostic test accuracy	1 (1.4)
Non-randomized interventional studies	7 (10)
Randomized controlled trials	6 (8.6)
Study Characteristics	**N (%)**
Longitudinal data presented	17 (24.3)
Control group included	34 (48.6)
Intervention	18 (25.7)
Participant Genotype	**N (%)**
Single genotype	24 (34.3)
Mixed known and unknown genotypes	35 (50)
Unknown genotype	11 (15.7)
Participant Characteristics	**N (%)**
Number of participants	37.0 people (range 2–239, SD 42.2)
Mean of mean ages (n = 64)	39.9 years (range 4.8–62, SD 14.9)

3.3. Clinical Outcome Assessments

3.3.1. Clinician Reported Outcome Measures

The Spastic Paraplegia Rating Scale (SPRS) was the most widely used CROM, reported in twenty-seven studies (Table 2). Although 12/27 included studies control groups, a comparison of SPRS scores in patients versus controls was performed in only two studies, both showing significant differences [28,29]. It is important to note that SPRS values from healthy controls are most relevant when comparing to pre-symptomatic HSP carriers rather than for comparison to individuals with symptomatic HSP. 8/27 studies were longitudinal, and only six of those studies showed disease progression over time with the SPRS (median follow-up time 12–31 months) [30–35]. The SPRS was used as an outcome measure in 4/18 interventional studies and showed a response to the intervention in only one of the four studies [36]. The SPRS was commonly used to correlate with other biomarkers or outcome measures (21/27 studies) (see Supplementary Material S2).

Table 2. Clinician-reported outcome measures.

Outcome Measure	Studies [1]	Control Group (Patient vs. Control Difference) [2]	Longitudinal (Disease Progression) [3]	Intervention (Response to Intervention) [4]
HSP-specific CROM				
SPRS	27	12 (2)	8 (6)	5 (1)
SPATAX-EUROSPA disability score	4	1 (0)	1 (1)	1 (0)
CROM for other neurological disorders				
SARA	3	1 (1)	1 (1)	0 (0)
ALSFRS-R	2	1 (0)	2 (1)	0 (0)
Unified Huntington's Disease Rating Scale Part IV	1	1 (0)	0 (0)	0 (0)
Multiple Sclerosis Impairment Scale	1	0 (0)	0 (0)	1 (0)
Generic CROM				
Functional questionnaire score	1	0 (0)	0 (0)	1 (0)
Modified Rankin Scale	1	1 (0)	1 (0)	0 (0)
Disability Score (DIS)	1	0 (0)	0 (0)	1 (0)
Functional Independence Measure (FIM)	1	0 (0)	0 (0)	0 (0)

[1] Total number of studies; [2] Number of studies that included a control group (Studies where outcome measure showed a difference between patients and controls); [3] Number of studies that had longitudinal data (studies where outcome measure was able to show disease progression); [4] Number of studies that included an intervention (Studies where outcome measure showed a response to the intervention). Details of the included studies are in Supplementary Material S2.

The SPATAX-EUROSPA disability score, another HSP-specific CROM, was less commonly used than the SPRS (Table 2). There were no control data and change over time was studied in only one study showing disease progression in 3/31 patients included in the study [32]. It was used in one interventional study [37] and did not show any significant change with intervention.

CROMs developed for other neurological conditions and generic functional CROMs were also used to assess patients with HSP. The Scale for Assessment and Rating of Ataxia (SARA) was used in three studies and showed a difference between patient versus controls in 1/3 studies [38] and longitudinal change in 1/3 studies [39]. The Amyotrophic Lateral Sclerosis rating scale revised (ALSFRS-R) was used in two studies [40,41], showing disease progression in one but not the other, and was shown to correlate with serum and CSF neurofilament heavy chain [40,41], though one paper included patients with ALS in their analysis (Supplementary Material S2).

3.3.2. Performance Outcome Measures

PerfOMs assessing motor function were widely used in the included studies, with twenty-one different outcome measures identified. The most commonly reported motor PerfOMs were the modified Ashworth Scale (MAS) (18 studies) and the 10 m walk test (10MWT), 6 min walk test (6MWT) and their variations (14 studies) (Table 3). Compared to CROMs, motor PerfOMs were more commonly used as outcome measures for interventional studies—MAS in 13/18 interventional studies, 10MWT/6MWT/variations in 9/18 interventional studies. Conversely, motor PerfOMs were less commonly used to correlate with other outcome measures or biomarkers, 2/18 studies for MAS and 3/14 studies for 10MWT and its variations (Supplementary Materials S2). Similar to the CROMs, there were very little longitudinal data—3/18 for MAS, 1/14 for 10MWT and variations.

Table 3. Performance outcome measures.

Outcome Measure	Studies [1]	Control Group (Patient vs. Control Difference) [2]	Longitudinal (Disease Progression) [3]	Intervention (Response to Intervention) [4]
Motor Function PerfOMS				
Modified Ashworth Scale	18	2 (0)	3 (1)	13 (11)
10MWT, 6MWT, 2MWT, 5MWT, 20MWT, 3-min endurance walk	14	3 (2)	1 (1)	9 (3)
Timed Up-and-Go test (TUG)	6	3 (2)	2 (2)	3 (1)
Medical Research Council muscle strength	4	0 (0)	0 (0)	2 (1)
Gross motor function measure (GMFM-66, GMFM-88)	2	0 (0)	0 (0)	2 (1)
Gross motor function classification score (GMFCS)	2	1 (0)	0 (0)	1 (0)
Falls Efficacy Scale-International (FES-I)	2	2 (1)	1 (0)	0 (0)
Berg balance scale	2	0 (0)	0 (0)	2 (1)
Composite cerebellar functional severity score (CCFSw)	2	1 (1)	1 (0)	0 (0)
Nine-hole pegboard test, click test, writing test, tapping test	2	1 (0)	0 (0)	1 (1)
Physiological Cost Index	2	1 (0)	1 (0)	2 (0)
Four-stage functional scale of motor disability (4SMD or 4FMS)	2	0 (0)	0 (0)	0 (0)
Activities specific Balance Confidence scale (ABC)	1	0 (0)	0 (0)	1 (0)
Modified version of Gillette Functional Assessment Questionnaire	1	0 (0)	0 (0)	1 (1)
Walking Handicap scale	1	0 (0)	0 (0)	1 (1)
Lower extremity subclass of Fugl–Meyer assessment	1	0 (0)	0 (0)	1 (0)
Ambulatory score (AMB)	1	0 (0)	0 (0)	1 (0)
AMBUS	1	0 (0)	1 (0)	0 (0)
Spasm Frequency Scale	1	0 (0)	1 (1)	1 (1)
Strength with microFET 2 hand-held dynamometer	1	0 (0)	0 (0)	1 (1)

Table 3. *Cont.*

Outcome Measure	Studies [1]	Control Group (Patient vs. Control Difference) [2]	Longitudinal (Disease Progression) [3]	Intervention (Response to Intervention) [4]
Motor Function PerfOMS				
Walking ability (landmarks of disability)	1	0 (0)	0 (0)	0 (0)
Cognitive Function PerfOMs				
Montreal Cognitive Assessment	4	4 (0)	1 (0)	0 (0)
Wechsler Adult Intelligence Scale—revised	3	1 (0)	1 (1)	0 (0)
Mini-Mental State Exam	2	2 (0)	0 (0)	0 (0)
Addenbrooke's Cognitive Exam	1	1 (0)	1 (0)	0 (0)
CANTAB assessment	1	1 (0)	0 (0)	0 (0)
VLMT, FWIT, TMT A/B, FAB, d2-R, RWT *	1	0 (0)	1 (1)	0 (0)

[1] Total number of studies; [2] Number of studies that included a control group (Studies where outcome measure showed a difference between patients and controls); [3] Studies that had longitudinal data (studies where outcome measure was able to show disease progression); [4] Studies that included an intervention (Studies where outcome measure showed a response to the intervention); * VLMT—Verbal Learning and Memory Test, FWIT—Farbe-Wort-Interferenz Test, TMT A/B—Trail Making Test Part A and B, FAB—Frontal Assessment Battery, d2-R—revised d2 Test of attention, RWT—Regensburg Word Fluency Test. Details of the included studies are in Supplementary Material S2.

PerfOMs measuring cognition were used in ten studies for descriptive purposes only rather than to measure outcomes in interventional studies.

3.3.3. Patient Reported Outcome Measures

Most PROMs used assessed quality of life (12/17), while others assessed fatigue, pain, and autonomic symptoms (Table 4). The most used PROM was the Short Form Health Survey-36 (SF-36) and its derivative, SF-12, in seven studies, followed by EuroQoL-5 Dimensions (EQ-5D) in three studies. PROMs were not commonly used in interventional studies (5/18 interventional studies). There was no longitudinal data for PROMs in the included studies. The SF-36 and its derivatives, EQ-5D, Becks Depression Inventory (BDI-V), Zung depression score, Brief Pain inventory, Modified Fatigue Impact Scale and multidimensional fatigue inventory showed differences between patients and controls in some but not all studies.

Table 4. Patient-Reported Outcome Measures.

Outcome Measure	Studies [1]	Control Group (Patient vs. Control Difference) [2]	Longitudinal (Disease Progression) [3]	Intervention (Response to Intervention) [4]
Quality of Life PROMs				
SF-36, SF-12, RAND 36-Item Health Survey	7	3 (2)	1 (0)	2 (1)
EQ-5D	3	3 (1)	0 (0)	0 (0)
Becks Depression Inventory (BDI-V)	2	2 (1)	0 (0)	0 (0)
Visual analogue score	2	0 (0)	0 (0)	2 (1)
Patient Health Questionnaire (PHQ-9)	1	1 (0)	0 (0)	0 (0)
Modified Goal Attainment Scale (mGAS)	1	0 (0)	0 (0)	1 (1)
International Consultation of Incontinence Questionnaire (ICIQ)—LUTSqol	1	0 (0)	0 (0)	0 (0)

Table 4. *Cont.*

Outcome Measure	Studies [1]	Control Group (Patient vs. Control Difference) [2]	Longitudinal (Disease Progression) [3]	Intervention (Response to Intervention) [4]
Cerebral Palsy QoL questionnaire (CPQoL)	1	0 (0)	0 (0)	1 (1)
Caregiver Priorities and Child Health Index of Life with Disabilities (CPCHILD)	1	0 (0)	0 (0)	0 (0)
Zung depression score	1	1 (1)	0 (0)	0 (0)
ICIQ-Short Form	1	0 (0)	0 (0)	0 (0)
Hospital Anxiety and Depression Scale	1	0 (0)	0 (0)	1 (1)
Other PROMs				
Brief pain inventory	3	2 (1)	0 (0)	1 (0)
Modified Fatigue Impact Scale (MFI)	2	1 (1)	0 (0)	1 (0)
Multidimensional fatigue inventory	1	1 (0)	0 (0)	0 (0)
Scale for Outcomes in Parkinson's Disease for Autonomic Symptoms (SCOPA-AUT)	1	0 (0)	0 (0)	0 (0)
Numeric rating scale for pain	1	0 (0)	0 (0)	1 (1)

[1] Total number of studies; [2] Number of studies that included a control group (Studies where outcome measure showed a difference between patients and controls); [3] Number of studies that had longitudinal data (Studies where outcome measure was able to show disease progression); [4] studies that included an intervention (Studies where outcome measure showed a response to the intervention). Details of the included studies are in Supplementary Material S2.

3.4. Biomarkers

3.4.1. Laboratory-Based Biomarkers

Serum and cerebrospinal fluid (CSF) neurofilament light chain (NfL) levels, and serum and CSF 25- and 27-hydroxycholesterol (25- and 27-OHC) levels were the two most studied biochemical biomarkers—included in six and four studies, respectively (Table 5). Serum and CSF NfL levels were able to differentiate patient vs. control groups or historical control values in all studies. Serum and CSF NfL levels were shown to correlate with SPRS scores in two studies [29,42] but this correlation was not present in the other two studies [32,43] (Supplementary Material S2). Plasma and CSF 25- and 27-OHC were significantly elevated in individuals with HSP-*CYP7B1* (SPG5) compared to controls or reported normative values in all four studies [35,43–45]. In the few longitudinal studies, there was no change over time in NfL levels [32] or 25- and 27-OHC levels [35,44]. NfL levels were not used as outcome measures in any interventional studies; however, 25- and 27-OHC levels showed a response to treatment with statins in interventional studies [35,44]. Other biochemical markers reported, such as lipidomics, amino acid levels, mitochondrial DNA levels and cell morphomics, were more commonly used as diagnostic biomarkers rather than to measure disease progression or response to the intervention.

3.4.2. Neuroimaging Biomarkers

Magnetic resonance imaging (MRI) of the brain and spine, with or without volumetric analysis, was used in seventeen studies, diffusion tensor imaging (DTI) in seven studies and magnetic resonance spectroscopy (MRS) in four studies (Table 6). MRI brain and spine differentiated patients vs. controls in six studies and showed longitudinal change in only one study [46]. No imaging parameters were used as outcome measures for interventional studies. MRI findings were found to correlate with SPRS scores in 3/4 of the studies, and DTI findings correlated with SPRS scores in 2/3 of the studies (Supplementary Materials S2).

Table 5. Biochemical Biomarkers.

Biomarker	Studies [1]	Control Group (Patient vs. Control Difference) [2]	Longitudinal (Disease Progression) [3]	Intervention (Response to Intervention) [4]
Serum and CSF NfL	6	6 (5)	1 (0)	0 (0)
Serum and CSF 25-OHC and 27-OHC	4	3 (2)	2 (0)	2 (2)
Blood(plasma) and CSF amino acids	2	1 (1)	0 (0)	1 (0)
Lipidomics: fibroblast and plasma	1	1 (1)	0 (0)	0 (0)
Citrulline	1	0 (0)	0 (0)	0 (0)
Glycosylceramide profile	1	0 (0)	0 (0)	0 (0)
Neurofilament heavy chain: CSF and serum	2	1 (1)	1 (0)	0 (0)
Autophagy-related protein (ATG9A) ratio	1	1 (1)	0 (0)	0 (0)
CSF Aβ 1–42, total tau, phospho tau	1	1 (0)	0 (0)	0 (0)
Mitochondrial DNA levels; Muscle biopsy	1	1 (1)	0 (0)	0 (0)
Cell morphomics	1	1 (1)	0 (0)	1 (1)
Scanning electron microscopy of hair shafts	1	1 (1)	0 (0)	0 (0)

[1] Total number of studies; [2] Number of studies that included a control group (Studies where outcome measure showed a difference between patients and controls); [3] Studies that had longitudinal data (Studies where outcome measure was able to show disease progression); [4] Studies that included an intervention (Studies where outcome measure showed a response to the intervention). Details of the included studies are in Supplementary Material S2.

Table 6. Neuroimaging Biomarkers.

Biomarker	Studies [1]	Control Group (Patient vs. Control Difference) [2]	Longitudinal (Disease Progression) [3]	Intervention (Response to Intervention) [4]
MRI brain and spine	17	8 (5)	4 (1)	1 (0)
DTI	7	7 (7)	2 (1)	0 (0)
MRS	4	3 (2)	1 (0)	0 (0)
Ioflupane Single Photon Emission Computed Tomography (SPECT)	1	0 (0)	0 (0)	0 (0)

[1] Total number of studies; [2] Number of studies that included a control group (Studies where outcome measure showed a difference between patients and controls); [3] Studies that had longitudinal data (studies where outcome measure was able to show disease progression); [4] Studies that included an intervention (Studies where outcome measure showed a response to the intervention). Details of the included studies are in Supplementary Material S2.

3.4.3. Neurophysiology Biomarkers

Motor evoked potentials (MEPs) and nerve conduction studies/electromyography (NCS/EMG) were the most widely used neurophysiological markers, being used in ten and nine studies, respectively (Table 7). Although MEPs were used as outcome measures in three interventional studies, no changes in MEP results were demonstrated in response to any of the interventions. NCS/EMG, somatosensory evoked potentials (SSEPs), visual evoked potentials (VEPs) and brainstem auditory evoked potentials (BAEPs) were mostly used as descriptive measures with few studies using these measures to compare patients versus controls, study longitudinal cohorts, or to evaluate interventions.

3.4.4. Other Biomarkers

We identified eight studies that utilized gait analysis—seven were laboratory-based, and one was a mobile system [34] (Table 8). Four studies described the use of an infrared multi-camera motion analysis system [28,47–49], one used pressure sensors [50], and another two did not provide details of the gait analysis system used [51,52]

(Supplementary Material S1). Four interventional studies used gait analysis as an outcome measure, with only two of those studies showing a response to the intervention [49,50]. Gait analysis was able to differentiate patients with HSP from patients with spastic diplegia [47], healthy controls [34,48] and pre-symptomatic HSP-*SPAST* carriers [28]. Gait parameters also correlate with SPRS scores [28,34] (Supplementary Table S2).

Table 7. Neurophysiology Biomarkers.

Biomarker	Studies [1]	Control Group (Patient vs. Control Difference) [2]	Longitudinal (Disease Progression) [3]	Intervention (Response to Intervention) [4]
MEPs	10	2 (1)	2 (0)	3 (0)
NCS/EMG	9	2 (0)	2 (0)	1 (0)
SSEP	5	1 (1)	0 (0)	0 (0)
VEP	2	0 (0)	0 (0)	0 (0)
BAEP	1	0 (0)	0 (0)	0 (0)

[1] Total number of studies; [2] Number of studies that included a control group (Studies where outcome measure showed a difference between patients and controls); [3] Studies that had longitudinal data (Studies where outcome measure was able to show disease progression); [4] Studies that included an intervention (Studies where outcome measure showed a response to the intervention). Details of the included studies are in Supplementary Material S2.

Table 8. Other Biomarkers.

Biomarker	Studies [1]	Control Group (Patient vs. Control Difference) [2]	Longitudinal (Disease Progression) [3]	Intervention (Response to Intervention) [4]
Laboratory and mobile gait analysis	8	4 (4)	1 (1)	4 (2)
Spectral-domain optical coherence tomography (SD-OCT)	4	0 (0)	2 (0)	0 (0)
Video-oculography and rotational chair testing	1	1 (1)	0 (0)	0 (0)
Goniometer	2	0 (0)	0 (0)	1 (1)
Instrumented dynamic balance assessment	1	0 (0)	0 (0)	1 (1)
Urodynamic assessment	1	0 (0)	0 (0)	0 (0)
Video supported posturography	1	0 (0)	1 (0)	0 (0)
Protocol for Evaluation of Acquired Speech Disorders (PADAF)	1	1 (1)	0 (0)	0 (0)

[1] Total number of studies; [2] Number of studies that included a control group (Studies where outcome measure showed a difference between patients and controls); [3] Studies that had longitudinal data (Studies where outcome measure was able to show disease progression); [4] Studies that included an intervention (Studies where outcome measure showed a response to the intervention). Details of the included studies are in Supplementary Material S2.

SD-OCT was investigated in four studies, with one study showing statistically significant retinal nerve fiber layer (RNFL) thinning in patients compared to normative values but no change over time (Table 8).

Video-oculography and rotational chair testing and speech assessment showed differences between patients vs. controls [53,54].

3.4.5. Genotype-Specific Biomarkers in HSP

We identified biomarkers that were specific to particular HSP genotypes (Table 9). The most widely studied were serum and CSF oxysterols, 25- and 27-hydroxycholesterol (25- and 27-OHC) levels, in HSP-*CYP7B1* or SPG5. 25- and 27-OHC levels were significantly elevated in the serum and CSF of patients compared to healthy controls. Plasma and serum oxysterol levels were used as outcome measures in two clinical trials showing reduced

levels with atorvastatin treatment but no clinical correlation [35,44]. Most genotype-specific biomarkers were reported in single studies, with some studies (citrulline, lipidomics, glycosylceramide profile, and scanning electron microscopy of hair shafts) having small patient numbers ($n \leq 5$).

Table 9. Genotype-specific biomarkers.

Biomarker	Studies	Genotype	Finding
Serum hydroxycholesterols [35,43–45]	4	HSP-*CYP7B1*	Higher 25- and 27-OHC levels in patients compared to controls
Plasma citrulline [55,56]	2	HSP-*ALDH18A1*	Low citrulline levels in patients (n = 3 and n = 4)
Lipidomics [57]	1	HSP-*PCYT2*	Accumulation of plasma phosphatidylcholine [O] etherphospholipids in patients (n = 3) compared to controls (n = 20)
Glycosylceramide profile [58]	1	HSP-*GBA2*	Elevated glycosylceramide levels in affected patient and carrier parent
ATG9A ratio (automated high-throughput imaging) [59]	1	HSP-*AP-4*	Increase in ATG9A ratio (intracellular distribution of ATG9A in trans-Golgi network compared to the remainder of the cell) in patient fibroblasts (n = 18) compared to asymptomatic carriers (n = 14)
mtDNA levels [60]	1	HSP-*SPG7*	Reduced mtDNA levels from whole blood in patients (n = 27) and carriers (n = 5) compared to controls (n = 17).
Scanning electron microscopy of hair shafts [61]	1	HSP-*FAHN*	Subtle to pronounced longitudinal grooves in hair shafts from 4/4 patients and adhesive plaques in 3/4 patients compared to controls.

3.5. Randomized Controlled Trials in HSP

We identified eight randomized controlled trials published over two decades (Supplementary Material S3). Of note, 5/8 RCTs we reviewed were not included in previously published reviews.

Six studies used a crossover design, while two studies were parallel randomized trials. Only two studies recruited patients with a single specific HSP genotype; the other six included mixed and unknown genotypes. All studies had small sample sizes (range 8–49). Two studies did not define a primary outcome measure [37,62]. Four studies showed a positive response to treatment. The outcome measures were heterogeneous, with no consistency between studies despite six studies aiming to evaluate spasticity in response to the intervention. The most common outcome measures used were the 10MWT and modified Ashworth score, with two positive studies showing a significant improvement in MAS with treatment [62,63]. Study durations were generally short—only one study had a duration of more than 6 months [64]. There was no clear association between the duration of the trial and response to the intervention.

4. Discussion

In this scoping review, we compiled a comprehensive list of outcome measures used in HSP (Figure 2) and categorized them according to construct (Tables 2–8), with information on key measurement properties. This is an important resource to inform the choice of outcome measures for future clinical trials in HSP. We identified eighty-three outcome measures highlighting the heterogeneity and inconsistency of outcome measures used. We identify a need for standardized outcome measures and recommendations for use in clinical trials, such as core outcome sets (COS) developed for other neurological conditions [19,65]. We identify common limitations of the included studies in this review (Figure 3) and list the advantages and disadvantages of commonly used outcome measures (Table 10).

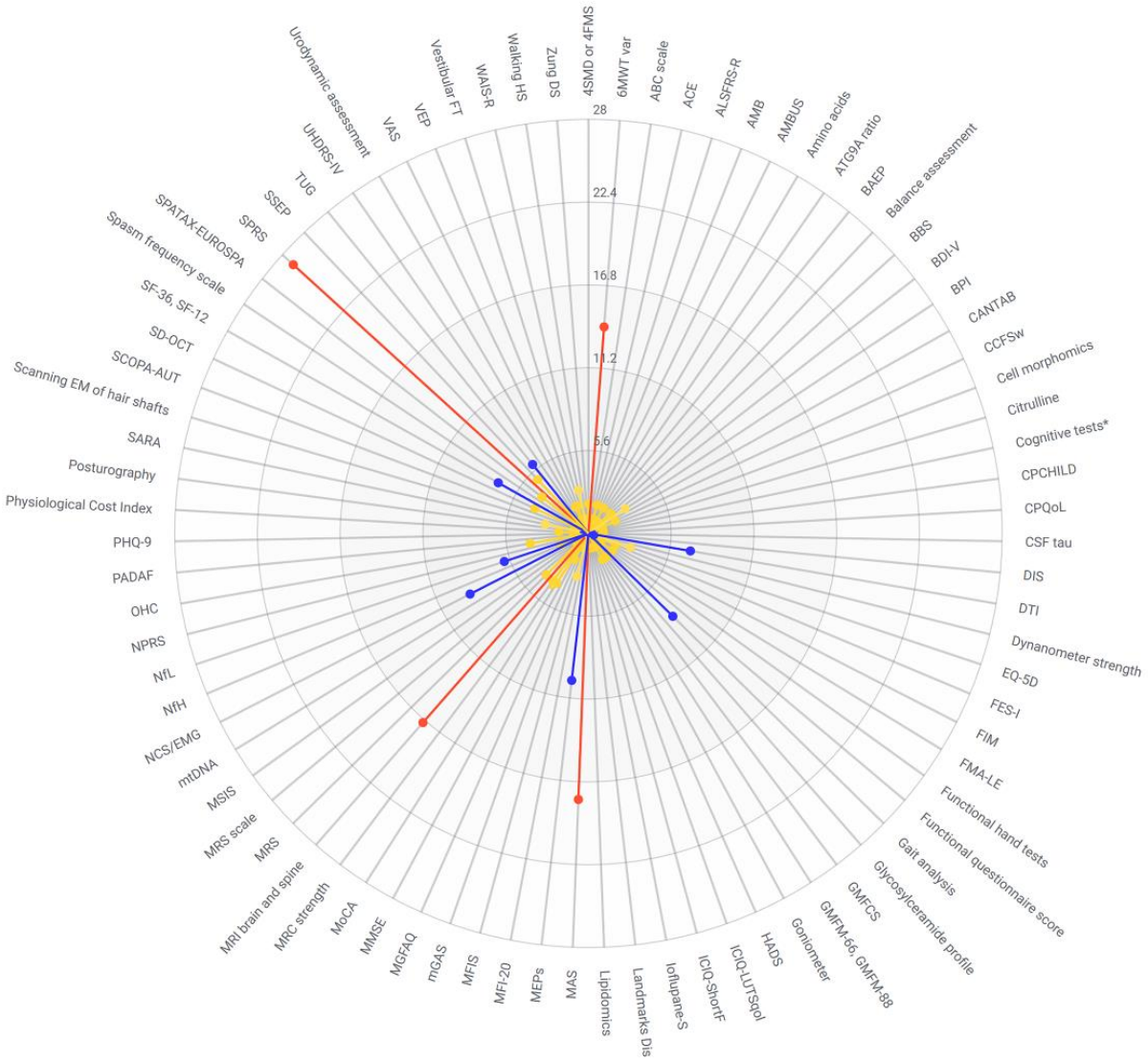


Figure 2. Outcome measures for hereditary spastic paraplegia according to frequency reported (Yellow 1–5 studies, Blue 6–10 studies, Red >10 studies). 4SMD or 4FMS—Four-stage functional scale of motor disability; 6MWT var—Six minute walk test and variations; ABC scale—Activities-specific Balance Confidence Scale; ACE—Addenbrooke's Cognitive Exam; ALSFRS-R—Amyotrophic Lateral Sclerosis Rating Scale Revised; AMB—Ambulatory score; AMBUS—Distance walked in meters walked in 5 s without help; ATG9A ratio—Autophagy-related protein 9A ratio; BAEP—brainstem auditory evoked potentials; BBS—Berg Balance Scale; BDI-V—Becks Depression Inventory; BPI—Brief Pain Inventory; CANTAB—CANTAB cognitive assessment; CCFSw—Composite Cerebellar Functional Severity Score; Cognitive tests*—Verbal Learning and Memory Test, Farbe-Wort-Interferenz Test, Trail Making Test Part A and B, Frontal Assessment Battery, revised d2 Test of attention, Regensburg Word Fluency Test; CPCHILD—Caregiver Priorities and Child Health Index of Life with Disabilities; CPQoL—Cerebral Palsy quality of life questionnaire; DIS—Disability score; DTI—diffusion tensor imaging; EQ-5D—EuroQoL 5 Dimensions; FES-I—Falls Efficacy Scale-International; FIM—Functional Independence Measure; FMA-LE—Lower extremity subclass of Fugl–Meyer assessment; GMFCS—Gross Motor Function Classification Score; GMFM—Gross Motor Function Measure; HADS—Hospital Anxiety and Depression Scale; ICIQ-LUTSqol—International Consultation of Incontinence Questionnaire lower urinary tract symptoms quality of life; ICIQ-ShortF—International Consultation of Incontinence Questionnaire Short Form; Ioflupane-S—Ioflupane-single photon emission computed tomography (SPECT); Landmarks

Dis—Landmarks of Disability; MAS—Modified Ashworth Scale; MEPs—motor evoked potentials; MFI-20—Multidimensional Fatigue Inventory; MFIS—Modified Fatigue Impact Scale; mGAS—Modified Goal Attainment Scale; MGFAQ—Modified version of Gillette Functional Assessment Questionnaire; MMSE—Mini-Mental State Exam; MoCA—Montreal Cognitive Assessment; MRC—Medical Research Council muscle strength; MRI—magnetic resonance imaging; MRS—magnetic resonance spectroscopy; MRScale—Modified Rankin Scale; MSIS—Multiple Sclerosis Impairment Scale; mtDNA—mitochondrial DNA load; NCS/EMG—nerve conduction studies/electromyography; NfH—Neurofilament heavy chain; NfL—Neurofilament light chain; NPRS—Numeric rating scale for pain; OHC—25 and 27 hydroxycholesterol; PADAF—protocol for evaluation of acquired speech disorder; PHQ-9—Patient Health Questionnaire; SARA—Scale for Assessment and Rating of Ataxia; scanning EM of hair shafts—scanning electron microscopy of hair shafts; SCOPA-AUT—Scale for Outcomes in Parkinson's Disease for Autonomic Symptoms; SD-OCT—spectral domain optical coherence tomography; SF-36, SF-12—Short Form 36, Short Form 12; SPRS—Spastic Paraplegia Rating Scale; SSEP—Somatosensory evoked potentials; TUG—Timed Up-and-Go test; UHDRS-IV—Unified Huntington's Disease Rating Scale Part IV; VAS—Visual Analogue Score; VEP—visual evoked potentials; Vestibular FT—video-oculography and rotational chair testing; WAIS-R—Wechsler Adult Intelligence Scale-revisited; Walking HS—Walking Handicap Scale; Zung DS—Zung Depression Scale.

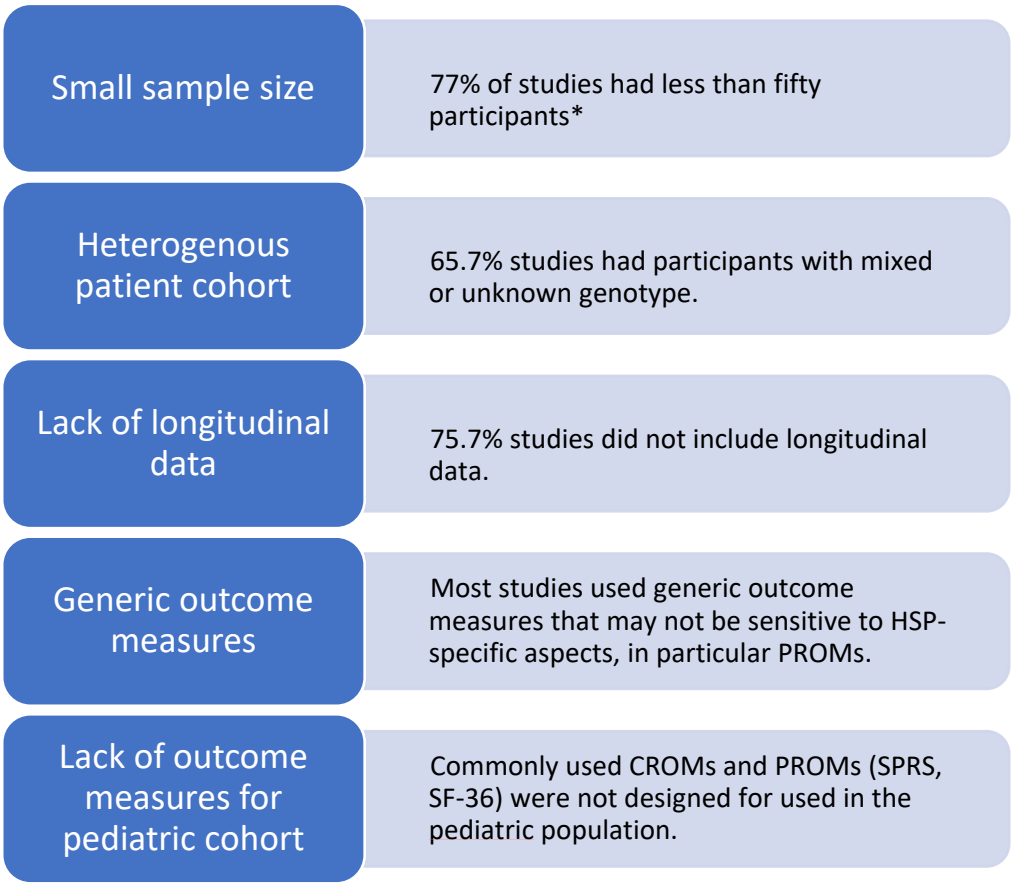


Figure 3. Common limitations of studies of HSP outcome measures. * [66] A sample size of less than fifty participants is defined as small in the COSMIN checklist. However, a smaller sample size may be justified in the study design. For example, a randomized controlled trial of 14 patients with *HSP-CYP7B1* calculated an estimate of the effect size of treatment using a pre-specified biomarker to determine the sample size required for adequate power [35].

Table 10. Advantages and disadvantages of commonly used outcome measures for hereditary spastic paraplegia.

Outcome Measure	Advantages	Disadvantages
SPRS	HSP-specific. Validated against other measures of disability. Cross-cultural validation [67,68]. Longitudinal measurement [30,33,35,69].	Not validated in a pediatric cohort. Inter-rater reliability only measured between two raters from same center [67]. Requires 10 m space and stairs to measure walking time.
SPATAX-EUROSPA disability score	HSP-specific.	Not validated. Does not measure pain, bladder or bowel function.
Modified Ashworth scale	Accessible. Moderate reliability [70].	Potential for inter-rater variation particularly for lower limb assessment [70]. Variation depends on when test is performed (e.g., the timing of anti-spasticity medication).
6MWT, 10MWT, TUG and variations	Accessible.	Not suitable for patients who are unable to mobilize. Potential variation depending on hallway lengths used due to time taken to change directions [71].
SF-36	Validated [72]. Published population norms [73]. Some versions easily accessible. Cross-cultural validity [73,74]. Demonstrated worse QoL in patients with HSP compared to controls [74,75].	Generic and does not address HSP-specific aspects of QoL.
EQ-5D	Validated [76]. Easily accessible. Cross-cultural validity [76]. Published population norms.	Generic and does not address HSP-specific aspects of QoL. Not as widely used as SF-36 in HSP population.
Serum and CSF neurofilament light chain (NfL)	Able to distinguish patients vs. controls, pre-symptomatic and symptomatic patients [29,77,78].	Non-specific, elevated in other conditions such as ALS, Alzheimer's dementia, Parkinson's Disease [79]. CSF collection requires an invasive procedure with potential adverse effects, such as a low-pressure headache.
27 and 25 hydroxycholesterol (OHC)	Specific to SPG5 (HSP-*CYP7B1*). Decrease in serum and plasma 27-OHC levels in response to atorvastatin [35,44]. Good diagnostic biomarker.	Biochemical response to treatment not reflected in clinical benefit as measured with SPRS. Role as prognostic or monitoring biomarker yet to be determined.
MRI brain and spine	Accessible. Volumetric analysis showed atrophy in certain parts of the brain or spine in patients with HSP [29,31,43,80].	Heterogenous findings between and within genotypes [81].
DTI	Abnormal in patients vs. controls (see results section). Correlate with other outcome measures [82–84]. Imaging can be performed with most MRI machines. Able to identify axonal damage not seen on MRI.	No longitudinal data. Requires analysis by experienced personnel.
MEPs	Able to measure upper motor neuron abnormalities seen in HSP [85]. Lower limb CMCT abnormal in 78% patients with HSP [86].	Inconsistent findings across various studies [86]. Requires specialized equipment and technical expertise. Some patients may find MEP studies uncomfortable.

Table 10. *Cont.*

Outcome Measure	Advantages	Disadvantages
NCS/EMG	Majority of patients with HSP-*SPG11* have axonal neuropathy [31,33].	Inconsistent findings across various studies.
Gait analysis	Able to differentiate patients vs. controls (see results). Able to demonstrate response to intervention [49,50]. Correlate with SPRS scores [28,34]. Mobile gait analysis system able to show disease progression over time [87].	Require expensive equipment. Data analysis can be complex depending on parameters used. Not suitable for participants who are unable to walk.
Retinal nerve fiber layer with OCT	Abnormal in 39% of patients with HSP [88].	No change in RNFL over time. No clinical correlation with SPRS. Inconsistent findings [88].

4.1. Recommendations for Future Research

4.1.1. Choice of Outcome Measure

This scoping review is the first comprehensive reference of outcome measures available for HSP according to outcome/construct. Based on our findings, we propose that a COS include a CROM—SPRS, PROM—SF36, and an objective biomarker—DTI, gait analysis or serum/CSF NfL.

We recommend the SPRS as it was the most commonly used CROM in HSP-related studies, has undergone psychometric testing in a cohort of individuals with HSP [67], has demonstrated cross-cultural validity [68], has been tested for responsiveness in longitudinal and interventional studies [30,33,35,62,64,69,89], and is a disease-specific outcome measure. In addition, it includes an assessment of motor function, and therefore, a performance outcome measure (PerfOM) for motor function is not required. However, we note that SPRS was not designed for use in the pediatric population, and therefore, we identify a need for a validated pediatric HSP-specific CROM.

We recommend the SF-36 as the most suitable PROM as it was commonly used in HSP-related studies, is well-validated in healthy controls and other conditions [72], has cross-cultural validity in the HSP population [73,74], and correlates with disease severity as measured with the SPRS [75,90,91] and gait analysis [34]. However, we note that the SF-36 is a generic QoL measure and may not be sensitive to smaller changes in HSP-specific symptoms, such as spasticity and bladder function, that are likely key targets for intervention in future HSP trials [92]. Therefore, we identify a need for an HSP-specific QoL scale to address the need for more sensitive tools, particularly when evaluating small changes in response to treatment in a slowly progressive condition. These findings are echoed by a recent study of CROMs and PROMs in HSP, identifying the SPRS as a suitable CROM to measure disease progression and the need for an HSP-specific PROM [89].

Diffusion tensor imaging, gait analysis, and neurofilament light chain levels are promising objective biomarkers likely to be suitable for use in clinical trials. However, further studies are needed to establish the sensitivity and specificity of these biomarkers in HSP, including longitudinal studies. Biomarkers identified through knowledge of disease pathways in specific HSP genotypes can aid in the diagnosis and measurement of treatment response. Abnormal plasma and CSF 25- and 27-OH levels are seen in individuals with HSP-*CYPB1* (SPG5) and responded to treatment with statins [35,44]. More recently, two groups used different approaches to differentiate individuals with HSP-*SPAST* (SPG4) from healthy controls by analyzing peripheral blood mononuclear cells. Our group demonstrated reduced levels of acetylated α-tubulin seen on flow cytometry [93], while another group showed increased distance between cell and nucleus centroids on automated image analysis [94]. Both studies used surrogate markers of microtubule dysfunction based on the known role of spastin in regulating microtubule dynamics in HSP-*SPAST*. These

findings highlight the need for further research to identify genotype-specific biomarkers that are more likely to be sensitive and specific for particular HSP genotypes. For the evaluation of existing outcome measures or the development of new outcome measures, we recommend referring to published guidelines [19] to ensure validity and reliability. Development of a COS for HSP should be informed by published recommendations, COS-STAD, to ensure that the included outcome measures meet minimum standards [95].

4.1.2. Recommendations for Trial Design

Recommendations for HSP clinical trial design will improve the quality and consistency of reporting of evidence to strengthen conclusions drawn from clinical trials. We identify a need for larger sample sizes in HSP studies, a particular challenge when studying rare conditions. International, multi-center collaborations are a potential solution to sample size challenges and have the added benefit of cross-cultural applicability of study results. It is important for outcome measures chosen to be available in different languages, relevant in different populations, and feasible for use in low-resource settings. Biomarkers that can be collected at recruitment sites and analyzed in a central facility are ideal for multi-center trials to improve the standardization of data. Although studies of single HSP genotypes are ideal for consistency within target populations, this may not be feasible when trying to attain large sample sizes. Therefore, a grouping of HSP genotypes or other neurodegenerative disorders according to similar underlying disease pathophysiology may allow for more efficient research of targeted therapeutic agents. Clinical trials for HSP may require longer periods of treatment and follow-up to demonstrate significant treatment effects, particularly in the more slowly progressive genotypes, such as HSP-*SPAST*. Using a combination of multiple outcome measures and biomarkers can account for phenotypic variability, even within the same genotype. It may improve the chance of detecting a treatment effect signal in heterogeneous patient cohorts.

4.1.3. Study Limitations

A limitation of this study was the use of the search terms "outcome measure" and "biomarker", which inevitably missed relevant studies [19]. When planning our study design, we considered the volume of search results from individual searches for each outcome, e.g., spasticity, mobility, neuroimaging, etc. and deemed that approach unfeasible.

Systematic reviews on specific outcome measures have been published previously [81,86,96,97] and provide information on the utility of these outcome measures. Due to the nature of a scoping review, we were unable to perform qualitative analysis of each outcome measure to assess validity and reliability. Therefore, a systematic review specific to an outcome measure is required to answer this question. Our review identifies outcome measures that require further validation and provides an overview of the currently available literature surrounding the identified outcome measures.

5. Conclusions

In this scoping review, we present a critical assessment of currently available outcome measures for use in HSP clinical trials. We discuss the benefits and limitations of commonly used outcome measures and propose areas for further research. Given the emergence of multiple candidate HSP therapies in recent years [35,44,98], there is an urgent need for further development of a core set of validated and standardized outcome measures for use in HSP clinical trials to test the efficacy of these therapies.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/genes14091756/s1; Supplementary Material S1: List of the included studies in scoping review; Supplementary Material S2: Table of HSP outcome measures and biomarkers; Supplementary Material S3: Comparison of Randomized controlled trials in HSP.

Author Contributions: Conceptualization, S.-F.S., G.W., K.R.K. and C.M.S.; methodology, S.-F.S.; results screening, S.-F.S., D.Y. and K.R.K.; data extraction, S.-F.S., D.Y., L.I.R. and F.J.; data analysis, S.-F.S., D.Y. and G.W.; writing—original draft preparation, S.-F.S.; writing—review and editing, all authors. All authors have read and agreed to the published version of the manuscript.

Funding: S.-F.S. is the recipient of the National Health and Medical Research Council and Australian and New Zealand Association of Neurologists Postgraduate Scholarships APP113610.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data supporting reported materials are available in the manuscript and Supplementary Materials.

Acknowledgments: The authors would like to thank Tess Aitken, Academic Liaison Librarian at the University of Sydney, for guidance in searching the databases. K.R.K. is supported by the Paul Ainsworth Family Foundation and the Medical Research Future Fund.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

All database searches performed on 9 August 2022.

Embase search
Embase Classic <1947 to 1973>
Embase <1974 to 9 August 2022>

1. spastic paraplegia/or spastic para*.mp. 11219
2. hereditary motor sensory neuropathy/or hereditary spastic parap*.mp. 13564
3. 1 or 2 21406
4. pharmacological biomarker/or biomarker*.mp. or biological marker/ 660089
5. patient-reported outcome/or outcome assessment/or "outcome measure*".mp. or "quality of life"/ 1469067
6. 4 or 5 2095172
7. 3 and 6 1267
8. remove duplicates from 7 1256

Medline search
Ovid MEDLINE(R) ALL <1946 to 9 August 2022>

1. Spastic Paraplegia, Hereditary/or "spastic para*".mp. 6979
2. Spastic Paraplegia, Hereditary/or "hereditary spastic para*".mp. or Paraparesis, Spastic/ 2875
3. biomarker*.mp. or Biomarkers, Pharmacological/or Biomarkers/ 719216
4. "outcome measure".mp. or Outcome Assessment, Health Care/ 147938
5. "patient reported outcome measure".mp. or Patient Reported Outcome Measures/ 13303
6. 1 or 2 7101
7. 3 or 4 or 5 873824
8. 6 and 7 199

Scopus search
TITLE-ABS-KEY (("hereditary spastic parap*" OR "spastic parap*") AND (biomarker* OR "outcome measure*"))
210 results

Web of Science search
("hereditary spastic parap*" OR "spastic parap*") AND (biomarker* OR "outcome measure*")
230 results

Central Cochrane Database
EBM Reviews—Cochrane Central Register of Controlled Trials <July 2022>

1. "hereditary spastic paraplegia".mp. or Spastic Paraplegia, Hereditary/ 35

References

1. Ruano, L.; Melo, C.; Silva, M.C.; Coutinho, P. The Global Epidemiology of Hereditary Ataxia and Spastic Paraplegia: A Systematic Review of Prevalence Studies. *Neuroepidemiology* **2014**, *42*, 174–183. [CrossRef]
2. Erichsen, A.K.; Koht, J.; Stray-Pedersen, A.; Abdelnoor, M.; Tallaksen, C.M.E. Prevalence of hereditary ataxia and spastic paraplegia in southeast Norway: A population-based study. *Brain* **2009**, *132*, 1577–1588. [CrossRef] [PubMed]
3. Coutinho, P.; Ruano, L.; Loureiro, J.L.; Cruz, V.T.; Barros, J.; Tuna, A.; Barbot, C.; Guimarães, J.; Alonso, I.; Silveira, I.; et al. Hereditary ataxia and spastic paraplegia in Portugal: A population-based prevalence study. *JAMA Neurol.* **2013**, *70*, 746–755. [CrossRef]
4. Vander Stichele, G.; Durr, A.; Yoon, G.; Schüle, R.; Blackstone, C.; Esposito, G.; Buffel, C.; Oliveira, I.; Freitag, C.; van Rooijen, S.; et al. An integrated modelling methodology for estimating global incidence and prevalence of hereditary spastic paraplegia subtypes SPG4, SPG7, SPG11, and SPG15. *BMC Neurol.* **2022**, *22*, 115. [CrossRef]
5. Kerstens, H.C.J.W.; Lith, B.J.H.v.; Nijkrake, M.J.; Swart, B.J.M.d.; Bemd, L.A.C.v.d.; Smeets, R.; Klemens, F.; Warrenburg, B.P.C.v.d.; Wees, P.J.v.d.; Geurts, A.C.H. Healthcare needs, expectations, utilization, and experienced treatment effects in patients with hereditary spastic paraplegia: A web-based survey in the Netherlands. *Orphanet J. Rare Dis.* **2021**, *16*, 1–283. [CrossRef]
6. Shribman, S.; Reid, E.; Crosby, A.H.; Houlden, H.; Warner, T.T. Hereditary spastic paraplegia: From diagnosis to emerging therapeutic approaches. *Lancet Neurol.* **2019**, *18*, 1136–1146. [CrossRef] [PubMed]
7. Meyyazhagan, A.; Orlacchio, A. Hereditary Spastic Paraplegia: An Update. *Int. J. Mol. Sci.* **2022**, *23*, 1697. [CrossRef]
8. Saputra, L.; Kumar, K.R. Challenges and Controversies in the Genetic Diagnosis of Hereditary Spastic Paraplegia. *Curr. Neurol. Neurosci. Rep.* **2021**, *21*, 15. [CrossRef]
9. Harding, A.E. Classification of the hereditary ataxias and paraplegias. *Lancet* **1983**, *1*, 1151–1155. [CrossRef]
10. Méreaux, J.-L.; Banneau, G.; Papin, M.; Coarelli, G.; Valter, R.; Raymond, L.; Kol, B.; Ariste, O.; Parodi, L.; Tissier, L.; et al. Clinical and genetic spectra of 1550 index patients with hereditary spastic paraplegia. *Brain* **2022**, *145*, 1029–1037. [CrossRef]
11. Wali, G.; Sutharsan, R.; Fan, Y.; Stewart, R.; Tello Velasquez, J.; Sue, C.M.; Crane, D.I.; Mackay-Sim, A. Mechanism of impaired microtubule-dependent peroxisome trafficking and oxidative stress in SPAST-mutated cells from patients with Hereditary Spastic Paraplegia. *Sci. Rep.* **2016**, *6*, 27004. [CrossRef]
12. Denton, K.R.; Lei, L.; Grenier, J.; Rodionov, V.; Blackstone, C.; Li, X.J. Loss of Spastin Function Results in Disease-Specific Axonal Defects in Human Pluripotent Stem Cell-Based Models of Hereditary Spastic Paraplegia. *Stem Cells* **2014**, *32*, 414–423. [CrossRef]
13. Fassier, C.; Tarrade, A.; Peris, L.; Courageot, S.; Mailly, P.; Dalard, C.; Delga, S.; Roblot, N.; Lefèvre, J.; Job, D.; et al. Microtubule-targeting drugs rescue axonal swellings in cortical neurons from spastin knockout mice. *Dis. Model. Mech.* **2013**, *6*, 72–83. [CrossRef]
14. Wali, G.; Liyanage, E.; Blair, N.F.; Sutharsan, R.; Park, J.S.; Mackay-Sim, A.; Sue, C.M. Oxidative Stress-Induced Axon Fragmentation Is a Consequence of Reduced Axonal Transport in Hereditary Spastic Paraplegia SPAST Patient Neurons. *Front. Neurosci.* **2020**, *14*, 401. [CrossRef]
15. Fan, Y.; Wali, G.; Sutharsan, R.; Bellette, B.; Crane, D.I.; Sue, C.M.; Mackay-Sim, A. Low dose tubulin-binding drugs rescue peroxisome trafficking deficit in patient-derived stem cells in Hereditary Spastic Paraplegia. *Biol. Open* **2014**, *3*, 494–502. [CrossRef]
16. Halley, M.C.; Smith, H.S.; Ashley, E.A.; Goldenberg, A.J.; Tabor, H.K. A call for an integrated approach to improve efficiency, equity and sustainability in rare disease research in the United States. *Nat. Genet.* **2022**, *54*, 219–222. [CrossRef] [PubMed]
17. Bellofatto, M.; De Michele, G.; Iovino, A.; Filla, A.; Santorelli, F.M. Management of Hereditary Spastic Paraplegia: A Systematic Review of the Literature. *Front. Neurol.* **2019**, *10*, 3. [CrossRef]
18. Chan, A.-W.; Tetzlaff, J.M.; Gøtzsche, P.C.; Altman, D.G.; Mann, H.; Berlin, J.A.; Dickersin, K.; Hróbjartsson, A.; Schulz, K.F.; Parulekar, W.R.; et al. SPIRIT 2013 explanation and elaboration: Guidance for protocols of clinical trials. *BMJ* **2013**, *346*, e7586. [CrossRef]
19. Prinsen, C.A.C.; Vohra, S.; Rose, M.R.; Boers, M.; Tugwell, P.; Clarke, M.; Williamson, P.R.; Terwee, C.B. How to select outcome measurement instruments for outcomes included in a "Core Outcome Set"—A practical guideline. *Curr. Control. Trials Cardiovasc. Med.* **2016**, *17*, 449. [CrossRef]
20. Trummer, B.; Haubenberger, D.; Blackstone, C. Clinical Trial Designs and Measures in Hereditary Spastic Paraplegias. *Front. Neurol.* **2018**, *9*, 1017. [CrossRef]
21. Williamson, P.R.; Altman, D.G.; Blazeby, J.M.; Clarke, M.; Devane, D.; Gargon, E.; Tugwell, P. Developing core outcome sets for clinical trials: Issues to consider. *Curr. Control. Trials Cardiovasc. Med.* **2012**, *13*, 132. [CrossRef] [PubMed]
22. Peters, M.D.J.; Godfrey, C.M.; Khalil, H.; McInerney, P.; Parker, D.; Soares, C.B. Guidance for conducting systematic scoping reviews. *Int. J. Evid. Based Healthc.* **2015**, *13*, 141–146. [CrossRef]
23. Peters, M.D.J.; Marnie, C.; Tricco, A.C.; Pollock, D.; Munn, Z.; Alexander, L.; McInerney, P.; Godfrey, C.M.; Khalil, H. Updated methodological guidance for the conduct of scoping reviews. *JBI Evid. Synth.* **2020**, *18*, 2119–2126. [CrossRef] [PubMed]
24. Veritas Health Innovation, Melbourne Australia. Covidence Systematic Review Software. Available online: www.covidence.org. (accessed on 12 July 2023).
25. U.S. Food and Drug Administration. Clinical Outcome Assessment (COA): Frequently Asked Questions. Available online: https://www.fda.gov/about-fda/clinical-outcome-assessment-coa-frequently-asked-questions#COADefinition (accessed on 2 June 2023).

26. Group OCfE-BMLoEW. The Oxford 2011 Levels of Evidence. Available online: http://www.cebm.net/index.aspx?o=56532011 (accessed on 12 July 2023).
27. Page, M.J.; McKenzie, J.E.; Bossuyt, P.M.; Boutron, I.; Hoffmann, T.C.; Mulrow, C.D.; Shamseer, L.; Tetzlaff, J.M.; Akl, E.A.; Brennan, S.E.; et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ* **2021**, *372*, n71. [CrossRef] [PubMed]
28. Lassmann, C.; Ilg, W.; Schneider, M.; Volker, M.; Haeufle, D.F.B.; Schule, R.; Giese, M.; Schols, L.; Rattay, T.W. Specific gait changes in prodromal hereditary spastic paraplegia type 4—preSPG4 study. *Medrxiv* **2022**, *21*. [CrossRef]
29. Rattay, T.W.; Volker, M.; Rautenberg, M.; Kessler, C.; Wurster, I.; Winter, N.; Haack, T.B.; Lindig, T.; Hengel, H.; Synofzik, M.; et al. The prodromal phase of hereditary spastic paraplegia type 4: The preSPG4 cohort study. *Brain* **2023**, *146*, 1093–1102. [CrossRef] [PubMed]
30. Cubillos-Arcila, D.M.; Machado, G.D.; Sehnem, L.; Burguez, D.; Zanardi, A.P.J.; Martins, V.F.; Peyre-Tartaruga, L.A.; Saute, J.A.M. Progression of Functional Gait in Hereditary Spastic Paraplegias. *Cerebellum* **2022**, *21*, 350–357. [CrossRef] [PubMed]
31. Faber, I.; Martinez, A.R.M.; de Rezende, T.J.R.; Martins, C.R.; Martins, M.P.; Lourenço, C.M.; Marques, W.; Montecchiani, C.; Orlacchio, A.; Pedroso, J.L.; et al. SPG11 mutations cause widespread white matter and basal ganglia abnormalities, but restricted cortical damage. *NeuroImage Clin.* **2018**, *19*, 848–857. [CrossRef]
32. Kessler, C.; Serna-Higuita, L.M.; Wilke, C.; Rattay, T.W.; Hengel, H.; Reichbauer, J.; Stransky, E.; Leyva-Gutierrez, A.; Mengel, D.; Synofzik, M.; et al. Characteristics of serum neurofilament light chain as a biomarker in hereditary spastic paraplegia type 4. *Ann. Clin. Transl. Neurol.* **2022**, *9*, 326–338. [CrossRef]
33. Musacchio, T.; Nohl, K.; Boelmans, K.; Maltese, V.; Zeller, D.; Isaias, I.; Volkmann, J.; Klebe, S. Biological course and natural history of hereditary spastic paraplegia type 11 (SPG11). *Mov. Disord.* **2018**, *33* (Suppl. 2), S225.
34. Regensburger, M.; Spatz, I.T.; Ollenschlager, M.; Martindale, C.F.; Lindeburg, P.; Kohl, Z.; Eskofier, B.; Klucken, J.; Schule, R.; Klebe, S.; et al. Inertial Gait Sensors to Measure Mobility and Functioning in Hereditary Spastic Paraplegia: A Cross-Sectional Multicenter Clinical Study. *Neurology* **2022**, *99*, e1079–e1089. [CrossRef] [PubMed]
35. Schols, L.; Rattay, T.W.; Martus, P.; Meisner, C.; Baets, J.; Fischer, I.; Jagle, C.; Fraidakis, M.J.; Martinuzzi, A.; Saute, J.A.; et al. Hereditary spastic paraplegia type 5: Natural history, biomarkers and a randomized controlled trial. *Brain* **2017**, *140*, 3112–3127. [CrossRef] [PubMed]
36. Paparella, G.; Vavla, M.; Bernardi, L.; Girardi, G.; Stefan, C.; Martinuzzi, A. Efficacy of a Combined Treatment of Botulinum Toxin and Intensive Physiotherapy in Hereditary Spastic Paraplegia. *Front. Neurosci.* **2020**, *14*, 111. [CrossRef] [PubMed]
37. Scheuer, K.H.; Svenstrup, K.; Jennum, P.; Rogvi-Hansen, B.; Werdelin, L.; Fenger, K.; Nielsen, J.E. Double-blind crossover trial of gabapentin in SPG4-linked hereditary spastic paraplegia. *Eur. J. Neurol.* **2007**, *14*, 663–666. [CrossRef]
38. Du Montcel, S.T.; Charles, P.; Ribai, P.; Goizet, C.; Le Bayon, A.; Labauge, P.; Guyant-Marechal, L.; Forlani, S.; Jauffret, C.; Vandenberghe, N.; et al. Composite cerebellar functional severity score: Validation of a quantitative score of cerebellar impairment. *Brain* **2008**, *131*, 1352–1361. [CrossRef]
39. Du Montcel, S.T.; Charles, P.; Goizet, C.; Marelli, C.; Ribai, P.; Vincitorio, C.; Anheim, M.; Guyant-Marechal, L.; Le Bayon, A.; Vandenberghe, N.; et al. Factors influencing disease progression in autosomal dominant cerebellar ataxia and spastic paraplegia. *Arch. Neurol.* **2012**, *69*, 500–508. [CrossRef]
40. Simonini, C.; Zucchi, E.; Bedin, R.; Martinelli, I.; Gianferrari, G.; Fini, N.; Sorau, G.; Liguori, R.; Vacchiano, V.; Mandrioli, J. CSF Heavy Neurofilament May Discriminate and Predict Motor Neuron Diseases with Upper Motor Neuron Involvement. *Biomedicines* **2021**, *9*, 1623. [CrossRef]
41. Zucchi, E.; Bedin, R.; Fasano, A.; Fini, N.; Gessani, A.; Vinceti, M.; Mandrioli, J. Cerebrospinal Fluid Neurofilaments May Discriminate Upper Motor Neuron Syndromes: A Pilot Study. *Neurodegener. Dis.* **2018**, *18*, 255–261. [CrossRef]
42. Wilke, C.; Rattay, T.W.; Hengel, H.; Zimmermann, M.; Brockmann, K.; Schols, L.; Jens, K.A.; Schule, R.; Synofzik, M. Serum neurofilament light chain is increased in hereditary spastic paraplegias. *Ann. Clin. Transl. Neurol.* **2018**, *5*, 876–882. [CrossRef]
43. Lin, Q.; Liu, Y.; Ye, Z.; Hu, J.; Cai, W.; Weng, Q.; Chen, W.J.; Wang, N.; Cao, D.; Lin, Y.; et al. Potential markers for sample size estimations in hereditary spastic paraplegia type 5. *Orphanet J. Rare Dis.* **2021**, *16*, 1–9. [CrossRef]
44. Marelli, C.; Lamari, F.; Rainteau, D.; Lafourcade, A.; Banneau, G.; Humbert, L.; Monin, M.L.; Petit, E.; Debs, R.; Castelnovo, G.; et al. Plasma oxysterols: Biomarkers for diagnosis and treatment in spastic paraplegia type 5. *Brain* **2018**, *141*, 72–84. [CrossRef] [PubMed]
45. Prestsaeter, S.; Koht, J.; Lamari, F.; Tallaksen, C.M.E.; Hoven, S.T.J.; Vigeland, M.D.; Selmer, K.K.; Rydning, S.L. Elevated hydroxycholesterols in Norwegian patients with hereditary spastic paraplegia SPG5. *J. Neurol. Sci.* **2020**, *419*, 117211. [CrossRef] [PubMed]
46. Sarret, C.; Lemaire, J.J.; Tonduti, D.; Sontheimer, A.; Coste, J.; Pereira, B.; Feschet, F.; Roche, B.; Boespflug-Tanguy, O. Time-course of myelination and atrophy on cerebral imaging in 35 patients with PLP1-related disorders. *Dev. Med. Child Neurol.* **2016**, *58*, 706–713. [CrossRef] [PubMed]
47. Bonnefoy-Mazure, A.; Turcot, K.; Kaelin, A.; De Coulon, G.; Armand, S. Full body gait analysis may improve diagnostic discrimination between hereditary spastic paraplegia and spastic diplegia: A preliminary study. *Res. Dev. Disabil.* **2013**, *34*, 495–504. [CrossRef]
48. Adair, B.; Rodda, J.; McGinley, J.L.; Graham, H.K.; Morris, M.E. Kinematic gait deficits at the trunk and pelvis: Characteristic features in children with hereditary spastic paraplegia. *Dev. Med. Child Neurol.* **2016**, *58*, 829–835. [CrossRef]

49. Zhang, Y.; Roxburgh, R.; Huang, L.; Parsons, J.; Davies, T.C. The effect of hydrotherapy treatment on gait characteristics of hereditary spastic paraparesis patients. *Gait Posture* **2014**, *39*, 1074–1079. [CrossRef]
50. Lith, B.J.H.v.; Boer, J.J.d.; Warrenburg, B.P.C.v.d.; Weerdesteyn, V.G.M.; Geurts, A.C.H. Functional effects of botulinum toxin type a in the hip adductors and subsequent stretching in patients with hereditary spastic paraplegia. *J. Rehabil. Med.* **2019**, *51*, 434–441. [CrossRef]
51. Pointon, R.; Whelan, H.; Raza, R.; Peacock, S.; Wilsmore, C.; Mulkeen, A.; Goodden, J.; Lodh, R. The use of intrathecal baclofen for management of spasticity in hereditary spastic paraparesis: A case series. *Eur. J. Paediatr. Neurol.* **2022**, *36*, 14–18. [CrossRef]
52. Henderson, G.; De Gheldere, A.; Gilman, J.; Hewart, P.; Clements, P.; Bliss, W.; Henman, P. A service evaluation of gait correction surgery for patients with hereditary spastic paraplegia. *Gait Posture* **2019**, *73* (Suppl. 1), 251–252. [CrossRef]
53. Milenkovic, I.; Klotz, S.; Zulehner, G.; Sycha, T.; Wiest, G. Slowed vertical saccades as a hallmark of hereditary spastic paraplegia type 7. *Ann. Clin. Transl. Neurol.* **2019**, *6*, 2127–2132. [CrossRef]
54. Olchik, M.; Scudeiro, L.; Dos Santos, V.; Ayres, A.; Rech, R.; Burguez, D.; Machado, G.; Gonzalez-Salazar, C.; Padovani, M.; Franca, M.; et al. Speech biomarkers in hereditary spastic paraplegia. *Mov. Disord.* **2021**, *36* (Suppl. 1), S490. [CrossRef]
55. Marelli, C.; Badiou, S.; Genestet, S.; Larrieu, L.; Damier, P.; Camu, W.; Planes, M.; Koenig, M.; Guissart, C. Autosomal dominant SPG9: Intrafamilial variability and onset during pregnancy. *Neurol. Sci.* **2020**, *41*, 1931–1933. [CrossRef] [PubMed]
56. Coutelier, M.; Goizet, C.; Durr, A.; Habarou, F.; Morais, S.; Dionne-Laporte, A.; Tao, F.; Konop, J.; Stoll, M.; Charles, P.; et al. Alteration of ornithine metabolism leads to dominant and recessive hereditary spastic paraplegia. *Brain* **2015**, *138*, 2191–2205. [CrossRef]
57. Vaz, F.M.; McDermott, J.H.; Alders, M.; Wortmann, S.B.; Kolker, S.; Pras-Raves, M.L.; Vervaart, M.A.T.; van Lenthe, H.; Luyf, A.C.M.; Elfrink, H.L.; et al. Mutations in PCYT2 disrupt etherlipid biosynthesis and cause a complex hereditary spastic paraplegia. *Brain* **2019**, *142*, 3382–3397. [CrossRef] [PubMed]
58. Kloth, K.; Cozma, C.; Bester, M.; Gerloff, C.; Biskup, S.; Zittel, S. Dystonia as initial presentation of compound heterozygous GBA2 mutations: Expanding the phenotypic spectrum of SPG46. *Eur. J. Med. Genet.* **2020**, *63*, 5. [CrossRef]
59. Ebrahimi-Fakhari, D.; Alecu, J.E.; Brechmann, B.; Ziegler, M.; Eberhardt, K.; Jumo, H.; D'Amore, A.; Habibzadeh, P.; Faghihi, M.A.; De Bleecker, J.L.; et al. High-throughput imaging of ATG9A distribution as a diagnostic functional assay for adaptor protein complex 4-associated hereditary spastic paraplegia. *Brain Commun.* **2021**, *3*, fcab221. [CrossRef]
60. De la Casa-Fages, B.; Fernandez-Eulate, G.; Gamez, J.; Barahona-Hernando, R.; Moris, G.; Garcia-Barcina, M.; Infante, J.; Zulaica, M.; Fernandez-Pelayo, U.; Munoz-Oreja, M.; et al. Parkinsonism and spastic paraplegia type 7: Expanding the spectrum of mitochondrial Parkinsonism. *Mov. Disord.* **2019**, *34*, 1547–1561. [CrossRef]
61. Rattay, T.W.; Lindig, T.; Baets, J.; Smets, K.; Deconinck, T.; Sohn, A.S.; Hortnagel, K.; Eckstein, K.N.; Wiethoff, S.; Reichbauer, J.; et al. FAHN/SPG35: A narrow phenotypic spectrum across disease classifications. *Brain* **2019**, *142*, 1561–1572. [CrossRef]
62. Ardolino, G.; Bocci, T.; Nigro, M.; Vergari, M.; Di Fonzo, A.; Bonato, S.; Cogiamanian, F.; Cortese, F.; Cova, I.; Barbieri, S.; et al. Spinal direct current stimulation (tsDCS) in hereditary spastic paraplegias (HSP): A sham-controlled crossover study. *J. Spinal Cord. Med.* **2021**, *44*, 46–53. [CrossRef]
63. Bastani, P.B.; Kordjazi, M.; Oveisgharan, S.; Abdi, S. A Randomized Controlled Trial of the Effect of Repetitive Transcranial Magnetic Stimulation of the Motor Cortex on Lower Extremity Spasticity in Hereditary Spastic Paraplegia. *J. Clin. Neurophysiol. Off. Publ. Am. Electroencephalogr. Soc.* **2021**, *40*, 173–179. [CrossRef]
64. de Lima, F.D.; Faber, I.; Servelhere, K.R.; Bittar, M.F.R.; Martinez, A.R.M.; Piovesana, L.G.; Martins, M.P.; Martins, C.R.; Benaglia, T.; Carvalho, B.D.; et al. Randomized Trial of Botulinum Toxin Type A in Hereditary Spastic Paraplegia—The SPASTOX Trial. *Mov. Disord.* **2021**, *36*, 1654–1663. [CrossRef]
65. Mitchell, J.W.; Noble, A.; Baker, G.; Batchelor, R.; Brigo, F.; Christensen, J.; French, J.; Gil-Nagel, A.; Guekht, A.; Jette, N.; et al. Protocol for the development of an international Core Outcome Set for treatment trials in adults with epilepsy: The EPilepsy outcome Set for Effectiveness Trials Project (EPSET). *Trials* **2022**, *23*, 943. [CrossRef] [PubMed]
66. Mokkink, L.B.; de Vet, H.C.W.; Prinsen, C.A.C.; Patrick, D.L.; Alonso, J.; Bouter, L.M.; Terwee, C.B. COSMIN Risk of Bias checklist for systematic reviews of Patient-Reported Outcome Measures. *Qual. Life Res.* **2018**, *27*, 1171–1179. [CrossRef] [PubMed]
67. SchÜLe, R.; Holland-Letz, T.; Klimpe, S.; Kassubek, J.; Klopstock, T.; Mall, V.; Otto, S.; Winner, B.; Schols, L. The Spastic Paraplegia Rating Scale (SPRS): A reliable and valid measure of disease severity. *Neurology* **2006**, *67*, 430–434. [CrossRef]
68. Servelhere, K.R.; Faber, I.; Coan, A.C.; França, J.M. Translation and validation into Brazilian Portuguese of the Spastic Paraplegia Rating Scale (SPRS). *Arq. De Neuro-Psiquiatr.* **2016**, *74*, 489–494. [CrossRef]
69. Montanaro, D.; Vavla, M.; Frijia, F.; Aghakhanyan, G.; Baratto, A.; Coi, A.; Stefan, C. Multimodal MRI Longitudinal Assessment of White and Gray Matter in Different SPG Types of Hereditary Spastic Paraparesis. *Front. Neurosci.* **2020**, *14*, 325. [CrossRef]
70. Meseguer-Henarejos, A.B.; Sánchez-Meca, J.; López-Pina, J.A.; Carles-Hernández, R. Inter- and intra-rater reliability of the Modified Ashworth Scale: A systematic review and meta-analysis. *Eur. J. Phys. Rehabil. Med.* **2018**, *54*, 576–590. [CrossRef]
71. ATS statement: Guidelines for the six-minute walk test. *Am. J. Respir. Crit. Care Med.* **2002**, *166*, 111–117. [CrossRef]
72. Stewart, A.; Sherbourne, C.D.; Ware, J.E.; Hays, R.D.; Wells, K.B.; Berry, S.H.; Kamberg, C.; Nelson, E.C.; Davies, A.R.; Rogers, W.H.; et al. *Measuring Functioning and Well Being: The Medical Outcomes Study Approach*; Duke University Press: Durham, UK; London, UK, 1992.

73. Lins, L.; Carvalho, F.M. SF-36 total score as a single measure of health-related quality of life: Scoping review. *SAGE Open Med.* **2016**, *4*, 2050312116671725. [CrossRef]
74. Braschinsky, M.; Rannikmae, K.; Krikmann, U.; Luus, S.M.; Raidvee, A.; Gross-Paju, K.; Haldre, S. Health-related quality of life in patients with hereditary spastic paraplegia in Estonia. *Spinal Cord.* **2011**, *49*, 175–181. [CrossRef]
75. Gassner, H.; List, J.; Martindale, C.F.; Regensburger, M.; Klucken, J.; Winkler, J.; Kohl, Z. Functional gait measures correlate to fear of falling, and quality of life in patients with Hereditary Spastic Paraplegia: A cross-sectional study. *Clin. Neurol. Neurosurg.* **2021**, *209*, 106888. [CrossRef] [PubMed]
76. Rabin, R.; de Charro, F. EQ-5D: A measure of health status from the EuroQol Group. *Ann. Med.* **2001**, *33*, 337–343. [CrossRef] [PubMed]
77. Kessler, C.; Serna-Higuita, L.M.; Rattay, T.W.; Maetzler, W.; Wurster, I.; Hayer, S.; Wilke, C.; Hengel, H.; Reichbauer, J.; Armbruster, M.; et al. Neurofilament light chain is a cerebrospinal fluid biomarker in hereditary spastic paraplegia. *Ann. Clin. Transl. Neurol.* **2021**, *8*, 1122–1131. [CrossRef]
78. Alecu, J.E.; Saffari, A.; Ziegler, M.; Jordan, C.; Tam, A.; Kim, S.; Leung, E.; Szczaluba, K.; Mierzewska, H.; King, S.D.; et al. Plasma Neurofilament Light Chain Is Elevated in Adaptor Protein Complex 4-Related Hereditary Spastic Paraplegia. *Mov. Disord.* **2023**. *online ahead of print*. [CrossRef] [PubMed]
79. Gaiottino, J.; Norgren, N.; Dobson, R.; Topping, J.; Nissim, A.; Malaspina, A.; Bestwick, J.P.; Monsch, A.U.; Regeniter, A.; Lindberg, R.L.; et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. *PLoS ONE* **2013**, *8*, e75091. [CrossRef]
80. Navas-Sanchez, F.J.; Fernandez-Pena, A.; de Blas, D.M.; Aleman-Gomez, Y.; Marcos-Vidal, L.; Guzman-de-Villoria, J.A.; Fernandez-Garcia, P.; Romero, J.; Catalina, I.; Lillo, L.; et al. Thalamic atrophy in patients with pure hereditary spastic paraplegia type 4. *J. Neurol.* **2021**, *268*, 2429–2440. [CrossRef]
81. Mishra, V.; Guo, X.; Delgado, M.R.; Huang, H. Toward tract-specific fractional anisotropy (TSFA) at crossing-fiber regions with clinical diffusion MRI. *Magn. Reson. Med.* **2015**, *74*, 1768–1779. [CrossRef]
82. Rezende, T.J.R.; de Albuquerque, M.; Lamas, G.M.; Martinez, A.R.M.; Campos, B.M.; Casseb, R.F.; Silva, C.B.; Branco, L.M.T.; D'Abreu, A.; Lopes-Cendes, I.; et al. Multimodal MRI-based study in patients with SPG4 mutations. *PLoS ONE* **2015**, *10*, e0117666. [CrossRef]
83. Martinuzzi, A.; Montanaro, D.; Vavla, M.; Paparella, G.; Bonanni, P.; Musumeci, O.; Brighina, E.; Hlavata, H.; Rossi, G.; Aghakhanyan, G.; et al. Clinical and Paraclinical Indicators of Motor System Impairment in Hereditary Spastic Paraplegia: A Pilot Study. *PLoS ONE* **2016**, *11*, e0153283. [CrossRef]
84. Groppa, S.; Oliviero, A.; Eisen, A.; Quartarone, A.; Cohen, L.G.; Mall, V.; Kaelin-Lang, A.; Mima, T.; Rossi, S.; Thickbroom, G.W.; et al. A practical guide to diagnostic transcranial magnetic stimulation: Report of an IFCN committee. *Clin. Neurophysiol.* **2012**, *123*, 858–882. [CrossRef]
85. Siow, S.-F.; Smail, R.C.; Ng, K.; Kumar, K.R.; Sue, C.M. Motor Evoked Potentials in Hereditary Spastic Paraplegia—A Systematic Review. *Front. Neurol.* **2019**, *10*, 967. [CrossRef]
86. Loris, E.; Ollenschläger, M.; Greinwalder, T.; Eskofier, B.; Winkler, J.; Gaßner, H.; Regensburger, M. Mobile digital gait analysis objectively measures progression in hereditary spastic paraplegia. *Ann. Clin. Transl. Neurol.* **2023**, *10*, 447–452. [CrossRef]
87. Vavla, M.; Paparella, G.; Papayannis, A.; Pascuzzo, R.; Girardi, G.; Pellegrini, F.; Capello, G.; Prosdocimo, G.; Martinuzzi, A. Optical Coherence Tomography in a Cohort of Genetically Defined Hereditary Spastic Paraplegia: A Brief Research Report. *Front. Neurol.* **2019**, *10*, 1193. [CrossRef] [PubMed]
88. Amprosi, M.; Indelicato, E.; Eigentler, A.; Fritz, J.; Nachbauer, W.; Boesch, S. Toward the Definition of Patient-Reported Outcome Measurements in Hereditary Spastic Paraplegia. *Neurol. Genet.* **2023**, *9*, e200052. [CrossRef] [PubMed]
89. Klimpe, S.; Schule, R.; Kassubek, J.; Otto, S.; Kohl, Z.; Klebe, S.; Klopstock, T.; Ratzka, S.; Karle, K.; Schols, L. Disease severity affects quality of life of hereditary spastic paraplegia patients. *Eur. J. Neurol.* **2012**, *19*, 168–171. [CrossRef] [PubMed]
90. Orsucci, D.; Petrucci, L.; Caldarazzo Ienco, E.; Chico, L.; Simi, P.; Fogli, A.; Baldinotti, F.; Simoncini, C.; Logerfo, A.; Carlesi, C.; et al. Hereditary spastic paraparesis in adults. A clinical and genetic perspective from Tuscany. *Clin. Neurol. Neurosurg.* **2014**, *120*, 14–19. [CrossRef] [PubMed]
91. Patrick, D.L.; Deyo, R.A. Generic and Disease-Specific Measures in Assessing Health Status and Quality of Life. *Med. Care* **1989**, *27*, S217–S232. [CrossRef]
92. Wali, G.; Siow, S.F.; Liyanage, E.; Kumar, K.R.; Mackay-Sim, A.; Sue, C.M. Reduced acetylated α-tubulin in SPAST hereditary spastic paraplegia patient PBMCs. *Front Neurosci.* **2023**, *17*, 1073516. [CrossRef]
93. Sardina, F.; Valente, D.; Fattorini, G.; Cioffi, E.; Zanna, G.D.; Tessa, A.; Trisciuoglio, D.; Soddu, S.; Santorelli, F.M.; Casali, C.; et al. New cellular imaging-based method to distinguish the SPG4 subtype of hereditary spastic paraplegia. *Eur. J. Neurol.* **2023**, *30*, 1734–1744. [CrossRef]
94. Kirkham, J.J.; Davis, K.; Altman, D.G.; Blazeby, J.M.; Clarke, M.; Tunis, S.; Williamson, P.R. Core Outcome Set-STAndards for Development: The COS-STAD recommendations. *PLoS Med.* **2017**, *14*, e1002447. [CrossRef]
95. da Graça, F.F.; de Rezende, T.J.R.; Vasconcellos, L.F.R.; Pedroso, J.L.; Barsottini, O.G.P.; França, J.M.C. Neuroimaging in Hereditary Spastic Paraplegias: Current Use and Future Perspectives. *Front. Neurol.* **2019**, *9*, 1117. [CrossRef]

96. Vavla, M.; Montanaro, D.; Pizzighello, S.; Frijia, F.; Arrigoni, F.; Baratto, A.; Piccoli, G.; Paparella, G.; Martinuzzi, A. Brain Magnetic Spectroscopy Imaging and Hereditary Spastic Paraplegia: A Focused Systematic Review on Current Landmarks and Future Perspectives. *Front. Neurol.* **2020**, *11*, 515. [CrossRef]
97. de Freitas, J.L.; Rezende Filho, F.M.; Sallum, J.M.F.; França, M.C.; Pedroso, J.L.; Barsottini, O.G.P. Ophthalmological changes in hereditary spastic paraplegia and other genetic diseases with spastic paraplegia. *J. Neurol. Sci.* **2020**, *409*, 116620. [CrossRef] [PubMed]
98. Wali, G.; Berkovsky, S.; Whiten, D.R.; Mackay-Sim, A.; Sue, C.M. Single cell morphology distinguishes genotype and drug effect in Hereditary Spastic Paraplegia. *Sci. Rep.* **2021**, *11*, 16635. [CrossRef] [PubMed]

1.4 Summary and Conclusion.

The literature review in section 1 provided an overview of HSP and discussed the need for standardised and validated outcome measures for HSP. I presented the challenges faced in developing outcome measures and performed a scoping review of pre-existing outcome measures used in HSP. The scoping review provided recommendations for choice of outcome measures in future HSP studies. I identified a need for (1) a core outcome set to standardise outcome measures across HSP clinical trials, (2) surrogate outcome measures like biomarkers that target underlying HSP disease pathophysiology, such as microtubule dysfunction in HSP-*SPAST*, and (3) an HSP-specific patient reported outcome measure (PROM). I addressed these identified needs in my thesis by (1) performing a systematic review on the utility of motor evoked potentials (MEP) as a biomarker for HSP, (2) investigating pre-existing outcome measures in a cohort of patients with HSP, (3) investigating acetylated-α tubulin as a biomarker for HSP-*SPAST*, and (4) designing an HSP-specific PROM.

1.5 Thesis Outline (Objectives by Chapter)

The aim of this thesis is to identify outcome measures and biomarkers for use in HSP clinical trials. In Chapter 2, I performed a systematic review of motor evoked potentials in HSP. I identified a need for standardisation of MEP protocols and found that MEP values were unlikely to be suitable biomarkers for HSP. To investigate pre-existing outcome measures and explore a potential core outcome set, I studied a cohort of patients with HSP with a standardised MEP protocol, generic PROM, an HSP-specific clinician-rated outcome measure, and ataxia-based clinician-rated outcome measure. I identified a need for a biomarker for HSP, and an HSP-specific PROM. In Chapter 3, I explored various methods to investigate a blood-based biomarker for HSP-*SPAST*, the most common HSP subtype. I developed and optimised a flow cytometry protocol to measure intracellular acetylated α-tubulin levels in viable peripheral blood mononuclear cells (PBMCs). I presented the results from the flow cytometry assay that showed lower acetylated α-tubulin levels in HSP-*SPAST* PBMCs compared to controls. In Chapter 4, I designed an HSP-specific PROM, the HSP Quality of Life Scale (HSPQoL). I modified a pre-existing widely used validated generic PROM, the Short Form-36, with HSP specific QoL questions. The HSP-specific questions were developed through a literature review, modified Delphi process, and cognitive interviewing. The HSPQoL was then validated in a large cohort of patients with HSP and

analysed for its psychometric properties. Finally in chapter 5, I discussed the pertinent findings from this thesis and made recommendations for future research.

1.6 References

1. Elsayed LEO, Eltazi IZ, Ahmed AE, et al. Insights into Clinical, Genetic, and Pathological Aspects of Hereditary Spastic Paraplegias: A Comprehensive Overview. *Frontiers in molecular biosciences* 2021; 8: 690899-690899. DOI: 10.3389/fmolb.2021.690899.
2. Kerstens HCJW, Lith BJHv, Nijkrake MJ, et al. Healthcare needs, expectations, utilization, and experienced treatment effects in patients with hereditary spastic paraplegia: a web-based survey in the Netherlands. *Orphanet journal of rare diseases* 2021; 16: 1-283. DOI: 10.1186/s13023-021-01915-0.
3. Bellofatto M, De Michele G, Iovino A, et al. Management of Hereditary Spastic Paraplegia: A Systematic Review of the Literature. *Frontiers in neurology* 2019; 10: 3-3. DOI: 10.3389/fneur.2019.00003.
4. Marelli C, Lamari F, Rainteau D, et al. Plasma oxysterols: Biomarkers for diagnosis and treatment in spastic paraplegia type 5. *Brain* 2018; 141(1): 72-84. DOI: https://dx.doi.org/10.1093/brain/awx297.
5. Fan Y, Wali G, Sutharsan R, et al. Low dose tubulin-binding drugs rescue peroxisome trafficking deficit in patient-derived stem cells in Hereditary Spastic Paraplegia. *Biol Open* 2014; 3: 494-502. 20140523. DOI: 10.1242/bio.20147641.
6. Trummer B, Haubenberger D and Blackstone C. Clinical Trial Designs and Measures in Hereditary Spastic Paraplegias. *Frontiers in neurology* 2018; 9: 1017-1017. DOI: 10.3389/fneur.2018.01017.
7. Walusinski O. A historical approach to hereditary spastic paraplegia. *Revue neurologique* 2020; 176: 225-234. DOI: 10.1016/j.neurol.2019.11.003.
8. Harding AE. Hereditary "pure" spastic paraplegia: a clinical and genetic study of 22 families. *Journal of neurology, neurosurgery and psychiatry* 1981; 44: 871-883. DOI: 10.1136/jnnp.44.10.871.
9. Harding AE. Classification of the hereditary ataxias and paraplegias. *The Lancet (British edition)* 1983; 1: 1151-1155.

10. Shribman S, Reid E, Crosby AH, et al. Hereditary spastic paraplegia: from diagnosis to emerging therapeutic approaches. *Lancet neurology* 2019; 18: 1136-1146. DOI: 10.1016/S1474-4422(19)30235-2.
11. Meyyazhagan A and Orlacchio A. Hereditary Spastic Paraplegia: An Update. *International journal of molecular sciences* 2022; 23: 1697. DOI: 10.3390/ijms23031697.
12. Saputra L and Kumar KR. Challenges and Controversies in the Genetic Diagnosis of Hereditary Spastic Paraplegia. *Curr Neurol Neurosci Rep* 2021; 21: 15. 20210228. DOI: 10.1007/s11910-021-01099-x.
13. Ruano L, Melo C, Silva MC, et al. The Global Epidemiology of Hereditary Ataxia and Spastic Paraplegia: A Systematic Review of Prevalence Studies. *Neuroepidemiology* 2014; 42: 174-183. DOI: 10.1159/000358801.
14. Erichsen AK, Koht J, Stray-Pedersen A, et al. Prevalence of hereditary ataxia and spastic paraplegia in southeast Norway: a population-based study. *Brain (London, England : 1878)* 2009; 132: 1577-1588. DOI: 10.1093/brain/awp056.
15. Coutinho P, Ruano L, Loureiro JL, et al. Hereditary ataxia and spastic paraplegia in Portugal: a population-based prevalence study. *JAMA Neurol* 2013; 70: 746-755. DOI: 10.1001/jamaneurol.2013.1707.
16. Vander Stichele G, Durr A, Yoon G, et al. An integrated modelling methodology for estimating global incidence and prevalence of hereditary spastic paraplegia subtypes SPG4, SPG7, SPG11, and SPG15. *BMC neurology* 2022; 22: 115-115. DOI: 10.1186/s12883-022-02595-4.
17. Braschinsky M, Rannikmae K, Krikmann U, et al. Health-related quality of life in patients with hereditary spastic paraplegia in Estonia. *Spinal Cord* 2011; 49(2): 175-181. DOI: https://dx.doi.org/10.1038/sc.2010.61.
18. Rattay TW, Boldt A, Völker M, et al. Non-motor symptoms are relevant and possibly treatable in hereditary spastic paraplegia type 4 (SPG4). *Journal of neurology* 2019; 267: 369-379. DOI: 10.1007/s00415-019-09573-w.
19. Murala S, Nagarajan E and Bollu PC. Hereditary spastic paraplegia. *Neurological sciences* 2021; 42: 883-894. DOI: 10.1007/s10072-020-04981-7.
20. Synofzik M and Schüle R. Overcoming the divide between ataxias and spastic paraplegias: Shared phenotypes, genes, and pathways. *Movement disorders* 2017; 32: 332-345. DOI: 10.1002/mds.26944.

21. Klebe S, Stevanin G and Depienne C. Clinical and genetic heterogeneity in hereditary spastic paraplegias: From SPG1 to SPG72 and still counting. *Revue neurologique* 2015; 171: 505-530. DOI: 10.1016/j.neurol.2015.02.017.
22. Lange LM, Gonzalez-Latapi P, Rajalingam R, et al. Nomenclature of Genetic Movement Disorders: Recommendations of the International Parkinson and Movement Disorder Society Task Force – An Update. *Movement disorders* 2022; 37: 905-935. DOI: 10.1002/mds.28982.
23. Pennings M, Schouten MI, van Gaalen J, et al. KIF1A variants are a frequent cause of autosomal dominant hereditary spastic paraplegia. *European journal of human genetics : EJHG* 2020; 28: 40-49. DOI: 10.1038/s41431-019-0497-z.
24. Esteves T, Durr A, Mundwiller E, et al. Loss of Association of REEP2 with Membranes Leads to Hereditary Spastic Paraplegia. *American journal of human genetics* 2014; 94: 268-277. DOI: 10.1016/j.ajhg.2013.12.005.
25. Kramer PL, de Leon D, Ozelius L, et al. Dystonia gene in Ashkenazi Jewish population is located on chromosome 9q32-34. *Annals of neurology* 1990; 27: 114. DOI: 10.1002/ana.410270203.
26. Heilig R, Dürr A, Brottier P, et al. Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. *Nature genetics* 1999; 23: 296-303. DOI: 10.1038/15472.
27. Marras C, Lohmann K, Lang A, et al. Fixing the broken system of genetic locus symbols: Parkinson disease and dystonia as examples. *Neurology* 2012; 78: 1016-1024. DOI: 10.1212/WNL.0b013e31824d58ab.
28. Connie M, Anthony EL, Bart PCvdW, et al. Nomenclature of genetic movement disorders: Recommendations of the international Parkinson and movement disorder society task force: Nomenclature of Genetic Movement Disorders. *Movement disorders* 2016; 31: 436-457. DOI: 10.1002/mds.26527.
29. da Graça FF, de Rezende TJR, Vasconcellos LFR, et al. Neuroimaging in Hereditary Spastic Paraplegias: Current Use and Future Perspectives. *Frontiers in neurology* 2019; 9: 1117-1117. DOI: 10.3389/fneur.2018.01117.
30. Karle KN, Schule R, Klebe S, et al. Electrophysiological characterisation of motor and sensory tracts in patients with hereditary spastic paraplegia (HSP). *Orphanet Journal of Rare Diseases* 2013; 8(1) (no pagination). DOI: https://dx.doi.org/10.1186/1750-1172-8-158.

31. Siow S-F, Smail RC, Ng K, et al. Motor Evoked Potentials in Hereditary Spastic Paraplegia—A Systematic Review. *Frontiers in neurology* 2019; 10: 967-967. DOI: 10.3389/fneur.2019.00967.

32. Teive HAG, Camargo CHF, Pereira ER, et al. Inherited metabolic diseases mimicking hereditary spastic paraplegia (HSP): a chance for treatment. *Neurogenetics* 2022; 23: 167-177. DOI: 10.1007/s10048-022-00688-3.

33. Boutry M, Morais S and Stevanin G. Update on the Genetics of Spastic Paraplegias. *Current neurology and neuroscience reports* 2019; 19: 18-19. DOI: 10.1007/s11910-019-0930-2.

34. Méreaux J-L, Banneau G, Papin M, et al. Clinical and genetic spectra of 1550 index patients with hereditary spastic paraplegia. *Brain (London, England : 1878)* 2022; 145: 1029-1037. DOI: 10.1093/brain/awab386.

35. Yeow D, Rudaks LI, Siow S-F, et al. Genetic Testing of Movements Disorders: A Review of Clinical Utility. *Tremor and other hyperkinetic movements (New York, NY)* 2024; 14: 2-2. DOI: 10.5334/tohm.835.

36. DeLuca GC, Ebers GC and Esiri MM. The extent of axonal loss in the long tracts in hereditary spastic paraplegia. *Neuropathology and applied neurobiology* 2004; 30: 576-584. DOI: 10.1111/j.1365-2990.2004.00587.x.

37. Blackstone C. Cellular Pathways of Hereditary Spastic Paraplegia. *Annual review of neuroscience* 2012; 35: 25-47. DOI: 10.1146/annurev-neuro-062111-150400.

38. Meyyazhagan A, Kuchi Bhotla H, Pappuswamy M, et al. The Puzzle of Hereditary Spastic Paraplegia: From Epidemiology to Treatment. *International journal of molecular sciences* 2022; 23: 7665. DOI: 10.3390/ijms23147665.

39. Scheuer KH, Svenstrup K, Jennum P, et al. Double-blind crossover trial of gabapentin in SPG4-linked hereditary spastic paraplegia. *European Journal of Neurology* 2007; Vol.14: 663-666p. DOI: https://doi.org/10.1111/j.1468-1331.2007.01812.x.

40. Uygunoglu U, Gunduz A, Tutuncu M, et al. The Effects of Dalfampridine on Hereditary Spastic Paraparesis. *European Neurology* 2016; 76(3-4): 152-153. DOI: https://dx.doi.org/10.1159/000449375.

41. de Lima FD, Faber I, Servelhere KR, et al. Randomized Trial of Botulinum Toxin Type A in Hereditary Spastic Paraplegia - The SPASTOX Trial. *Movement Disorders* 2021; 36: 1654-1663. DOI: 10.1002/mds.28523.

42. Paparella G, Vavla M, Bernardi L, et al. Efficacy of a Combined Treatment of Botulinum Toxin and Intensive Physiotherapy in Hereditary Spastic Paraplegia. *Frontiers in Neuroscience* 2020; 14 (no pagination). DOI: https://dx.doi.org/10.3389/fnins.2020.00111.
43. Lith BJHv, Boer JJd, Warrenburg BPCvd, et al. Functional Effects of Botulinum Toxin Type A in the Hip Adductors and Subsequent Stretching in Patients with Hereditary Spastic Paraplegia. *Journal of rehabilitation medicine* 2019; 51: 434-441. DOI: 10.2340/16501977-2556.
44. Schols L, Rattay TW, Martus P, et al. Hereditary spastic paraplegia type 5: Natural history, biomarkers and a randomized controlled trial. *Brain* 2017; 140(12): 3112-3127. DOI: https://dx.doi.org/10.1093/brain/awx273.
45. Bertolucci F, Di Martino S, Orsucci D, et al. Robotic gait training improves motor skills and quality of life in hereditary spastic paraplegia. *NeuroRehabilitation* 2015; 36(1): 93-99. DOI: https://dx.doi.org/10.3233/NRE-141196.
46. Zhang Y, Roxburgh R, Huang L, et al. The effect of hydrotherapy treatment on gait characteristics of hereditary spastic paraparesis patients. *Gait & posture* 2014; 39: 1074-1079. DOI: 10.1016/j.gaitpost.2014.01.010.
47. Margetis K, Korfias S, Boutos N, et al. Intrathecal baclofen therapy for the symptomatic treatment of hereditary spastic paraplegia. *Clinical Neurology and Neurosurgery* 2014; 123: 142-145. DOI: https://dx.doi.org/10.1016/j.clineuro.2014.05.024.
48. Pointon R, Whelan H, Raza R, et al. The use of intrathecal baclofen for management of spasticity in hereditary spastic paraparesis: A case series. *European Journal of Paediatric Neurology* 2022; 36: 14-18. DOI: https://dx.doi.org/10.1016/j.ejpn.2021.11.003.
49. Kai M, Yongjie L and Ping Z. Long-term results of selective dorsal rhizotomy for hereditary spastic paraparesis. *Journal of Clinical Neuroscience* 2014; 21(1): 116-120. DOI: https://dx.doi.org/10.1016/j.jocn.2013.04.020.
50. Park TS, Joh S, Walter DM, et al. Selective Dorsal Rhizotomy for Treatment of Hereditary Spastic Paraplegia-Associated Spasticity in 37 Patients. *Curēus (Palo Alto, CA)* 2021; 13: e17690-e17690. DOI: 10.7759/cureus.17690.
51. Ardolino G, Bocci T, Nigro M, et al. Spinal direct current stimulation (tsDCS) in hereditary spastic paraplegias (HSP): A sham-controlled crossover study. *Journal of Spinal Cord Medicine* 2021; 44(1): 46-53. DOI: https://dx.doi.org/10.1080/10790268.2018.1543926.
52. Antczak J, Pera J, aogon, et al. The Effect of Repetitive Transcranial Magnetic Stimulation on Motor Symptoms in Hereditary Spastic Paraplegia. *Neural plasticity* 2019; 2019. DOI: https://doi.org/10.1155/2019/7638675.

53. Bastani PB, Kordjazi M, Oveisgharan S, et al. A Randomized Controlled Trial of the Effect of Repetitive Transcranial Magnetic Stimulation of the Motor Cortex on Lower Extremity Spasticity in Hereditary Spastic Paraplegia. *Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society* 2021; 23. DOI: https://dx.doi.org/10.1097/WNP.0000000000000874.

Chapter 2. Utility of pre-existing outcome measures in Hereditary Spastic Paraplegia

2.1 Introduction

In chapter 1, I identified a need for standardised outcome measures for HSP clinical trials and explored the heterogeneity and variable utility of outcome measures used in HSP studies with a scoping review. I discussed the challenges with identifying suitable outcome measures for HSP due to the phenotypic heterogeneity, rarity, and generally slowly progressive nature of HSP. A set of outcome measures targeting multiple domains of interest and inclusion of a targeted biomarker able to sensitively detect changes over time or in response to treatment, are key to successfully measuring treatment outcomes for HSP. Therefore, I proposed a core outcome set consisting of a clinician-rated outcome measure (CROM), patient-reported outcome measure (PROM) and an objective biomarker. In this chapter, I investigate the utility of pre-existing CROMs, PROM and motor evoked potentials as a potential biomarker for HSP.

2.1.1 Spastic Paraplegia Rating Scale – Clinician Rated Outcome Measure

The Spastic Paraplegia Rating Scale (SPRS) is a 13-item scale designed for clinicians to rate functional impairment in patients with HSP. [1] Each item is scored from 0 to 4 yielding an overall score ranging from 0 (no functional impairment) to 52 (severe impairment). It was validated in a group of patients from Germany and was found to be reliable, have high inter-rater agreement between two examiners, and correlated with other measures of functional disability. [1] It has been translated and validated in a Brazilian-Portugese Cohort, [2] used as an outcome measure in clinical trials, [3-5] and has demonstrated longitudinal disease progression. [3, 6-8] The SPRS was used to measure disease severity when evaluating potential biomarkers, including diffusion tensor imaging (DTI), [9] serum neurofilament light chain[10] and serum 27-hydroxycholesterol. [3, 11] It is available online and can be administered in a clinical setting without requiring special equipment. Overall, the SPRS is the most widely used HSP-specific CROM that has been validated in several HSP patient cohorts.

2.1.2 Scale for Assessment and Rating of Ataxia – Clinician Reported Outcome Measure

The Scale for Assessment and Rating of Ataxia (SARA) is an 8-item scale used to rate severity of ataxia symptoms. [12] SARA has been used to quantify disease severity and measure longitudinal disease progression in HSP-*SPG7*. [13, 14] The SARA has been shown to be reliable when used in a telehealth setting [15] and has been translated and validated in other languages, such as French and Portuguese. [16, 17] I decided to evaluate the SARA as HSP-*SPG7* was the second most common genotype in our patient cohort. As HSP-*SPG7* is known to be associated with an ataxia phenotype and the SPRS does not evaluate symptoms of ataxia, I proposed the SARA as a complementary CROM to the SPRS.

2.1.3 Short Form 36 – Patient reported outcome measure

The Short Form 36 (SF-36) was developed as part of the Medical Outcomes Study to measure health related quality of life for patients with chronic conditions. [18] The SF-36 has been widely used in health research and normative values for the general population are available. [19, 20] There are several versions of the SF-36 including SF-36v1.0, RAND-36 and SF-36v2.0 with differences in wording and scoring methods. Use of and scoring of SF-36v2.0 requires a license and paid software whilst the other two versions are freely available with published scoring instructions online. [21] The surveys have been used in multiple countries and translated into other languages. [19, 22] The SF-36 has been used to demonstrate worse quality of life in patients with HSP compared to the general population. [22-24] Worse quality of life as measured with SF-36 was found to correlate with increasing disease severity as measured with the SPRS. [25, 26] One study found that physical quality of life measured with the SF-12, an abbreviated version of the SF-36, moderately correlated with gait parameters measured with mobile gait analysis. [24] The SF-36 showed improvement in 4 domains (role limitations due to physical health, social functioning, role limitations due to emotional problems, emotional wellbeing) in 13 patients with pure HSP treated with robotic gait training [27] but no effect of repetitive TMS in a group of 8 patients with HSP. [28] Overall, SF-36 was the most widely used PROM in previous HSP clinical trials and has been used to measure quality of life impairment in patients with HSP.

2.1.4 Motor Evoked Potentials

Motor evoked potentials have been studied in HSP for several decades. [29] Motor evoked potentials (MEP) are used to assess central motor pathways using transcranial magnetic stimulation of the brain. The central motor conduction time (CMCT) is the most reported MEP parameter in HSP. CMCT is the time taken for the action potential to travel from the motor cortex to the spinal cord where it synapses with the lower motor neurons. Therefore, prolongation of CMCT is used as a marker for upper motor neuron abnormalities causing abnormal conduction. [30]

As the underlying pathophysiology of HSP involves length-dependent degeneration of neurons, MEP has been proposed as a potential biomarker for HSP. The Royal North Shore Hospital Neurophysiology Department is one of the few specialised services in Sydney, Australia, that offer assessment of MEP. In the next section, I conducted a systematic review of the literature to clarify the utility of MEP in HSP. In Section 2.3, I investigate a curated set of pre-existing outcome measures, SPRS, SARA, SF-36 and MEP in a cohort of patients with HSP to understand the utility of these outcome measures in HSP.

2.2 Motor evoked potentials in HSP Systematic Review.

Siow SF, Cameron Smail R, Ng K, Kumar KR, Sue CM. Motor Evoked Potentials in Hereditary Spastic Paraplegia-A Systematic Review. Front Neurol. 2019 Sep 18;10:967. doi: 10.3389/fneur.2019.00967. PMID: 31620065.

frontiers in Neurology

SYSTEMATIC REVIEW
published: 18 September 2019
doi: 10.3389/fneur.2019.00967

Motor Evoked Potentials in Hereditary Spastic Paraplegia—A Systematic Review

Sue-Faye Siow[1,2,3]*, *Ruaridh Cameron Smail*[2,3], *Karl Ng*[2,3,4†], *Kishore R. Kumar*[1,4,5,6,7†] and *Carolyn M. Sue*[1,2,3,4]*

[1] Department of Neurogenetics, Kolling Institute, Royal North Shore Hospital, St Leonards, NSW, Australia, [2] Department of Neurology and Neurophysiology, Royal North Shore Hospital, St Leonards, NSW, Australia, [3] Northern Clinical School, Kolling Institute, Royal North Shore Hospital, St Leonards, NSW, Australia, [4] Sydney Medical School, University of Sydney, Sydney, NSW, Australia, [5] Department of Neurology, Concord Hospital, Sydney, NSW, Australia, [6] Kinghorn Centre for Clinical Genomics, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia, [7] Molecular Medicine Laboratory, Concord Hospital, Sydney, NSW, Australia

Background: Hereditary Spastic Paraplegia (HSP) is a slowly progressive neurodegenerative disorder with no disease modifying treatment. Potential therapeutic approaches are emerging and large-scale clinical drug trials for patients with HSP are imminent. A sensitive biomarker to measure the drug efficacy in these trials is required. Motor evoked potentials (MEPs) are a potential biomarker for HSP as they assess the central motor pathways and can be standardized with set protocols and guidelines.

Objectives: We performed a systematic review to investigate the utility of MEPs as a diagnostic and disease severity biomarker for HSP.

Search Methods: Systematic searches of PubMed, Embase, Medline, and Scopus were performed.

Selection Criteria: Studies reporting on central motor conduction time measured with MEPs in adult and pediatric patients with HSP were included. We excluded studies in non-HSP patient cohorts, not in English, not original research, and unpublished journal articles.

Data Collection and analysis: Search results were de-duplicated and screened according to the inclusion and exclusion criteria. The included papers were reviewed independently by two reviewers and data was collected on patient cohorts, test methods, results, and study quality. Results were analyzed using descriptive methods.

Results: Of the 882 search results, 32 studies were included in the review. The most common finding was absent or prolonged lower limb (LL) central motor conduction time (CMCT) in patients with HSP (78% of patients studied). Quality assessment revealed variability in study methodology and reporting of results. Variations included patient cohorts of various genotypes as well as variations in equipment and techniques used. Aside from CMCT, none of the MEP parameter measures correlated with disease severity and many did not show significant difference between HSP patients and controls.

Conclusion: Systematic review of MEP studies in HSP patient cohorts demonstrated mixed findings. Lower limb CMCT was the most promising parameter in terms of differentiating HSP patients from controls, with one study demonstrating a weak

OPEN ACCESS

Edited by:
Jonas Alex Morales Saute, Federal University of Rio Grande Do Sul, Brazil

Reviewed by:
Stephan Klebe, Essen University Hospital, Germany
Filippo M. Santorelli, Fondazione Stella Maris (IRCCS), Italy

***Correspondence:**
Sue-Faye Siow
suefaye.siow@health.nsw.gov.au
Carolyn M. Sue
carolyn.sue@sydney.edu.au

[†] *These authors have contributed equally to this work*

Specialty section:
This article was submitted to Neurogenetics, a section of the journal Frontiers in Neurology

Received: *27 May 2019*
Accepted: *23 August 2019*
Published: *18 September 2019*

Citation:
Siow S-F, Cameron Smail R, Ng K, Kumar KR and Sue CM (2019) Motor Evoked Potentials in Hereditary Spastic Paraplegia—A Systematic Review. Front. Neurol. 10:967. doi: 10.3389/fneur.2019.00967

correlation with clinical disease severity. It is possible that the lack of consistency in study methodologies and small patient cohorts have contributed to the variable findings. A longitudinal study of MEPs in a large cohort of HSP patients with the same genotype will help clarify the utility of MEPs as a biomarker for disease severity and use in clinical trials.

Keywords: hereditary spastic paraplegia, motor evoked potentials, systematic review, biomarker, clinical trials

INTRODUCTION

Hereditary spastic paraplegia (HSP) encompasses a group of neurodegenerative conditions that result in lower limb spasticity and weakness. Despite causing significant disability, there is no available cure for this progressive condition (1). The underlying pathophysiology of HSP remains poorly understood and it is likely that this varies according to genotype (2). There are >64 HSP associated genes and 13 HSP associated loci identified to date and this number will continue to grow with the advent of next generation sequencing (3, 4).

Recent research using stem cell models to study HSP disease pathogenesis, including SPG4 (HSP-*SPAST*), SPG3A (HSP-*atlastin-1*), and SPG11 (HSP-*spatacsin*) (5–7), have revealed several potential treatment options targeting the underlying disease mechanisms (8). There is a paucity of clinical trials for HSP drug treatment options, the most recent trials targeting SPG5 (HSP-*CYP7B1*) showed improvement of biological markers but no discernible clinical improvement (1, 9, 10).

Biomarkers of disease severity and progression are vital components of establishing a clinical trial for therapeutic agents in HSP. Although several different biomarkers have been used in the small number of clinical trials published to date, a standardized biomarker for use across all clinical trials has not been defined (9–11). Promising biomarkers for disease severity in HSP include the Spastic Paraplegia Rating Scale (SPRS) (10), gait analysis (12), motor evoked potentials (MEPs) (13), diffusion tensor imaging (DTI) (14), and genotype-specific biochemical markers, such as 27-hydroxycholesterol in SPG5 (9, 10). Ideally, a disease biomarker for HSP should be easily accessible, able to be standardized across different institutions, affordable and minimally invasive. Challenges to developing a standardized biomarker include heterogeneity of HSP genotypes, broad spectrum of associated features and the typically slow progression of HSP.

Motor evoked potentials (MEPs) elicited through transcranial magnetic or electric stimulation have been proposed as a biomarker for disease severity in HSP (13). Prolongation of the central motor conduction time (CMCT) is used as a marker for upper motor neuron abnormalities causing slowing of conduction (15). There are clear guidelines for measurement of MEPs allowing standardization across multiple centers, a useful feature for a disease biomarker (16). In order to evaluate the utility of MEPs as a biomarker for HSP, we performed a systematic review on the measurement of MEPs in HSP patient cohorts. Our aim was to evaluate CMCT as a diagnostic tool and as a biomarker for disease severity in HSP.

METHODS

We performed a systematic search of PubMed, Embase, Medline and Scopus. The full search terms are included in **Appendix 1**. In short, the terms used were "hereditary spastic paraplegia" AND ["motor evoked potentials" OR "transcranial magnetic stimulation" OR "central motor conduction time"].

The search results were exported to EndNote and de-duplicated. The first screen was of the titles and abstracts according to the inclusion and exclusion criteria. The second screen was of the full article. The included studies were then reviewed independently by two reviewers.

Inclusion criteria were full text articles only, English articles, humans only.

Exclusion criteria were conditions that were not hereditary spastic paraplegia, articles that did not measure central motor conduction time using transcranial stimulation, not original research.

Data were collected on patient demographics, neurophysiological techniques, and study results using a pre-set form that populated a database (**Appendix 2**). Assessment of methodological quality was performed with the NIH Study Quality Assessment Tool (17), scored out of 12 points for case control studies and 9 points for case series; as well as a 24 point checklist for assessing methodological quality of transcranial magnetic stimulation studies (18) (**Appendix 3**). Any discrepancies between the reviewers were resolved with discussion and if required, a third reviewer was involved.

Data was analyzed with descriptive methods and presented in tables and graphs. Meta-analysis could not be performed due to the differing methodologies and heterogeneity of the results reported by the studies.

RESULTS

Search Results and Screening Process (PRISMA Diagram)

There were 882 search results with a total of 675 individual studies identified after duplicates were removed. Two studies were identified through the reference lists of relevant studies. Thirty-two studies were included after the screening process. The search process is illustrated in the PRISMA flow chart (**Figure 1**).

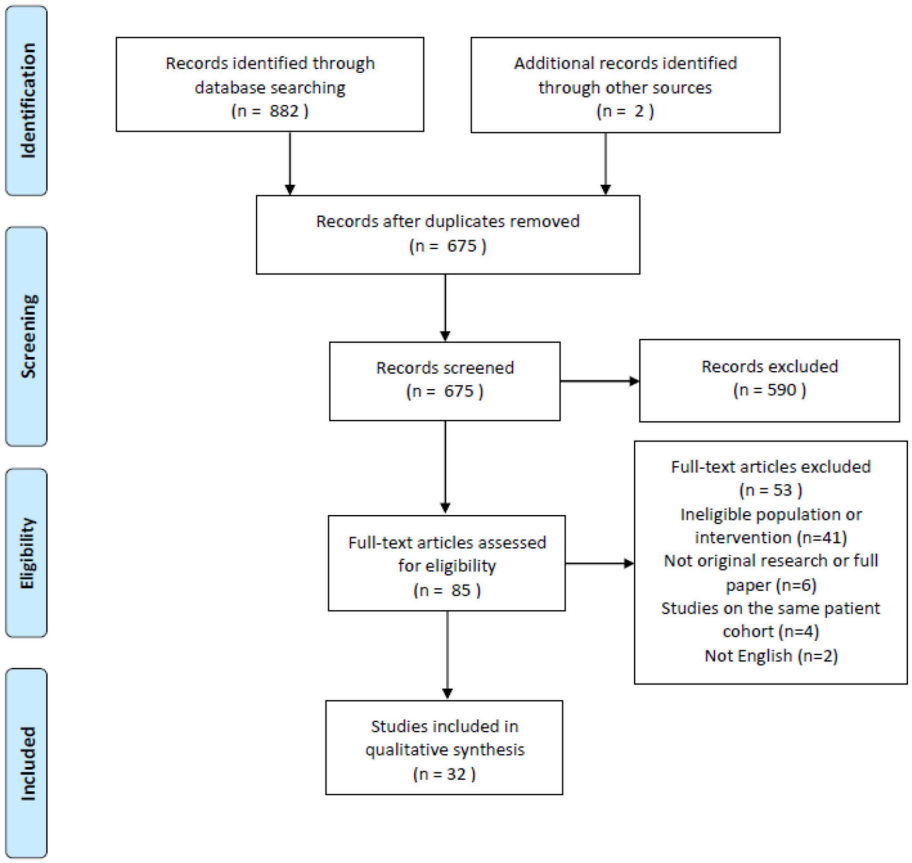


FIGURE 1 | PRISMA flow chart of search results (19).

Study Characteristics

Thirty-two studies were included after the screening process. Studies were published between 1987 and 2016. Study types include 12 case controls, 19 case series/reports and 1 cohort retrospective study.

Studies were grouped according to their primary aims (see **Figure 2**).

Patient Demographics

Patient sample sizes ranged between 1 and 128 patients in each study. The mean number of patients who underwent MEPs per study was 14.88. 31/32 (97%) studies included fewer than 50 patients, 16/32 (48%) studies included 10 or fewer patients. Overall, there were a total of 476 patients with HSP who underwent MEPs in the studies reviewed.

Information on patient genotypes was included for 20/32 (63%) studies. 16/32 (50%) studies included patients with a single genotype, most commonly SPG4. 10/32 (31%) studies did not specify the patients' genotypes or only included patients with unknown genotypes. Six (19%) studies included patients with mixed genotypes and unknown genotypes. Several studies were performed before genetic testing was available, and the inheritance pattern was determined when possible.

The proportion of patients with pure and complicated phenotypes was described in 24/32 studies. Overall, 234 (76%) patients with pure HSP and 73 (24%) patients with complicated HSP were studied. Of the 23 studies that commented on the presence of peripheral neuropathy, either on examination or nerve conduction studies, there was a total of 130 out of 355 (37%) patients who had peripheral neuropathy.

The ratio of male:female patients was available for 21/32 studies, the overall ratio calculated from the 21 studies was 1.3. Mean age was available for 23/32 papers with an overall mean age of 40.93 years at the time of the study. Additionally, some papers included age at onset and disease duration. Thirteen out of 32 studies included patients from the same family.

Neurophysiological Techniques

Information on the type of stimulator used, type of coil used, stimulus intensity, number of stimuli administered, and muscles studied are provided in **Table 1**. Three studies used transcranial electrical stimulation whereas the remainder used transcranial magnetic stimulation (20–22).

CMCT calculation method was specified for 24/32 papers; 11 papers used the F-wave method, 11 papers used the spinal stimulation method and 2 studies used both methods (23,

FIGURE 2 | Studies grouped according to the aim/objective of the study. Group 1—Studies investigating the role of MEPs in HSP; Group 2—Studies where MEPs were performed to characterize the phenotype of HSP; Group 3—Studies where MEPs were performed as an adjunct to another intervention or investigation in HSP.

TABLE 1 | Details of neurophysiological techniques used in MEP studies.

Details of Neurophysiological techniques	Number of studies with available information	Details
Stimulator used	18/32	Dantec Maglite, MagStim 200, Twin top, Digitimer, interconnected electrodes
Coil used	17/32	Circular, double cone/parabolic/figure of 8
Stimulus intensity to elicit MEP	17/32	Most common 120% resting motor threshold but wide range used
Number of stimuli	11/32	Range from 3 stimuli to "at least 20"
Muscles studied	27/32	Most common tibialis anterior (16/26), abductor pollicis brevis (9/26), first dorsal interosseus (8/26), adductor digiti minimi (7/26), abductor hallucis (5/26)

24). Two studies used the F-wave method, however detailed a different peripheral motor conduction time calculation formula to previously published guidelines (13, 16, 25).

Clinical Rating Scales

Clinical rating scales were used in 15/32 studies with the most common being the modified Ashworth scale (5 studies) (23, 26–29), SPRS (4 studies) (13, 28, 30, 31) and MRC (4 studies) (27–30). Other rating scales used included functional grading scales (29, 32), disability staging scores (33–35), Functional Independence Measure (28), Barthel index (26), Behan-Maia modified scale (29), Abbreviated Mental Test Score (26), revised Wechsler Adult Intelligence Scale (28), Esame Neuropsicologico Breve 2 (28), Cambridge Cognition Examination (36, 37), and 6 min walk test (28).

Gait analysis was performed in one study investigating gait patterns in HSP (27).

MEP Results

Central Motor Conduction Time

Overall, lower limb CMCT was more likely to be abnormal in patients with HSP compared to upper limb CMCT. Most (96%) studies that investigated lower limb CMCT reported abnormalities, with LL CMCT abnormalities in 308/393 (78%) patients with HSP that were studied (excluding 4 studies with unspecified UL/LL CMCT).

Fifty nine percent of studies that investigated upper limb CMCT reported abnormalities, with 93/282 HSP patients studied showing UL CMCT abnormalities (see **Table 4.1** in Appendix 4).

Upper limb CMCT results were reported for 22/32 (67%) studies and not studied in 7 papers. UL CMCT was found to be normal in all patients studied in 9/22 (41%) papers and abnormal in 13/22 (59%) studies (**Chart 1**, **Tables 4.1**, **4.2** in Appendix 4). In the 9 studies that reported normal UL CMCT in all patients, 5 studies were in exclusively SPG4 patient cohorts (23, 25, 30, 31, 34), 3 in HSP patient cohorts with unknown genotype (21, 38) and 1 in a patient with SPG31 (39) HSP. Overall, 93/359 (26%) patients studied had abnormal UL CMCT.

Sensitivity of upper limb CMCT abnormalities is 0.3.

Lower limb CMCT results were reported for 26/32 (81%) studies and not studied in 3 papers. Only 1 study of 16 patients from 4 families with chromosome 2p linked HSP (SPG4) reported LL CMCT within the normal range for all patients although there was a tendency for delay in the lower limbs (34). LL CMCT was prolonged or absent for all patients in 8/26 studies and in some patients for 15/26 studies (**Chart 2**, **Table 4.3** in Appendix 4). Overall, 308/393 (78%) patients studied had abnormal LL CMCT.

Sensitivity of lower limb CMCT abnormalities to diagnose HSP is 0.8, allowing for bias of only studying affected patients. This is the same as the result reached by Di Lazzaro et al. (24).

Four studies that did not distinguish between UL and LL CMCT all showed varying abnormalities in CMCT (**Table 4.4** in Appendix 4).

Chart 3 illustrates the summative CMCT results across all studies reviewed.

Amplitude

MEP amplitude was reported for UL in 8 papers, with lower amplitudes found in 5/8 studies and normal amplitudes in 3/8 studies (22, 25, 29, 30, 35, 40–42). LL MEP amplitude was studied in 12 papers with absent or reduced LL MEP amplitudes reported for some or all patients in all 12 studies (13, 20, 22, 23, 27–29, 33, 35, 40, 43, 44).

Resting Motor Threshold (RMT)

Resting motor threshold was reported in 7 studies. LL RMT was reported in 5 studies and found to be increased in all. UL RMT was reported in 4 studies and was noted to be normal in all.

Correlation of CMCT With Other Variables

Correlation between CMCT and other variables were investigated in 10/32 studies. In one paper, upper and lower limb CMCT was found to significantly correlate with SPRS scores ($r = 0.176$ and $r = 0.234$) and the spastic subscore ($r = 0.241$ and $r = 0.300$) whilst LL CMCT correlated with disease duration ($r = 0.231$) (13). Another study that included only 2 patients found that the more clinically severe patient had a prolonged CMCT (41). A further study found that tibialis anterior derived LL CMCT correlated weakly with disability but only in the early onset (<20 years of age) HSP subgroup (32).

Eight studies found no correlation between CMCT and other variables including disease severity, clinical signs, disease duration, age, age of onset, gait abnormalities, modified Ashworth score, and gender (23, 27–29, 32, 34, 35, 45).

Other Results

Cortical silent period (CSP) was measured using transcranial magnetic stimulation (TMS) in four studies, three of which showed no difference in HSP patients compared to controls (25, 30, 45). One study showed significantly reduced CSP for tibialis anterior, and this correlated with spasticity as measured by the Modified Ashworth Score (23).

Short-interval intracortical inhibition (SICI) was measured in three studies. One showed no difference in HSP patients compared to controls (30), another showed reduced SICI in SPG4 patients but not SPG7 (45) and the last was a case report of a SPG11 patient that demonstrated delayed transcallosal inhibition (44).

Two studies investigating MEP recruitment curves showed no difference in HSP patients compared to controls (25, 31). One study of TMS modulation of the soleus H reflex showed abnormal modulation in a pure HSP cohort (29).

One study not included in our analysis evaluated CMCT to the external anal sphincter in the SPG4 cohort described in Nielsen et al. (34). This demonstrated that a prolonged CMCT to the external anal sphincter correlated with lower urinary tract symptoms in this SPG4 cohort. Subjects without symptoms had similar CMCT compared to controls (46).

Results by Genotype

SPG4

Thirteen papers included patients with *SPAST* (SPG4) mutations. Two studied just lower limb MEPs, two studied just upper limb, nine studied both. Nine studies provided an overall result for the whole SPG4 subgroup whilst the rest (four) provided individual patient results (at least in graph format). Eight included quantitative data on CMCTs (including graphed data) whilst the rest reported either "normal" or "prolonged" CMCT.

Of the 13 UL studies, 9 reported no difference in the CMCT compared to controls. A minority of abnormal cases were reported in the 4 other studies. Overall, 10/149 patients were reported to have abnormal UL CMCT.

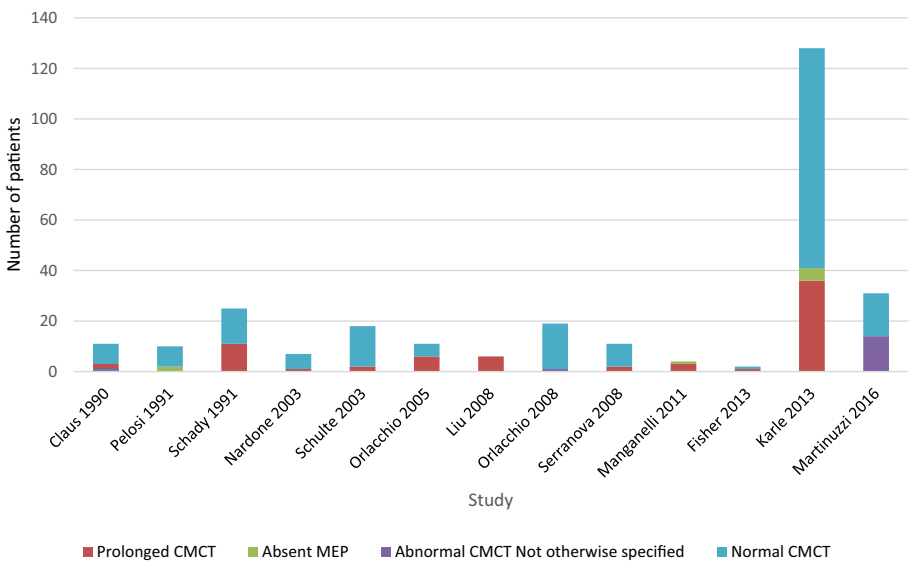


Chart 1 | Studies of upper limb MEPs. Data available in **Table 4.1**, Appendix 4.

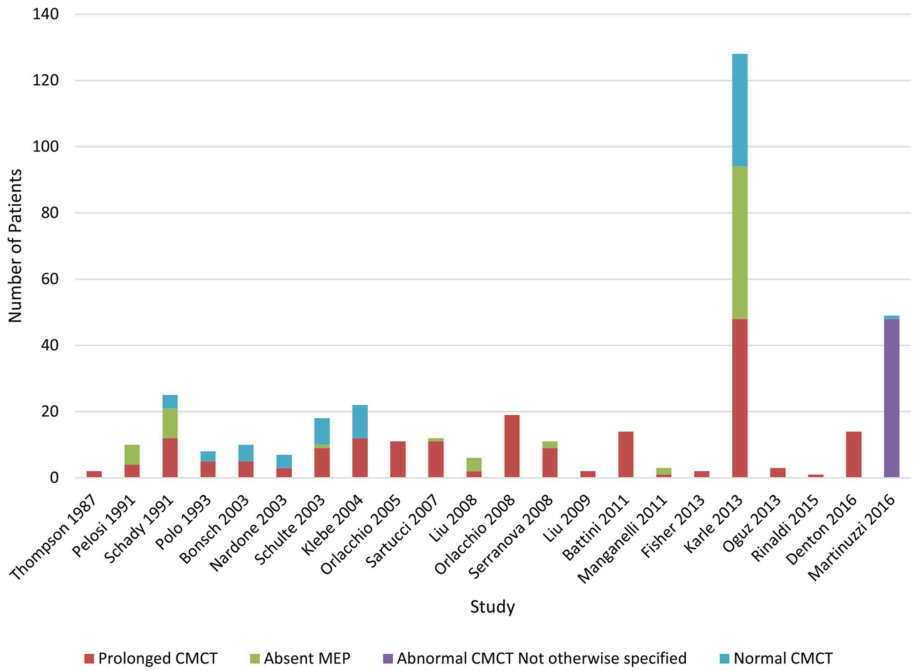


Chart 2 | Studies of lower limb MEPs. Data available in **Table 4.3**, Appendix 4.

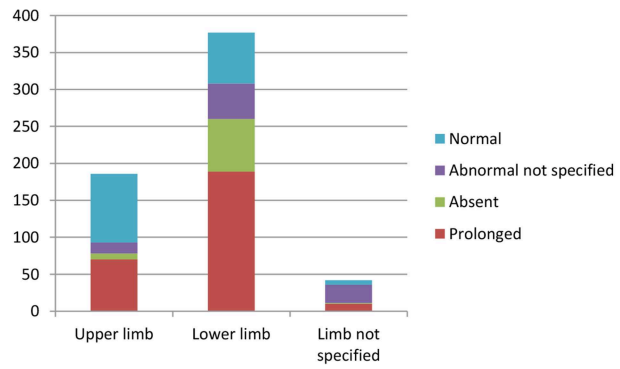


Chart 3 | Summation of studies demonstrating number of patients with abnormal vs. normal central motor conduction times. Data available in **Tables 4.1–4.4** in Appendix 4.

Of the 12 LL studies, all reported prolonged CMCT in all or at least some of the patients studied. For the papers which reported individual results, the proportion of abnormality ranged from 10 to 100%. Although we cannot summate the results due to methodological differences and methods of reporting results, ∼60–70% patients demonstrated abnormal results.

Notably, large variation was seen, despite mutations in the same gene. Bonsch et al. demonstrated abnormality in a family with an in-frame deletion of exons 13 to 16, but not in a family with a single base pair deletion resulting in a premature stop codon in exon 9 (25). Orlacchio et al. found significantly prolonged CMCTs in the males of a family with the same mutation, whereas the females had normal CMCTs (36). Karle et al. noted that mutation type influenced CMCT findings: SPG4 missense mutations were associated with shorter CMCT compared to patients with SPG4 splice site mutations, premature stop codon or in-frame deletions (13).

Three studies compared SPG4 patients with other genotypes. Schulte and colleagues compared SPG4 and non-SPG4 patients, finding MEP abnormalities were milder for SPG4 patients despite no clinical differences (47). Karle et al. found that SPG4 patients were more likely to have a "pure" HSP phenotype compared to non-SPG4 patients (60% vs. 36%) (13). In the cohort of Karle et al., UL and LL CMCTs were normal in most SPG4 patients but significantly longer in non-SPG4 HSP patients. Nardone et al. compared SPG4 and SPG7 and found no significant difference between the two patient groups aside from reduced short interval ICI in chromosome 2p linked HSP compared to normal ICI in chromosome 16q linked HSP (45).

Other Genotypes

Martinuzzi et al. performed a battery of clinical and neurophysiological tests (including MEPs) in a large cohort of genotypically characterized HSP patients (28). It was not specified how many of each HSP genotype underwent MEPs. Though the authors noted some correlations between SPRS and disease duration for some genotypes (SPG5, 7, 10), no correlation was found with MEPs. The authors did not draw any conclusions regarding an association of CMCT to any single HSP genotype.

Regarding other genotypes, it is hard to draw conclusions due to small numbers and incomplete reporting of results.

Though most data were only qualitative, there were several mentions of *severely* prolonged CMCT in some cases of non-SPG4 patients which may imply a demyelinating rather than axonal pathophysiology (13, 47). SPG5/5A, 6, 7 and 11 tended to be prolonged, though not in *all* cases (13, 35, 37, 40, 47, 48).

Methodological Quality Assessment: Risk of Bias

The studies were methodologically evaluated by two reviewers and the full results can be found in **Appendix 3**. Validated tools for clinical series and case control studies were used. Using the NIH Study Quality Assessment Tool, case series studies were scored between 2 and 8 out of 8 criteria (17). For case-control studies, the two criteria relating to a study "exposure" were not appropriate for HSP and were discounted. We deemed that studies scored between 3 and 6 out of a possible 9. It was found that the aims and objectives of the studies were adequately published in most cases, though some papers were only used to report a clinical phenotype, rather than answer a more specific question related to MEPs (see **Figure 2**). The study methodology was reported variably, and poorly in some cases. Though some papers used concurrent controls, others relied upon laboratory values or historical controls. Only some papers mentioned efforts to match the controls for age, sex, and other variables (24–26, 30, 46). The nature of the cases meant that risk of bias was high, and selection of patients from a proband may have resulted in bias. Furthermore, no study mentioned that the MEP assessment was blinded to the diagnosis, and prior knowledge of the diagnosis may have introduced bias.

Similarly, the results of the studies were variably reported. Some papers only mentioned prolongation of CMCTs in a single sentence, whereas others provided tables and graphs of individual CMCT results, as well as their comparative control results. 15/32 studies reported individual results (some on a graph), 15/32 reported results for the group, and 1 study reported both. 17/32 studies provided quantitative results, whereas 16/32 were only qualitative (e.g., "prolonged" CMCT). This variability in reporting of results prevented amalgamation of the data, precluding us from performing a meta-analysis.

The TMS methodology checklist demonstrated significant variability between studies (18). Studies scored between 0 and 22 of the 24 criteria. Studies focused on MEPs often outlined their methodology in detail (including muscles studied, stimulator, coil, and stimuli given). Other studies which were only using MEPs as an ancillary test, and those concentrating on other aspects of HSP, did not include sufficient detail (28, 33, 35–37, 39, 40, 44, 47–50). Only a few studies mentioned the presence of possible confounders such as medications and other medical conditions (29, 32, 42, 44).

DISCUSSION

This systematic review showed that prolonged or absent lower limb CMCT is a potential diagnostic biomarker for HSP. However, only one study showed a weak correlation between upper and lower limb CMCT and clinical disease severity. Hence, the utility of CMCT as a prognostic biomarker in HSP remains uncertain.

CMCT as a Diagnostic Biomarker for HSP

Most (96%) of studies reported prolongation of CMCT or absence of MEPs in the lower limbs with 78% of patients studied demonstrating lower limb CMCT abnormalities, whilst only 59% of studies reported abnormalities in the upper limbs. The gold standard for the diagnosis of HSP was considered to be appropriate findings on clinical exam, appropriate exclusion of other disorders, and genetic testing. Compared to this standard, this review found that prolongation of lower limb CMCT had a sensitivity of 0.8.

The underlying disease pathophysiology of HSP is length-dependent axonal degeneration (2). CMCT measured from MEPs are thought to reflect neuronal integrity and are therefore a potential surrogate marker of disease severity. In this systematic review, CMCT was more likely to be abnormal in the lower limb (78%) vs. the upper limb (26%) consistent with more neuronal damage in the longer motor tracts to the lower limbs. This was also seen when HSP was compared to other motor neuron diseases such as hereditary motor and sensory neuropathy (HMSN) types 1 and 2, amyotrophic lateral sclerosis (ALS) and primary lateral sclerosis (PLS), where CMCT to the upper limbs were more likely to be normal in HSP compared to these other conditions (30, 41, 42). This finding was also confirmed in a study using the triple stimulation technique, thought to be the most accurate neurophysiological measure of upper motor neuron integrity, which found normal central motor conduction to the upper limbs in 15 patients with pure HSP (13 had SPG4 HSP) (51).

However, there was a significant proportion of patients with normal CMCT to the upper and lower limbs despite severe signs of spasticity. In fact, most studies did not find any correlation of CMCT abnormalities with disease severity, disease duration or age of onset (23, 27–29, 32, 34, 35, 45). There are several possible explanations for this finding. One could be that although not significantly prolonged compared to controls, the CMCT of affected patients may be prolonged compared to their baseline before developing symptoms. Two studies showed borderline or mild prolongation in CMCT in patients with HSP compared to controls although these were reported as normal as the prolongation was not significant (30, 34). A longitudinal study of MEPs in patients with HSP, ideally from presymptomatic to symptomatic stage might shed light on the theory that increasing CMCT occurs in the presymptomatic stage and may not change once the patients is symptomatic, indicative of a ceiling effect of the test (see later).

Another explanation is that although symptoms are associated with neuronal damage, there may be enough residual nerve fibers that are intact to conduct the action potentials induced with transcranial magnetic stimulation or transcranial electrical stimulation. There are limited neuropathological studies in HSP as it typically does not reduce life span. One study

looked at cervical and lumbar spinal cord sections from two patients with HSP and found axonal swellings in the long descending corticospinal (CST) axons, more prevalent in the dorsal column (52). Another looked at post-mortem tissue from six HSP patients and showed a significant reduction in axonal density and number of axons of CST and sensory tracts compared to controls (53). The latter study demonstrated a reduction of both larger and smaller diameter fibers equally within the CST, however the integrity of the remaining neurons was not described and these could be enough to maintain central motor conduction without sufficiently demonstrating slow conduction in a grouped axon test.

It is also likely that clinical motor impairment in HSP is due to more global cerebral impairment than just the motor tracts. A study using magnetoencephalography to assess the connectivity of brain networks found changes that suggested global network rearrangement due to changes beyond motor impairment (54). One study looking at 118 patients with SPG4 HSP found 10% had psychiatric comorbidities and 3.5% had memory impairment, possible reflecting more global cerebral impairment (55).

There are several limitations of MEPs as a clinical biomarker. A recent paper investigating the use of MEP measures in movement disorders, specifically Parkinson's disease, dystonia, Tourette syndrome, Huntington Disease and essential tremor, found discrepancies between studies reporting "canonical" MEP findings for these conditions (56). Similar to our findings, the authors found inconsistencies in methodology, diagnostic criteria for inclusion, study participants' disease stages, and small sample sizes contributed to weaker evidence for the use of MEPs for diagnosis and differential diagnosis in movement disorders. See below for limitations of MEPs as a biomarker in HSP.

Limitations of MEPs as a biomarker in HSP

- Limitations of MEPs
  - Low to moderate reliability of some MEP measures, e.g., MEP recruitment, cortical silence period, intracortical facilitation (56)
  - Peripheral neuropathy causing CMCT prolongation due to slowing in the cauda equina (16)
  - Cognitive impairment may affect patient's ability to actively participate
- HSP-related factors
  - Slow progression of disease
  - Low prevalence
  - Clinically and genetically heterogenous
  - No previously known biomarker
- Limitations of reviewed studies
  - Variability in study methodology
  - Small sample size of patient cohorts studied
  - Clinically and genetically heterogeneous patient samples

In studies where HSP was investigated along with other neurodegenerative conditions, CMCT changes in HSP were found to be milder than in multiple sclerosis, myelopathy, stroke and motor neuron disease (20, 21, 24). Therefore, characteristic changes of prolonged or absent LL CMCT with normal or mildly prolonged UL CMCT are more likely to suggest a diagnosis of HSP, whilst patients with grossly abnormal UL and LL CMCT are more likely to be seen in other types of motor neuron disease. However, it is important to note that these changes are not specific to HSP and can be seen in other motor neuron conditions (20).

In summary, although useful in diagnosis of HSP, CMCT is best used to confirm the clinical examination findings or the results of genetic testing.

CMCT as a Measure of Treatment Response for HSP

Only 30% of studies that investigated correlation between CMCT and other clinical variables reported a mild correlation with disease severity. One study only included two patients whilst the other only showed a correlation in patients with disease onset before 20 years old (32, 41). It is difficult to draw conclusions from these results, but the current evidence does not support a strong correlation with disease severity.

MEPs have not been studied longitudinally in HSP and therefore, the ability to assess changes in disease severity over time remains to be established. It is possible that individual patients may show changes from their baseline MEPs over time although some may not be in the abnormal range, whilst in others, their MEPs may become absent before their CMCT is in the abnormal range. Patients may exhibit a "ceiling" effect where after a certain degree of motor neuron damage, CMCT measurements remain unchanged or absent. Studies investigating the change in MEP parameters longitudinally in HSP patients across different disease stages will help clarify the ability of MEPs to reflect disease progression.

None of the studies reviewed included presymptomatic patients with confirmed genetic diagnoses. MEP studies in this specific group of patients will help shed light on the utility of CMCT to predict future motor impairment. MEPs may be more sensitive to changes early in the disease process, before significant motor neuron damage limits variation in MEP measures.

Overall, longitudinal studies of MEPs in patients with HSP, including presymptomatic and various stages of the disease, are required to establish the utility of CMCT as a prognostic biomarker. Similarly, information from such studies will reveal the natural history of upper motor neuron damage in HSP.

Impact of HSP Genotype on MEP Findings

Fifty two percent of studies reviewed included patients from a single genotype, however, most of these were case reports or case series. The largest studies of MEP in HSP included a heterogeneous cohort of patients with multiple genotypes and unknown genotypes (13, 23). In addition, these studies did not clearly delineate MEP changes according to each genotype, with the exception of the study by Karle

and colleagues which performed subgroup analysis on SPG4 patients as well as providing values for other genotypes tested (13). Therefore, it is difficult to draw conclusions on MEP findings specific to each genotype although some patterns do emerge.

The largest cohorts that studied genotypically confirmed SPG4 patients noted that SPG4 patients had normal or only mildly prolonged MEPs (13, 34). These papers hypothesized that a very prolonged CMCT makes an SPG4 genotype unlikely, and may indicate SPG5, 6, 7, or 11. A study by Schulte et al. suggested that MEPs were a useful way of differentiating SPG4 from non-SPG4 HSP (47). Orlacchio et al. provides a caveat to this: all males in the SPG4 family studied had very prolonged CMCTs, whereas the women did not (36). This finding has not been replicated and the mechanism underlying this observation is uncertain.

For the other genotypes, the study numbers were too small to draw any firm conclusions. Though a significantly prolonged CMCT may point to certain HSP genotypes, patients will still need to undergo genetic testing to identify the mutation.

Future Recommendations

Further studies of MEPs in HSP with standardized methodology, strict inclusion criteria, adequate sample sizes and standardized assessment of clinical disease severity will clarify the role of MEP measures in HSP diagnosis and monitoring of disease severity. Our systematic review does not strongly support the use of MEPs as a sole biomarker in HSP although it remains useful when combined with other biomarkers, including clinical rating scales and diffusion tensor imaging.

There remains a need for a biomarker for use in future HSP clinical therapeutic trials. An ideal biomarker will be able to measure small changes in disease severity seen in HSP to account for the relatively short duration of clinical trials (1–2 years). Future studies of other potential biomarkers, such as neuroimaging and biological fluid-based biomarkers, are needed.

CONCLUSION

In summary, MEPs are not superior to clinical examination and genetic testing for diagnosis for HSP. However, MEP findings may help confirm a clinical diagnosis in suspected patients. Prospective longitudinal studies in presymptomatic and symptomatic patients with known genotypes are needed to clarify the utility of MEPs as a prognostic biomarker for HSP. Overall, this systematic review has revealed variation in MEP findings in patients in HSP with the most consistent finding being prolonged lower limb CMCT over upper limb abnormalities. In fact, the presence of greater lower limb involvement may be more likely to signify the presence of HSP when compared to other upper motor neuron disorders. Study quality assessment has shown inconsistencies in study methodology and reporting of results, perhaps contributing to the variation in results and preventing meta-analysis of available data. Current studies are insufficient to establish the validity of MEPs as a prognostic marker or a measure of disease severity as most were not designed for this purpose. Nevertheless, no clear correlation was found between MEP abnormalities and disease severity or duration, but there was some tendency for certain subtypes (e.g., SPG4) to be less affected. Future longitudinal studies in HSP patients with known genotypes, investigating the correlation of MEP parameters with standardized measures of clinical disease severity will help clarify the use of MEPs as a surrogate marker for disease severity for use in future clinical drug trials.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS

S-FS and KK conceived the study. S-FS performed the database search, initial screening, and developed the data extraction form. S-FS and RC extracted and analyzed the data and drafted the manuscript. KK, KN, and CS provided advice, critically reviewed the manuscript, and approved the manuscript for publication.

FUNDING

S-FS is the recipient of an NHMRC Postgraduate Scholarship and an ANZAN E&RF Scholarship (APP1133610). CS is a NHMRC Practitioner Fellow (APP1136800).

ACKNOWLEDGMENTS

We would like to thank Yulia Ulyannikova for her assistance with searching the various databases and Peter McInnis for his assistance with proofreading.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2019.00967/full#supplementary-material

REFERENCES

1. Bellofatto M, De Michele G, Iovino A, Filla A, Santorelli FM. Management of hereditary spastic paraplegia: a systematic review of the literature. *Front Neurol*. (2019) 10:3. doi: 10.3389/fneur.2019.00003
2. Blackstone C. Cellular pathways of hereditary spastic paraplegia. *Ann Rev Neurosci*. (2012) 35:25–47. doi: 10.1146/annurev-neuro-062111-150400
3. Boutry M, Morais S, Stevanin G. Update on the genetics of spastic paraplegias. *Curr Neurol Neurosci Rep*. (2019) 19:18. doi: 10.1007/s11910-019-0930-2
4. Kumar KR, Blair NF, Sue CM. An update on the hereditary spastic paraplegias: new genes and new disease models. *Mov Disord Clin Pract*. (2015) 2:213–23. doi: 10.1002/mdc3.12184

5. Denton KR, Xu C, Shah H, Li XJ. Modeling axonal defects in hereditary spastic paraplegia with human pluripotent stem cells. *Front Biol*. (2016) 11:339–54. doi: 10.1007/s11515-016-1416-0
6. Wali G, Sutharsan R, Fan Y, Stewart R, Tello Velasquez J, Sue CM, et al. Mechanism of impaired microtubule-dependent peroxisome trafficking and oxidative stress in SPAST-mutated cells from patients with Hereditary Spastic Paraplegia. *Sci Rep*. (2016) 6:27004. doi: 10.1038/srep27004
7. Wali G, Sue CM, Mackay-Sim A. Patient-derived stem cell models in SPAST HSP: disease modelling and drug discovery. *Brain Sci*. (2018) 8:E142. doi: 10.3390/brainsci8080142
8. Fan Y, Wali G, Sutharsan R, Bellette B, Crane DI, Sue CM, et al. Low dose tubulin-binding drugs rescue peroxisome trafficking deficit in patient-derived stem cells in Hereditary Spastic Paraplegia. *Biol Open*. (2014) 3:494–502. doi: 10.1242/bio.20147641
9. Marelli C, Lamari F, Rainteau D, Lafourcade A, Banneau G, Humbert L, et al. Plasma oxysterols: biomarkers for diagnosis and treatment in spastic paraplegia type 5. *Brain*. (2018) 141:72–84. doi: 10.1093/brain/awx297
10. Schöls L, Rattay TW, Martus P, Meisner C, Baets J, Fischer I, et al. Hereditary spastic paraplegia type 5: natural history, biomarkers and a randomized controlled trial. *Brain*. (2017) 140:3112–27. doi: 10.1093/brain/awx273
11. Scheuer KH, Svenstrup K, Jennum P, Rogvi-Hansen Bá, Werdelin L, Fenger K, et al. Double-blind crossover trial of gabapentin in SPG4-linked hereditary spastic paraplegia. *Eur J Neurol*. (2007) 14:663–6. doi: 10.1111/j.1468-1331.2007.01812.x
12. Martino G, Ivanenko Y, Serrao M, Ranavolo A, Draicchio F, Casali C, et al. Locomotor coordination in patients with Hereditary Spastic Paraplegia. *J Electromyogr Kinesiol*. (2019) 45:61–9. doi: 10.1016/j.jelekin.2019.02.006
13. Karle KN, Schüle R, Klebe S, Otto S, Frischholz C, Liepelt-Scarfone I, et al. Electrophysiological characterisation of motor and sensory tracts in patients with hereditary spastic paraplegia (HSP). *Orphan J Rare Dis*. (2013) 8:158. doi: 10.1186/1750-1172-8-158
14. Sadeghi N, Arrigoni F, D'Angelo MG, Thomas C, Irfanoglu MO, Hutchinson EB, et al. Tensor-based morphometry using scalar and directional information of diffusion tensor MRI data (DTBM): application to hereditary spastic paraplegia. *Hum Brain Mapp*. (2018) 39:4643–51. doi: 10.1002/hbm.24278
15. Groppa S, Oliviero A, Eisen A, Quartarone A, Cohen LG, Mall V, et al. A practical guide to diagnostic transcranial magnetic stimulation: report of an IFCN committee. *Clin Neurophysiol*. (2012) 123:858–82. doi: 10.1016/j.clinph.2012.01.010
16. Rossini PM, Burke D, Chen R, Cohen LG, Daskalakis Z, Di Iorio R, et al. Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin Neurophysiol*. (2015) 126:1071–107. doi: 10.1016/j.clinph.2015.02.001
17. National Heart, Lung, and Blood Institute; National Institutes of Health; U.S. Department of Health and Human Services. *Study Quality Assessment Tools*. (2019). Available online at: https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools.
18. Chipchase L, Schabrun S, Cohen L, Hodges P, Ridding M, Rothwell J, et al. A checklist for assessing the methodological quality of studies using transcranial magnetic stimulation to study the motor system: an international consensus study. *Clin Neurophysiol*. (2012) 123:1698–704. doi: 10.1016/j.clinph.2012.05.003
19. Moher D, Liberati A, Tetzlaff J, Altman DG, PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol*. (2009) 62:1006–12. doi: 10.1016/j.jclinepi.2009.06.005
20. Sue CM, Yiannikas C, Clouston PD, Lim CL, Graham S. Transcranial cortical stimulation in disorders of the central motor pathways. *J Clin Neurosci*. (1997) 4:19–25. doi: 10.1016/S0967-5868(97)90005-9
21. Thompson PD, Day BL, Rothwell JC, Dick JP, Cowan JM, Asselman P, et al. The interpretation of electromyographic responses to electrical stimulation of the motor cortex in diseases of the upper motor neurone. *J Neurol Sci*. (1987) 80:91–110. doi: 10.1016/0022-510X(87)90224-3
22. Pelosi L, Lanzillo B, Perretti A, Santoro L, Blumhardt L, Caruso G. Motor and somatosensory evoked potentials in hereditary spastic paraplegia. *J Neurol Neurosurg Psychiatry*. (1991) 54:1099–102. doi: 10.1136/jnnp.54.12.1099
23. Sartucci F, Tovani S, Murri L, Sagliocco L. Motor and somatosensory evoked potentials in Autosomal Dominant Hereditary Spastic Paraparesis (ADHSP) linked to chromosome 2p, SPG4. *Brain Res Bull*. (2007) 74:243–9. doi: 10.1016/j.brainresbull.2007.06.018
24. Di Lazzaro V, Oliviero A, Profice P, Ferrara L, Saturno E, Pilato F, et al. The diagnostic value of motor evoked potentials. *Clin Neurophysiol*. (1999) 110:1297–307. doi: 10.1016/S1388-2457(99)00060-7
25. Bönsch D, Schwindt A, Navratil P, Palm D, Neumann C, Klimpe S, et al. Motor system abnormalities in hereditary spastic paraparesis type 4 (SPG4) depend on the type of mutation in the spastin gene. *J Neurol Neurosurg Psychiatry*. (2003) 74:1109–12. doi: 10.1136/jnnp.74.8.1109
26. Denton A, Bunn L, Hough A, Bugmann G, Marsden J. Superficial warming and cooling of the leg affects walking speed and neuromuscular impairments in people with spastic paraparesis. *Ann Phys Rehabil Med*. (2016) 59:326–32. doi: 10.1016/j.rehab.2016.04.006
27. Klebe S, Stolze H, Kopper F, Lorenz D, Wenzelburger R, Volkmann J, et al. Gait analysis of sporadic and hereditary spastic paraplegia. *J Neurol*. (2004) 251:571–8. doi: 10.1007/s00415-004-0366-7
28. Martinuzzi A, Montanaro D, Vavla M, Paparella G, Bonanni P, Musumeci O, et al. Clinical and paraclinical indicators of motor system impairment in hereditary spastic Paraplegia: a pilot study. *PLoS ONE*. (2016) 11:e0153283. doi: 10.1371/journal.pone.0153283
29. Serranová T, Valls-Solé J, Muñoz E, Genís D, Jech R, Seeman P. Abnormal corticospinal tract modulation of the soleus H reflex in patients with pure spastic paraparesis. *Neurosci Lett*. (2008) 437:15–19. doi: 10.1016/j.neulet.2008.03.068
30. Geevasinga N, Menon P, Sue CM, Kumar KR, Ng K, Yiannikas C, et al. Cortical excitability changes distinguish the motor neuron disease phenotypes from hereditary spastic paraplegia. *Eur J Neurol*. (2015) 22:826–58. doi: 10.1111/ene.12669
31. Ginanneschi F, Carluccio MA, Mignarri A, Tessa A, Santorelli FM, Rossi A, et al. Hand muscles corticomotor excitability in hereditary spastic paraparesis type 4. *Neurol Sci*. (2014) 35:1287–91. doi: 10.1007/s10072-014-1707-7
32. Schady W, Dick JP, Sheard A, Crampton S. Central motor conduction studies in hereditary spastic paraplegia. *J Neurol Neurosurg Psychiatry*. (1991) 54:775–9. doi: 10.1136/jnnp.54.9.775
33. Battini R, Fogli A, Borghetti D, Michelucci A, Perazza S, Baldinotti F, et al. Clinical and genetic findings in a series of Italian children with pure hereditary spastic paraplegia. *Eur J Neurol*. (2011) 18:150–7. doi: 10.1111/j.1468-1331.2010.03102.x
34. Nielsen JE, Krabbe K, Jennum P, Koefoed P, Jensen LN, Fenger K, et al. Autosomal dominant pure spastic paraplegia: a clinical, paraclinical, and genetic study. *J Neurol Neurosurg Psychiatry*. (1998) 64:61–6. doi: 10.1136/jnnp.64.1.61
35. Liu SG, Zhao JJ, Zhuang MY, Li FF, Zhang QJ, Huang SZ, et al. Clinical and genetic study of SPG6 mutation in a Chinese family with hereditary spastic paraplegia. *J Neurol Sci*. (2008) 266:109–14. doi: 10.1016/j.jns.2007.09.024
36. Orlacchio A, Kawarai T, Gaudiello F, Totaro A, Schillaci O, Stefani A, et al. Clinical and genetic study of a large SPG4 Italian family. *Mov Disord*. (2005) 20:1055–9. doi: 10.1002/mds.20494
37. Orlacchio A, Patrono C, Gaudiello F, Rocchi C, Moschella V, Floris R, et al. Silver syndrome variant of hereditary spastic paraplegia: a locus to 4p and allelism with SPG4. *Neurology*. (2008) 70:1959–66. doi: 10.1212/01.wnl.0000294330.27058.61
38. Polo JM, Calleja J, Combarros O, Berciano J. Hereditary "pure" spastic paraplegia: a study of nine families. *J Neurol Neurosurg Psychiatry*. (1993) 56:175–81. doi: 10.1136/jnnp.56.2.175
39. Liu SG, Che FY, Heng XY, Li FF, Huang SZ, Lu DG, et al. Clinical and genetic study of a novel mutation in the REEP1 gene. *Synapse*. (2009) 63:201–5. doi: 10.1002/syn.20602
40. Manganelli F, Pisciotta C, Dubbioso R, Iodice R, Criscuolo C, Ruggiero L, et al. Electrophysiological characterisation in hereditary spastic paraplegia type 5. *Clin Neurophysiol*. (2011) 122:819–22. doi: 10.1016/j.clinph.2010.10.025
41. Cruz Martínez A, Tejada J. Central motor conduction in hereditary motor and sensory neuropathy and hereditary spastic paraplegia. *Electromyogr ClinNeurophysiol*. (1999) 39:331–5.

42. Claus D, Waddy HM, Harding AE, Murray NM, Thomas PK. Hereditary motor and sensory neuropathies and hereditary spastic paraplegia: a magnetic stimulation study. *Ann Neurol*. (1990) 28:43–9. doi: 10.1002/ana.410280109
43. Oguz KK, Sanverdi E, Has A, Temuçin Ç, Türk S, Doerschner K. Tract-based spatial statistics of diffusion tensor imaging in hereditary spastic paraplegia with thin corpus callosum reveals widespread white matter changes. *Diagn Interv Radiol*. (2013) 19:181–6. doi: 10.5152/dir.2013.046
44. Winner B, Gross C, Uyanik G, Schulte-Mattler W, Lürding R, Marienhagen J, et al. Thin corpus callosum and amyotrophy in spastic paraplegia-case report and review of literature. *Clin Neurol Neurosurg*. (2006) 108:692–8. doi: 10.1016/j.clineuro.2005.06.007
45. Nardone R, Tezzon F. Transcranial magnetic stimulation study in hereditary spastic paraparesis. *European neurology*. (2003) 49:234–7. doi: 10.1159/000070193
46. Jennum P, Neerup Jensen L, Fenger K, Nielsen JE, Fuglsang-Frederiksen A, Nielsen JE. Motor evoked potentials from the external anal sphincter in patients with autosomal dominant pure spastic paraplegia linked to chromosome 2p. *J Neurol Neurosurg Psychiatry*. (2001) 71:561–2. doi: 10.1136/jnnp.71.4.561
47. Schulte T, Miterski B, Börnke C, Przuntek H, Epplen JT, Schöls L. Neurophysiological findings in SPG4 patients differ from other types of spastic paraplegia. *Neurology*. (2003) 60:1529–32. doi: 10.1212/01.WNL.0000058769.75218.69
48. Schüle R, Brandt E, Karle KN, Tsaousidou M, Klebe S, Klimpe S, et al. Analysis of CYP7B1 in non-consanguineous cases of hereditary spastic paraplegia. *Neurogenetics*. (2009) 10:97–104. doi: 10.1007/s10048-008-0158-9
49. Roos P, Svenstrup K, Danielsen ER, Thomsen C, Nielsen JE. CYP7B1: novel mutations and magnetic resonance spectroscopy abnormalities in hereditary spastic paraplegia type 5A. *Acta Neurol Scand*. (2014) 129:330–4. doi: 10.1111/ane.12188
50. Rinaldi F, Bassi MT, Todeschini A, Rota S, Arnoldi A, Padovani A, et al. A novel mutation in motor domain of KIF5A associated with an HSP/axonal neuropathy phenotype. *J Clin Neuromuscul Dis*. (2015) 16:153–8. doi: 10.1097/CND.0000000000000063
51. Lang N, Optenhoefel T, Deuschl G, Klebe S. Axonal integrity of corticospinal projections to the upper limbs in patients with pure hereditary spastic paraplegia. *Clin Neurophysiol*. (2011) 122:1417–20. doi: 10.1016/j.clinph.2010.12.033
52. Kasher PR, De Vos KJ, Wharton SB, Manser C, Bennett EJ, Bingley M, et al. Direct evidence for axonal transport defects in a novel mouse model of mutant spastin-induced hereditary spastic paraplegia (HSP) and human HSP patients. *J Neurochem*. (2009) 110:34–44. doi: 10.1111/j.1471-4159.2009.06104.x
53. Deluca GC, Ebers GC, Esiri MM. The extent of axonal loss in the long tracts in hereditary spastic paraplegia. *Neuropathol Appl Neurobiol*. (2004) 30:576–84. doi: 10.1111/j.1365-2990.2004.00587.x
54. Rucco R, Liparoti M, Jacini F, Baselice F, Antenora A, De Michele G, et al. Mutations in the SPAST gene causing hereditary spastic paraplegia are related to global topological alterations in brain functional networks. *Neurol Sci*. (2019) 40:979–84. doi: 10.1007/s10072-019-3725-y
55. Chelban V, Tucci A, Lynch DS, Polke JM, Santos L, Jonvik H, et al. Truncating mutations in SPAST patients are associated with a high rate of psychiatric comorbidities in hereditary spastic paraplegia. *J Neurol Neurosurg Psychiatry*. (2017) 88:681–7. doi: 10.1136/jnnp-2017-315796
56. Latorre A, Rocchi L, Berardelli A, Bhatia KP, Rothwell JC. The interindividual variability of transcranial magnetic stimulation effects: implications for diagnostic use in movement disorders. *Mov Disord*. (2019) 34:936–49. doi: 10.1002/mds.27736

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

2.3 Choice of outcome measures for Hereditary Spastic Paraplegia clinical trials: Insights from an Australian cohort

2.3.1 Introduction

The results from this study are published in the Journal of Neurological Sciences as "Siow SF, Waters A, Coward S, Wali G, Ng K, Sue CM, Kumar KR. Outcome measures for hereditary spastic paraplegia clinical trials: Learnings from an Australian HSP center. J Neurol Sci. 2024 Jun 20:123100." I have included a more detailed explanation of this study in my thesis and attached the publication as an appendix.

In this study, I evaluated the utility of clinician rated outcome measures (SPRS and SARA), a patient reported outcome measure (SF-36), and a neurophysiological biomarker (motor evoked potentials) in a cohort of patients with HSP in Australia. To my knowledge, this is the first study to systematically evaluate and compare clinician and patient reported outcome measures, and a neurophysiological biomarker in a well-characterised cohort of patients with HSP to inform choice of outcome measures for HSP clinical trials.

2.3.2 Methods

2.3.2.1 Patient Recruitment

Patients were recruited from the Neurogenetics Clinic at Royal North Shore Hospital, Sydney, NSW, Australia. The inclusion criteria were a clinical and/or genetic diagnosis of HSP, age over 18 years, and ability to complete surveys in English. Exclusion criteria were the presence of any contraindications to transcranial magnetic stimulation including pregnancy, presence of metal in the body, and inability to lie on examination couch for procedure. Patients were recruited between April 2017 and January 2018. A total of 21 eligible patients were included. A sample size of convenience was used due to rarity of HSP and low population density. The diagnosis of HSP was made based on accepted diagnostic criteria of lower limb spasticity and weakness, [31] and the exclusion of other causes.

This study was approved by the local Institutional Ethics Committee (Reference number HREC/17/HAWKE/21).

2.3.2.2 Clinical data and outcome measures

Clinical data was collected after written consent was provided by all subjects. Standardised clinical data included demographic information, age of onset, disease duration, presence of weakness and spasticity, and associated features in complex HSP. Patients were assessed by a neurologist experienced in management of patients with HSP (authors SFS or KRK) and were administered the SPRS and SARA. The SPRS consists of 13 questions, each question has a possible score from 0-4 (normal to most severe), with a maximum possible total score of 52. [1] SARA is a clinician-rated outcome measure for symptoms of cerebellar ataxia. [12] It consists of 8 items, each item with a possible score from 0-4, with a maximum total score of 32. The SARA was included as ataxia is known to overlap with spastic paraplegia and the SPRS does not include items to assess severity of ataxia. Patients self-completed the SF-36, a generic widely used patient reported outcome measure employed to evaluate health-related quality of life. [21] SF-36 values were calculated as 8 subscales, and a summary mental health and physical component score were calculated according to published methods. [32, 33]

2.3.2.3 Motor evoked potentials

Motor evoked potentials were performed for all patients on the right abductor digiti minimi, tibialis anterior, and adductor hallucis. Motor evoked potentials were performed by experienced neurophysiology technicians (AW, SC) and neurologists (SFS, KN). In patients who were unable to be tested on their right limbs, e.g. joint deformity, their left limbs were tested. A standardised protocol was designed according to published guidelines for motor evoked potentials. [30]

Patients were examined lying supine. Nerve conduction studies were performed to calculate the peripheral motor conduction time for abductor digiti minimi, tibialis anterior, and adductor hallucis. Electrodes were applied to the muscle and the relevant nerves were stimulated. Peripheral compound muscle action potential latency was calculated when maximal amplitude was reached. A series of 8-10 measurements were made at supramaximal stimulation with the shortest latency used to calculate the F-wave. Peripheral motor conduction time is calculated as [F-wave + compound muscle action potential latency - 1]/2.

Transcranial magnetic stimulation was performed with a MagStim 200 stimulator using a double coil cone. Recording electrodes were placed over the muscle bellies for tibialis anterior, adductor hallucis and abductor digiti minimi. Resting motor threshold was measured with the patient at rest. The hotspot was determined by starting at the contralateral motor cortex for the leg area, and 5cm lateral to the contralateral motor cortex for the hand area. The

coil was moved vertically, then horizontally, in 1 cm increments until the maximal response is seen for the same level of stimulation. The stimulator output is set at 35% for upper limb and 50% for lower limb. The output is increased in 5% increments then lowered in steps of 1% until the % maximum stimulator output required to produce 50uV (peak-to-peak amplitude) response in 50% of 20 stimuli is determined. This is defined as the resting motor threshold.

To measure the total motor conduction time, recording electrodes were positioned over the muscles studied. The coil was positioned over the hotspot. The stimulator was set to 120% resting motor threshold. Stimulation was applied with muscles activated at maximum effort. Measurements were repeated 5-6 times. The shortest latency was taken as the total motor conduction time. Motor evoked potential amplitude was calculated from the largest motor evoked potential out of 5-6 measurements, where the compound muscle action potential amplitude was more than 15% of the peripherally elicited compound muscle action potential.

Central motor conduction time was calculated by subtracting the peripheral motor conduction time from total motor conduction time. Lab normative values were used to determine abnormal central motor conduction time values.

2.3.2.4 Statistical analysis

Patient demographics, outcome measure scores, and motor evoked potential results were evaluated descriptively. Differences of outcome measures between HSP genotype groups were investigated with one way ANOVA. An independent t-test was used to compare means of motor evoked potential measures between groups with different clinical severities. Pearson correlation coefficient was used to evaluate correlation between outcome measures. A p value of <0.05 was considered significant.

2.3.3 Results

2.3.3.1 Patient Characteristics

Twenty-one patients were recruited for this study. This included eleven female, ten male, with a mean age of 54.3 years (SD ±13.8 years). Ten had a diagnosis of HSP-*SPAST*, four HSP-*SPG7*, five without a genetic diagnosis, and one each HSP-*ATL1* and HSP-*KIF1A*. Most patients had a complicated phenotype (n=15, 71.4%) compared to pure phenotype (n=6, 28.6%).

Patients with HSP-*SPAST* had a mixture of pure and complicated phenotypes whilst patients with HSP-*SPG7* all had complicated phenotypes (Table 1). Patients with unknown genotype, HSP-*ATL1*, and HSP-*KIF1A* were grouped together as the rarer forms and for comparison purposes. The other/unknown genotype group were therefore heterogeneous in terms of phenotype and genotype.

Table 1**.** Patient demographics

Genotype	**HSP-*SPAST***	**HSP-*SPG7***	**Other (Unknown genotype, HSP-*ATL1*, HSP-*KIF1A*)**	**Total**
No. of patients N (%)	10 (47%)	4 (19%)	7 (33%)	21 (100%)
Female:Male	5:5	2:2	4:3	11:10
Age (years) Mean (SD)	57.1 (SD 12.36)	59.8 (SD 4.91)	47.1 (SD 17.3)	54.3 (SD 13.8)
Pure phenotype N (%)	5 (50%)	0	1 (14.3%)	6 (28.6%)
Complicated phenotype N (%)	5 (50%)	4 (100%)	6 (86%)	15 (71.4%)
Disease duration (years) Mean (range)	23.9 (12-42)	19.5 (14-24)	29.3 (6-50)	25.3 (6-50)

2.3.3.2 Clinician Rated Outcome Measures

Mean SPRS and SARA scores are presented in Table 2. I grouped the patients according to genotype, HSP-*SPG7* (n=4), HSP-*SPAST* (n=10), and other/unknown genotypes (n=7), for comparison. Shapiro-Wilk test and Levene's test showed normality and homogeneity

assumptions were met for all three groups. One-way ANOVA test was used to compare means between the groups. (Appendix)

Table 2**.** Spastic Paraplegia Rating Scale (SPRS) and Scale for Assessment and Rating of Ataxia (SARA) scores

	HSP-*SPAST* **N=10**	**HSP-*SPG7*** **N=4**	**Other/Unknown** **N=7**	**Total** **N=21**
SPRS score **Mean (SD)** **Range**	18.1 (8.21) Range 2-27	25.25 (6.40) Range 16-30	17.71 (5.71) Range 13-29	19.33 (SD 7.41) Range 2-30
SARA score **Mean (SD)** **Range**	6.65 (4.07) Range 0-12	15.25 (3.93) Range 10-18	6.71 (3.39) Range 3-13	8.31 (SD 5.01) Range 0-18

There were no significant differences between HSP-*SPAST*, HSP-*SPG7* and other/unknown subgroups for mean SPRS scores (p=0.213). There was a trend towards worse (higher) SPRS scores in HSP-*SPG7* compared to HSP-*SPAST* and other genotypes however this was not significant (Figure 1).

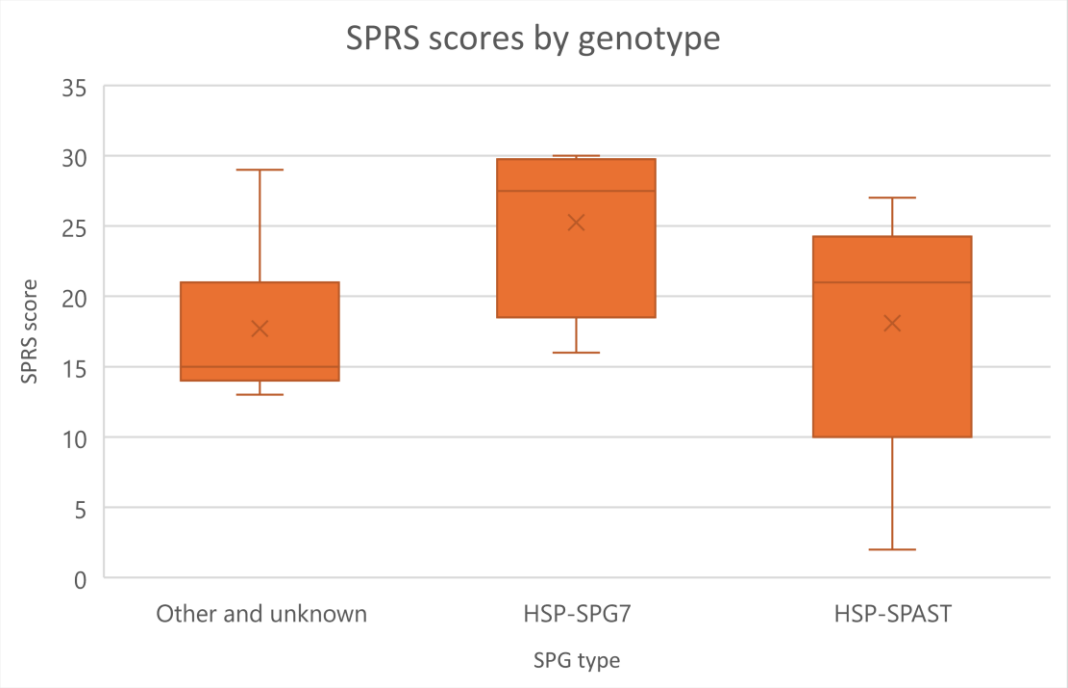


Figure 1. Spastic Paraplegia Rating Scale scores by genotype.

There was a significant difference between mean SARA scores between groups (p=0.003) (Figure 2).

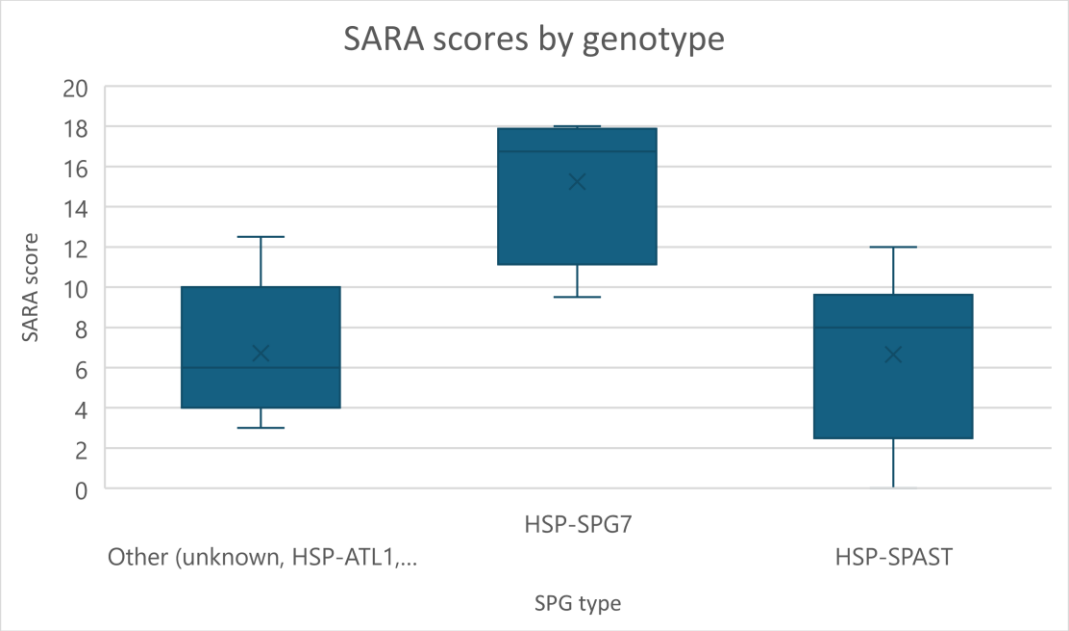


Figure 2. Scale for Assessment and Rating of Ataxia scores by genotype.

Tukey post hoc test showed significantly higher scores for the HSP-*SPG7* subgroup when compared to HSP-*SPAST* and other/unknown genotype subgroup (unknown genotypes, HSP-*ATL1*, HSP-*KIF1A*). (Table 3)

Table 3. Tukey post hoc test comparison of CROM scores between genotype groups (* represents significant difference)

Dependent Variable	**(I) SPG_type**	**(J) SPG_type**	**Sig.**
SPRS	Other/unknown	4	.993
		7	.241
	4	Other/unknown	.993

		7	.238
	7	Other/unknown	.241
		4	.238
SARA	Other/unknown	4	.999
		7	.006*
	4	Other/Unknown	.999
		7	.004*
	7	Other/Unknown	.006*
		4	.004*

2.3.3.3 Patient Rated Outcome Measures

Mean SF-36 subscores and component summary scores are presented in Table 4. Means of all eight SF-36 subscores, Mental Health Component Scores and Physical Component Scores were significantly lower (unpaired t-test two-tailed p value 0.0002 for Physical Component Score, 0.0229 for Mental Health Component Score) for our HSP cohort compared to the Australian population norms,[20] corresponding with a worse quality of life.

Shapiro-Wilk test and Levene's test showed normality and homogeneity assumptions were met for all three groups across SF-36 subscores and component summary scores, except for emotional limitations subscore for HSP-*SPAST* group which did not meet Shapiro-Wilk test for normality. One-way ANOVA test was used to compare means between the groups excluding the SF-36 emotional limitations subgroup. There were no significant differences in SF-36 subscore or summary component scores between the three groups. (Appendix)

Table 4. SF-36 subscores and component summary scores

	HSP-*SPAST* N=10	**HSP-*SPG7* N=4**	**Unknown/Other (including 1 HSP-*ATL1*, 1 HSP-*KIF1A*) N=7**	**Total N=21**	**Australian population norm (ABS 1995) [20] N=18,468**
SF-36 physical	41.50 (28.39)	17.50 (15.00)	40.71 (21.10)	36.67 (24.92)	82.6 (23.9)

function subscore Mean (SD)					
SF-36 physical limitations subscore Mean (SD)	42.50 (42.57)	25.00 (20.41)	28.57(36.60)	34.54 (36.64)	79.9 (35.1)
SF-36 emotional limitations subscore Mean (SD)	66.67 (47.14)	49.99 (43.04)	52.37 (46.58)	58.73 (44.61)	82.9 (32.3)
SF-36 energy subscore Mean (SD)	51.50 (23.69)	38.75 (14.93)	51.43 (16.26)	49.05 (19.79)	64.5 (19.8)
SF-36 emotional subscore Mean (SD)	72.00 (20.40)	60.00 (28.66)	70.86 (19.14)	69.33 (21.03)	75.9 (17.0)
SF-36 social subscore Mean (SD)	72.50 (26.87)	43.75 (26.02)	71.43 (18.7)	66.67 (25.72)	85.0 (22.5)
SF-36 pain subscore Mean (SD)	71.50 (29.77)	65.00 (19.04)	62.14 (19.55)	67.14 (24.23)	76.8 (25.0)
SF-36 general health subscore Mean (SD)	50.50 (13.42)	42.50 (17.08)	55.71 (18.58)	50.71 (15.83)	71.6 (20.3)

Mental Health Component Summary Score Mean (SD)	47.52 (16.45)	39.12 (16.60)	45.14 (13.38)	45.13 (15.06)	50.1 (10.0)
Physical Component Summary Score Mean (SD)	42.73 (9.28)	39.40 (3.67)	40.39 (4.20)	41.42 (6.93)	49.8 (10.2)

2.3.3.4 Motor evoked potentials

Motor evoked potential results are presented in Table 5. Abductor digiti minimi central motor conduction time was normal in all patients (normal <6.8 sec). Tibialis anterior central motor conduction time was prolonged (normal <14.24 sec) in 15 patients (71.4%), and borderline in 1 patient (4.8%). Adductor hallucis central motor conduction time was prolonged (normal <13sec) in 13 patients (61.9%), absent in 6 patients (28.6%), and normal in 2 patients (9.5%).

Table 5. Motor Evoked Potential results

	HSP-*SPAST* N=10 Mean (SD)	**HSP-*SPG7* N=4**	**Unknown, HSP-*ATL1*, HSP-*KIF1A* N=7**	**Total**
Abductor digiti minimi CMCT (msec)	4.77 (1.34) N=10	4.88 (1.89) N=3	4.29 (1.88) N=6	4.64 (1.53) **N=19**
Abductor digiti minimi amp (mV)	0.57 (0.13) N=10	0.65 (0.14) N=3	0.79 (0.12) N=6	0.65 (0.16) **N=19**

Adductor hallucis CMCT (ms)	15.84 (2.41) N=7	15.72 (2.40) N=3	14.80 (3.49) N=5	15.47 (2.65) **N=15**
Adductor hallucis amp (mV)	0.25 (0.14) N=7	0.23 (0.10) N=3	0.33 (0.20) N=5	0.27 (0.15) **N=15**
Tibialis anterior CMCT (ms)	16.03 (3.09) N=10	18.18 (1.60) N=4	16.96 (4.05) N=7	16.75 (3.21) **N=21**
Tibialis anterior amp (mV)	0.61 (0.16) N=10	0.56 (0.36) N=4	0.61 (0.28) N=7	0.60 (0.24) **N=21**

*Central motor conduction time - CMCT

Tibialis anterior central motor conduction time was prolonged in 6/10 (60%) HSP-*SPAST* patients and adductor hallucis central motor conduction time was prolonged or absent in 7/10 (70%) HSP-*SPAST* patients. Mean adductor hallucis central motor conduction time were similar across the various genotypes. Mean tibialis anterior central motor conduction time was more prolonged in the HSP-*SPG7* group compared to HSP-*SPAST* and non-HSP-*SPG7* patients. Mean abductor digiti minimi, adductor hallucis, and tibialis anterior amplitudes were not significantly different across all genotypes.

Shapiro-Wilk test and Levene's test showed normality and homogeneity assumptions were met for motor evoked potential measures, except for adductor hallucis amplitude in HSP-*SPAST* subgroup which did not satisfy Shapiro-Wilk test for normality and tibialis anterior amplitude which did not satisfy Levene's test for homogeneity. One way ANOVA excluding those two measures did not identify between group differences for upper or lower limb central motor conduction times and lower limb amplitude. I compared the means of central motor conduction time between patients with mild disease severity (SPRS scores 0-17) and moderate disease severity (17-34) using an independent t-test which did not identify any significant differences. (Appendix)

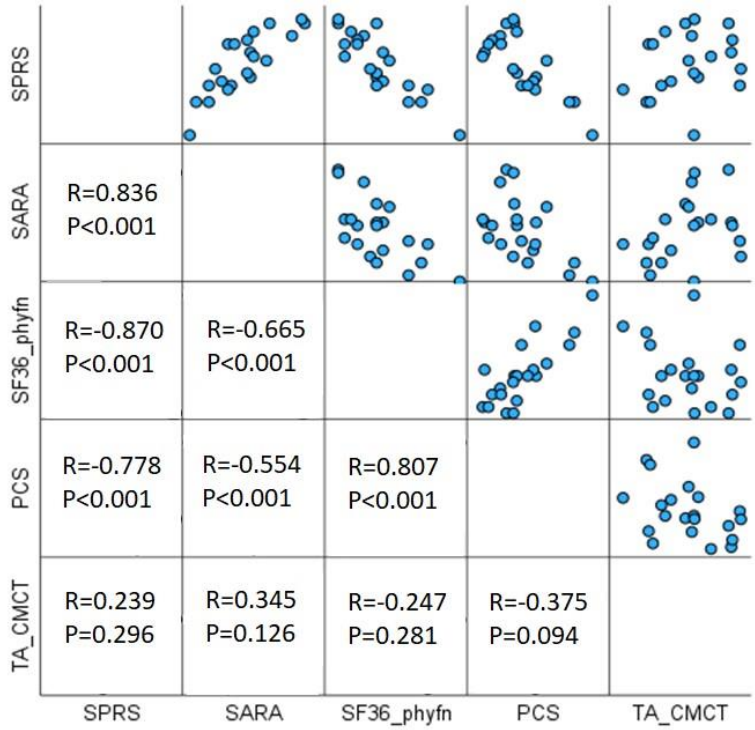
2.3.3.5 Correlation between patient and clinician-rated outcome measures: SPRS, SARA, SF36

There was significant correlation between SPRS and SARA scores (r = 0.836, p<0.001), SPRS Scores and SF36 physical function subscale (r = -0.870, p<0.001), and SARA scores and SF36 physical function subscale (r = -0.665, p=0.001). The SPRS and SARA scores also correlated with the SF36 Physical component summary score (r=-0.778, p<0.001, and r=-0.554, p-0.009). There was no significant correlation between the SPRS and SARA with the other SF36 subscales. (Figure 3)

2.3.3.6 Correlation between patient and clinician-rated outcome measures and motor evoked potentials

There was no significant correlation between the SPRS, SARA, or SF36 scores with any of the motor evoked potential measures in the whole HSP cohort. (Figure 3)

Figure 3. Correlation between clinician and patient-rated outcome measures, and motor evoked potential measures.



2.3.4 Discussion

There is an urgent need for standardised outcome measures and biomarkers to evaluate treatment efficacy in HSP clinical trials. [34] The findings of this study inform choice of outcome measures for HSP studies.

SPRS was able to differentiate patients across a spectrum of severities with SPRS scores ranging from 2 to 30 (mild to moderate) in our patient cohort. SPRS showed a trend towards higher scores in the HSP-*SPG7* subgroup though larger studies are needed to determine if SPRS can differentiate between HSP subtypes. Overall, the SPRS was a comprehensive clinical disease severity measure encompassing relevant symptomatic features of HSP.

SARA scores were significantly higher for patients with HSP-*SPG7* compared to other genotypes. This finding could be due to SARA being more sensitive to severity of ataxic symptoms involving upper limb and bulbar function, [12] that can be seen in patients with complex HSP but are not assessed in SPRS. Therefore, SARA is recommended in addition to SPRS for HSP studies which include HSP genotypes with ataxic symptoms, such as HSP-*SPG7*. Sensitivity to change in ataxic symptoms are relevant in assessing efficacy of treatment options for HSP.

SF-36 scores were significantly lower (worse quality of life) in our patient cohort compared to population normative values. This is in keeping with previously reported studies of worse quality of life as measured with SF-36 in HSP. [22-24] Despite this finding, there was no correlation with overall disease severity as measured with the SPRS. This could be due to the lack of HSP-specific items in the SF-36 such as bladder and bowel symptoms, spasticity and spasms, and frequency of falls. [35] My findings suggest that generic measures such as SF-36 lack sensitivity to quality of life changes related to HSP and an HSP-specific quality of life scale is needed.

There was no correlation between SF-36 subscores, aside from the physical function subscore, and clinical disease severity. This suggests that the non-physical components of quality of life, such as mood and general wellbeing, are not always aligned with clinical disease severity. Quality of life is likely influenced by other non-HSP related factors which can be important when assessing outcomes in clinical trials, such as the psychosocial impact of a potential treatment. Therefore, inclusion of a quality of life measure is important in the design of HSP clinical trials.

There was a significant correlation between the SF36 physical function subscore with SPRS and SARA scores in the whole group analysis. This demonstrates concordance of patient-reported disease severity with clinician-rated measures. Therefore, I propose SF-36 physical function subscore as a patient-reported measure of clinical disease severity. This could increase accessibility of clinical trials for patients who are unable to travel to attend trials centers for a clinician assessment.

There was no correlation between motor evoked potential measures and clinical disease severity or quality of life. This adds to the growing literature of motor evoked potentials in HSP with most studies showing no correlation with clinician reported outcome measures. [36-38] Although a single study reported a weak correlation between lower limb central motor conduction time and SPRS scores, [39] this finding has not been reproduced. [37] In addition, lower limb central motor conduction time values did not differentiate mild from moderate clinical severity as measured with the SPRS. I did not find any correlation between motor evoked potential measures and SF-36 scores. The relationship between motor evoked potential values and patient-reported outcome measures have not been studied previously. My scoping review of outcome measures revealed only one study which looked at the correlation between a patient rated outcome measure and an objective biomarker, gait analysis, which showed a correlation between gait parameters and the SF-36 Physical Component Summary score. [24, 34] Overall, the relationship between patient-reported outcome measures and objective biomarkers in HSP has not been widely studied. It is likely that there will not be strong correlations between patient reported outcome measures and objective biomarkers as health-related quality of life is a complex measure including non-physical factors. This highlights the importance of including a patient reported outcome measure in addition to an objective biomarker to include the patient perspective in assessing patient outcomes.

There were several limitations in this study. I had a small patient cohort due to the rarity of the condition and low population density in Australia. However, this patient cohort represented a range of HSP genotypes and disease severities. As I have investigated the relationship between the various outcome measures, these findings remain relevant to the greater HSP community. Larger studies will help validate these findings. Multicenter, international, collaborations are key to facilitate larger sample sizes in the study of rare disease, such as HSP.

I was unable to recruit patients with severe disease due to mobility issues precluding motor evoked potentials which required patients to lie on an examination couch. This may have limited representation of patients with severe disease. However, this finding is important when considering the choice of biomarker for HSP clinical trials as measures such as gait analysis and diffusion tensor imaging may not be accessible by patients with limited mobility. Therefore, inclusion of these measures may discriminate against more severely affected patients for enrolment in HSP clinical trials.

Due to time and resource limitations, I was unable to collect longitudinal data. This would have been helpful to determine responsiveness of the studied clinician reported outcome measures, patient reported outcome measure, and motor evoked potentials to change over time. Further longitudinal studies are crucial to determine optimal trial duration, a challenge in a slowly progressive disorder like HSP.

I was only able to investigate a single objective biomarker, motor evoked potentials. I acknowledge that there are several more promising biomarkers, including gait analysis, diffusion tensor imaging, and serum neurofilament light chain. Due to resource limitations, I was unable to include these biomarkers in this study. However, there are studies in the literature that have investigated the correlation of these biomarkers with clinical measures such as the SPRS. [6, 10, 11, 24, 38, 40-42] A systematic review of the literature will clarify the utility of these biomarkers and identify any gaps needed to be addressed.

2.3.5 Study Conclusion

In this study, I compared clinician and patient reported outcome measures, and an objective biomarker to identify suitable outcome measures for use in HSP studies. The results of this study inform choice of outcome measures to evaluate treatment candidates in HSP clinical trials.

2.4 Discussion and Conclusion

In this chapter, I evaluated a set of outcome measures for HSP. I found that the SPRS was a suitable CROM for HSP as it was easy to administer and able to stratify varying disease severities (Section 2.3). However, the SPRS does not evaluate additional features associated

with complex HSP subtypes such as ataxia or cognition. Therefore, I evaluated SARA as a complementary CROM and found that it was particularly useful in HSP-*SPG7*, which is often associated with ataxia symptoms.

Consistent with previous literature, I found that the SF-36 demonstrated worse quality of life in this HSP patient cohort compared to general population norms. However, there was no correlation between worse quality of life and clinical disease severity. Although this finding could be attributed to the fact that the SF-36 and SPRS/SARA are measuring different domains, it is also likely that generic quality of life measures such as the SF-36 may not be sensitive to HSP-related quality of life changes. In Chapter 4, I develop an HSP-specific quality of life measure to address this gap.

My systematic review and cohort study confirmed that prolonged central motor conduction time measured with MEPs can be used as a diagnostic biomarker for HSP. However, there was no correlation between MEP values and clinician or patient-reported disease severity. In addition, I found that MEPs required specialised equipment and trained personnel, and could cause patient discomfort. Overall, my study findings do not support the use of MEPs as a monitoring or prognostic biomarker in HSP clinical trials. In Chapter 3, I investigate a potential blood-based biomarker for HSP-*SPAST* to address the need for a standardised, easily accessible, HSP-specific biomarker suitable for international multi-site clinical trials.

2.5 References

1. Schüle R, Holland-Letz T, Klimpe S, et al. The Spastic Paraplegia Rating Scale (SPRS) : A reliable and valid measure of disease severity. *Neurology* 2006; 67: 430-434. DOI: 10.1212/01.wnl.0000228242.53336.90.
2. Servelhere KR, Faber I, Coan AC, et al. Translation and validation into Brazilian Portuguese of the Spastic Paraplegia Rating Scale (SPRS). *Arquivos de neuro-psiquiatria* 2016; 74: 489-494. DOI: 10.1590/0004-282X20160047.
3. Schols L, Rattay TW, Martus P, et al. Hereditary spastic paraplegia type 5: Natural history, biomarkers and a randomized controlled trial. *Brain* 2017; 140(12): 3112-3127. DOI: https://dx.doi.org/10.1093/brain/awx273.
4. Ardolino G, Bocci T, Nigro M, et al. Spinal direct current stimulation (tsDCS) in hereditary spastic paraplegias (HSP): A sham-controlled crossover study. *Journal of Spinal Cord Medicine* 2021; 44(1): 46-53. DOI: https://dx.doi.org/10.1080/10790268.2018.1543926.

5. de Lima FD, Faber I, Servelhere KR, et al. Randomized Trial of Botulinum Toxin Type A in Hereditary Spastic Paraplegia - The SPASTOX Trial. *Movement Disorders* 2021; 36: 1654-1663. DOI: 10.1002/mds.28523.
6. Montanaro D, Vavla M, Frijia F, et al. Multimodal MRI Longitudinal Assessment of White and Gray Matter in Different SPG Types of Hereditary Spastic Paraparesis. *Frontiers in neuroscience* 2020; 14: 325-325. DOI: 10.3389/fnins.2020.00325.
7. Musacchio T, Nohl K, Boelmans K, et al. Biological course and natural history of hereditary spastic paraplegia type 11 (SPG11). *Movement Disorders* 2018; 33(Supplement 2): S225.
8. Cubillos-Arcila DM, Machado GD, Sehnem L, et al. Progression of Functional Gait in Hereditary Spastic Paraplegias. *Cerebellum* 2022; 21(3): 350-357. DOI: https://dx.doi.org/10.1007/s12311-021-01302-2.
9. Rezende TJR, de Albuquerque M, Lamas GM, et al. Multimodal MRI-based study in patients with SPG4 mutations. *PloS one* 2015; 10: e0117666-e0117666. DOI: 10.1371/journal.pone.0117666.
10. Kessler C, Serna-Higuita LM, Wilke C, et al. Characteristics of serum neurofilament light chain as a biomarker in hereditary spastic paraplegia type 4. *Annals of Clinical and Translational Neurology* 2022; 9(3): 326-338. DOI: https://dx.doi.org/10.1002/acn3.51518.
11. Lin Q, Liu Y, Ye Z, et al. Potential markers for sample size estimations in hereditary spastic paraplegia type 5. *Orphanet Journal of Rare Diseases* 2021; 16(1) (no pagination). DOI: https://dx.doi.org/10.1186/s13023-021-02014-w.
12. Schmitz-HÜBsch T, Tezenas Du Montcel S, Kremer B, et al. Scale for the assessment and rating of ataxia : Development of a new clinical scale. *Neurology* 2006; 66: 1717-1720. DOI: 10.1212/01.wnl.0000219042.60538.92.
13. De la Casa-Fages B, Fernandez-Eulate G, Gamez J, et al. Parkinsonism and spastic paraplegia type 7: Expanding the spectrum of mitochondrial Parkinsonism. *Movement Disorders* 2019; 34(10): 1547-1561. DOI: https://dx.doi.org/10.1002/mds.27812.
14. Traschütz A, Adarmes-Gómez AD, Anheim M, et al. Responsiveness of the Scale for the Assessment and Rating of Ataxia and Natural History in 884 Recessive and Early Onset Ataxia Patients. *Ann Neurol* 2023; 94: 470-485. 20230612. DOI: 10.1002/ana.26712.
15. Reoli R, Therrien A, Millar J, et al. The Scale for Assessment and Rating of Ataxia Is Reliable and Valid in the Telehealth Setting for Patients With Cerebellar Ataxia. *Phys Ther* 2024; 104. DOI: 10.1093/ptj/pzad166.

16. Bourcier D, Bélair N, Pedneault-Tremblay É A, et al. French Translation and Cross-cultural Adaptation of the Scale for the Assessment and Rating of Ataxia. *Cerebellum* 2023; 22: 1118-1122. 20221008. DOI: 10.1007/s12311-022-01484-3.
17. Braga-Neto P, Godeiro-Junior C, Dutra LA, et al. Translation and validation into Brazilian version of the Scale of the Assessment and Rating of Ataxia (SARA). *Arq Neuropsiquiatr* 2010; 68: 228-230. DOI: 10.1590/s0004-282x2010000200014.
18. Anita Stewart CDS, John E. Ware, Ron D. Hays, Kenneth B. Wells, Sandra H. Berry, Caren Kamberg, Eugene C. Nelson, Allyson Ross Davies, William H. Rogers, et al. *Measuring Functioning and Well Being: The Medical Outcomes Study Approach.* Durham and London: Duke University Press., 1992.
19. Lins L and Carvalho FM. SF-36 total score as a single measure of health-related quality of life: Scoping review. *SAGE Open Medicine* 2016; 4: 2050312116671725-2050312116671725. DOI: 10.1177/2050312116671725.
20. Statistics ABo. National Health Survey: SF-36 Population Norms. Australia: Asutralian Bureau of Statistics, 1995.
21. Hays RD, Sherbourne CD and Mazel RM. The rand 36-item health survey 1.0. *Health economics* 1993; 2: 217-227. DOI: 10.1002/hec.4730020305.
22. Braschinsky M, Rannikmae K, Krikmann U, et al. Health-related quality of life in patients with hereditary spastic paraplegia in Estonia. *Spinal Cord* 2011; 49(2): 175-181. DOI: https://dx.doi.org/10.1038/sc.2010.61.
23. Gassner H, List J, Martindale CF, et al. Functional gait measures correlate to fear of falling, and quality of life in patients with Hereditary Spastic Paraplegia: A cross-sectional study. *Clinical Neurology and Neurosurgery* 2021; 209 (no pagination). DOI: https://dx.doi.org/10.1016/j.clineuro.2021.106888.
24. Regensburger M, Spatz IT, Ollenschlager M, et al. Inertial Gait Sensors to Measure Mobility and Functioning in Hereditary Spastic Paraplegia: A Cross-Sectional Multicenter Clinical Study. *Neurology* 2022; 06. DOI: https://dx.doi.org/10.1212/WNL.0000000000200819.
25. Klimpe S, Schule R, Kassubek J, et al. Disease severity affects quality of life of hereditary spastic paraplegia patients. *European Journal of Neurology* 2012; 19(1): 168-171. DOI: https://dx.doi.org/10.1111/j.1468-1331.2011.03443.x.
26. Orsucci D, Petrucci L, Caldarazzo Ienco E, et al. Hereditary spastic paraparesis in adults. A clinical and genetic perspective from Tuscany. *Clinical Neurology and Neurosurgery* 2014; 120: 14-19. DOI: https://dx.doi.org/10.1016/j.clineuro.2014.02.002.

27. Bertolucci F, Di Martino S, Orsucci D, et al. Robotic gait training improves motor skills and quality of life in hereditary spastic paraplegia. *NeuroRehabilitation* 2015; 36(1): 93-99. DOI: https://dx.doi.org/10.3233/NRE-141196.
28. Bastani PB, Kordjazi M, Oveisgharan S, et al. A Randomized Controlled Trial of the Effect of Repetitive Transcranial Magnetic Stimulation of the Motor Cortex on Lower Extremity Spasticity in Hereditary Spastic Paraplegia. *Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society* 2021; 23. DOI: https://dx.doi.org/10.1097/WNP.0000000000000874.
29. Thompson PD, Day BL, Rothwell JC, et al. The interpretation of electromyographic responses to electrical stimulation of the motor cortex in diseases of the upper motor neurone. *Journal of the neurological sciences* 1987; 80: 91-110. DOI: 10.1016/0022-510X(87)90224-3.
30. Groppa S, Oliviero A, Eisen A, et al. A practical guide to diagnostic transcranial magnetic stimulation: Report of an IFCN committee. *Clinical neurophysiology* 2012; 123: 858-882. DOI: 10.1016/j.clinph.2012.01.010.
31. Fink JK. Hereditary spastic paraplegia: clinico-pathologic features and emerging molecular mechanisms. *Acta neuropathologica* 2013; 126: 307-328. DOI: 10.1007/s00401-013-1115-8.
32. Ware JE, Kosinski M, Bayliss MS, et al. Comparison of Methods for the Scoring and Statistical Analysis of SF-36 Health Profile and Summary Measures: Summary of Results from the Medical Outcomes Study. *Medical care* 1995; 33: AS264-AS279. DOI: 10.1097/00005650-199501001-00005.
33. Charles T, Karlsson J and Marianne S. Do SF-36 Summary Component Scores Accurately Summarize Subscale Scores? *Quality of life research* 2001; 10: 395-404. DOI: 10.1023/A:1012552211996.
34. Siow S-F, Yeow D, Rudaks LI, et al. Outcome Measures and Biomarkers for Clinical Trials in Hereditary Spastic Paraplegia: A Scoping Review. *Genes* 2023; 14: 1756. DOI: 10.3390/genes14091756.
35. Kerstens HCJW, Lith BJHv, Nijkrake MJ, et al. Healthcare needs, expectations, utilization, and experienced treatment effects in patients with hereditary spastic paraplegia: a web-based survey in the Netherlands. *Orphanet journal of rare diseases* 2021; 16: 1-283. DOI: 10.1186/s13023-021-01915-0.

36. Siow S-F, Smail RC, Ng K, et al. Motor Evoked Potentials in Hereditary Spastic Paraplegia—A Systematic Review. *Frontiers in neurology* 2019; 10: 967-967. DOI: 10.3389/fneur.2019.00967.
37. Brighente SF, Vicuna P, Louzada ALR, et al. Evoked potentials as biomarkers of hereditary spastic paraplegias: A case-control study. *PLoS ONE* 2021; 16(11) (no pagination). DOI: https://dx.doi.org/10.1371/journal.pone.0259397.
38. Martinuzzi A, Montanaro D, Vavla M, et al. Clinical and Paraclinical Indicators of Motor System Impairment in Hereditary Spastic Paraplegia: A Pilot Study. *PloS one* 2016; 11: e0153283-e0153283. DOI: 10.1371/journal.pone.0153283.
39. Karle KN, Schule R, Klebe S, et al. Electrophysiological characterisation of motor and sensory tracts in patients with hereditary spastic paraplegia (HSP). *Orphanet Journal of Rare Diseases* 2013; 8(1) (no pagination). DOI: https://dx.doi.org/10.1186/1750-1172-8-158.
40. Lassmann C, Ilg W, Schneider M, et al. Specific gait changes in prodromal hereditary spastic paraplegia type 4 - preSPG4 study. *medRxiv* 2022; 21. DOI: https://dx.doi.org/10.1101/2022.04.20.22274071.
41. Rattay TW, Volker M, Rautenberg M, et al. The prodromal phase of hereditary spastic paraplegia type 4: the preSPG4 cohort study. *Brain : a journal of neurology* 2022; 26. DOI: https://dx.doi.org/10.1093/brain/awac155.
42. Wilke C, Rattay TW, Hengel H, et al. Serum neurofilament light chain is increased in hereditary spastic paraplegias. *Annals of Clinical and Translational Neurology* 2018; 5: 876-882. DOI: 10.1002/acn3.583.

Chapter 3 Developing a biomarker targeted to underlying disease pathophysiology of HSP-*SPAST*

3.1 Introduction

In Chapter 1, I proposed a core outcome set for HSP that included a biomarker for HSP to sensitively measure treatment response in HSP clinical trials. I reviewed and investigated motor evoked potentials in Chapter 2 and found that it was not a suitable biomarker for HSP. In this chapter, I explore a potential biomarker for HSP-*SPAST*, the most common form of HSP. I hypothesised that acetylated α-tubulin, an intracellular protein that is reduced in HSP-*SPAST* patient-derived neuronal cell models, would also be reduced in patient blood samples. *In vitro* studies have shown that restoring levels of acetylated α-tubulin levels rescued the downstream effects of oxidative stress induced axonal degeneration. These findings highlighted the possibility of using acetylated α-tubulin levels as a potential pharmacodynamic biomarker to test the effect of tubulin-modulating drugs in HSP-*SPAST.* In this chapter, I investigated if acetylated α-tubulin levels are reduced in HSP-*SPAST* patient PBMCs compared to controls. I evaluated acetylated α-tubulin levels in peripheral blood mononuclear cells (PBMCs) as an easily accessible patient sample.

3.1.1 Biochemical biomarkers for HSP and their limitations

In my scoping review of biomarkers for HSP, I reviewed biochemical, neuroimaging, neurophysiology, and other types of biomarkers. [1] When considering potential biomarkers for HSP, I explored biochemical biomarkers identified through knowledge of underlying disease pathophysiological mechanisms as these biomarkers had the potential for high specificity and sensitivity and could be measured in easily collected patient samples, such as blood or urine. Biochemical biomarkers for HSP reported in the literature, including blood and cerebrospinal fluid amino acid levels, lipidomics, glycosylceramide profile, neurofilament heavy chain levels, autophagy-related protein (ATG9A) ratio, cerebrospinal fluid tau levels, mitochondrial DNA levels, cell morphometry, and scanning electron microscopy of hair shafts, either did not clearly differentiate between patients and controls, or were not evaluated longitudinally to measure disease progression or response to intervention. [1] In the past decade, only two

biochemical biomarkers have been found to be consistently abnormal in patients with HSP – serum neurofilament light chain levels, and cerebrospinal fluid/plasma 25-hydroxycholesterol levels. [1]

Neurofilaments are axonal proteins that are used as surrogate markers for axonal damage. [2] Neurofilament light chains are released, following neuronal damage, into the cerebrospinal fluid and subsequently into blood. [3] Serum neurofilament light chain levels are elevated in several conditions that involve neuronal damage, such as multiple sclerosis, motor neuron disease, Parkinson's disease and Alzheimer's dementia. [2, 4, 5] Serum neurofilament light chain levels have also been shown to be elevated in patients with HSP compared to controls. [6-11] However, neurofilament light chain levels have not been demonstrated to measure longitudinal change or response to interventions in HSP. A longitudinal study of patients with HSP-*SPAST* showed that there was no change in serum neurofilament light chain levels over a 2-year period. [6] In addition, there was no correlation between serum neurofilament light chain levels and clinical disease severity as measured with the Spastic Paraplegia Rating Scale (SPRS). [6] Therefore, serum neurofilament light chain is a non-specific measure for neuronal degeneration and its sensitivity to change over time or in response to intervention in HSP requires further investigation.

HSP-*CYP7B1* (SPG5) are due to mutations in the *CYP7B1* gene which encodes cytochrome P-450 oxysterol 7a-hydroxylase. These mutations impair enzymatic function and result in increased oxysterol substrates, 27-hydroxycholesterol (27-OHC) and 25-hydroxycholesterol (25-OHC). [12, 13] Individuals with HSP-*CYP7B1* were shown to have elevated 25-OHC and 27-OHC levels compared to controls. [13] However, correlation between 27-OHC levels with clinical disease severity as measured with the SPRS was inconsistent with one study reporting a correlation[14] which was subsequently not replicated. [13] Two clinical trials showed decrease in 27-OHC levels in response to atorvastatin however there was no demonstrable clinical benefit. [13, 14] 25-OHC and 27-OHC levels are potential diagnostic biomarkers for HSP-*CYP7B1* but are not likely to be relevant to other HSP subtypes. The role of 25-OHC and 27-OHC levels as prognostic or monitoring biomarkers remain unknown.

3.1.2 HSP-*SPAST* cellular biology and its relation to acetylated α-tubulin

HSP-*SPAST* is the most common HSP subtype, accounting for 15-40% of HSP cases depending on ethnic background. [15] Patients with HSP-*SPAST* tend to have pure or uncomplicated HSP though there are a subset who have complex HSP. [16] HSP-*SPAST* tends to have a later age of onset (mean 29 +/- 17 years) with significant clinical variability, even within families with identical *SPAST* mutations. [17]

HSP-*SPAST* is due to mutations in the *SPAST* gene, which encodes spastin, a microtubule severing ATPase responsible for regulating microtubule breakdown which is an essential component of axonal transport. This regulation of microtubule dynamics is critical to axonal transport, and reduction in or loss of spastin function has been shown to slow axonal transport. [18]

Studies in HSP-*SPAST* patient-derived stem cell models (olfactory neurosphere-derived cells and cortical neurons differentiated from induced pluripotent stem cells), showed that the patient cells had reduced spastin, a microtubule severing protein, and reduced acetylated α-tubulin leading to slower microtubule-dependent axonal transport and subsequent increase in oxidative stress and neuronal degeneration. [18] The observed changes were reversed with tubulin-binding drugs, epitholone D and noscapine. [19-21] These findings have led to the proposal of tubulin-binding drugs, such as epitholone D and noscapine, as potential therapeutic agents for HSP-*SPAST*.

Previous studies have demonstrated no correlation between spastin levels and disease severity in HSP-*SPAST*. [22] However, there has been no studies of the level of microtubule dysfunction in patients with HSP-*SPAST*. Therefore, I postulated that patients with HSP-*SPAST* have abnormal levels of markers of microtubule dysfunction as reflected in aforementioned *in vitro* studies.

3.1.3 Acetylated alpha tubulin as a surrogate marker for microtubule dysfunction

Microtubules are polymers made up of alpha and beta tubulin subunits that form part of the cell cytoskeleton and are essential to intracellular organelle transport. Specific tubulin isotypes are differentially expressed in various tissues though specific expression patterns of each isotype are not completely understood. [23] β3-tubulin is a marker for neuronal microtubules that has been identified in development and adulthood, with highest expression

during axonal guidance and maturation. [24] α-tubulin isotypes play roles in neuronal migration and skeletal muscle cytoskeletal function. [25, 26]

Post-translational modifications of tubulin are reversible changes used by cells to alter microtubule structure and function. Types of post-translational modifications include acetylation, tyrosination, detyrosination, and polyglutamylation of tubulin. All α-tubulin isotypes, except TUBA8, undergo acetylation on lysine 40. [27] Acetylation of α-tubulin at lysine 40 has been shown to restrict motion of that part of the α-tubulin subunit resulting in increased resistance of the microtubule to stress, and subsequently increased microtubule stability. [28] Therefore, levels of acetylated α-tubulin have been used as surrogate marker for microtubule stability. [29] Tyrosination and detyrosination of α-tubulin isotypes play a role in neuronal differentiation. Although spastin has been shown to be activated by long glutamate side chains, [30] the role of polyglutamylation remains unclear. [23]

I investigated acetylated α-tubulin as a biomarker for HSP-*SPAST* as it is a marker for microtubule dysfunction, and *in vitro* studies of HSP-*SPAST* neurons have demonstrated microtubule dysfunction and reduced levels of acetylated α-tubulin. In addition, *in vitro* studies have demonstrated restoration of acetylated α-tubulin levels in response to tubulin-binding drugs and therefore acetylated α-tubulin is a potential pharmacodynamic biomarker to measure the effect of tubulin-binding drugs in a clinical trial.

3.1.4 Measurement of acetylated α-tubulin levels in PBMCs

PBMCs were chosen as the patient sample as it was easy to access, transport, and process. As microtubules are known to be present in all human cells, I postulated that the reduced levels of acetylated α-tubulin seen in patient-derived neuronal cell models [18, 21] would also be seen in peripheral blood mononuclear cells. When planning our approach to quantify acetylated α-tubulin levels in PBMCs, I considered sensitivity, accuracy, cost, time needed, equipment required, and capabilities for processing large numbers of samples. PBMCs are non-adherent and consist of multiple cell sub-types. Therefore, I considered assays that were able to measure levels in intact cell suspensions.

Flow cytometry is a well-established tool used to identify and characterise cell populations. Cells are labelled with fluorescent antibodies that can target intracellular and extracellular proteins. This allows classification of specific cell subtypes based on cellular surface

markers, as well as quantification of intra- and/or extra-cellular proteins of interest. Flow cytometry has been used to quantify acetylated α-tubulin levels in airway epithelial cells demonstrating a feasible method to quantify intracellular acetylated α-tubulin levels in various cell subtypes. [31] The ability to process intact cell suspensions and distinguish between cell subtypes supported the use of flow cytometry for our study. In section 3.2, I present a protocol for quantification of acetylated α-tubulin levels in PBMCs using flow cytometry. I used flow cytometry to characterise and quantify intracellular levels of acetylated α-tubulin in PBMC subtypes in section 3.3.

There have been several other published methods of measuring acetylated α-tubulin levels. One of the earliest methods is Western blotting, using anti-acetylated tubulin antibody to identify and compare the optical density of the acetylated tubulin band with an unacetylated sample. [32] This approach is semiquantitative and requires control samples with known levels of acetylated tubulin to compare and infer acetylated tubulin levels in the sample of interest. In addition, the error margin (~10%) may not detect smaller changes in acetylated tubulin levels in patient samples. [32] The analysis of large numbers of patient samples with Western blotting is challenging and may not be suitable in large scale clinical trials. Western blotting was not chosen for our samples as it required cell lysis and did not distinguish between cell subtypes.

Immunostaining of acetylated α-tubulin in live cells and imaging with live cell microscopy for the visualisation of acetylated α-tubulin levels in live neurons is another semiquantitative method of measurement. [19] Although this method can differentiate patients and controls, accurate measurements of acetylated α-tubulin levels with this method have not been developed. Therefore, immunofluorescence live cell microscopy may be more suited to mechanistic studies of disease pathogenesis rather than large scale biomarker detection. On the other hand, high content microscopy analysis allows analysis of larger sample sizes as cells are seeded in a 96 well plate, stained, and fixed before imaging and analysis with a High Content Screening System. [33] However, standardised reference ranges for acetylated α-tubulin levels have not been established. High content microscopy analysis remains a potential quantitative measure for acetylated α-tubulin levels. Both these methods are not ideal for processing PBMCs which are non-adherent.

Mass spectrometry has been used to quantify acetylated α-tubulin levels in small tissue volume and at low limit of quantitation. Various methods to estimate acetylated α-tubulin

levels have been published including stable-isotope standard and capture by anti-peptide antibody (SISCAPA) liquid chromatography-mass spectrometry (LC-MS), and multiple reaction monitoring-mass spectrometry assay (MRM-MS). [33, 34] However, these methods require expensive equipment and complex workflows. In addition, cells are digested and reported levels may not reflect levels of intracellular acetylated α-tubulin levels in live intact cells. Unreported results from Shah *et al* found that the proteomics assay was not sensitive enough to detect low levels of acetylated α-tubulin in PBMCs.

Sandwich enzyme-linked immunosorbent assay (ELISA) has been used to quantify α-tubulin isotypes. This method is quick, simple and does not require complex equipment. [35] ELISA, like mass spectrometry, requires cell lysates and therefore does not allow measurement of acetylated α-tubulin in intact cells. Unfortunately, I was unable to reliably detect acetylated α-tubulin levels with the available acetylated α-tubulin ELISA kit (Cell Signalling Technology Catalogue #7204C).

Homogenous Time Resolved Fluorescence (HTRF) is another simple method to quantify acetylated α-tubulin levels. HTRF relies on a donor and acceptor antibody binding to the protein of interest, acetylated α-tubulin. When in close proximity, a light source is used to excite the donor antibody to trigger a Fluorescence Resonance Energy Transfer to the acceptor antibody that will then fluoresce at a specific wavelength. Therefore, the presence of a target protein in a sample will generate a fluorescence signal in proportion to the number of antigen-antibody complexes formed reflecting the acetylated α-tubulin concentration. [36] HTRF is performed with cell lysates and is often used for high throughput testing as it can be performed in 384 and 1536 well plates. [36] HTRF was found to be a promising assay and used by members of my research team with their results presented in section 3.3.

3.2 Protocol for acetylated α-tubulin flow cytometry

Manuscript in preparation

Title: Measurement of intracellular acetylated α-tubulin levels in human peripheral blood mononuclear cells with flow cytometry

Sue Faye Siow, Gautam Wali[†], Carolyn Sue[†]

[1]Northern Clinical School, Kolling Institute, St Leonards, NSW, 2065, Australia

[2]Department of Clinical Genetics, Royal North Shore Hospital, St Leonards, NSW, 2065, Australia

[3]Department of Neurology, Royal North Shore Hospital, St Leonards, NSW, 2065, Australia

[†] These authors share senior authorship.

Technical contact: Sue Faye Siow

Lead contact: Gautam Wali

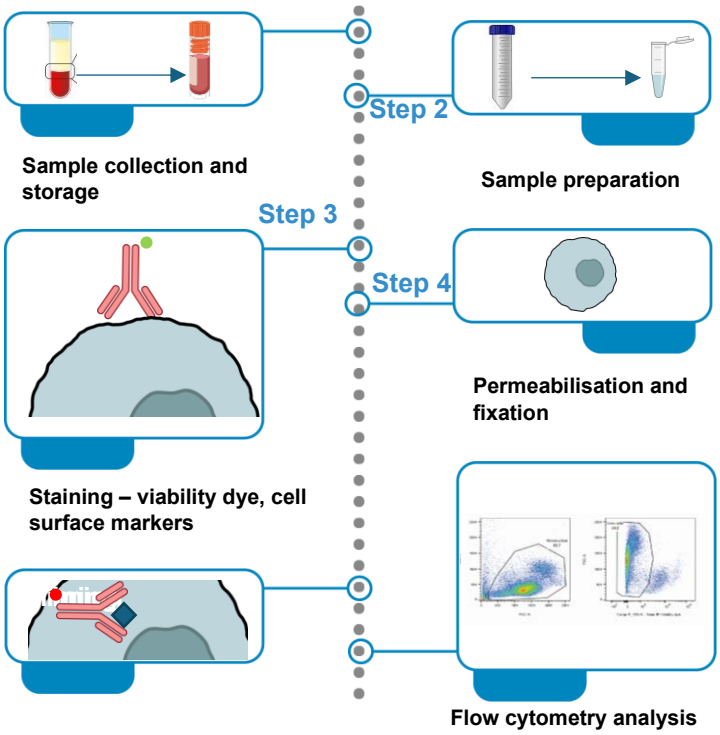
*Correspondence: suefaye.siow@health.nsw.gov.au

**Correspondence: g.wali@unsw.edu.au

Summary

Acetylated α-tubulin plays an important role in microtubule structure and has been used as a surrogate marker for microtubule stability (Eshun-Wilson et al., 2019). To date, Western blotting has been used as a semi-quantitative measurement of acetylated α-tubulin (Chesta et al., 2013). Mass spectrometry has been demonstrated to quantify total acetylated α-tubulin levels in digested tissue (Yang et al., 2018). However, these techniques require cell lysis and are unable to characterise intracellular acetylated α-tubulin levels in various cell types. We present a protocol for measurement of intracellular acetylated α-tubulin using flow cytometry. This technique allows the characterisation of acetylated α-tubulin levels in human peripheral blood mononuclear cell (PBMC) subtypes and selection of viable cells to more accurately measure acetylated α-tubulin levels to reflect in vivo findings. Using flow cytometry, we determine the acetylated α-tubulin levels in each PBMC subtype.

Graphical abstract



Before you begin

The protocol below describes the specific steps for measurement of intracellular acetylated α tubulin with flow cytometry in human peripheral blood mononuclear cells (PBMCs). The results are as seen in Wali G, Siow SF, Liyanage E, Kumar KR, Mackay-Sim A and Sue CM (2023) Reduced acetylated α-tubulin in *SPAST* hereditary spastic paraplegia patient PBMCs. *Front. Neurosci*. 17:1073516. doi: 10.3389/fnins.2023.1073516. We have also used this protocol for human fibroblast cells.

Institutional permissions

All experiments on human cells have been approved by the Northern Sydney Local Health District Human Research Ethics Committee (reference number: RESP/15/314) and written consent was obtained from all participants. All experiments conform to relevant regulatory standards. Permissions from your local Human Research Ethics Committee are required for any experiments on human samples.

Sample collection, storage and preparation

Timing: [2 hours]

1. PBMC isolation and storage.
    a. Collect ten 4mL vials of blood from participant using 4mL BD Vacutainer Cell Preparation Tube with Sodium Citrate (BD biosciences REF 362760).
    b. Centrifuge within 2 hours. Centrifuge at 1500-1800 rcf for 20 minutes.
    c. Invert the unopened tube 5 to 10 times to resuspend PBMCs.
    d. Pour out top layer from each tube into individual 15mL Conical Centrifuge tubes (ten Conical Centrifuge tubes for 10 CPT tubes).
    e. Centrifuge tubes at 300 rcf for 15 minutes.
    f. Check for cell pellet. Remove supernatant.

    CRITICAL: Check for cell pellet before removing supernatant. Be careful not to disturb cell pellet.
    g. Add 1mL CryoStor cell cryopreservation media (Sigma-Aldrich C2874) per tube to cell pellet and mix well.
        - Note: 10uL of the cell mixture can be used to measure cell count and viability with a cell counter
    h. Transfer PBMC mixture to 1.0mL cryogenic vials.
        - Note: depending on the cell count, the PBMC mixture can be divided amongst two or three cryogenic vials aiming for ~2 million cells per vial.
    i. Freeze cryogenic vials in Mr. Frosty Freezing Container (ThermoFisher Scientific Catalog number: 5100-0001) at -80ºC.
    j. Transfer vials to Liquid Nitrogen Freezer once frozen for longer term storage (more than 2 weeks).
2. Thawing PBMCs
    a. Prepare media: RPMI Medium (see “Materials and Equipment” for details
        - Note: media can be used for two to three weeks once prepared, stored in sealed container in 4ºC fridge
    b. Pre-warm RPMI medium in a 37ºC water bath.

c. To thaw a vial of PBMCs, remove the frozen vial from the cryo tank and quickly thaw cells in a water bath at 37^{o}C by gently swirling the vial for 1-2 minutes.
    - Note: Thaw 1 to 2 vials at a time to avoid cells sitting at room temperature for too long.
d. Transfer the 1mL cell suspension to a 15mL conical centrifuge tube and dilute the cell suspension with 7mL of pre-warmed RPMI medium
e. Centrifuge the cell suspension at 500 x g for 5 min at room temperature.
f. Gently remove the supernatant using a glass pipette on vacuum suction leaving behind an undisturbed cell pellet. Resuspend cell pellet in 1mL media. **CRITICAL**: Check for cell pellet before removing supernatant. Be careful not to disturb cell pellet.
g. Add 5mL pre-warmed RPMI medium and incubate in conical centrifuge tube with cap loosely on (to allow for gas exchange) at 37^{o}C for at 1 hour.

3. Harvest PBMCs
    a. Centrifuge the conical centrifuge tube with incubated cells suspended in media at 500 x g for 5 minutes at room temperature.
    b. Check for cell pellet before carefully removing supernatant with a glass pipette on vacuum suction.
    c. Resuspend cell pellet in 1mL of PBS.
    d. Transfer cell suspension to 1.5mL microcentrifuge tube.
    e. Centrifuge microcentrifuge tube at 500 x g for 5 minutes at room temperature.
    f. Check for cell pellet then gently remove supernatant with pipette and discard, leaving behind the cell pellet. Resuspend the cell pellet in 1mL of PBS.

<u>Prepare reagents</u>

Timing: [1 hour] (Perform before or concurrent with the staining or fixation steps below)

Note: Check product information booklet for updated instructions

4. Prepare viability dye.
    a. Thaw one vial of viability dye and stock tube of DMSO.
    b. Dilute one vial of dye with 50µL DMSO.
    c. Store viability dye solution in -20^{o}C freezer, thaw before use.

5. Prepare CytoPerm/Wash solution.
    a. Dilute 10X CytoPerm/Wash with miliQ water to make up 1X (Aim fo ~3-5mL of CytoPerm/Wash per sample being tested).

Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-human CD2 antibody PE	Biolegend	Cat no 309208
Mouse anti-human CD19 antibody APC	Biolegend	Cat no 302212
Mouse anti-human CD14 antibody BV421	Biolegend	Cat no 301830
Anti-acetylated α Tubulin conjugated antibody AF488	Santa Cruz	Cat no sc-23950 AF488
Biological samples		
Patient blood samples	Neurogenetics Clinic, Royal North Shore Hospital, Australia	
Chemicals, peptides, and recombinant proteins		
LIVE/DEAD Fixable Near IR Viability dye	ThermoFisher Scientific	Cat no L34975
CytoFix/Perm Kit **CRITICAL: contains 1-10% formaldehyde Hazards: may cause an allergic skin reaction, suspected of causing genetic defects, may cause cancer, harmful to aquatic life.** **Handling: Wash promptly with soap and water if skin is exposed. Do not eat, drink or smoke when using. Read and follow manufacturer's recommendations. Use personal protective equipment as required. Store in tightly closed original container in dry, cool, well-ventilated place.**	BD Biosciences	Cat no 554714

Materials and equipment

PBMC culture medium

Reagent	**Final concentration**	**Amount**

RPMI 1640	-	445mL
FBS	10%	50mL
Penicillin-Streptomycin	1%	5 mL
Total		**500mL**

Store at 4°C

BSA blocking agent

Reagent	**Final concentration**	**Amount**
30% BSA	0.3%	100 uL
PBS	-	9.9mL
Total		**10mL**

CytoPerm/Wash solution

Reagent	**Final concentration**	**Amount**
CytoPerm/Wash 10X		5 mL
MiliQ water		45 mL
Total		**50 mL**

Step-by-step method details

Note: Prepare compensation controls, fluorescence minus one and unstained controls alongside samples. Cells treated with TSA can be used as positive controls.

Planning control samples

Note: Any additional PBMC samples surplus to needs can be used as controls. All controls processed with the usual protocol excluding specific staining steps as specified below.

Compensation controls

1. Prepare 5 vials of PBMCs stained with single dye or antibody as below:
    a. 1 vial stained with only Near IR viability dye
    b. 1 vial stained with only anti-human CD2 antibody PE
    c. 1 vial stained with only anti-human CD19 antibody APC
    d. 1 vial stained with only anti-human CD14 antibody BV421
    e. 1 vial stained with only anti-acetylated α-tubulin conjugate antibody AF488

Fluorescence minus one controls

- Prepare 5 vials of PBMCs stained will all dyes and antibodies excluding one as below:
    a. 1 vial without Near IR viability dye
    b. 1 vial without anti-human CD2 antibody PE
    c. 1 vial without anti-human CD19 antibody APC
    d. 1 vial without anti-human CD14 antibody BV421
    e. 1 vial without anti-acetylated α-tubulin conjugate antibody AF488

Unstained control

1. Prepare 1 vial of unstained PBMCs

Staining with viability dye

Timing: [45 minutes]

This step allows identification of viable cells for analysis. Non-viable cells are excluded from final flow cytometric analysis.

1. Add 1µL of ThermoFisher LIVE/DEAD Fixable Near IR Viability dye (prepared as per "Prepare Reagents" section) to 1mL of prepared PBMC cell suspension from "Sample collection, storage and preparation (Viability Dye concentration 1:1,000), mix well.
2. Incubate on ice, protected from light, for 15 minutes.
3. Centrifuge tube at 500 x g for 5 minutes. Check for cell pellet and carefully remove supernatant with a pipette, leaving behind the cell pellet.
4. Wash: Add 1000µL PBS cell pellet in tube and mix well. Centrifuge tube at 500 x g for 5 minutes. Check for cell pellet and carefully remove supernatant with a pipette, leaving the cell pellet behind.

Staining with cell surface markers

Timing: [45 minutes]

This step allows identification of CD2-positive T cells, CD19-positive B cells, and CD14-positive monocytes.

1. Prepare surface antibody staining mixture: add 5µL each of CD2, CD19 and CD14 antibodies to 100µL of 0.3% BSA blocking agent (see "Materials and Equipment") for each sample tested. Concentration of surface antibodies are 1:20 each (Check product information and perform optimisation studies to confirm ideal concentration).
2. Add 115µL of cell surface marker mixture to each sample. Mix well.
3. Incubate on ice, protected from light, for 30 minutes.
4. Wash: Add 1000µL PBS to tube. Centrifuge at 500 x g for 5 minutes, check for cell pellet then gently remove supernatant, leaving behind the cell pellet.

Permeabilisation and Fixation, Blocking Step

Timing: [45 minutes]

This step permeabilises the cell for staining of intracellular acetylated α tubulin.

1. Add 250µL CytoFix/Perm to each sample. Mix well. Incubate on ice for 20 minutes.
2. Wash: Add 1000µL CytoPerm/Wash (See "Materials and Equipment") to each sample. Centrifuge at 500 x g for 5 minutes, check for cell pellet, then gently remove supernatant, leaving behind the cell pellet.
3. Blocking: Re-suspend the cell pellet in 300µL CytoPerm/Wash. Incubate on ice, protected from light, for 30 minutes.
4. Centrifuge tube at 500 x g for 5 minutes, check for cell pellet and gently remove supernatant, leaving behind the cell pellet.

Staining for intracellular acetylated α-tubulin

Timing: [45 minutes]

This step stains intracellular acetylated α-tubulin.

1. Prepare acetylated α-tubulin staining mixture. Dilute acetylated α tubulin antibody with CytoPerm/Wash solution to a concentration of 1:50 (note this may vary depending on antibody used and optimisation steps). Prepare 300µL of acetylated α tubulin antibody solution per sample tested.
2. Add 300µL of 1:50 acetylated α tubulin antibody mixture to each sample. Mix well.
3. Incubate on ice, protected from light, for 30 min.

4. Wash: Add 1000µL of CytoPerm/Wash to each tube. Centrifuge at 500 x g for 5 minutes, check for cell pellet and gently remove supernatant, leaving cell pellet behind.
5. Resuspend cells in 500µL PBS. (if high cell count, >3million cells per tube, can suspend in 1mL PBS)
6. Filter cell suspension with 70um cell filters into 5mL round bottom polystyrene tubes for flow cytometry tubes.

Flow cytometric analysis

Timing: [45 minutes]

This step includes instructions for set up of flow cytometer (BD LSRFortessa flow cytometer used for this experiment).

1. Set the voltages/gains for each channel.
2. Load the stained samples on the machine and begin acquiring events with slow flow rate.
3. Start recording. Flow rate can be increased, aiming for acquisition rate of ~1000 cells per second.
4. Create a scatter plot graph of FSC-A and SSC-A.
5. Create a scatter plot graph of FSC-H and FSC-A to gate for single cells and doublets.
6. Run single fluorescence compensation controls to calculate compensation for each fluorochrome (PE, APC, BV421, Near IR, AF488).
7. Run fluorescence minus one controls to gate viable cells and various cell types, and as negative control for AF488.
8. Run unstained control.
9. Use compensation controls to adjust for fluorescence spillover.
10. Aim to record 20,000 events for controls and 100,000 events for samples.
11. Data analysis performed with FlowJo.

Note: Samples should be analysed on flow cytometer as quickly as possible, no longer than 24 hours following completion of staining.

Expected outcomes

Using this protocol, we expect the relative levels of intracellular levels in T cells, B cells and monocytes to be measurable with flow cytometric analysis. We have used this protocol to measure flow cytometric readings of acetylated α tubulin levels in HSP-SPAST patient compared to healthy controls and response to TSA treatment.

Quantification and statistical analysis (optional)

FlowJo is used to calculate compensation controls. Mean fluorescence of AF488 in the various cell subtypes are compared between samples and controls. Fluoroscence minus one controls are used to gate viable cells and as negative control for AF488 levels.

Limitations

This protocol measures the relative levels of AAT between various PBMC subtypes and between different samples however is unable to quantify the exact AAT levels for each cell. The mean fluorescence for AF488 will vary between runs. Therefore, we advise using the same sample on both runs to standardise the results if samples both runs are to be analysed together.

Due to the complexity of the protocol, it is advisable to limit the number of samples per run for time management and to avoid mistakes. Results may be skewed by differences in viable cell count between each sample.

Troubleshooting

Problem 1:Low or high cell count

Potential solution:

The sample collection protocol presented in this paper should yield between 3 to 9 million cells per CPT tube. This results in sufficient cells at the end of the protocol to achieve an acquisition rate of ~1000 cells per second. If the cell count is low at the beginning of the experiment, consider diluting the final sample in a lower volume of PBS (minimum volume varies depending on type of flow cytometry tube) for a greater cell concentration. If the cell count is high at the beginning of the experiment, consider dividing the cells into two or three

tubes. The extra tubes can be used to prepare the controls (unstained, fluorescence minus one and compensation).

Problem 2: Difficulty gating cell populations
Potential solution:
We recommend using unstained controls and fluorescence minus one controls to gait positive and negative populations, for example for viable vs. non-viable cells. Only viable cells are analysed.

Problem 3: Weak fluorescence intensity
Potential solution:
Check antibody production date, may have lost fluorescence over time. Apply compensation controls for accurate measurement of fluorescence.

3.3 Acetylated α-tubulin levels in HSP-*SPAST* patients vs controls

The results from my flow cytometry experiments to measure acetylated α-tubulin levels in HSP-*SPAST* patients vs control samples with methodology detailed in Section 3.2 are presented in the following paper. Other results presented including animal studies and HTRF analysis were from experiments performed by other members of the research team.

Wali G, Siow SF, Liyanage E, Kumar KR, Mackay-Sim A, Sue CM. Reduced acetylated α-tubulin in *SPAST* hereditary spastic paraplegia patient PBMCs. Front Neurosci. 2023 Mar 28;17:1073516. doi: 10.3389/fnins.2023.1073516. PMID: 37144097.

frontiers | Frontiers in Neuroscience

TYPE Brief Research Report
PUBLISHED 28 March 2023
DOI 10.3389/fnins.2023.1073516

OPEN ACCESS

EDITED BY
David Lynch,
University of Pennsylvania,
United States

REVIEWED BY
Magda Matos Santana,
University of Coimbra,
Portugal
Filippo M. Santorelli,
Stella Maris Foundation (IRCCS),
Italy
Mariana Santos,
Universidade do Porto,
Portugal

*CORRESPONDENCE
Gautam Wali
✉ g.wali@neura.edu.au

†These authors have contributed equally to this work

‡These authors share senior authorship

SPECIALTY SECTION
This article was submitted to Neurodegeneration, a section of the journal Frontiers in Neuroscience

RECEIVED 18 October 2022
ACCEPTED 06 March 2023
PUBLISHED 28 March 2023

CITATION
Wali G, Siow SF, Liyanage E, Kumar KR, Mackay-Sim A and Sue CM (2023) Reduced acetylated α-tubulin in *SPAST* hereditary spastic paraplegia patient PBMCs. *Front. Neurosci.* 17:1073516. doi: 10.3389/fnins.2023.1073516

Reduced acetylated α-tubulin in *SPAST* hereditary spastic paraplegia patient PBMCs

Gautam Wali[1,2,3*†‡], Sue-Faye Siow[2,3†], Erandhi Liyanage[1,2], Kishore R. Kumar[2,4,5], Alan Mackay-Sim[3,6‡] and Carolyn M. Sue[1,2,3‡]

[1]Neuroscience Research Australia and University of New South Wales, Sydney, NSW, Australia, [2]Kolling Institute for Medical Research and Department of Medicine, University of Sydney, Sydney, NSW, Australia, [3]Northern Clinical School, Sydney Medical School, University of Sydney, Sydney, NSW, Australia, [4]Molecular Medicine Laboratory and Department of Neurology, Concord Repatriation General Hospital, Concord Clinical School, University of Sydney, Concord, NSW, Australia, [5]Garvan Institute of Medical Research, Darlinghurst, NSW, Australia, [6]Griffith Institute for Drug Discovery, Griffith University, Brisbane, QLD, Australia

HSP-*SPAST* is the most common form of hereditary spastic paraplegia (HSP), a neurodegenerative disease causing lower limb spasticity. Previous studies using HSP-*SPAST* patient-derived induced pluripotent stem cell cortical neurons have shown that patient neurons have reduced levels of acetylated α-tubulin, a form of stabilized microtubules, leading to a chain of downstream effects eventuating in increased vulnerability to axonal degeneration. Noscapine treatment rescued these downstream effects by restoring the levels of acetylated α-tubulin in patient neurons. Here we show that HSP-*SPAST* patient non-neuronal cells, peripheral blood mononuclear cells (PBMCs), also have the disease-associated effect of reduced levels of acetylated α-tubulin. Evaluation of multiple PBMC subtypes showed that patient T cell lymphocytes had reduced levels of acetylated α-tubulin. T cells make up to 80% of all PBMCs and likely contributed to the effect of reduced acetylated α-tubulin levels seen in overall PBMCs. We further showed that mouse administered orally with increasing concentrations of noscapine exhibited a dose-dependent increase of noscapine levels and acetylated α-tubulin in the brain. A similar effect of noscapine treatment is anticipated in HSP-*SPAST* patients. To measure acetylated α-tubulin levels, we used a homogeneous time resolved fluorescence technology-based assay. This assay was sensitive to noscapine-induced changes in acetylated α-tubulin levels in multiple sample types. The assay is high throughput and uses nano-molar protein concentrations, making it an ideal assay for evaluation of noscapine-induced changes in acetylated α-tubulin levels. This study shows that HSP-*SPAST* patient PBMCs exhibit disease-associated effects. This finding can help expedite the drug discovery and testing process.

KEYWORDS

hereditary spastic paraplegia, microtubule, noscapine, biomarkers, neurodegenerative disease

Introduction

Hereditary Spastic Paraplegia (HSP) is a neurodegenerative disorder caused by disease-causing variants in any one of at least 80 genes (Méreaux et al., 2022). HSP-*SPAST* (also known as SPG4; Lange et al., 2022) is caused by disease-causing *SPAST* variants and is the most common form of autosomal dominant HSP (59% of autosomal dominant cases; Méreaux et al., 2022). Patients with HSP-*SPAST* undergo degeneration of the corticospinal motor neurons leading to

lower limb spasticity and gait disturbances. *SPAST* encodes spastin, a microtubule regulating protein (Kasher et al., 2009). We have previously evaluated the disease-mechanism of HSP-*SPAST* using patient-derived stem cell models: olfactory neurosphere-derived stem cells and induced pluripotent stem (iPS) cell derived-neurons (Abrahamsen et al., 2013; Fan et al., 2014; Wali et al., 2016, 2018, 2020). Patient-derived neuronal cells have reduced spastin levels causing reduced levels of acetylated α-tubulin, a form of stabilized microtubules (Abrahamsen et al., 2013; Wali et al., 2020). Reduced levels of acetylated α-tubulin in patient neurons leads to downstream effects of reduced microtubule-dependent axonal transport, increased oxidative stress and increased vulnerability to axonal degeneration (Wali et al., 2016, 2020). The downstream effects can be rescued by restoring acetylated α-tubulin levels in patient neurons with noscapine and other tubulin-binding drugs (Fan et al., 2014; Wali et al., 2020). In summary, our *in vitro* study findings have established that we can protect HSP-*SPAST* patient neurons from being vulnerable to degeneration by restoring acetylated α-tubulin levels.

Noscapine is an opium poppy alkaloid that does not bind to opioid receptors. It is non-narcotic, therefore non-addictive and has no effect on pain (Rida et al., 2015). It is approved in several countries as an antitussive (Rida et al., 2015). It makes a good candidate for re-purposing as a treatment for HSP-*SPAST* as it can cross the blood brain barrier and *in vitro* studies show that it rescues the downstream effects of reduced acetylated α-tubulin levels in HSP-*SPAST* patient neurons (Wali et al., 2020). At present, it is not known if orally administered noscapine can increase the cellular content of acetylated α-tubulin in the brain *in vivo*.

Identifying disease-associated effects in the blood can help expedite the drug discovery and testing process. Microtubule expression is not restricted to neuronal cells that are degenerated in HSP-*SPAST* patients. Based on this, we hypothesized that acetylated α-tubulin levels are also reduced in non-neuronal patient cells. The first aim of this study was to compare the expression of acetylated α-tubulin between HSP-*SPAST* patients and healthy controls in peripheral blood mononuclear cells (PBMCs).

The second aim of this study was to test if noscapine can cross the blood–brain barrier and increase the content of acetylated α-tubulin in the brain. For this, we orally administered mouse with increasing doses of noscapine and measured the levels of noscapine and acetylated α-tubulin in the mouse brain. Acetylated α-tubulin was measured in mouse brain homogenate protein samples using the highly sensitive Homogeneous Time Resolved Fluorescence (HTRF) assay technology (Degorce et al., 2009). Human brain cells are relatively large (cortical neurons extend up to 1 m in length) and subsequently have higher expression of microtubules compared to smaller cell types especially PBMCs (that are about 50–100 μm long). The third aim of the study was to test if the HTRF assay can detect acetylated α-tubulin expression and noscapine induced changes in acetylated α-tubulin expression in human PBMCs. This assay will be relevant to future studies testing noscapine drug treatment effects on acetylated α-tubulin expression in non-neuronal cells for HSP-*SPAST* and other diseases.

Overall, we investigated if HSP-*SPAST* patient PBMCs reflect the disease associated pathological effects of reduced acetylated α-tubulin seen in neuronal cells, and if noscapine can cross the blood brain barrier to increase the levels of acetylated α-tubulin in the brain. Finally, we investigate HTRF as a highly sensitive high throughput assay to measure the effects of noscapine treatment in mouse brain (*in vivo*) and human PBMCs (*in vitro*).

Methods

Human ethics compliance and patient recruitment

Experiments involving human PBMCs were approved by the Northern Sydney Local Health District Human Research Ethics Committee, Australia (Reference number: RESP/15/314) and written informed consent was obtained from all participants. Twenty participants, i.e., 9 *SPAST* patients and 11 healthy controls were recruited to this study from the Neurogenetics Clinic, Royal North Shore Hospital, Sydney, Australia. The patients in this study were examined by movement disorder specialists (CS, SFS, and KRK) and had a confirmed diagnosis of HSP-*SPAST* on genetic testing. Disease-severity of the patients was measured using the HSP clinical rating scale, Spastic Paraplegia Rating Scale (SPRS; Schüle et al., 2006). Details of the study participants are presented in Table 1.

Blood sample collection, PBMC isolation, and PBMC acetylated α-tubulin measurement using flow cytometry

Blood collection

Fifty milli liter (mL) of peripheral blood was collected for each participant in 5 × 10 mL heparin tubes. Samples were processed for PBMCs within 4 h of collection.

PBMC isolation

PBMCs were isolated from whole blood collected in 10 mL heparin blood tubes using Ficoll–Hypaque density gradient (Fuss et al., 2009). For each 10 mL blood sample, blood sample was diluted with Phosphate-buffered saline (PBS) + 2% Fetal Bovine Serum (1:1 volume ratio) to form a 20 mL diluted sample solution. 15 mL volume of Ficoll–Hypaque density gradient solution was added to a 50 mL falcon tube. The diluted sample was layered on top of the Ficoll–Hypaque solution. The tube was centrifuged at 900 g for 20 min with the brakes off. The upper plasma layer was removed and the mononuclear cells at the interphase were harvested (the layer above the Ficoll–Hypaque solution). Mononuclear cells were washed twice in PBS buffer +2% Fetal Bovine Serum solution. Finally, PBMCs were counted and frozen down in CryoStor® CS10 freezing media (Catalog# 07930, Stem cell technologies) and stored in cryo tanks for long term storage.

PBMC immunostaining

Frozen PBMCs were thawed in RPMI cell culture media: RPMI media (Catalog# 11875-093, Thermo Fisher scientific) with 10% FBS. To identify viable cells, PBMCs were incubated with (Live/Dead Fixable Near IR Viability Dye, Catalog# L34975 Thermo Fisher Scientific) at a concentration of 1:1,000 for 15 min. The cells were then washed twice with PBS. To identify PBMC cell sub-types, PBMCs were incubated with a cocktail of surface antibodies—CD14 (1:20, Catalog# 301830, BioLegend), CD2 (1:20, Catalog# 309208

TABLE 1 Details of individuals with HSP-*SPAST* (*SPG4*) and healthy controls.

Sample	Gender	Age	Diagnosis	SPRS	*SPAST* variant (NM_014946.4)
Healthy controls					
19/009	F	58	Control		
19/048	M	34	Control		
19/056	M	33	Control		
12/013	F	47	Control		
19/007	F	34	Control		
19/052	F	56	Control		
19/059	F	22	Control		
19/058	M	37	Control		
19/014	F	31	Control		
19/054	F	31	Control		
19/053	F	40	Control		
Individuals with HSP-*SPAST* (SPG4)					
08/088	M	81	HSP-*SPAST*	22	c.444G>A (p.Trp148Ter)
06/042	F	58	HSP-*SPAST*	49	c.1392A>T (p.Glu464Asp)*
09/010	F	53	HSP-*SPAST*	10	c.1291C>T (p.Arg431Ter)
18/268	M	58	HSP-*SPAST*	23	c.1413+1G>T
09/017	F	73	HSP-*SPAST*	24	Duplication of exon 16*
04/011	M	73	HSP-*SPAST*	22	c.1196C>T (p.Ser399Leu)*
17/114	F	36	HSP-*SPAST*	2	c.451_454del (p.Lys151fs)
14/074	F	51	HSP-*SPAST*	1	c.1291C>T (p.Arg431Ter)
09/016	M	46	HSP-*SPAST*	20	Duplication of exon 16*.

SPRS, Spastic paraplegia Rating Scale. *(Vandebona et al., 2012).

BioLegend) and CD19 (1:20, Catalog# 302212, BioLegend) for 30 min. The cells were then washed twice with PBS. PBMCs were fixed and permeabilised using the Cytofix/Cytoperm Fixation/Permeabilization solution kit (Catalog# 554714, BD Biosciences), as described before (Wali et al., 2021). PBMCs were fixed using the CytoFix reagent for 25 min, followed by two CytoPerm reagent washes. PBMCs were permeabilised using the CytoPerm reagent for 30 min. PBMCs were immunostained by incubating PBMCs with the conjugated anti-acetylated α tubulin antibody at a dilution of 1:50 for 30 min [(Bonser et al., 2021), Catalog# sc-23950, Santacruz], followed by two CytoPerm reagent washes. After immunostaining, PBMCs were resuspended in 500 µL PBS and filtered through a 70 µm cell strainer.

Flow cytometry and data analysis

The PBMC sample fluorescence levels were measured using the BD LSRFortessa™ flow cytometer. Data analysis was performed using FlowJo™, a flow cytometry data analysis software. Compensation controls for PE stained anti-CD2 antibody, APC stained anti-CD19 antibody, BV421 stained anti-CD14 antibody and AF488 stained anti-acetylated α-tubulin antibody were used to adjust for fluorophore spill over. Fluorescence minus one (FMO) controls for Near IR and AF488 were used to gate for viable cells and set the threshold for positive acetylated α-tubulin readings (Maecker and Trotter, 2006). FMO control for AF488 is presented in Figures 1H,K,M as the negative control for acetylated α-tubulin. Acetylated α-tubulin fluorescence was measured in all viable PBMCs, CD2 positive T cells, CD19 positive B cells, and CD14 positive monocytes for each sample. The acetylated α-tubulin geometric mean fluorescence intensity was measured from individual samples for group level comparisons.

Animal ethics compliance

Study involving mouse was conducted in accordance with the guidelines set out in the National Health and Medical Research Council, Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 8th edition, 2013 (1). The study was assessed and approved by the University of Queensland Animal Ethics Committee and assigned the following project code: TETRAQ/330/19/388/18/M 'Noscapine HCL (Noscapine): Tissue Distribution and Pharmacokinetic Analysis of Novel Compounds in Rodents'.

Mouse noscapine treatment, blood and brain sample preparation and mouse brain protein extraction

Mouse type

C57BL/6 J mouse are an acceptable and commonly used strain and species for pharmacokinetic and pharmacodynamic studies. The

10.3389/fnins.2023.1073516

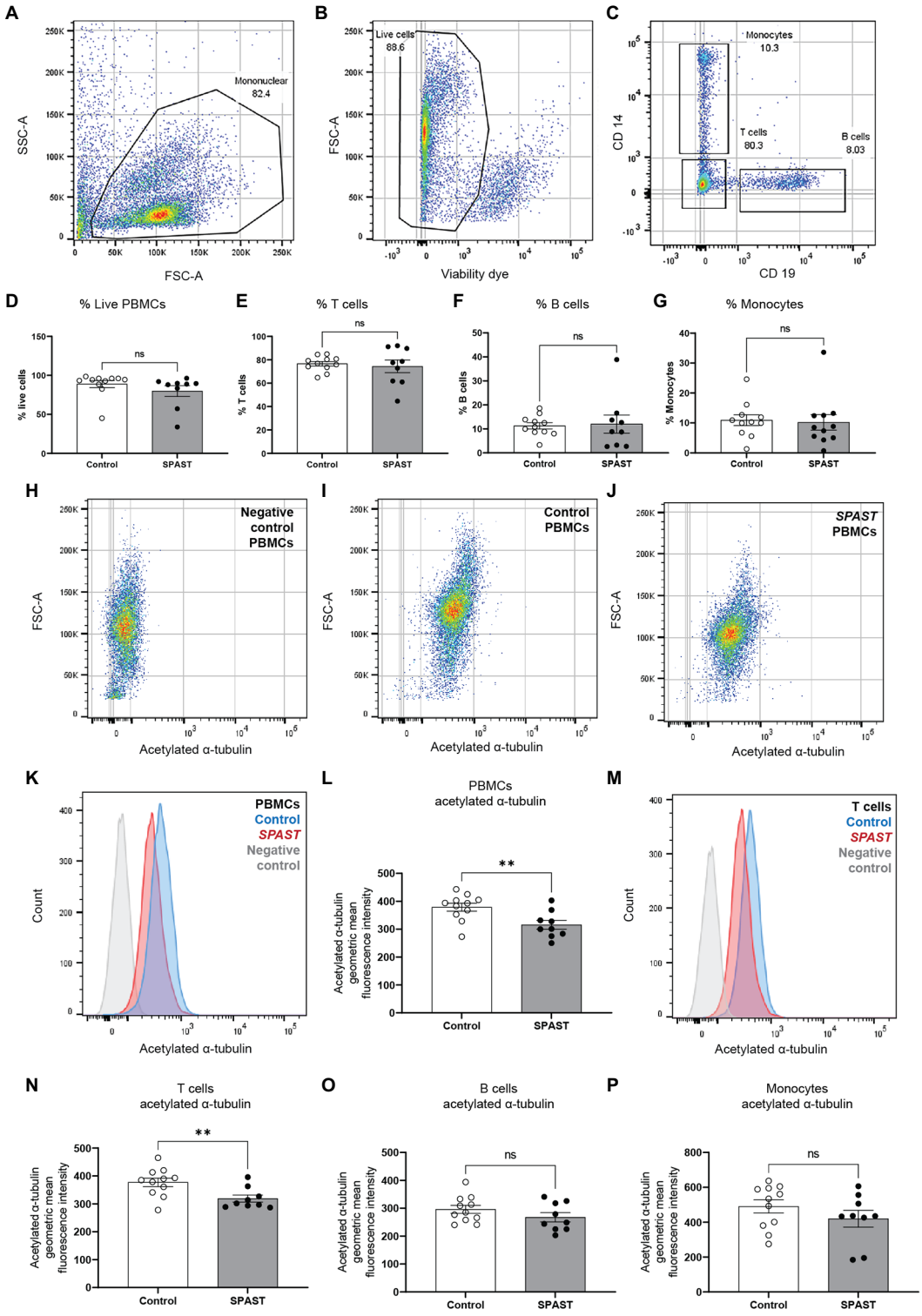


FIGURE 1
Acetylated α-tubulin levels in *SPAST* HSP patient and control PBMCs. Flow cytometry was used to measure acetylated α-tubulin in overall PBMCs and PBMC cell sub-types. **(A)** The forward-scatter and side-scatter (FSC/SSC) gated plots represent the total mononuclear cells. **(B)** Live PBMCs were identified using a cell viability dye. Viable cells have a relatively lower fluorescence level of the viability dye and vice versa for non-viable cells. Gating was applied to identify the proportion of viable cells. **(C)** CD2, CD19, and CD14 cell surface antibodies were used to identify PBMC cell sub-types T cells, B cells and monocytes. The scatter plot shows CD14 monocytes and CD19 B cells separated using the surface antibodies. The proportion of live PBMCs **(D)** and the proportion of T cells **(E)**, B cells **(F)** and monocytes **(G)** is shown for all HSP-*SPAST* patients and healthy controls. **(H–J)** Levels of acetylated α-tubulin in PBMCs were measured in patient and control PBMC samples. Scatter plot of an acetylated α-tubulin negative control sample **(H)** and representative control **(I)** and patient **(J)** samples are shown. **(K)** Histogram plot showing acetylated α-tubulin levels in representative patient and control PBMC samples. **(L)** Patient and control group level comparison based on acetylated α-tubulin geometric mean fluorescence intensity in all PBMCs. **(M)** Histogram plot showing acetylated α-tubulin levels in representative patient and control T cells. Patient and control group level comparison of T cells **(N)**, B cells **(O)**and monocytes **(P)** based acetylated α-tubulin geometric mean fluorescence intensity. Mean ± SEM.

TABLE 2 Mouse noscapine treatment study plan.

Study group	Noscapine dose (mg/kg)	Route	C57BL/6 J mouse	Terminal samples collection time points
1	100	Oral	4	60 min post-dose
2	200	Oral	4	60 min post-dose
3	400	Oral	4	60 min post-dose

mice used were 7–8 weeks old and they weighed 20.83–23.13 g. Mouse were subject to a 5-day acclimation period prior to noscapine administration. Only animals without visible signs of illness were used for the study.

Animal housing conditions

Animals were housed in their study groups with up to four animals per individually ventilated cage (IVC). The IVC units had a minimum of 15 air changes per hour. Environmental enrichment was added to each IVC. These items included chewsticks, rodent hutches and nesting material. The animal housing temperature was 22.5°C ± 2.5°C and humidity of 50% ± 20%. The animal house had a 12-h light/dark cycle.

Study design

The study design is summarized in Table 2. On completion of the acclimation period, mouse were administered with noscapine (3 groups, $n = 4$ mouse per group, Table 2). Noscapine treatment was administered as a bolus oral dose by gavage in a volume of 10 mL/kg.

Mouse plasma processing

Blood samples were collected via the inferior vena cava and transferred into tubes containing K3-EDTA which had been previously cooled on ice. Whole blood was transferred to a separate tube and red blood cells lysed using Red Blood Cell Lysis Buffer (ab204733, Abcam) to collect plasma. The processed plasma was stored at −80°C.

Mouse brain tissue processing

Mouse were euthanised by an overdose of pentobarbitone (administered via intraperitoneal injection). Immediately following euthanasia, the entire brain was collected and homogenized in ice-cold phosphate buffered saline containing 1× protease inhibitor cocktail (Sigma-Aldrich, Catalog# P8340). The brain tissue was homogenized using the Retsch mixer mill mm 400 homogenizer and two stainless steel grinding balls (3 mm, Retsch) using these conditions: 10 Hz for 2 min at 4°C + 30 Hz for 7 min at 4°C. The resulting homogenized brain sample was stored at −80°C.

Mouse brain homogenate protein extraction

One hundred micro liter of the brain homogenate was mixed with 300 µL RIPA lysis buffer (Sigma, Catalog# R0278-50ML) supplemented with 1x Protease Inhibitor Cocktail (Thermo Fisher scientific, Catalog# 78429). The sample mixture was mixed by pipetting and vortexing. The sample mixture was kept on ice and agitated on a shaker for 2 h. The sample mixture was centrifuged for 20 min at 4°C at 16,000 rcf. Supernatant containing the protein was collected and protein concentration was measured using the BCA protein estimation kit (Thermo Fisher scientific, Catalog# 23225).

Measuring noscapine concentrations in mouse plasma and brain using liquid chromatography tandem mass spectrometry

Twenty micro liter (µL) aliquots of mouse plasma and brain homogenate were spiked with 1-Hydroxymidazolam internal standard, and the samples were then analyzed using the liquid chromatography tandem mass spectrometry (LC-MS/MS) method. Plasma and brain homogenate concentrations of noscapine were back calculated from calibration curves. The lower limit of quantification (LLOQ) for this method was 1 ng/mL. The LC-MS/MS chromatograms of plasma and brain homogenate with and without noscapine (150 ng/mL) and internal standard 1-Hydroxymidazolam (1 µg/mL) are shown in Supplementary Figures 2, 3.

In vitro PBMCs noscapine treatment and PBMC protein extraction

The human PBMCs used to test *in-vitro* effects of noscapine treatment ($n = 8$ PBMC cell lines) were purchased from Stemcell technologies (Catalog#: 70025.1). The PBMCs were cultured in RPMI cell culture media: RPMI media (Catalog# 11875-093, Thermo Fisher scientific) with 10% FBS at 37°C with 5% CO_2. The PBMCs were treated with 10 µM noscapine (Cayman chemical, Catalog# 17255) for 1 h.

Human PBMC protein extraction

PBMCs cultured in RPMI media were transferred to a 15 mL falcon tube and centrifuged at 500 g for 10 min. PBMC cell pellet was resuspended in 50 µL of cell lysis reagent (Sigma, Catalog# C3228) supplemented with 1x Protease Inhibitor Cocktail (Thermo Fisher scientific, Catalog# 78429). The sample mixture was mixed by pipetting and vortexing. Then, the sample was kept on ice and agitated on a shaker for 20 min. The sample was then centrifuged at 4°C at 16,000 rcf. Supernatant containing the protein was collected and protein concentration was measured using the BCA protein estimation kit (Thermo Fisher scientific, Catalog# 23225).

Acetylated α-tubulin measurement in mouse brain and human PBMCs using the HTRF acetylated α-tubulin assay

We used the HTRF Alpha-Tubulin acetyl-K40 kit (Perkin Elmer, Catalog# 63ADK072PEG) to measure the relative levels of acetylated α-tubulin. In this study, we have measured the levels of acetylated α-tubulin in the mouse brain and human PBMCs. The expression levels of acetylated α-tubulin can vary between different tissue types. A serial protein dilution was used to identify the optimal protein

concentration required to measure acetylated α-tubulin for each tissue sample type. For both mouse brain and human PBMC samples, a dilution series was prepared for the protein samples in pure water: 10, 5, 1, 0.1, 0.05, 0.01, and 0.001 ng/16 μL. 4 μL of the antibody mixture, i.e., 2 μL of d2-antibody solution and 2 μL of Cryptate-antibody solution was added to each protein dilution solution (both the antibodies bind to acetylated α-tubulin and subsequently generating Fluorescence resonance energy transfer (FRET)). The total reaction volume per sample is 20 μL. The assay was performed in a 384 well plate. Each well can hold 20 μL sample. The sample solution was incubated for 2 h at room temperature. The fluorescence was measured using the HTRF compatible fluorescence plate reader (Nivo, Perkin Elmer). 5 and 0.01 ng/μL were identified to be the optimal protein concentration to measure acetylated α-tubulin in human PBMCs and mouse brain samples, respectively. The average time required for this assay from protein sample to data analysis is 4 h.

Statistical analysis

Prism (GraphPad) was used to analyze data and plot graphs. Analysis of data presented was performed using one-way analysis of variance with Tukey's multiple comparisons test, Student's *t*-test and Person's correlation coefficient analysis.

Results

HSP-*SPAST* patient PBMCs have reduced acetylated α-tubulin

To test if non-neuronal HSP-*SPAST* patient cells have disease-associated pathology of reduced acetylated α-tubulin, we compared the expression of acetylated α-tubulin in PBMCs from 9 HSP-*SPAST* patients and 11 healthy control PBMCs. Acetylated α-tubulin levels were measured using flow cytometry in overall PBMCs (Figures 1A,B) and in PBMC cell sub-types, i.e., CD2 positive T-cell lymphocytes, CD19 positive B-cell lymphocytes and CD14 positive monocytes (Figure 1C).

First, we tested the proportion of PBMC cell sub-types between the patient and control groups. There was no difference in the proportion of PBMC cell sub-types between the HSP-*SPAST* patients and healthy controls. Both patient and control groups had about 90% live PBMCs (Figure 1D). Of these live PBMCs, about 80% of PBMCs were T-cells (Figure 1E), 10% were B-cells (Figure 1F) and 10% were monocytes (Figure 1G). The proportion of the different PBMC cell subtypes was as expected and not affected by disease status.

Figures 1H–J show acetylated α-tubulin expression dot plots of a representative negative control (Figure 1H), healthy control PBMC sample (Figure 1I) and patient PBMC sample (Figure 1J). The same data is presented as a histogram in Figure 1K. Acetylated α-tubulin levels in the HSP-*SPAST* patient PBMCs was significantly lower than control PBMCs (Figures 1L, $p < 0.01$). Assessment of acetylated α-tubulin levels in the PBMC cell sub-types showed that patient T-cells had significantly lower acetylated α-tubulin levels compared to control T-cells (Figures 1M,N). Patient B cells (Figure 1O) and monocytes (Figure 1P) showed a trend of reduced acetylated α-tubulin levels, but this effect was not statistically significant.

The average age of the 9 HSP-*SPAST* patients (4 males and 5 females) and 11 healthy controls (3 males and 8 females) is 58.78 and 38.45 years, respectively. Correlation analysis did not identify any correlation between the age of the control and patient participants vs. their levels of acetylated α-tubulin (Supplementary Figure 1; controls: $R^2 = 0.03$ and HSP-*SPAST*: $R^2 = 0.22$).

Correlation analysis did not identify a significant correlation between the SPRS and acetylated α-tubulin levels in HSP-*SPAST* patients (R^2: 0.041).

Orally administered noscapine was detected in mouse brain

To test if orally administered noscapine can cross the blood brain barrier, three groups of mouse ($n = 4$ mouse per group) were orally administered with increasing concentrations of noscapine, i.e., 100, 200, and 400 mg/kg. No morbidity or mortality was observed during the acclimation and the study period. Body weight was recorded once during the acclimation period and on the day of dosing. No changes in body weight were observed post-dosage. Body weight (g) ± SD of noscapine treated mouse: 100 mg/kg—22.39 ± 1.07, 200 mg/kg mouse—22.05 ± 0.92, and 400 mg/kg mouse—21.83 ± 0.87. Noscapine was measured using Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) in the mouse plasma and brain following oral administration of noscapine at 60 min post-dose. Noscapine treatment increased the levels of noscapine in the mouse plasma and brain (Figures 2A,B). ANOVA indicated a significant effect of noscapine treatment in the mouse plasma (Figure 2A, $p < 0.01$) and brain (Figure 2B, $p < 0.01$). Tukey's *post-hoc* multiple comparisons of both mouse plasma and brain noscapine levels indicated that the noscapine levels in 100 mg/kg noscapine treated mouse was significantly different from 200 mg/kg noscapine treated mouse (Figures 2A,B, $p < 0.05$) and 400 mg/kg noscapine treated mouse (Figures 2A,B $p < 0.01$). Noscapine treatment showed a tissue dependent affect. Comparison of noscapine levels in mouse plasma and brain showed that noscapine concentration in the brain is about 12-fold lower than in plasma (for example, at 100 mg/kg dosage, brain noscapine average: 573 ng/mL vs. plasma noscapine average: 7327 ng/mL). There was a significant correlation between noscapine levels in plasma and brain samples. Spearman correlation coefficient analysis showed a significant correlation in noscapine levels in plasma and brain samples for the 100 and 200 mg/kg treatment groups (Table 3).

Noscapine dose-dependent increase of acetylated α-tubulin levels

Acetylated α-tubulin was measured in homogenized brain lysates using the highly sensitive HTRF Alpha-Tubulin acetyl-K40 assay. Brain samples from mouse treated with noscapine showed a dose-dependent increase in the levels of acetylated α-tubulin (Figure 3). ANOVA indicated a significant effect of noscapine treatment on the levels of acetylated α-tubulin in the mouse brain ($p < 0.001$). Tukey's *post-hoc* multiple comparisons of the mouse brain acetylated α-tubulin levels indicate that the acetylated α-tubulin in the untreated mouse was significantly different from mouse treated with 400 mg/kg noscapine ($p < 0.01$; Figure 3). The level of acetylated α-tubulin in 100

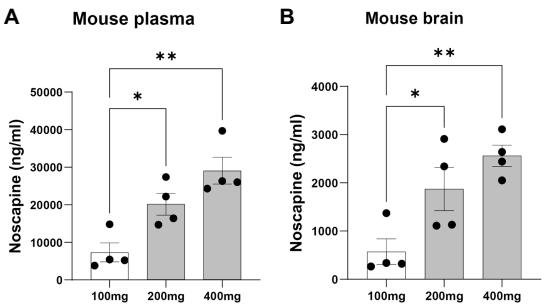


FIGURE 2
Plasma and brain noscapine levels in noscapine treated mouse. Mouse was orally administered with increasing concentrations of noscapine: 100, 200, and 400 mg/kg. Noscapine was measured in mouse plasma **(A)** and brain homogenate **(B)** at 60 min post-dose using Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS). *N* = 4 mouse per group. One way ANOVA confirmed a significant noscapine treatment effect in the mouse plasma ($p < 0.01$) and brain ($p < 0.01$). Tukey's *post-hoc* multiple comparisons showed group differences (*$p < 0.05$ and **$p < 0.01$). Mean ± SEM.

TABLE 3 Correlation of noscapine levels in plasma and brain of noscapine treated mouse.

Noscapine treatment in mouse	Pearson correlation coefficient	
	R squared	*p*-Value
100 mg/kg	0.969	0.015
200 mg/kg	0.97	0.015
400 mg/kg	0.675	0.177

mg/kg noscapine treated mouse was significantly different from mouse treated with 200 mg/kg ($p < 0.05$) and 400 mg/kg ($p < 0.001$). These results show that orally administered noscapine can increase the levels of acetylated α-tubulin in the mouse brain. This effect can be achieved at a noscapine dosage of 400 mg/kg or higher.

HTRF assay can detect noscapine-induced acetylated α-tubulin changes in human PBMCs

To test if the HTRF Alpha-Tubulin acetyl-K40 assay can detect changes in acetylated α-tubulin levels in human PBMCs, we treated PBMCs with 10 μM noscapine *in-vitro* for 1 h. Acetylated α-tubulin levels in the noscapine treated PBMCs was 1.5 fold higher than untreated PBMCs ($p < 0.0001$; Figure 4).

Discussion

In this study, we show that acetylated α-tubulin level is reduced in HSP-*SPAST* patient PBMCs compared to healthy control PBMCs. This finding supports our hypothesis that non-neuronal cells, PBMCs, from patients with HSP-*SPAST* show the disease-associated effect of reduced acetylated α-tubulin, similar to previous findings in patient-derived neuronal cells (Abrahamsen et al., 2013; Wali et al., 2020).

We investigated acetylated α-tubulin levels in multiple PBMC subtypes, i.e., T and B cell lymphocytes and monocytes to understand how acetylated α-tubulin levels differed in each subtype. The levels of acetylated α-tubulin in patient T cell lymphocytes were significantly lower compared to control T cell lymphocytes. But the levels of acetylated α-tubulin were comparable between patient and control B cell lymphocytes and monocytes. It is likely that the high proportion of T cells in the PBMCs (80% of all PBMCs) contributed to the patient vs. control differences seen in the overall PBMCs.

The expression of microtubules is not restricted to neuronal cells, that are degenerated in HSP-*SPAST* patients, but are also expressed in non-neuronal cell types. Therefore, it is plausible for the HSP-*SPAST* disease associated effect of reduced acetylated α-tubulin to be present in non-neuronal cell types. Our results show that PBMCs, an easily accessible source of cells exhibit HSP-*SPAST* disease-associated effects, thus showing that HSP-*SPAST* PBMCs can be used to study disease pathology and potentially develop a biomarker for the disease. To our knowledge, this is the first study showing HSP disease-associated effects in PBMCs. Similar to our findings, disease-associated effects have been shown in PBMCs for multiple neurodegenerative diseases. For example, evaluation of PBMCs from patients with Parkinson's disease and healthy controls showed that patient PBMCs have increased levels of mitochondrial reactive oxygen species and reduced levels of the antioxidant superoxide dismutase (Smith et al., 2018). Other blood components have been used to study disease-associated effects in HSP. Serum neurofilament light chain (sNfL) is an indicator of neuronal damage. Evaluation of sNfL levels in 93 patients with HSP-*SPAST* and 60 controls showed that sNfL levels was increased in the patients (Wilke et al., 2018). However, increased sNfL has been observed in multiple neurodegenerative disorders including motor neuron disease (Nardo et al., 2011) and multiple sclerosis (Benkert et al., 2022) and is not HSP-*SPAST* disease specific.

As described in the introduction, we have previously evaluated the disease-mechanism and identified a possible drug treatment candidate for HSP-*SPAST* using patient-derived stem cell models: olfactory neurosphere-derived stem cells and iPS cell derived-neurons (Abrahamsen et al., 2013; Fan et al., 2014; Wali et al., 2016, 2018, 2020). We have shown that tubulin-binding drug noscapine can increase the levels of acetylated α-tubulin in patient neurons and rescue their vulnerability to degeneration. The second aim of the study was to test if orally administered noscapine can cross the blood brain barrier and increase the level of acetylated α-tubulin in the brain. Previous studies have shown that when taken orally, noscapine can cross the blood brain barrier (Rahmanian-Devin et al., 2021). In humans, mean plasma concentration after a single 150 mg oral dose reaches a peak at 1 h (Dahlström et al., 1982). Similar kinetics were observed after 100, 200, and 300 mg doses (Karlsson et al., 1990). The terminal half-life in humans was estimated at 4.5 h or longer and bioavailability calculated to be 30% with 3.6-fold inter-individual variation (Dahlström et al., 1982; Karlsson et al., 1990). Similar to humans, in mouse administered with 75, 150, and 300 mg/kg oral noscapine, the mean plasma noscapine concentrations peaked at 1.12, 1.50, and 0.46 h, respectively (Aneja et al., 2007). The mean percent oral bioavailability of noscapine was 22.76%, 45.05%, and 26.6% at 75, 150, and 300 mg/kg, respectively (Aneja et al., 2007). In our experiments, we dosed adult mouse by gavage with 100, 200, 400 mg/kg of noscapine. At 1 h after oral gavage there was a dose-dependent increase in noscapine levels in the mouse plasma and brain as well as

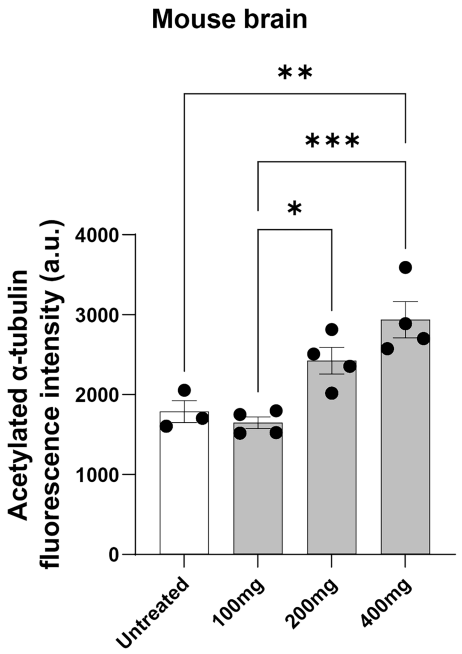


FIGURE 3
Acetylated α-tubulin levels in noscapine treated mouse. Acetylated α-tubulin was measured in the brain of the mouse dosed with noscapine. Mouse was orally administered with increasing concentrations of noscapine: 100, 200, and 400 mg/kg. One way ANOVA confirmed a significant noscapine treatment effect on acetylated α-tubulin in the mouse brain ($p < 0.01$). Tukey's *post-hoc* multiple comparisons showed group differences (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). $N = 3$ or 4 mouse per group. a.u. = arbitrary unit. Mean ± SEM.

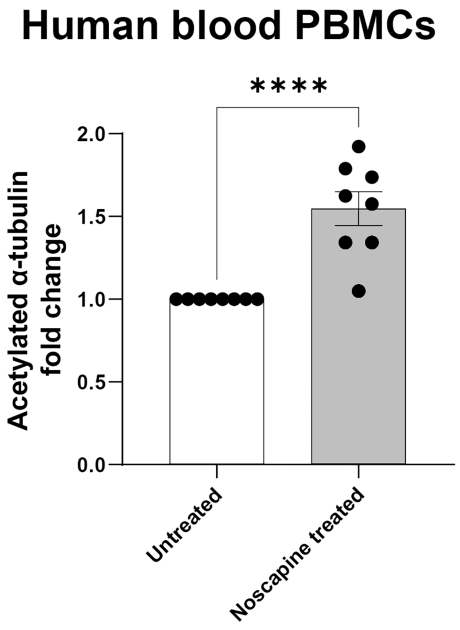


FIGURE 4
Acetylated α-tubulin levels in noscapine treated human PBMCs. Acetylated α-tubulin was measured in human PBMCs treated with noscapine (*in vitro*) at 10 μM for 1 h. Student's t-test confirmed a significant noscapine treatment effect on acetylated α-tubulin levels (****$p < 0.0001$). $N = 8$ PBMC samples per untreated and noscapine treated groups. Mean ± SEM.

a dose-dependent increase in the levels of acetylated α-tubulin in mouse brain. Our study provides evidence that orally administered noscapine can increase the levels of acetylated α-tubulin in the brain. This *in vivo* result supports the use of noscapine as a potential therapeutic agent for HSP-*SPAST*.

Noscapine-induced changes in acetylated α-tubulin levels in the mouse brain (*in vivo* treatment) and human PBMCs (*in vitro* treatment) were detected using a HTRF based assay. This assay is shown to be highly sensitive and most importantly can be miniaturized into the 384 well plate format (Degorce et al., 2009). The HTRF assay uses nanogram protein concentrations and the total reaction volume per sample is 20 μL. The low protein concentration required and the ability to perform this assay in a 384 well plate makes this assay ideal for evaluation of acetylated α-tubulin in large batches of samples, for example clinical trials where multiple samples collected at multiple different timepoints can be analyzed together avoiding any batch-to-batch effects. Therefore, we propose this assay as a sensitive high-throughput assay to measure the effect of noscapine treatment.

One of the limitations of this study is the lack of appropriately age and gender matched healthy controls. The recruitment of study participants for this study was affected by the COVID-19 global pandemic. However, correlation analysis did not identify any correlation between the age of the study participants vs. their levels of acetylated α-tubulin, suggesting that the reduced levels of acetylated α-tubulin levels in the patient PBMCs is a disease-associated effect. Evaluation of a larger cohort with more appropriately age matched controls and disease-controls, particularly including mutations associated with microtubule function (for example: KIF1A/SPG30, ATL1/SPG3A) are required to establish the reduced levels of acetylated α-tubulin in PBMCs as a HSP-*SPAST* specific disease biomarker.

In summary, we show that (a) easily accessible patient PBMCs show HSP-*SPAST* disease-associated pathology of reduced levels of acetylated α-tubulin (b) orally administered noscapine can increase levels of acetylated α-tubulin in mouse brain and (c) the HTRF acetylated α-tubulin assay can detect noscapine induced changes in acetylated α-tubulin levels in multiple tissue types. Therefore, we propose measurement of acetylated α-tubulin levels using HTRF to quantify the treatment effects of noscapine in patients with HSP-*SPAST* at the cellular level.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The experiments involving human PBMCs was approved by the Northern Sydney Local Health District Human Research Ethics Committee, Australia (Reference number: RESP/15/314) and written informed consent was obtained from all participants. The patients/participants provided their written informed consent to participate in

this study. Experiments involving mice were assessed and approved by the University of Queensland Animal Ethics Committee and assigned the following project code: TETRAQ/330/19/388/18/M 'Noscapine HCL (Noscapine): Tissue Distribution and Pharmacokinetic Analysis of Novel Compounds in Rodents'.

Author contributions

GW, AM-S, and CS designed the study. S-FS, KK, and CS recruited patients to the study. S-FS, GW, and EL performed the experiments measuring acetylated α-tubulin. GW performed data analysis. GW, AM-S, and CS provided funding. CS provided facilities for the experiments. AM-S and GW wrote and edited the first draft. All authors contributed to the article and approved the submitted version.

Funding

We are most grateful to the Hereditary Spastic Research Foundation Inc. and the Spastic Paraplegia Foundation Inc. for funding the study.

Acknowledgments

We are grateful to Drew Brockman at TetraQ for the mouse experiments, noscapine assay and preparation of mouse tissues. S-FS is the recipient of an NHMRC Postgraduate Scholarship and an ANZAN E&RF Scholarship (APP1133610). CS is a MRFF NHMRC Practitioner Fellow (APP 1136800).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1073516/full#supplementary-material

References

Abrahamsen, G., Fan, Y., Matigian, N., Wali, G., Bellette, B., Sutharsan, R., et al. (2013). A patient-derived stem cell model of hereditary spastic paraplegia with SPAST mutations. *Dis. Model. Mech.* 6, 489–502. doi: 10.1242/dmm.010884

Aneja, R., Dhiman, N., Idnani, J., Awasthi, A., Arora, S. K., Chandra, R., et al. (2007). Preclinical pharmacokinetics and bioavailability of noscapine, a tubulin-binding anticancer agent. *Cancer Chemother. Pharmacol.* 60, 831–839. doi: 10.1007/s00280-007-0430-y

Benkert, P., Meier, S., Schaedelin, S., Manouchehrinia, A., Yaldizli, Ö., Maceski, A., et al. (2022). Serum neurofilament light chain for individual prognostication of disease activity in people with multiple sclerosis: a retrospective modelling and validation study. *Lancet Neurol* 21, 246–257. doi: 10.1016/S1474-4422(22)00009-6

Bonser, L. R., Koh, K. D., Johansson, K., Choksi, S. P., Cheng, D., Liu, L., et al. (2021). Flow-Cytometric analysis and purification of airway epithelial-cell subsets. *Am. J. Respir. Cell Mol. Biol.* 64, 308–317. doi: 10.1165/rcmb.2020-0149MA

Dahlström, B., Mellstrand, T., Löfdahl, C. G., and Johansson, M. (1982). Pharmakokinetic properties of noscapine. *Eur. J. Clin. Pharmacol.* 22, 535–539. doi: 10.1007/BF00609627

Degorce, F., Card, A., Soh, S., Trinquet, E., Knapik, G. P., and Xie, B. (2009). HTRF: a technology tailored for drug discovery—a review of theoretical aspects and recent applications. *Curr Chem Genomics* 3, 22–32. doi: 10.2174/1875397300903010022

Fan, Y., Wali, G., Sutharsan, R., Bellette, B., Crane, D. I., Sue, C. M., et al. (2014). Low dose tubulin-binding drugs rescue peroxisome trafficking deficit in patient-derived stem cells in hereditary spastic paraplegia. *Biol Open* 3, 494–502. doi: 10.1242/bio.20147641

Fuss, I. J., Kanof, M. E., Smith, P. D., and Zola, H. (2009). Isolation of whole mononuclear cells from peripheral blood and cord blood. *Curr. Protoc. Immunol.* 85, 7.1.1–7.1.8. doi: 10.1002/0471142735.im0701s85

Karlsson, M. O., Dahlstrm, B., Eckerns, S., Johansson, M., and Tufvesson Alm, A. (1990). Pharmacokinetics of oral noscapine. *Eur. J. Clin. Pharmacol.* 39, 275–279. doi: 10.1007/BF00315110

Kasher, P., De Vos, K., Wharton, S., Manser, C., Bennett, E., Bingley, M, et al. (2009). Direct evidence for axonal transport defects in a novel mouse model of mutant spastin-induced hereditary spastic paraplegia (HSP) and human HSP patients. *J. Neurochem.* 110, 34–44. doi: 10.1111/j.1471-4159.2009.06104.x

Lange, L. M., Gonzalez-Latapi, P., Rajalingam, R., Tijssen, M. A. J., Ebrahimi-Fakhari, D., Gabbert, C., et al. (2022). Nomenclature of genetic movement disorders: recommendations of the International Parkinson and Movement Disorder Society task force – an update. *Mov. Disord.* 37, 905–935. doi: 10.1002/mds.28982

Maecker, H. T., and Trotter, J. (2006). Flow cytometry controls, instrument setup, and the determination of positivity. *Cytometry A* 69A, 1037–1042. doi: 10.1002/cyto.a.20333

Méreaux, J.-L., Banneau, G., Papin, M., Coarelli, G., Valter, R., Raymond, L., et al. (2022). Clinical and genetic spectra of 1550 index patients with hereditary spastic paraplegia. *Brain* 145, 1029–1037. doi: 10.1093/brain/awab386

Nardo, G., Pozzi, S., Pignataro, M., Lauranzano, E., Spano, G., Garbelli, S., et al. (2011). Amyotrophic lateral sclerosis multiprotein biomarkers in peripheral blood mononuclear cells. *PLoS One* 6:e25545. doi: 10.1371/journal.pone.0025545

Rahmanian-Devin, P., Baradaran Rahimi, V., Jaafari, M. R., Golmohammadzadeh, S., Sanei-far, Z., and Askari, V. R. (2021). Noscapine, an emerging medication for different diseases: a mechanistic review. *Evid. Based Complement. Alternat. Med.* 2021:8402517. doi: 10.1155/2021/8402517

Rida, P. C. G., LiVecche, D., Ogden, A., Zhou, J., and Aneja, R. (2015). The noscapine chronicle: a Pharmaco-historic biography of the opiate alkaloid family and its clinical applications. *Med. Res. Rev.* 35, 1072–1096. doi: 10.1002/med.21357

Schüle, R., Holland-Letz, T., Klimpe, S., Kassubek, J., Klopstock, T., Mall, V, et al. (2006). The spastic paraplegia rating scale (SPRS). *Neurology* 67, 430–434. doi: 10.1212/01.wnl.0000228242.53336.90

Smith, A. M., Depp, C., Ryan, B. J., Johnston, G. I., Alegre-Abarrategui, J., Evetts, S., et al. (2018). Mitochondrial dysfunction and increased glycolysis in prodromal and early Parkinson's blood cells. *Mov. Disord.* 33, 1580–1590. doi: 10.1002/mds.104

Vandebona, H., Kerr, N. P., Liang, C., and Sue, C. M. (2012). SPAST mutations in Australian patients with hereditary spastic paraplegia. *Intern. Med. J.* 42, 1342–1347. doi: 10.1111/j.1445-5994.2012.02941.x

Wali, G., Berkovsky, S., Whiten, D. R., Mackay-Sim, A., and Sue, C. M. (2021). Single cell morphology distinguishes genotype and drug effect in hereditary spastic paraplegia. *Sci. Rep.* 11:16635. doi: 10.1038/s41598-021-95995-4

Wali, G., Liyanage, E., Blair, N. F., Sutharsan, R., Park, J. S., Mackay-Sim, A., et al. (2020). Oxidative stress-induced axon fragmentation is a consequence of reduced axonal transport in hereditary spastic paraplegia SPAST patient neurons. *Front. Neurosci.* 14:820. doi: 10.3389/fnins.2020.00401

Wali, G., Sue, C., and Mackay-Sim, A. (2018). Patient-derived stem cell models in SPAST HSP: disease modelling and drug discovery. *Brain Sci.* 8:142. doi: 10.3390/brainsci8080142

Wali, G., Sutharsan, R., Fan, Y., Stewart, R., Tello Velasquez, J., Sue, C. M., et al. (2016). Mechanism of impaired microtubule-dependent peroxisome trafficking and oxidative stress in SPAST-mutated cells from patients with hereditary spastic paraplegia. *Sci. Rep.* 6:27004. doi: 10.1038/srep27004

Wilke, C., Rattay, T. W., Hengel, H., Zimmermann, M., Brockmann, K., Schöls, L., et al. (2018). Serum neurofilament light chain is increased in hereditary spastic paraplegias. *Ann. Clin. Transl. Neurol.* 5, 876–882. doi: 10.1002/acn3.583

3.4 Discussion and Conclusion

In this chapter, I discussed the rationale behind acetylated α-tubulin as a biomarker for HSP-*SPAST*. I developed a flow cytometry protocol for measurement of intracellular levels of acetylated α-tubulin in peripheral blood mononuclear cell (PBMC) subtypes. I used the flow cytometry protocol to demonstrate reduced levels of acetylated α-tubulin levels in HSP-*SPAST* patient PBMCs compared to controls. Based on our findings, I propose PBMC acetylated α-tubulin levels as a potential biochemical biomarker for HSP-*SPAST*.

This study is the first step in biomarker discovery with further studies needed for analytical and clinical validation of PBMC acetylated α-tubulin levels as a biomarker. Further studies are needed to evaluate the suitability of acetylated α-tubulin levels as a diagnostic, prognostic, predictive or monitoring biomarker. [37] Evaluation of biomarker performance to determine sensitivity, specificity, positive and negative predictive values, discrimination, and calibration is needed. This can be achieved through a study with larger sample size, age and gender-matched controls, and patients with different HSP-subtypes or neurodegenerative conditions. Clinical validity and clinical utility can be established by correlating detailed phenotyping of patients and changes in clinical disease severity over time with acetylated α-tubulin levels. [37]

It is likely that abnormal acetylated α-tubulin levels will be specific to HSP subtypes with tubulin dysfunction as the underlying pathophysiology, such as HSP-*SPAST* and HSP-*ATL1* (SPG3A). However, α-tubulin dysfunction has been reported in other neurodegenerative disorders including *TUBA4A*-associated amyotrophic lateral sclerosis and Parkinson's Disease. [38, 39] Therefore, PBMC acetylated α-tubulin levels could be a potential biomarker for other neurodegenerative disorders with similar underlying pathophysiology to HSP-*SPAST*.

The results from animal experiments in Section 3.3 showed increased levels of acetylated α-tubulin in mouse brain with administration of noscapine. Noscapine is a tubulin-binding drug that has been demonstrated to have anti-tumour activity and relatively low side-effect profile. [40] *In vitro* studies have demonstrated reversal of reduced acetylated α-tubulin levels with noscapine treatment in patient-derived neuronal cell models at a lower dose than that used in anti-tumour studies. [20] Therefore, PBMC acetylated α-tubulin could be a biomarker for monitoring drug effects, such as noscapine, in therapeutic clinical trials.

In section 3.3, my research group presented a high throughput acetylated α-tubulin protein assay for measurement of acetylated α-tubulin levels in mouse brain and human PBMCs.

Although flow cytometry was able to quantify intracellular acetylated α-tubulin levels in human PBMCs, it involved a complex workflow that would be challenging to perform with a large sample size such as in a multicenter clinical trial. HTRF on the other hand is a high throughput assay that would be ideal for processing a large number of samples. Therefore, the flow cytometry assay is useful to characterise the pattern and distribution of acetylated α-tubulin levels in various cell subtypes whilst the HTRF assay is more practical for real-world use to quantify acetylated α-tubulin levels in large clinical trials. Moving forward, validation of the HTRF acetylated α-tubulin assay in a larger cohort of patients with HSP will be necessary to clarify the suitability of PBMC acetylated α-tubulin levels as a biomarker for HSP clinical trials.

3.5 References

1. Siow S-F, Yeow D, Rudaks LI, et al. Outcome Measures and Biomarkers for Clinical Trials in Hereditary Spastic Paraplegia: A Scoping Review. *Genes* 2023; 14: 1756. DOI: 10.3390/genes14091756.
2. Gaiottino J, Norgren N, Dobson R, et al. Increased neurofilament light chain blood levels in neurodegenerative neurological diseases. *PloS one* 2013; 8: e75091-e75091. DOI: 10.1371/journal.pone.0075091.
3. Ning L and Wang B. Neurofilament light chain in blood as a diagnostic and predictive biomarker for multiple sclerosis: A systematic review and meta-analysis. *PLoS One* 2022; 17: e0274565. 20220914. DOI: 10.1371/journal.pone.0274565.
4. Srpova B, Uher T, Hrnciarova T, et al. Serum neurofilament light chain reflects inflammation-driven neurodegeneration and predicts delayed brain volume loss in early stage of multiple sclerosis. *Mult Scler* 2021; 27: 52-60. 20200121. DOI: 10.1177/1352458519901272.
5. Sampedro F, Pérez-González R, Martínez-Horta S, et al. Serum neurofilament light chain levels reflect cortical neurodegeneration in de novo Parkinson's disease. *Parkinsonism Relat Disord* 2020; 74: 43-49. 20200415. DOI: 10.1016/j.parkreldis.2020.04.009.
6. Kessler C, Serna-Higuita LM, Wilke C, et al. Characteristics of serum neurofilament light chain as a biomarker in hereditary spastic paraplegia type 4. *Annals of Clinical and Translational Neurology* 2022; 9(3): 326-338. DOI: https://dx.doi.org/10.1002/acn3.51518.

7. Kessler C, Serna-Higuita LM, Rattay TW, et al. Neurofilament light chain is a cerebrospinal fluid biomarker in hereditary spastic paraplegia. *Annals of Clinical & Translational Neurology* 2021; 8: 1122-1131. DOI: https://dx.doi.org/10.1002/acn3.51358.
8. Rattay TW, Volker M, Rautenberg M, et al. The prodromal phase of hereditary spastic paraplegia type 4: the preSPG4 cohort study. *Brain : a journal of neurology* 2022; 26. DOI: https://dx.doi.org/10.1093/brain/awac155.
9. Lassmann C, Ilg W, Schneider M, et al. Specific gait changes in prodromal hereditary spastic paraplegia type 4 - preSPG4 study. *medRxiv* 2022; 21. DOI: https://dx.doi.org/10.1101/2022.04.20.22274071.
10. Lin Q, Liu Y, Ye Z, et al. Potential markers for sample size estimations in hereditary spastic paraplegia type 5. *Orphanet Journal of Rare Diseases* 2021; 16(1) (no pagination). DOI: https://dx.doi.org/10.1186/s13023-021-02014-w.
11. Wilke C, Rattay TW, Hengel H, et al. Serum neurofilament light chain is increased in hereditary spastic paraplegias. *Annals of Clinical and Translational Neurology* 2018; 5: 876-882. DOI: 10.1002/acn3.583.
12. Arnoldi A, Crimella C, Tenderini E, et al. Clinical phenotype variability in patients with hereditary spastic paraplegia type 5 associated with CYP7B1 mutations. *Clin Genet* 2012; 81: 150-157. 20110131. DOI: 10.1111/j.1399-0004.2011.01624.x.
13. Marelli C, Lamari F, Rainteau D, et al. Plasma oxysterols: Biomarkers for diagnosis and treatment in spastic paraplegia type 5. *Brain* 2018; 141(1): 72-84. DOI: https://dx.doi.org/10.1093/brain/awx297.
14. Schols L, Rattay TW, Martus P, et al. Hereditary spastic paraplegia type 5: Natural history, biomarkers and a randomized controlled trial. *Brain* 2017; 140(12): 3112-3127. DOI: https://dx.doi.org/10.1093/brain/awx273.
15. Solowska JM and Baas PW. Hereditary spastic paraplegia SPG4: what is known and not known about the disease. *Brain : a journal of neurology* 2015; 138: 2471-2484. DOI: 10.1093/brain/awv178.
16. Shoukier M, Neesen J, Sauter SM, et al. Expansion of mutation spectrum, determination of mutation cluster regions and predictive structural classification of SPAST mutations in hereditary spastic paraplegia. *Eur J Hum Genet* 2009; 17: 187-194. 20080813. DOI: 10.1038/ejhg.2008.147.
17. Santorelli FM, Patrono C, Fortini D, et al. Intrafamilial variability in hereditary spastic paraplegia associated with an SPG4 gene mutation. *Neurology* 2000; 55: 702-705. DOI: 10.1212/wnl.55.5.702.

18. Abrahamsen G, Fan Y, Matigian N, et al. A patient-derived stem cell model of hereditary spastic paraplegia with SPAST mutations. *Disease models & mechanisms* 2013; 6: 489-502. DOI: 10.1242/dmm.010884.
19. Wali G, Sutharsan R, Fan Y, et al. Mechanism of impaired microtubule-dependent peroxisome trafficking and oxidative stress in SPAST-mutated cells from patients with Hereditary Spastic Paraplegia. *Scientific reports* 2016; 6: 27004-27004. DOI: 10.1038/srep27004.
20. Wali G, Liyanage E, Blair NF, et al. Oxidative Stress-Induced Axon Fragmentation Is a Consequence of Reduced Axonal Transport in Hereditary Spastic Paraplegia SPAST Patient Neurons. *Front Neurosci* 2020; 14: 401. 20200507. DOI: 10.3389/fnins.2020.00401.
21. Wali G, Sue C and Mackay-Sim A. Patient-Derived Stem Cell Models in SPAST HSP: Disease Modelling and Drug Discovery. *Brain Sciences* 2018; 8: 142. DOI: 10.3390/brainsci8080142.
22. Solowska JM, D'Rozario M, Jean DC, et al. Pathogenic mutation of spastin has gain-of-function effects on microtubule dynamics. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2014; 34: 1856-1867. DOI: 10.1523/JNEUROSCI.3309-13.2014.
23. Sferra A, Nicita F and Bertini E. Microtubule Dysfunction: A Common Feature of Neurodegenerative Diseases. *Int J Mol Sci* 2020; 21 20201005. DOI: 10.3390/ijms21197354.
24. Jiang YQ and Oblinger MM. Differential regulation of beta III and other tubulin genes during peripheral and central neuron development. *J Cell Sci* 1992; 103 (Pt 3): 643-651. DOI: 10.1242/jcs.103.3.643.
25. Keays DA, Tian G, Poirier K, et al. Mutations in alpha-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. *Cell* 2007; 128: 45-57. DOI: 10.1016/j.cell.2006.12.017.
26. Stanchi F, Corso V, Scannapieco P, et al. TUBA8: A new tissue-specific isoform of alpha-tubulin that is highly conserved in human and mouse. *Biochem Biophys Res Commun* 2000; 270: 1111-1118. DOI: 10.1006/bbrc.2000.2571.
27. Howes SC, Alushin GM, Shida T, et al. Effects of tubulin acetylation and tubulin acetyltransferase binding on microtubule structure. *Mol Biol Cell* 2014; 25: 257-266. 20131113. DOI: 10.1091/mbc.E13-07-0387.
28. Eshun-Wilson L, Zhang R, Portran D, et al. Effects of α-tubulin acetylation on microtubule structure and stability. *Proc Natl Acad Sci U S A* 2019; 116: 10366-10371. 20190509. DOI: 10.1073/pnas.1900441116.

29. Niu X, Mao CX, Wang S, et al. α-Tubulin acetylation at lysine 40 regulates dendritic arborization and larval locomotion by promoting microtubule stability in Drosophila. *PLoS One* 2023; 18: e0280573. 20230224. DOI: 10.1371/journal.pone.0280573.
30. Lacroix B, van Dijk J, Gold ND, et al. Tubulin polyglutamylation stimulates spastin-mediated microtubule severing. *J Cell Biol* 2010; 189: 945-954. 20100607. DOI: 10.1083/jcb.201001024.
31. Bonser LR, Koh KD, Johansson K, et al. Flow-Cytometric Analysis and Purification of Airway Epithelial-Cell Subsets. *Am J Respir Cell Mol Biol* 2021; 64: 308-317. DOI: 10.1165/rcmb.2020-0149MA.
32. Chesta ME, Carbajal A, Bisig CG, et al. Quantification of acetylated tubulin. *Cytoskeleton (Hoboken)* 2013; 70: 297-303. 20130508. DOI: 10.1002/cm.21111.
33. Shah AK, Wali G, Sue CM, et al. Antibody-Free Targeted Proteomics Assay for Absolute Measurement of α-Tubulin Acetylation. *Anal Chem* 2020; 92: 11204-11212. 20200727. DOI: 10.1021/acs.analchem.0c01683.
34. Yang X, Naughton SX, Han Z, et al. Mass Spectrometric Quantitation of Tubulin Acetylation from Pepsin-Digested Rat Brain Tissue Using a Novel Stable-Isotope Standard and Capture by Anti-Peptide Antibody (SISCAPA) Method. *Anal Chem* 2018; 90: 2155-2163. 20180124. DOI: 10.1021/acs.analchem.7b04484.
35. Dráberová E, Stegurová L, Sulimenko V, et al. Quantification of α-tubulin isotypes by sandwich ELISA with signal amplification through biotinyl-tyramide or immuno-PCR. *J Immunol Methods* 2013; 395: 63-70. 20130710. DOI: 10.1016/j.jim.2013.07.001.
36. Degorce F, Card A, Soh S, et al. HTRF: A technology tailored for drug discovery - a review of theoretical aspects and recent applications. *Curr Chem Genomics* 2009; 3: 22-32. 20090528. DOI: 10.2174/1875397300903010022.
37. Ou FS, Michiels S, Shyr Y, et al. Biomarker Discovery and Validation: Statistical Considerations. *J Thorac Oncol* 2021; 16: 537-545. 20210202. DOI: 10.1016/j.jtho.2021.01.1616.
38. Mazzetti S, Giampietro F, Calogero AM, et al. Linking acetylated α-Tubulin redistribution to α-Synuclein pathology in brain of Parkinson's disease patients. *npj Parkinson's Disease* 2024; 10: 2. DOI: 10.1038/s41531-023-00607-9.
39. Ganne A, Balasubramaniam M, Ayyadevara H, et al. In silico analysis of TUBA4A mutations in Amyotrophic Lateral Sclerosis to define mechanisms of microtubule disintegration. *Sci Rep* 2023; 13: 2096. 20230206. DOI: 10.1038/s41598-023-28381-x.

40. Ye K, Ke Y, Keshava N, et al. Opium alkaloid noscapine is an antitumor agent that arrests metaphase and induces apoptosis in dividing cells. *Proc Natl Acad Sci U S A* 1998; 95: 1601-1606. DOI: 10.1073/pnas.95.4.1601.

Chapter 4. Developing and validating an HSP-specific patient reported outcome measure with consumer consultation

4.1 Introduction

In Chapter 1, a scoping review of outcome measures for HSP found that patient-reported outcome measures (PROM) were not used in most HSP clinical trials and there was no consensus on choice of PROM to measure quality of life (QoL). In chapter 2, I used the Short Form 36 (SF-36), a generic PROM, in a cohort of patients with HSP and found that although the SF-36 demonstrated impaired QoL compared to population norms, it did not include HSP-specific QoL items and was unlikely to be sensitive to QoL changes in HSP. Therefore, I identified a need for an HSP-specific QoL measure. In this chapter, I describe how I designed and validated an HSP-specific PROM, the Hereditary Spastic Paraplegia Quality of Life scale (HSPQoL). I report the HSPQoL results in a cohort of patients with HSP.

4.1.1 Impact of HSP on QoL.

Patients with HSP have been demonstrated to have worse QoL than the general population. [1-3] A qualitative study of how HSP impacts patients in the Netherlands revealed four themes "I stumble, I struggle, I feel ashamed, I need support". [4] Patients reported specific symptoms related to their HSP including pain, stiffness, fatigue, balance and gait disturbances, and symptom fluctuations. The impact of their symptoms on their daily activities, their feelings of being judged, feeling ashamed, fear, and frustration; and varying degrees of healthcare support were reported as relevant and important to their QoL and management. [4] Patients with HSP and HSP health care providers in South West England reported the challenges with conveying the diagnosis, potential stigma of HSP, symptom management, importance of psychological and emotional support, gaps and limited access to services, the importance of carers and their needs, and the need for care coordination. [5] Surveys of patients with HSP revealed findings of lower life satisfaction, lower mental well-being, limited social support,

limited access to health care, poorer memory and sleep, muscle stiffness, cramps and weakness, bladder and bowel problems, fatigue, pain, and loss of mobility. [6-10]

4.1.2 PROMs used in HSP.

My scoping review in Chapter 1 showed a broad range of PROMs used in HSP (n=17). [11] However, there was little consistency of PROMs used amongst HSP studies. The various PROMs used measured different domains including health related QoL, anxiety and depression symptoms, and specific symptoms including pain, fatigue, incontinence, and autonomic symptoms. PROMs previously used in HSP studies were either generic PROMs or designed for non-HSP conditions, such as cerebral palsy or Parkinson's disease. Therefore, there was no HSP-specific PROM for measurement of health related QoL.

4.1.3 Rationale for HSPQoL and study design.

Many HSP-specific symptoms and concerns that impact QoL described in section 4.1.1 are not assessed in generic QoL measures. SF-36, the most commonly used PROM in HSP does not include items on bladder or bowel function, spasticity or cramps, sleep quality, or access to health care. [12] The limitation of a generic QoL measure is demonstrated by testing of the Pediatric Quality of Life Inventory (PedsQL 4.0), a generic QoL measure, in children with spinal cord injuries demonstrating poor internal consistency due to lack of condition-specific items. [13] Therefore, an HSP-specific QoL measure is needed to accurately assess QoL for HSP. An HSP-specific PROM will be used to monitor the patient well-being in routine clinical care, and patient-reported outcomes in clinical trials.

In designing the HSPQoL, I supplemented the SF-36 with additional HSP-specific items, similar to the design of the Multiple Sclerosis Quality of Life survey (MSQoL). [14] Additional HSP-specific items were developed using methods previously reported, including (1) item generation through a literature review; (2) a modified Delphi process with an international HSP expert panel to establish content validity and consensus for item development; and (3) rigorously testing the items through cognitive interviews with consumers for relevance and appropriateness. [15-19] An alternative method considered for item generation with in-depth qualitative interviews of individuals with HSP would limit participant input to English-

speaking individuals living in Australia whereas the combination of a literature review and international expert panel will incorporate broader patient experience. [20]

The original Delphi process and the modified Delphi process are commonly used techniques in healthcare research to aid survey design, and to achieve consensus amongst a group. [21] In a modified Delphi, the initial list of items is developed by the researchers based on pre-existing literature and expert knowledge to replace the initial round of the original Delphi where an item list is generated by the panel. The advantage of using the modified version is to reduce the time taken for the entire process and therefore potentially increase participation and completion rates. [22] Panel members remain anonymous to each other and the degree of consensus for inclusion, modification or exclusion of any item is predetermined. [23] This method allows the incorporation of both clinician and patient input whilst reducing response bias. The ability to perform the whole process online also facilitates inclusion of panel members unable to participate due to geographical location, pandemic lockdowns and mobility issues.

Cognitive interviewing is a qualitative research method used to establish content validity in the development of PROMs. [21, 24, 25] The cognitive process, item comprehension and relevance of items are assessed, enabling survey developers to identify and revise questions that respondents find difficult to interpret. Two approaches are described, “think aloud”, which involves encouraging the interviewees to verbalise their thought process when answering an item, and “verbal probing”, which involves using cognitive probes to evaluate a particular aspect of an item. [21] Each method has its own benefits and limitations, whilst combining both allows for efficient and targeted responses with room for unexpected issues not identified by the survey developers.

Consumer involvement is an important component of health and medical research[26] and there is a published statement on consumer involvement by the Australian National Health and Medical Research Council and Consumers Health Forum of Australia. [27] A scoping review of patient involvement in the development of PROMs found that there were no patients involved in 25.9% of PROM development studies, raising concerns regarding the validity of those PROMs and how they capture patients’ perspectives. [28] In my study, the patient perspective was an essential component of item development with patient participation in the modified Delphi process and further item refinement with a cognitive interviewing approach.

In Section 4.2, I present the development and validation of the HSPQoL. I present the results of the HSPQoL participant responses in section 4.3.

4.2 Development and validation of the HSPQoL

Available on the medRxiv preprint repository as Siow SF, Fleming J, Barlow-Stewart K, Kumar KR, Sue CM. Designing and validating a Hereditary Spastic Paraplegia-specific Quality of Life Rating Scale (HSPQoL), medRxiv 2024.07.23.24310834; doi: https://doi.org/10.1101/2024.07.23.24310834 and is under review.

Title: Designing and validating a Hereditary Spastic Paraplegia-specific Quality of Life Rating Scale (HSPQoL)

Authors: Sue Faye Siow[1,2], Jane Fleming[1], Kristine Barlow-Stewart[2], Kishore R Kumar[2,3,4,5], Carolyn M Sue[5,6,7].

Affiliations:

1. Department of Clinical Genetics, Royal North Shore Hospital, St Leonards, NSW, Australia
2. Northern Clinical School, Faculty of Medicine and Health, University of Sydney, NSW, Australia
3. Translational Neurogenomics Group, Genomic and Inherited Disease Program, Garvan Institute of Medical Research, Darlinghurst 2010, Australia.
4. Molecular Medicine Laboratory and Department of Neurology, Concord Hospital, Concord 2139, Australia.
5. School of Clinical Medicine, UNSW Medicine & Health, University of New South Wales, Kensington 2052, Australia.
6. Neuroscience Research Australia, University of New South Wales, Randwick, NSW, Australia
7. Department of Neurology, Prince of Wales Hospital, Randwick, NSW, Australia

Corresponding author:

Sue Faye Siow, suefaye.siow@health.nsw.gov.au or sue-faye.siow@sydney.edu.au

Department of Clinical Genetics, Royal North Shore Hospital, Reserve Road, St Leonards NSW 2065, Australia.

Abstract

Background: Patients with Hereditary Spastic Paraplegia (HSP) report reduced quality of life (QoL) compared to the general population. Generic QoL measures do not address disease-specific aspects such as spasticity, access to specialty HSP clinics, and bladder symptoms. We designed and validated an HSP-specific QoL scale (HSPQoL), intended for use in standard clinical settings and clinical trials.

Methods: HSP-specific items were added to the RAND 36-Item Short Form Health Survey (SF-36) to form HSPQoL. Following literature review/expert input, 23 items were presented to a panel of HSP clinicians, patients, and patient representatives (n=12) using a modified Delphi process. Items were ranked for clarity and relevance (inclusion criteria: 80% consensus). Interviews with patients (n=5) assessed suitability, comprehension, clarity, and response options to additional items. Patients completed the HSPQoL and EQ5D-5L for evaluation of construct validity (correlation, exploratory factor analysis) and test-retest reliability.

Results: Following the modified Delphi process, 21/23 items met the inclusion criteria. Based on cognitive interview results, items were modified (n=4), removed (n=7), or added (n=3). Sixty-one participants completed the HSPQoL and EQ5D-5L. The HSPQoL was repeated with 19 patients: 15/17 additional items moderately to strongly correlated with pre-existing SF-36 subscores (Spearman correlation 0.319-0.771, $p<0.05$). Exploratory factor analyses showed high percentage of variance in the first component (>45%). HSPQoL demonstrated good internal consistency (Cronbach alpha 0.94), test-retest reliability (ICC 0.957), and convergent validity with EQ5D-5L (r=0.725).

Conclusions: Demonstrated validity and reliability of the HSPQoL confirms consideration of its use for assessing specific QoL in individuals with HSP.

Key words: hereditary spastic paraplegia, quality of life, patient reported outcome measure, modified Delphi, cognitive interview

Introduction

Hereditary Spastic Paraplegia (HSP) refers to a group of inherited neurodegenerative disorders characterized by lower limb spasticity and increased reflexes. [1] Studies exploring the experience of patients with HSP have identified features specific to HSP that influence overall quality of life (QoL), including spasticity, cramps, impaired mobility, pain, fatigue, bladder symptoms, poor sleep, access to treatment and support, and depression. [2-8] Generic health related QoL measures have been used to demonstrate reduced QoL in patients with HSP. [2, 9, 10] However, these measures QoL do not address HSP specific QoL aspects and may not fully represent the patient experience.

There is no curative treatment for HSP although there are several drug candidates that are ready for clinical trials. [11] Patient reported outcome measures (PROMs) have not been consistently used in HSP clinical trials. [12] Generic QoL measures, such as the 36-item short form health survey (SF-36) and the EuroQoL-5 Dimensions (EQ-5D), have previously been used in some clinical trials for patients with HSP, but only as secondary outcome measures. [13, 14]

A recent study investigating clinician and patient reported outcomes in a cohort of patients with HSP demonstrated that the generic QoL rating scale, EQ-5D was not sensitive to change over time and did not correlate with disease severity. [9] The SF-36 demonstrated poorer QoL in patients with HSP compared to the general population[10, 15] and correlation with disease severity. [15, 16] So, while the SF-36 is a widely used, standardized and validated health related QoL measure, it has not been tested in the HSP population for sensitivity to change over time and internal consistency. As a generic measure, the SF-36 is likely to be less sensitive than an HSP-specific QoL survey as it does not capture aspects specific to HSP. There was no HSP-specific QoL scale at the time of study conception, therefore we aimed to develop the first

HSP-specific QoL scale, HSPQoL. Since then, TreatHSP-QoL[17], an HSP-specific QoL scale has been published and comparisons can therefore be made between the two scales.

Methods

Ethics

This study was approved by Northern Sydney Local Health District Human Research Ethics Committee, ethics approval number 2019/ETH13187. All participants provided informed written or verbal consent.

HSPQoL design

As the SF-36 has been used in HSP patient cohorts, for our study we combined it with additional HSP-specific questions. Such an amendment to the SF-36 was used to develop the Multiple Sclerosis QoL scale (MSQoL) which has subsequently been validated in various populations. [18, 19] We chose to use the RAND-36 version of the SF-36 for supplementation with HSP specific items, as it is freely available, has a published scoring system, population norms, and changes are permitted. [20]

Literature Review

A comprehensive literature review was performed to identify common themes related to QoL in patients with HSP. The PubMed database was searched using the terms “hereditary spastic paraplegia”, “quality of life”, and “patient reported outcome measures”. Inclusion criteria were original full text research articles, published in peer reviewed journals, and written in English. Additional relevant articles from the reference lists of included articles were also reviewed. Common themes were identified from the reviewed articles. Additional items to supplement the SF36 were designed to address identified themes.

Modified Delphi process

Panel selection: Potential panel participants were identified through the network of HSP specialists in Australia and internationally, patients of the Neurogenetics Clinic in Royal North Shore Hospital, NSW, Australia, and representatives of the HSP Research Foundation, an Australian-based HSP patient support group with members from across the world. Invitations were sent out via email and potential participants were informed of expected time for completion and number of rounds. The modified Delphi process was performed between January 2020 to May 2020.

Round 1: The additional items were distributed to the expert panel in a two-round modified Delphi process [21] via email. Responses were collected using REDCap electronic data capture tools hosted at the University of Sydney. [22] The modified Delphi was conducted online with email invites sent to individual panel members with blinding of the identity of panel members to each other. In the first round, the items were grouped by theme and presented with the rationale for each item. Members of the panel were asked to rate the relevance and clarity of each item on a 5-point Likert scale. Free-text boxes were included to provide reasons and suggestions for modification. Participant responses were used to calculate a relevance and clarity score for each item (Supplementary Material File 1). Participant feedback in the free-text boxes was summarized.

Round 2: The scores from round 1 and summarized feedback were presented to the panel who were asked to select (1) if an item should be included, (2) if the item was not to be included, (3) if a modified version was to be included and if they agreed with the proposed modified item. The level of consensus rate for inclusion in or modification of each item was pre-determined at 80%.

Cognitive interview process

Recruitment: Patients were recruited through the Neurogenetics Clinic, Royal North Shore Hospital, New South Wales, Australia. Eligibility criteria were a clinical diagnosis of HSP; aged older than 18 years and able to speak and read English. Eligible patients were identified through a review of the clinic patient database and contacted via phone call. Patients who participated in the modified Delphi process were not invited for the cognitive interview. Study details were emailed if patients expressed interest in participating. Patients who wanted to participate contacted the study coordinator to arrange an interview time and verbal informed consent was recorded at the beginning of the interview. Cognitive interviews were conducted between August and September 2022.

Interviews and analysis: Interviews were conducted by SFS, a specialist physician with experience consulting and counselling patients with neurological and genetic conditions. SFS was trained and supervised by a genetic counsellor and researcher with extensive experience in qualitative research methods. Structured patient interviews were conducted via phone call and audio-recorded with consent. A template for the interview transcript is available upon request. A combination of 'think-aloud' and 'verbal probing' techniques were used and an open-ended question for overall feedback accompanied each item.[23] Interviews were transcribed verbatim for analysis. Interview responses were compiled for each item and coded into the relevant themes. [24] Items that had issues identified by two or more respondents were revised and additional definitions were added where required.

Final validation step

The final HSPQoL consisting of 54 items (36 items from SF-36 and 18 additional items) was distributed via RedCap to individuals with HSP from the Neurogenetics Clinic at Royal North Shore Hospital, Sydney, Australia, and members of the HSP Research Foundation who responded to an email invitation to participate. Survey distribution and data collection was

performed between August to December 2023. Relevant demographic information was collected, and the EQ5D-5L[25] scale was included to test convergent validity. Participants were then invited to repeat the HSPQoL two weeks later for test-retest reliability. RedCap responses were collated and data checking and clean up was performed. SPSS version 29,[26] and Microsoft Excel were used for all statistical analyses. Demographics and survey scores were analyzed descriptively. The correlation of additional HSP-specific items with pre-existing SF-36 subscores was studied with Spearman correlation coefficients. Exploratory factor analysis was performed to test construct validity of SF-36 subscores with additional items. Convergent validity of HSPQoL with EQ5D-5L was tested with Pearson correlation. Cronbach alpha was used to test for internal consistency. Test-retest reliability was calculated with intraclass coefficient of scores from initial HSPQoL and repeat HSPQoL.

Results

Literature Review

Ten articles were reviewed, and the common themes identified. The study team designed 23 items (data available upon request) to address the five themes identified that were unique to those already assessed by the SF-36 survey: HSP specific symptoms, Visibility of HSP, Progressive nature of HSP, Access to specialized health care for HSP, Genetic nature of HSP (Supplementary Material 2- Table 1).

Modified Delphi process

Round 1: 16 email invitations were sent and the final panel consisted of 12 members (response rate 75%): HSP specialist neurologists (n=4), clinical nurse specialist (n=1), HSP patient representative (n=1), patients with HSP (n=5) and one carer for a patient with HSP. All panel members completed the modified Delphi process (Response rate 100%). The

relevance and clarity scores for each question are presented in Supplementary Material 2 - Table 2. In summary, most items were ranked as moderate to high relevance (17 items with >70% relevance score), however most items were thought to be unclear (13 items with <70% clarity score). Feedback included better definition of terms used, relevance of items to respondents, relevance of respondent's demographics, clarity of intent, and order of items.

Nine additional demographic questions were included to provide context for participant responses (Supplementary File 1 – List of items presented in modified Delphi round 2). Ten additional items were added based on panel feedback, and a total of 33 items were presented to the panel for Round 2 of the modified Delphi process (Figure 1).

Round 2: Response rate was 100%. 11 items that did not meet the 80% consensus rate were removed with a total of 22 items remaining (Supplementary Material 2 - Table 3). At least one item from each section met the criteria for inclusion. Feedback included review of wording, clarity of question intent, grouping together of similar questions, and standardization of response options.

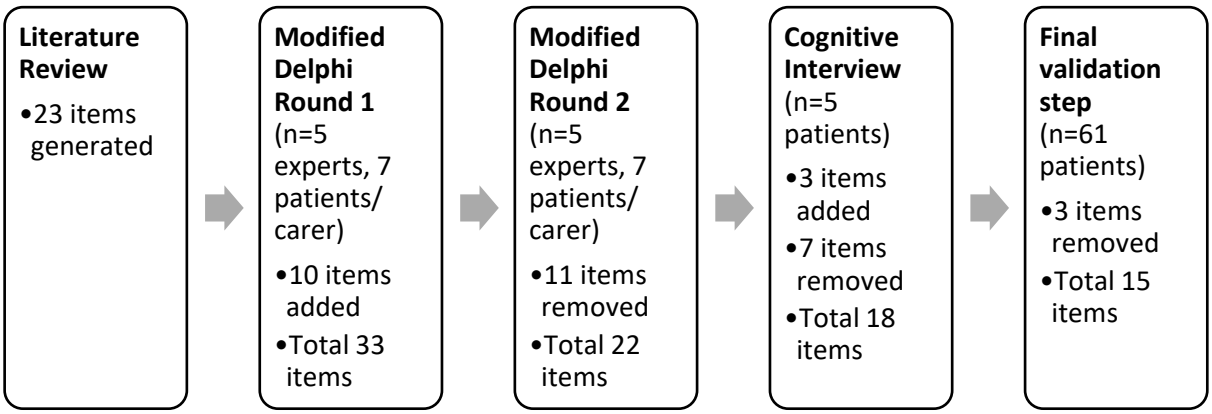


Fig. 1 Study design. Twenty-three items were generated from a literature review and underwent a modified Delphi process. Twenty-two items following the modified Delphi

process were presented to study participants for feedback through a cognitive interview. Eighteen items following the cognitive interview underwent a final validation step with a final total of fifteen additional HSP items for addition to the SF36 to form the HSPQoL.

Cognitive interview

Five patients with HSP were recruited, patient demographics are listed in Table 1. Mean interview duration was 36 minutes (range 22 to 42 minutes). 22 items were presented to the interview participants for feedback. The findings of the interviews are presented in Supplementary Material 2 - Table 4. Overall, the participants reported that the additional questions covered most relevant issues. Additional comments suggested inclusion of a free-text box at the end of the survey, inclusion of information for patient support groups, inclusion of a contact person on the form as some concepts may be confusing or confronting, changes to wording of items, changes to grouping of symptoms, and modification of response options for items that may not be relevant to some respondents. Based on the results of the cognitive interview, three items were added, seven items removed, five questions were modified, and three questions were kept unchanged based on participant feedback (Figure 1). A total of 18 HSP-specific items remained for evaluation in the final validation step.

Table 1 Demographics of cognitive interview participants

Subject ID	**Gender**	**Age (years)**	**SPRS Score**	**Location**
1	M	77	22	Metropolitan
2	M	61	23	Regional

3	F	36	13	Metropolitan
4	F	59	21	Regional
5	F	76	24	Metropolitan

M - male, F – female, SPRS – Spastic Paraplegia Rating Scale.

Final validation step

Of 64 individuals with HSP who responded to the email invitation to participate in the study, 61 completed the survey. Response rate was not calculated as invitations were distributed through multiple sources and the exact number of invitations received could not be determined. Invitations were then sent to 21 participants who had completed the initial HSPQoL for a re-test two weeks later with 19/21 repeat HSPQoL surveys completed (90% response rate). Participant demographics are presented in Table 2.

Table 2. HSPQoL validation participant demographics.

Total n	61 (100%)	
Age (years)	Mean 57.51 Range 28-78	
Gender n (%)	Female 30 (49.2%)	Male 31 (50.8%)
Place of residence n (%)	Metropolitan 53 (86.9%)	Rural 8 (13.1%)

	Yes n (%)	**No n (%)**

Genetic diagnosis of HSP	44 (72.1%)	14 (23%) (3 don't know, 4.9%)
Partner	42 (68.9%)	19 (31.1%)
Children	40 (65.6%)	21 (34.4%)
Children who have a diagnosis of HSP	8 (13.1%)	33 (54.1%)
Relatives with HSP	34 (55.7%)	27 (44.3%)
Able to walk	37 (60.7%)	24 (39.3%)
Access to specialised HSP clinic	23 (37.7%)	38 (62.3%)
Other non-HSP related health issues	33 (54.1%)	28 (45.9%)
Impact of non-HSP related health issues on QoL	20 (32.8%)	13 (21.3%)

18 additional HSP-specific items were analyzed using Spearman correlation to map these items to the existing SF-36 subscores (Supplementary Material 2 – Table 5). All items moderately to strongly correlated with a corresponding SF36 subscores (Supplementary Material 2 - Table 5) except for items 44, 45 and 53. Those three items addressed the impact of bladder/bowel symptoms on work and lifestyle, and regarding difficulty accessing specialised healthcare for HSP. Item 54, which asked respondents to rate the impact of access to healthcare on their QoL, moderately correlated with multiple subscores but was included with the General Health subscore as theoretically this was considered the best fit.

Exploratory factor analysis was performed to determine if the additional items fit within pre-existing SF-36 subscores (Supplementary Material 2 - Table 6). All additional items had

factor loadings of at least 0.40 except for items 51, which assessed how much of the time respondents wanted to hide their symptoms, and 52, which assessed their concern for passing on HSP to their children. However, these items were retained in this subscore as there was a moderate-strong correlation with the Mental Health subscore and were thought to be a good theoretical fit. Item 53, which asked if respondents had difficulty accessing health care for their HSP, could not be included in the factor analysis as it determined if respondents were able to answer item 54, which assessed how much access to health care impacted their QoL. Therefore, item 53 was moved to demographics. Items 44 and 45, which assessed the impact of bladder or bowel symptoms on work and lifestyle, did not fit with any of the SF36 subscores using Spearman correlation or exploratory factor analysis and were therefore removed. All exploratory factor analysis showed high percentage of variance in the first component (>45%) with a sharp drop off in the second component (<19.77%) demonstrating that the items best fit within their allocated subscores (Supplementary Material 2 - Table 6). Comparison of exploratory factor analysis of pre-existing SF36 subscores with and without additional items showed minimal change in percentage variance explained by first component with the addition of the HSP-specific items (Supplementary Material 2 - Table 6). This finding demonstrates that the construct of the HSPQoL remains intact and supports enrichment of the scale with the additional items. Final allocation of additional items to pre-existing SF36 subscores is presented in Table 3.

Table 3. SF36 subscores and additional items allocated to each subscore based on Spearman correlation and exploratory factor analysis. For specific items, please refer to HSPQoL survey available in Appendices.

SF36 subscore	Original SF36 Items	Additional Items	Themes of additional items
Physical Functioning	3-12	37-41	Frequency of HSP-specific symptoms: loss of balance, falls, leg weakness, leg stiffness, spasms/cramps
Social Functioning	20, 32	42-43	Impact of HSP-specific symptoms and bladder/bowel function on QoL
Pain	21,22	46	Impact of pain on sleep
Energy/Fatigue (vitality)	23, 27, 29, 31	47-49	Impact of HSP-specific symptoms and bladder/bowel function on sleep
Emotional well-being (mental health)	24, 25, 26, 28, 30	50-52	Progressive nature, visibility, and genetic nature of HSP
General Health	1, 33, 34, 35, 36	54	Impact of access to specialized health care on QoL
Role limitations due to physical health	13-16	Nil	

Role limitations due to emotional problems	17-19	Nil	
Health change	2	Nil	

Confirmatory factor analysis was not performed as the small sample size (n=61) was insufficient for simultaneous latent modelling. Further studies with larger sample sizes will be required to determine factor loadings, which may be used to calculate summary scores, such as the Mental Health Component Score and Physical Health Component Score [27].

Convergent validity was determined using Pearson correlation for additional HSP-specific items and EQ5D-5L scores. EQ5D-5L scores were calculated using the Australian value set as scores are calculated based on population norms [28]. The EQ Index scores and EQ Visual Analogue Scores were strongly correlated with total score of additional HSP items (Pearson correlation coefficient 0.725 and 0.549 respectively, $p<0.001$) demonstrating convergent validity.

Test-retest reliability was calculated using intraclass coefficient (ICC) scores from 19 participants who completed both the initial HSPQoL and repeat HSPQoL two weeks later. ICC was 0.957 ($p<0.001$), demonstrating good test-retest reliability.

The Flesch reading ease score was 70.3 and Flesch-Kincaid grade level score was 7.2 for the HSPQoL, consistent with a "fairly easy" level of readability. [29] This is similar to the readability scores of the SF36 survey which were 70.3 and 6.7; and equivalent to text suitable for grade 7. [30]

In summary, 18 additional items were included in the final validation step. Three items were removed based on Spearman correlation and exploratory factor analysis results. The final HSPQoL consisted of 51 items (36 items from SF36 and 15 additional HSP-specific items).

Discussion

In this study, we present the design and validation of the HSPQoL, an HSP-specific patient reported outcome measure intended for use in standard clinical practice and clinical trials. The HSPQoL was developed through a rigorous process of expert consensus, consumer engagement, and psychometric testing. We demonstrate content validity, construct validity, internal consistency, convergent validity, and test-retest reliability of the HSPQoL. The patient perspective was an integral component of item development with patient participation in the modified Delphi process and further item refinement with a patient cognitive interviewing approach. [31, 32]

HSP-specific symptoms represented the majority of additional items in the HSPQoL (11/15 items). This included items addressing the frequency of lower limb spasticity, cramps, mobility, balance, bladder or bowel symptoms, and the impact of these symptoms on patients' QoL (Table 3). By enriching a pre-existing validated health-related QoL measure with HSP-specific items, we hope to maximize the sensitivity of the HSPQoL to changes in HSP-related QoL over time. This is particularly helpful in an HSP clinical trial where the primary outcome measure should be improvement of HSP-related symptoms. The HSPQoL is a validated patient reported outcome measure that is designed to complement an HSP-specific clinician outcome measure, such as the Spastic Paraplegia Rating Scale (SPRS)[33], in a clinical trial.

Although genetics of HSP was identified as relevant to QoL in individuals with HSP (Supplementary Material 2 - Table 1 and 2), several cognitive interview participants found

items regarding hereditability of HSP difficult or confronting to answer in a survey setting (Supplementary Material 2 - Table 4). This finding highlights the importance of the cognitive interview process to elicit patient feedback regarding sensitive survey items and potential impacts of those items, including potential for response bias and psychological harm. [23] We were able to identify the importance of discussing the genetics of HSP in a clinical setting with support from a qualified health professional. If conducted prior to an appointment, PROMs such as the HSPQoL could facilitate the discussion of sensitive topics which may not have otherwise been raised. [34]

The progressive nature of HSP is well recognized though can be difficult to quantify, particularly in subtypes of HSP with slow progression. [35] We included an item to assess change in HSP symptoms, "I am able to continue enjoying leisure activities despite my HSP symptoms" in the hope of capturing subtle changes in patients' QoL that may not be reflected on clinical rating scales, such as the SPRS, but may have an impact on their social functioning. Similarly, the visibility of HSP is another aspect that impacts patients' QoL but is not routinely assessed in patient reported outcome measures. [17, 36]

Despite the majority of respondents residing in a metropolitan area, most did not have access to specialized HSP services. Inclusion of an item measuring the impact of poor access to healthcare in HSP is particularly important to identify patients who will benefit from assistance linking them in with appropriate healthcare services. [37] In addition, measuring the impact of access to healthcare on QoL can be relevant to measures of acceptability and feasibility for interventions in clinical trials. The HSPQoL demonstrated "fairly easy" readability as measured with Flesch-Kincaid readability tests. The final survey consists of 51 items which is comparable to the MSQoL which has 54 items. [19] Overall, the HSPQoL is likely to accessible to most respondents or their carers.

TreatHSP-QoL is a recently published HSP-specific 25-item patient reported outcome measure[17] developed and validated in a large HSP patient cohort (n=298). The items are grouped into five domains: General QoL and attitude to the disease, Mobility and leisure time, Medical care, Social life and occupation/work, Associated Symptoms. Although there were similar items in both the TreatHSP-QoL and HSPQoL, there were some differences. TreatHSP-QoL may be suitable for younger patient cohorts of working age and patients with complex HSP as it included items on employment, finances, and complex symptoms including speech, upper limb symptoms, and memory. On the other hand, HSPQoL may be relevant to a broader age range and varied employment statuses, and patients who are family planning as items were relevant to respondents who were working, retired, or unemployed, and addressed the genetics of HSP, but did not include items for complex HSP symptoms. As HSPQoL incorporates a pre-existing validated QoL scale, population norms for SF-36 are available for comparison with HSP patient scores and may assist stratification of HSPQoL scores. In addition, future studies using the HSPQoL as an outcome measure can use the SF-36 portion of the scale to compare and potentially collate their results with previous HSP studies that used the generic scale. TreatHSP-QoL was developed and validated in German whilst HSPQoL was developed and validated in English. The choice between TreatHSP-QoL or HSPQoL would rely on the target participants and intended outcomes of any future HSP clinical trial.

Limitations

There are several limitations to this study. The modified Delphi panel members and patients interviewed were native English speakers who lived in developed countries - most participants were from Australia, whilst two participants lived in Europe. Therefore, clinician and patient perspectives were limited to experiences of stakeholders from developed Western countries and healthcare systems. All patients interviewed had moderate disease severity as

measured by the SPRS. Therefore, the perspective of mildly or severely affected individuals was not captured. However, the reviewed literature included perspectives of patients with varying disease severities, from different countries and language backgrounds. There may be a selection bias of participants with a particular interest in HSP research and QoL that may have influenced the results of the study.

Some participants reported that certain QoL aspects, for example concern regarding the impact of HSP on other family members and the fluctuating nature of their symptoms, were too complex to explore in a survey. We plan to include a free-text box at the end of each survey for patients to report relevant issues that are not included in the survey. This feedback will be important if HSPQoL is used as a pre-appointment PROM to inform patient management in clinic. The HSPQoL is not suitable for use in the pediatric population as it incorporates the SF-36 which is intended for use in adults. [38] In addition, the development of the additional HSP-specific questions involved only adult participants and clinicians who treat adults with HSP. Testing validity and reliability of the HSPQoL in various countries, and languages will clarify its suitability for use internationally. Longitudinal use of the HSPQoL will test for sensitivity to change over time.

Conclusion

We have designed an HSP-specific QoL survey, the HSPQoL. Content validity was established through expert consensus and consumer engagement. Survey validity and reliability established through comprehensive psychometric testing. We intend for the HSPQoL to be used in routine clinical practice to promote discussion of patient wellbeing, and in clinical trials to measure the patient perspective of treatment outcomes.

Acknowledgements

We thank the patients and their carers, clinicians and HSP Research Foundation for their participation in this study. The authors acknowledge the Statistical Consulting Service provided by Dr Stanislaus Stadlmann from the Sydney Informatics Hub, a Core Research Facility of the University of Sydney.

Declarations

Funding

Sue Faye Siow is the recipient of a National Health and Medical Research Council Postgraduate Scholarship and Australian and New Zealand Association of Neurologists Education and Research Foundation Scholarship. No other funding was received for conducting this study.

Competing interests

The authors have no competing interests to declare that are relevant to the content of this article. The authors declare they have no financial interests.

Ethics approval

This study was approved by the Northern Sydney Local Health District Human Research Ethics Committee (Ethics approval number: 2019/ETH13187).

Consent

Informed consent was obtained from all individual participants included in the study.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Author Contributions

All authors contributed to the study conception and design. Data collection, analysis, and writing of the first draft of the manuscript were performed by Sue-Faye Siow. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Harding AE. Classification of the hereditary ataxias and paraplegias. *Lancet* 1983; 1: 1151-1155. 1983/05/21. DOI: 10.1016/s0140-6736(83)92879-9.
2. Rattay TW, Boldt A, Volker M, et al. Non-motor symptoms are relevant and possibly treatable in hereditary spastic paraplegia type 4 (SPG4). *J Neurol* 2020; 267: 369-379. 20191023. DOI: 10.1007/s00415-019-09573-w.
3. van Lith BJH, Kerstens H, van den Bemd LAC, et al. Experienced complaints, activity limitations and loss of motor capacities in patients with pure hereditary spastic paraplegia: a web-based survey in the Netherlands. *Orphanet J Rare Dis* 2020; 15: 64. 20200304. DOI: 10.1186/s13023-020-1338-4.
4. Kerstens H, Satink T, Nijkrake MJ, et al. Stumbling, struggling, and shame due to spasticity: a qualitative study of adult persons with hereditary spastic paraplegia. *Disabil Rehabil* 2020; 42: 3744-3751. 20190513. DOI: 10.1080/09638288.2019.1610084.
5. Servelhere KR, Faber I, Saute JA, et al. Non-motor symptoms in patients with hereditary spastic paraplegia caused by SPG4 mutations. *Eur J Neurol* 2016; 23: 408-411. 2016/01/26. DOI: 10.1111/ene.12839.
6. Fjermestad KW, Kanavin OJ, Naess EE, et al. Health survey of adults with hereditary spastic paraparesis compared to population study controls. *Orphanet J Rare Dis* 2016; 11: 98. 20160713. DOI: 10.1186/s13023-016-0469-0.
7. Andrews JA, Paganoni S, Braastad C, et al. Disease burden in upper motor neuron syndromes: a survey of patient perspectives. *J Clin Neuromuscul Dis* 2014; 16: 104-105. DOI: 10.1097/cnd.0000000000000051.
8. Grose J, Freeman J and Marsden J. Service delivery for people with hereditary spastic paraparesis living in the South West of England. *Disabil Rehabil* 2014; 36: 907-913. 20130814. DOI: 10.3109/09638288.2013.824032.
9. Amprosi M, Indelicato E, Eigentler A, et al. Toward the Definition of Patient-Reported Outcome Measurements in Hereditary Spastic Paraplegia. *Neurol Genet* 2023; 9: e200052. 20230110. DOI: 10.1212/NXG.0000000000200052.
10. Braschinsky M, Rannikmae K, Krikmann U, et al. Health-related quality of life in patients with hereditary spastic paraplegia in Estonia. *Spinal Cord* 2011; 49: 175-181. 20100525. DOI: 10.1038/sc.2010.61.
11. Bellofatto M, De Michele G, Iovino A, et al. Management of Hereditary Spastic Paraplegia: A Systematic Review of the Literature. *Front Neurol* 2019; 10: 3. 20190122. DOI: 10.3389/fneur.2019.00003.
12. Siow SF, Yeow D, Rudaks LI, et al. Outcome Measures and Biomarkers for Clinical Trials in Hereditary Spastic Paraplegia: A Scoping Review. *Genes (Basel)* 2023; 14 20230903. DOI: 10.3390/genes14091756.
13. Scheuer KH, Svenstrup K, Jennum P, et al. Double-blind crossover trial of gabapentin in SPG4-linked hereditary spastic paraplegia. *Eur J Neurol* 2007; 14: 663-666. 2007/06/02. DOI: 10.1111/j.1468-1331.2007.01812.x.
14. Bastani PB, Kordjazi M, Oveisgharan S, et al. A Randomized Controlled Trial of the Effect of Repetitive Transcranial Magnetic Stimulation of the Motor Cortex on Lower Extremity Spasticity in Hereditary Spastic Paraplegia. *J Clin Neurophysiol* 2023; 40: 173-179. 20211123. DOI: 10.1097/WNP.0000000000000874.
15. Klimpe S, Schule R, Kassubek J, et al. Disease severity affects quality of life of hereditary spastic paraplegia patients. *Eur J Neurol* 2012; 19: 168-171. 20110601. DOI: 10.1111/j.1468-1331.2011.03443.x.

16. Orsucci D, Petrucci L, Ienco EC, et al. Hereditary spastic paraparesis in adults. A clinical and genetic perspective from Tuscany. *Clin Neurol Neurosurg* 2014; 120: 14-19. 20140217. DOI: 10.1016/j.clineuro.2014.02.002.
17. Malina J, Huessler EM, Jöckel KH, et al. Development and validation of TreatHSP-QoL: a patient-reported outcome measure for health-related quality of life in hereditary spastic paraplegia. *Orphanet J Rare Dis* 2024; 19: 2. 20240102. DOI: 10.1186/s13023-023-03012-w.
18. Vickrey BG, Hays RD, Harooni R, et al. A health-related quality of life measure for multiple sclerosis. *Qual Life Res* 1995; 4: 187-206. DOI: 10.1007/bf02260859.
19. Heiskanen S, Merilainen P and Pietila AM. Health-related quality of life-testing the reliability of the MSQOL-54 instrument among MS patients. *Scand J Caring Sci* 2007; 21: 199-206. 2007/06/15. DOI: 10.1111/j.1471-6712.2007.00456.x.
20. Hays RD, Sherbourne CD and Mazel RM. The RAND 36-Item Health Survey 1.0. *Health Econ* 1993; 2: 217-227. DOI: 10.1002/hec.4730020305.
21. Linstone HA and Turoff M. *The Delphi method : techniques and applications*. Reading, Mass: Addison-Wesley Pub. Co., Advanced Book Program, 1975.
22. Harris PA, Taylor R, Minor BL, et al. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform* 2019; 95: 103208. 20190509. DOI: 10.1016/j.jbi.2019.103208.
23. Willis GB. *Cognitive interviewing : a tool for improving questionnaire design.*: Sage Publications, 2005.
24. Braun V and Clarke V. Using thematic analysis in psychology. *Qualitative research in psychology* 2006; 3: 77-101. DOI: 10.1191/1478088706qp063oa.
25. Herdman M, Gudex C, Lloyd A, et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual Life Res* 2011; 20: 1727-1736. 20110409. DOI: 10.1007/s11136-011-9903-x.
26. Corp I. IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp, Released 2023.
27. Taft C, Karlsson J and Sullivan M. Do SF-36 summary component scores accurately summarize subscale scores? *Qual Life Res* 2001; 10: 395-404. DOI: 10.1023/a:1012552211996.
28. Norman R, Mulhern B, Lancsar E, et al. The Use of a Discrete Choice Experiment Including Both Duration and Dead for the Development of an EQ-5D-5L Value Set for Australia. *Pharmacoeconomics* 2023; 41: 427-438. 20230131. DOI: 10.1007/s40273-023-01243-0.
29. Calderón JL, Morales LS, Liu H, et al. Variation in the readability of items within surveys. *Am J Med Qual* 2006; 21: 49-56. DOI: 10.1177/1062860605283572.
30. Paz SH, Liu H, Fongwa MN, et al. Readability estimates for commonly used health-related quality of life surveys. *Qual Life Res* 2009; 18: 889-900. 20090710. DOI: 10.1007/s11136-009-9506-y.
31. Gunatillake T, Shadbolt C, Gould D, et al. Embedding consumer and community involvement within an established research centre: moving from general recommendations to an actionable framework. *Res Involv Engagem* 2020; 6: 64. 20201027. DOI: 10.1186/s40900-020-00241-2.
32. National Health and Medical Research Council CHFoA. Statement on Consumer and Community involvement in Health and Medical Research. 2016.
33. SchÜLe R, Holland-Letz T, Klimpe S, et al. The Spastic Paraplegia Rating Scale (SPRS) : A reliable and valid measure of disease severity. *Neurology* 2006; 67: 430-434. DOI: 10.1212/01.wnl.0000228242.53336.90.
34. Campbell R, Ju A, King MT, et al. Perceived benefits and limitations of using patient-reported outcome measures in clinical practice with individual patients: a systematic review

of qualitative studies. *Qual Life Res* 2022; 31: 1597-1620. 20210927. DOI: 10.1007/s11136-021-03003-z.
35. Cubillos-Arcila DM, Machado GD, Sehnem L, et al. Progression of Functional Gait in Hereditary Spastic Paraplegias. *Cerebellum* 2022; 21(3): 350-357. DOI: https://dx.doi.org/10.1007/s12311-021-01302-2.
36. Rabin R and de Charro F. EQ-5D: a measure of health status from the EuroQol Group. *Ann Med* 2001; 33: 337-343. DOI: 10.3109/07853890109002087.
37. Kerstens HCJW, Lith BJHv, Nijkrake MJ, et al. Healthcare needs, expectations, utilization, and experienced treatment effects in patients with hereditary spastic paraplegia: a web-based survey in the Netherlands. *Orphanet journal of rare diseases* 2021; 16: 1-283. DOI: 10.1186/s13023-021-01915-0.
38. Villalonga-Olives E, Kawachi I, Almansa J, et al. Pediatric health-related quality of life: a structural equation modeling approach. *PLoS One* 2014; 9: e113166. 20141121. DOI: 10.1371/journal.pone.0113166.

4.3 Findings from HSPQoL

In this section, I present the participant responses from the final validation step of HSPQoL (n=61) as described in section 4.2. Participant demographics were presented in section 4.2.

4.3.1 HSPQoL scores compared to population norms

The range, mean, 95% confidence interval and standard deviation of SF-36 subscores are presented in Table 1. Mean scores for all SF-36 subscores were significantly lower in our HSP cohort compared to published Australian population norms (one-sample t-test two-sided $p < 0.05$).

Table 1. Distribution of participant response to HSPQoL (n=61)

	SF-36			Australian population norms (ABS 1995) [29]
SF-36 subscores	**range**	**Mean (SD)**	**95% CI**	**mean(SD)**
Physical functioning	0-95	32.70 (29.56)	25.49-40.74	82.6 (23.9)
Role limitations due to physical health	0-100	35.66 (41.95)	25.01-46.71	79.9 (35.1)
Role limitations due to emotional problems	0-100	58.47 (44.58)	47.54-70.49	82.9 (32.3)
Energy/fatigue (vitality)	5-95	46.89 (19.90)	41.97-51.72	64.5 (19.8)
Emotional well-being (mental health)	16-96	67.74 (18.24)	63.28-72.26	75.9 (17.0)
Social functioning	12.5-100	57.99 (23.83)	52.05-64.34	85.0 (22.5)
Pain	22.5-100	68.61 (23.58)	62.59-74.38	76.8 (25.0)

General Health	5-90	49.43 (20.13)	44.67-54.51	71.6 (20.3)
Additional HSP items	16.25-90.60	54.43 (19.31)	49.54-59.14	-

4.3.2 Participant responses to additional HSP-related HSPQoL items

Most participants reported experiencing HSP-related symptoms including lower limb weakness, stiffness, and balance difficulties (Figure 1) with 73% reporting an impact of their lower limb and balance symptoms on their QoL (Figure 2). 64% of respondents reported bowel or bladder symptoms affecting their QoL (Figure 2) and 62% of respondents made lifestyle modifications to manage their bladder or bowel symptoms (Figure 3).

Figure 1. Participant responses to additional items 37-41

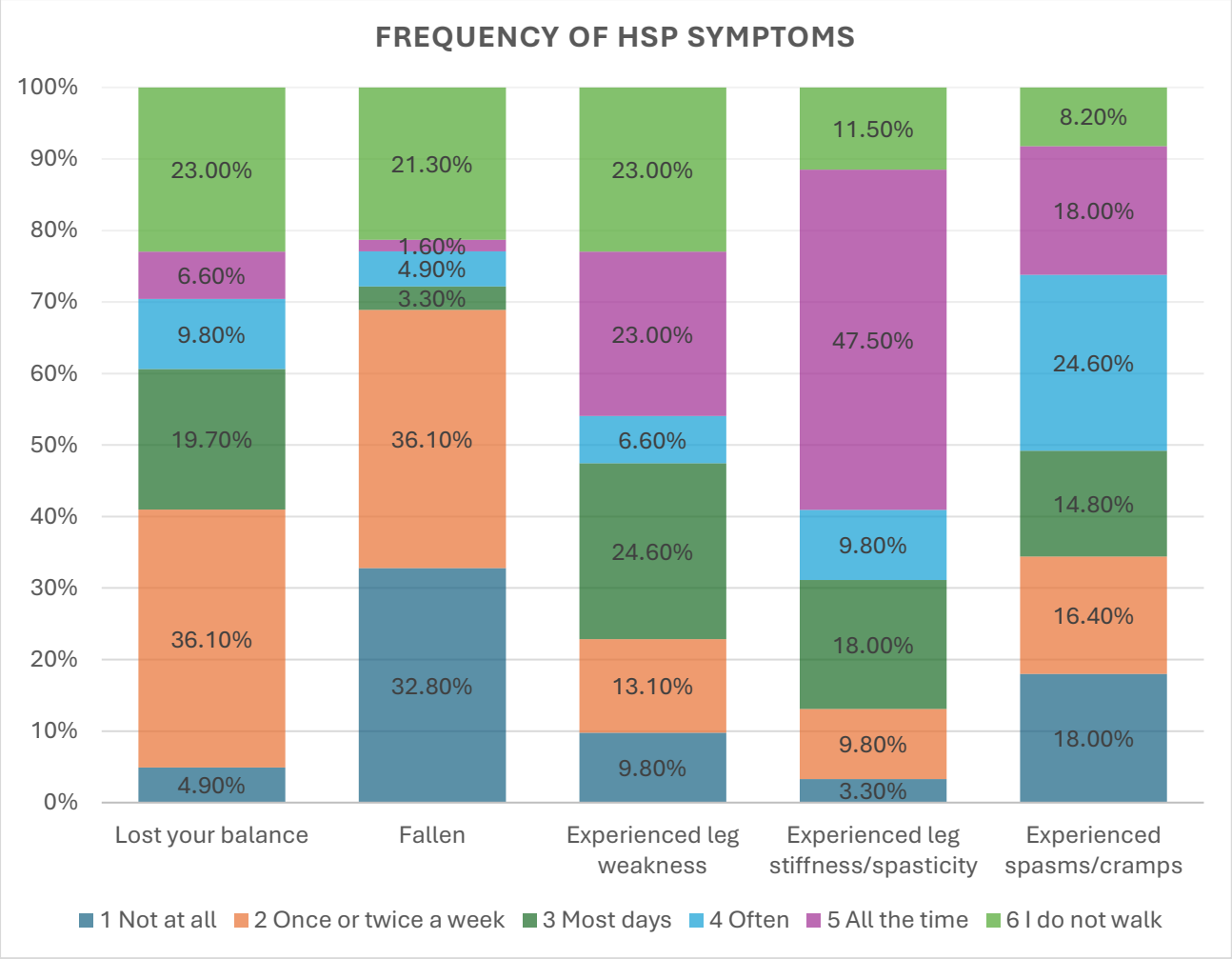


Figure 2. Participant responses to items 42-43

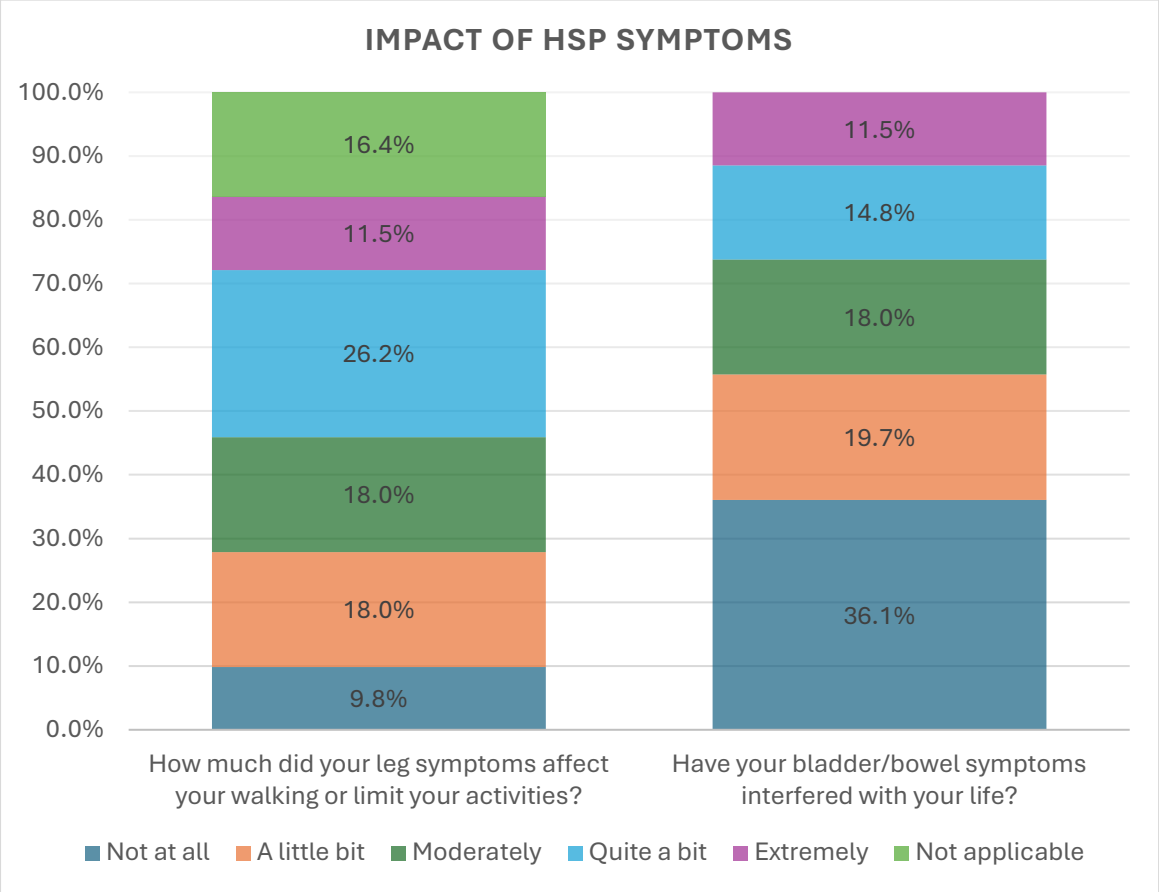
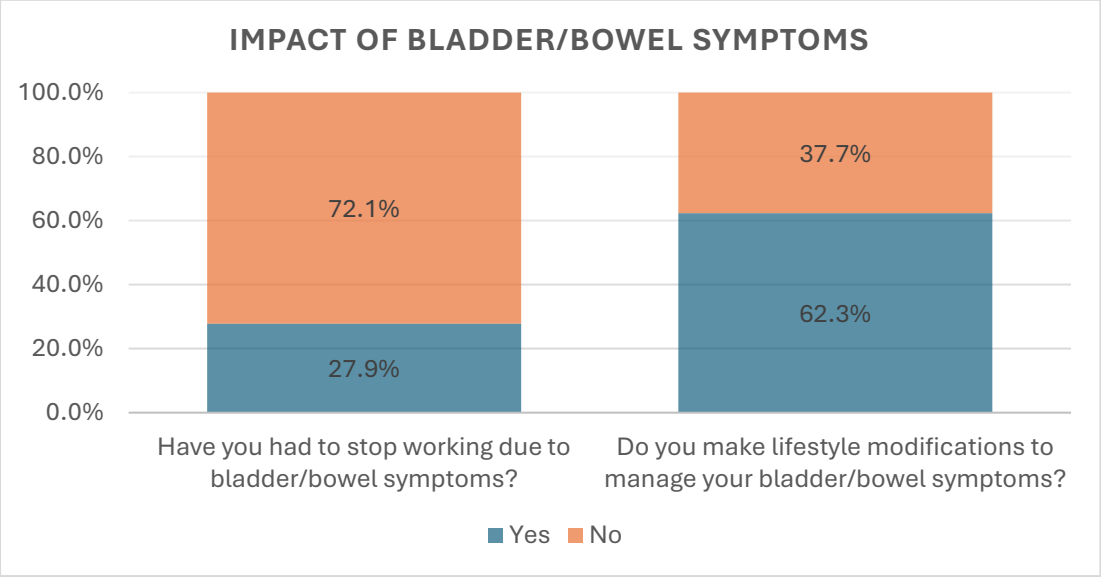
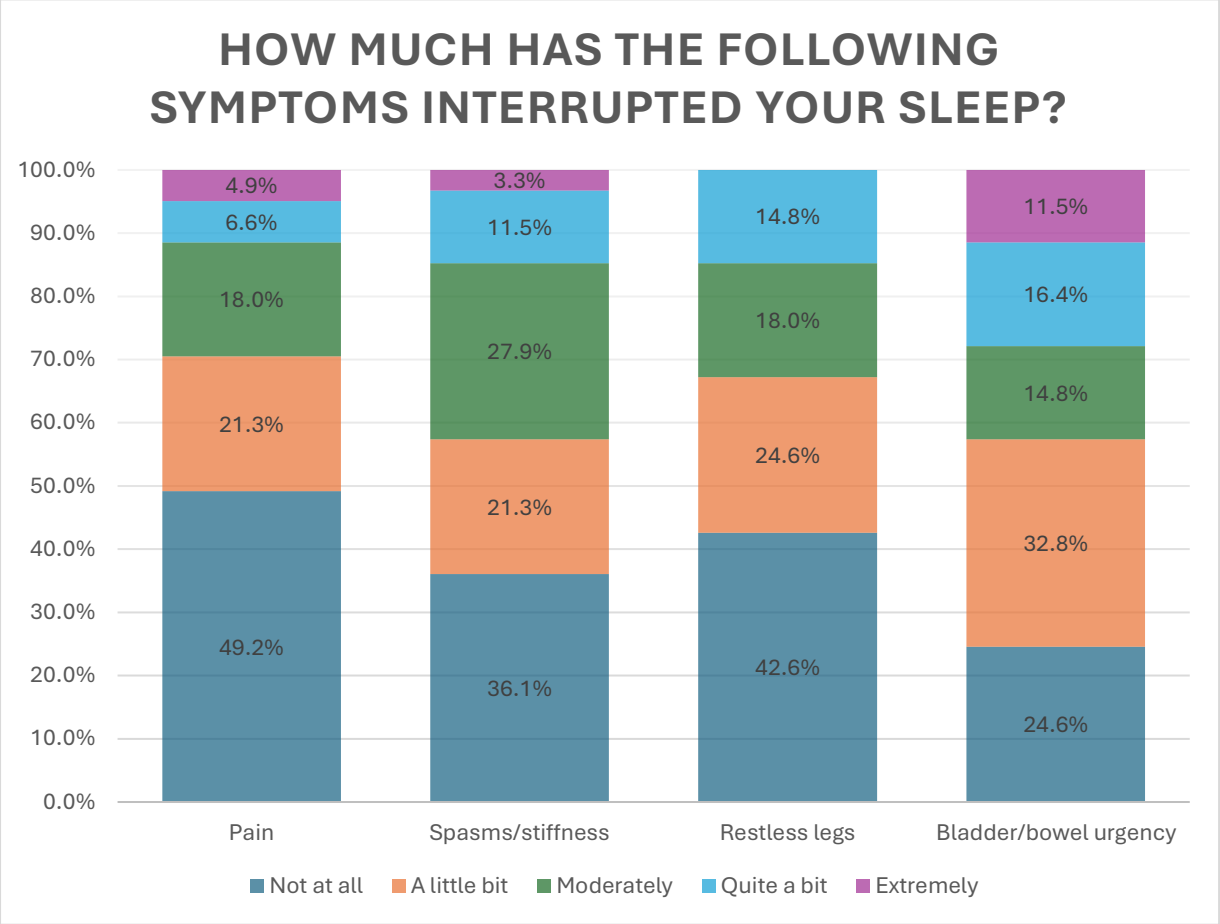


Figure 3. Participant responses to items 44-45



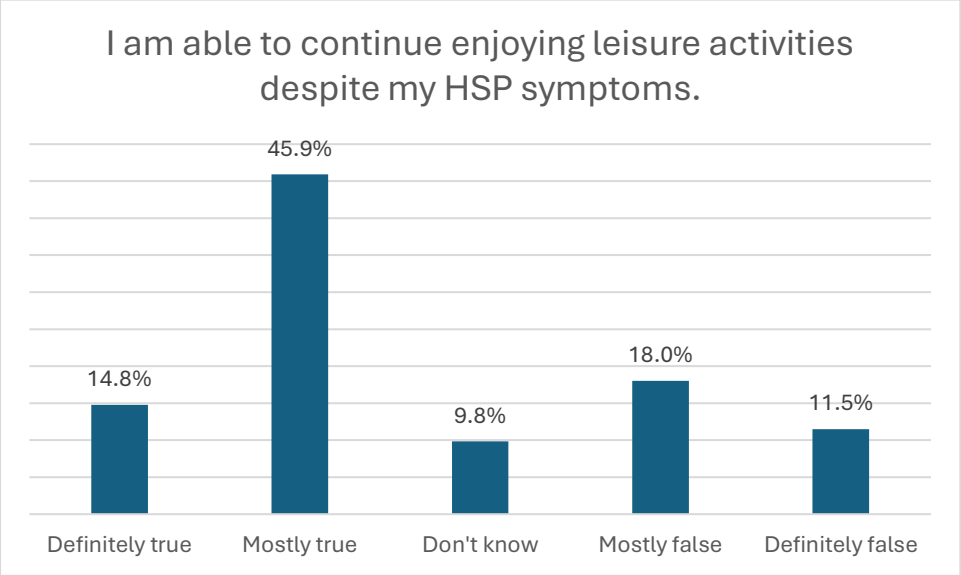
28% of respondents had to stop work due to their bladder or bowel symptoms. Sleep disruption from HSP-related symptoms including pain, leg spasms or stiffness, restless legs, and bladder or bowel symptoms were common with at least 50% respondents reporting this (Figure 4).

Figure 4. Participant responses to items 46-49



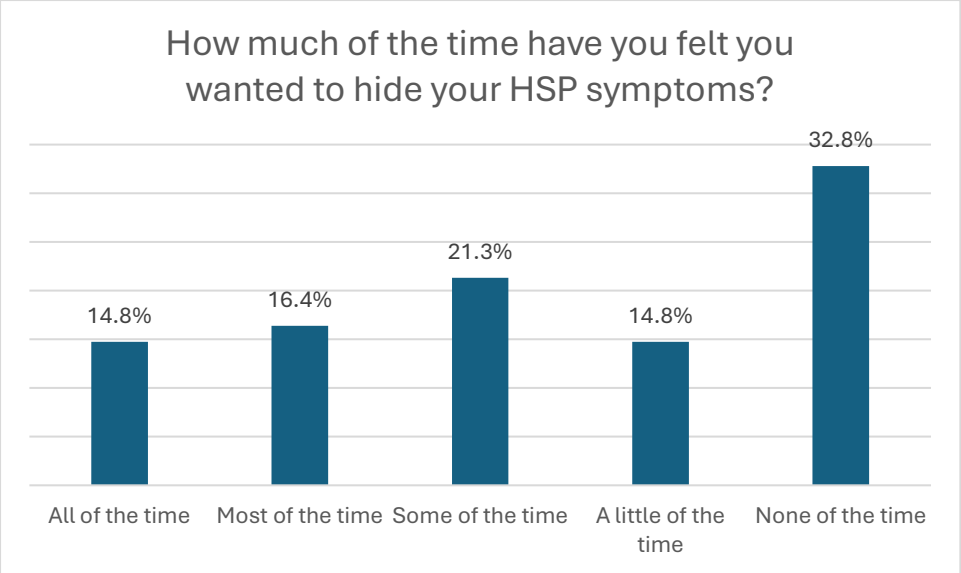
Despite most respondents reporting HSP-related symptoms disrupting their QoL, the majority (60.7%) of respondents were able to continue enjoying leisure activities despite their symptoms (Figure 5).

Figure 5. Participant responses to item 50



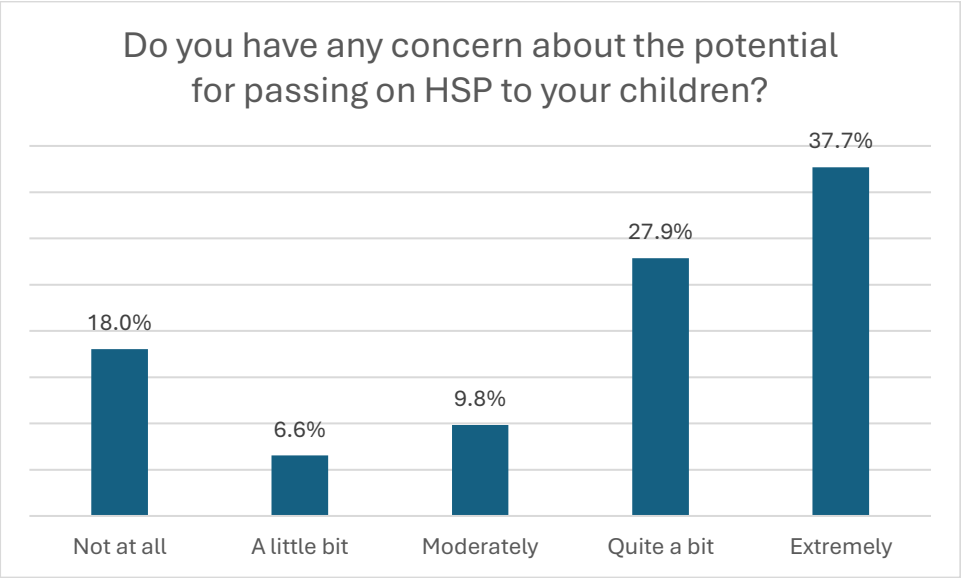
Feelings of shame and being judged were reflected by 67% of respondents who felt they wanted to hide their symptoms (Figure 6).

Figure 6. Participant responses to item 51



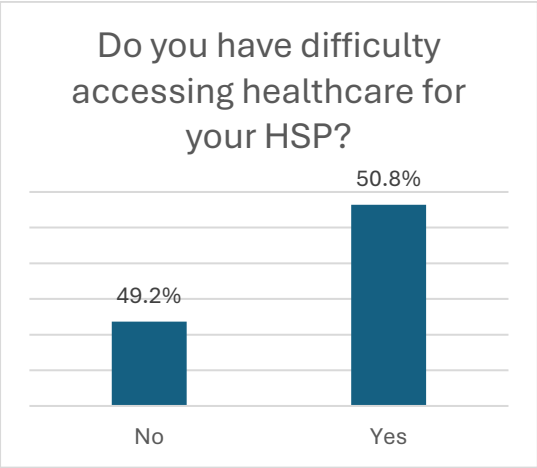
82% of respondents expressed concern about the chance of passing on HSP to their children (Figure 7).

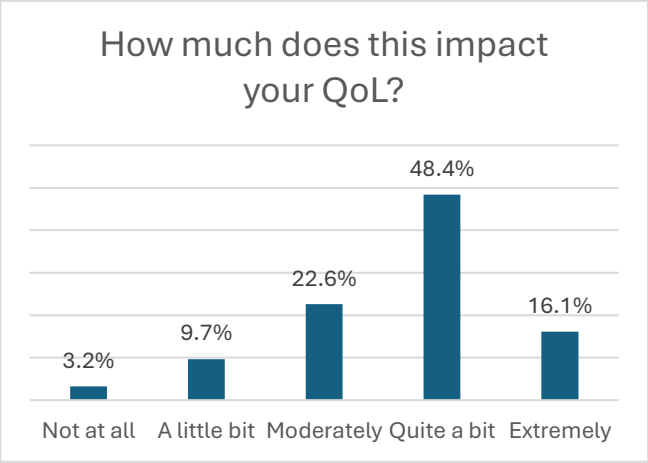
Figure 7. Participant responses to item 52



Of the 51% of respondents who had difficulty accessing specialised health care for their HSP, 97% felt that poor access to healthcare impacted their QoL (Figure 8).

Figure 8. Participant responses to item 53 and 54

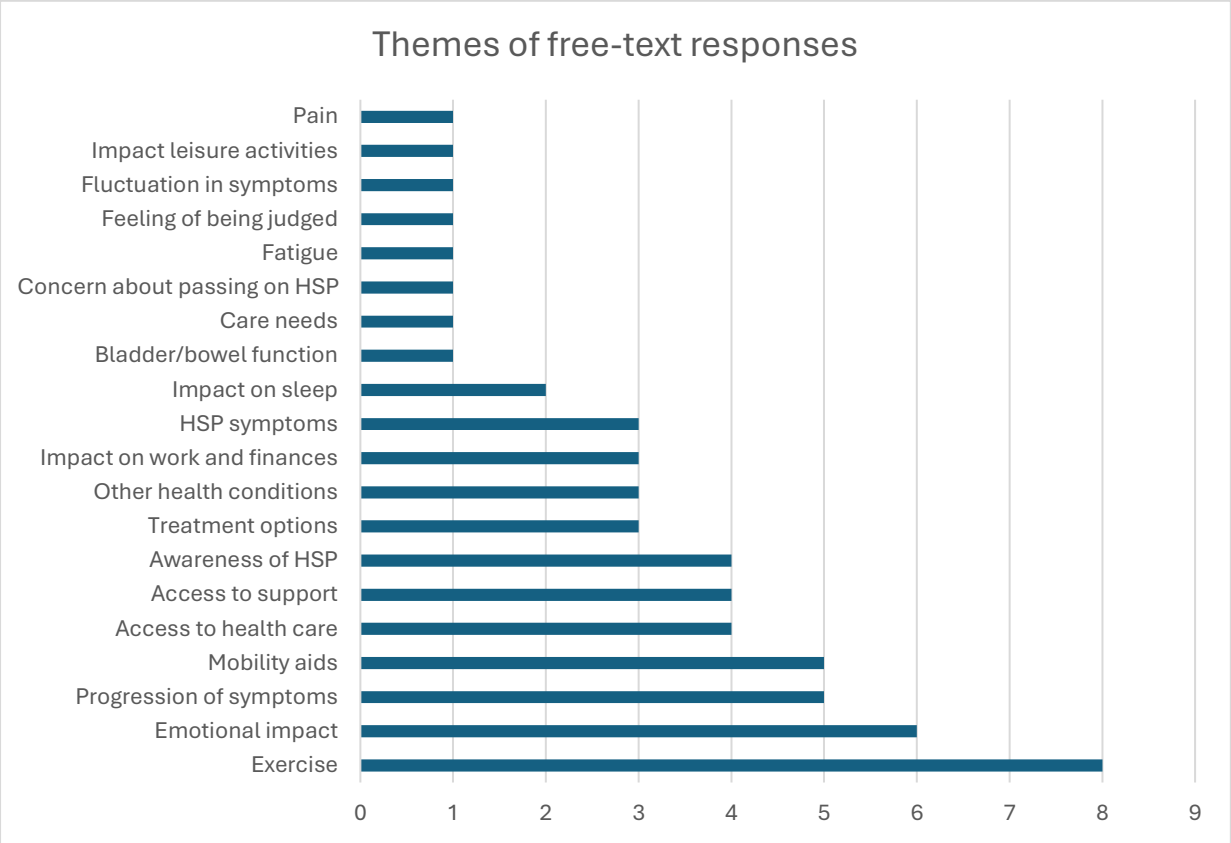




4.3.3 HSPQoL free-text responses

39/61 (64%) of respondents filled in the free text box at the end of the survey (Figure 9). Many participants mentioned the role exercise plays in prevention of their symptom progression. The emotional impact of HSP was reported by six participants including feelings of depression, helplessness, and frustration. Symptom progression was described by five participants and five participants described using mobility aids. Poor access to healthcare and community supports was mentioned by several participants and lack of treatment options described by three participants. Respondents described the impact of HSP on their employment and finances. Overall, the free text comments aligned with the findings from the HSPQoL item generation process including the results of the literature review, modified Delphi process, and cognitive interviews. The free text box provided respondents with the option to elaborate on their personal experiences and identify specific care areas that could be addressed in a clinical setting. Some respondents expressed that the survey was unable to capture the complexity of their symptoms and that in-depth interviews will allow them to communicate their experience better.

Figure 9. Themes of patient responses in free-text box. X-axis - Number of patients who provided responses. Y-axis – Theme.



4.4 Discussion and Conclusion

In this chapter, I discussed the well-recognised impact of HSP on patients' QoL and the importance of the patient perspective for both clinical care and clinical trial outcomes. I developed the HSPQoL to better represent the patient experience.

HSP-specific symptoms such as lower limb weakness, spasticity, spasms, bladder or bowel symptoms, and balance difficulties were a prominent finding at all stages of the HSPQoL design process, from literature review, to modified Delphi, cognitive interview, and survey responses. 5/18 of the additional HSP-specific items in the HSPQoL addressed these issues. I hope that inclusion of these items will increase the sensitivity of this HSP-specific PROM to changes in patient symptoms in response to treatment in future clinical trials.

Patient engagement was paramount at every step of the study design and validation process. I endeavored to identify issues that were important to patients to accurately represent the patient experience. The HSPQoL might be a useful tool in a patient-centred approach to clinical care, providing a template for discussion of relevant and potentially sensitive issues that may not have been addressed otherwise. In a clinical trial setting, the HSPQoL allows the patient perspective to be represented in measurement of treatment outcomes and determination of treatment success.

However, the HSPQoL does not replace direct patient-clinician communication. Several respondents expressed the limitations of a survey in capturing the complexities of their symptoms and experiences. Some respondents preferred an in-depth interview to discuss their experience. Some respondents found answering sensitive topics, such as concerns about passing on HSP to their children and bladder or bowel symptoms, challenging to answer in a survey setting. This could have introduced response bias. Therefore, careful wording is required whilst some issues may require consultation with their clinician or experienced professionals, such as genetic counsellors, to adequately meet patient needs.

HSP can co-exist with other co-morbidities and it may be difficult to differentiate the impact of separate conditions on patients' QoL. HSP-specific items such as items on spasticity, balance, and concerns regarding the hereditability of HSP, can provide some distinction between HSP-specific impacts on QoL. However, it will be important to note the presence of other co-morbidities and account for these when analysing HSPQoL results.

The HSPQoL is intended to be used as a PROM in international multi-site clinical trials. Therefore, validation in various patient cohorts including other countries and languages is required. HSPQoL responses from a larger patient cohort are required for confirmatory factor analysis to determine factor loadings which will be used for calculation of summary scores. The sensitivity of the HSPQoL to changes over time will need to be determined with longitudinal studies. In addition, it would be useful to assess the correlation of HSPQoL scores with clinical disease severity as measured with established clinician-rated outcome measures such as the Spastic Paraplegia Rating Scale (SPRS).

The patient responses to the additional HSP items in the HSPQoL, as discussed in section 4.3, are consistent with my findings from the literature as described in section 4.2. Most patients reported moderate to extreme impact of HSP symptoms on their QoL (Figure 2). This finding emphasizes the impact of balance difficulties, weakness, and spasticity in patients with HSP and the need for therapeutic agents that target these symptoms. Bladder, bowel, and sleep symptoms were reported to have less of an impact than mobility and balance issues, though most patients still reported being at least mildly affected (Figure 4). Most patients felt they wanted to hide their symptoms (Figure 6) and were worried about the implications of their HSP for their children (Figure 7). The HSPQoL allows patients the opportunity to discuss these issues which may not usually be discussed in a routine checkup appointment with their healthcare providers. Limited access to healthcare for their HSP was a common finding and is an area of need in HSP clinical care. The patient responses in the free-text box provided an overview of issues that were most concerning to the study participants and forms a guide for future interventions to improve QoL for patients with HSP.

In conclusion, I established the validity and reliability of the HSPQoL as a PROM for use in clinical care and clinical trials. I hope that the HSPQoL will be used to represent the patient experience and empower patient involvement in their care and clinical research.

4.5 References

1. Amprosi M, Indelicato E, Eigentler A, et al. Toward the Definition of Patient-Reported Outcome Measurements in Hereditary Spastic Paraplegia. *Neurology Genetics* 2023; 9: e200052-e200052. DOI: 10.1212/NXG.0000000000200052.

2. Rattay TW, Boldt A, Völker M, et al. Non-motor symptoms are relevant and possibly treatable in hereditary spastic paraplegia type 4 (SPG4). *Journal of neurology* 2019; 267: 369-379. DOI: 10.1007/s00415-019-09573-w.

3. Braschinsky M, Rannikmae K, Krikmann U, et al. Health-related quality of life in patients with hereditary spastic paraplegia in Estonia. *Spinal Cord* 2011; 49(2): 175-181. DOI: https://dx.doi.org/10.1038/sc.2010.61.

4. Kerstens H, Satink T, Nijkrake MJ, et al. Stumbling, struggling, and shame due to spasticity: a qualitative study of adult persons with hereditary spastic paraplegia. *Disabil Rehabil* 2020; 42: 3744-3751. 20190513. DOI: 10.1080/09638288.2019.1610084.

5. Grose J, Freeman J and Marsden J. Service delivery for people with hereditary spastic paraparesis living in the South West of England. *Disabil Rehabil* 2014; 36: 907-913. 20130814. DOI: 10.3109/09638288.2013.824032.

6. Fjermestad KW, Kanavin Ø J, Næss EE, et al. Health survey of adults with hereditary spastic paraparesis compared to population study controls. *Orphanet J Rare Dis* 2016; 11: 98. 20160713. DOI: 10.1186/s13023-016-0469-0.

7. van Lith BJH, Kerstens H, van den Bemd LAC, et al. Experienced complaints, activity limitations and loss of motor capacities in patients with pure hereditary spastic paraplegia: a web-based survey in the Netherlands. *Orphanet J Rare Dis* 2020; 15: 64. 20200304. DOI: 10.1186/s13023-020-1338-4.

8. Schneider SA, Beckinger VE, Moller B, et al. Urinary symptoms, quality of life, and patient satisfaction in genetic and sporadic hereditary spastic paraplegia. *Journal of Neurology* 2019; 266(1): 207-211. DOI: https://dx.doi.org/10.1007/s00415-018-9129-8.

9. Servelhere KR, Faber I, Coan AC, et al. Translation and validation into Brazilian Portuguese of the Spastic Paraplegia Rating Scale (SPRS). *Arquivos de neuro-psiquiatria* 2016; 74: 489-494. DOI: 10.1590/0004-282X20160047.

10. Andrews JA, Paganoni S, Braastad C, et al. Disease burden in upper motor neuron syndromes: a survey of patient perspectives. *J Clin Neuromuscul Dis* 2014; 16: 104-105. DOI: 10.1097/cnd.0000000000000051.

11. Siow S-F, Yeow D, Rudaks LI, et al. Outcome Measures and Biomarkers for Clinical Trials in Hereditary Spastic Paraplegia: A Scoping Review. *Genes* 2023; 14: 1756. DOI: 10.3390/genes14091756.

12. Hays RD, Sherbourne CD and Mazel RM. The rand 36-item health survey 1.0. *Health economics* 1993; 2: 217-227. DOI: 10.1002/hec.4730020305.

13. Oladeji O, Johnston TE, Smith BT, et al. Quality of life in children with spinal cord injury. *Pediatr Phys Ther* 2007; 19: 296-300. 2007/11/16. DOI: 10.1097/PEP.0b013e31815a12ef.
14. Vickrey BG, Hays RD, Harooni R, et al. A health-related quality of life measure for multiple sclerosis. *Qual Life Res* 1995; 4: 187-206. DOI: 10.1007/bf02260859.
15. Hwang M, Zebracki K, Vogel LC, et al. Development of the Pediatric Quality of Life Inventory Spinal Cord Injury (PedsQL SCI) module: qualitative methods. *Spinal Cord* 2020; 58: 1134-1142. 20200408. DOI: 10.1038/s41393-020-0450-6.
16. Heuzenroeder L, Ibrahim F, Khadka J, et al. A Delphi study to identify content for a new questionnaire based on the 10 Principles of Dignity in Care. *J Clin Nurs* 2022; 31: 1960-1971. 20200907. DOI: 10.1111/jocn.15462.
17. Panepinto JA, Torres S and Varni JW. Development of the PedsQL Sickle Cell Disease Module items: qualitative methods. *Qual Life Res* 2012; 21: 341-357. 20110603. DOI: 10.1007/s11136-011-9941-4.
18. Varni JW, Kay MT, Limbers CA, et al. PedsQL gastrointestinal symptoms module item development: qualitative methods. *J Pediatr Gastroenterol Nutr* 2012; 54: 664-671. 2011/10/20. DOI: 10.1097/MPG.0b013e31823c9b88.
19. Nutakki K, Varni JW, Steinbrenner S, et al. Development of the pediatric quality of life inventory neurofibromatosis type 1 module items for children, adolescents and young adults: qualitative methods. *J Neurooncol* 2017; 132: 135-143. 20170111. DOI: 10.1007/s11060-016-2351-2.
20. Peto V, Jenkinson C and Fitzpatrick R. PDQ-39: a review of the development, validation and application of a Parkinson's disease quality of life questionnaire and its associated measures. *J Neurol* 1998; 245 Suppl 1: S10-14. DOI: 10.1007/pl00007730.
21. Willis GB. *Cognitive interviewing : a tool for improving questionnaire design.*: Sage Publications, 2005.
22. Stewart D, Gibson-Smith K, MacLure K, et al. A modified Delphi study to determine the level of consensus across the European Union on the structures, processes and desired outcomes of the management of polypharmacy in older people. *PLoS One* 2017; 12: e0188348. 20171120. DOI: 10.1371/journal.pone.0188348.
23. Diamond IR, Grant RC, Feldman BM, et al. Defining consensus: a systematic review recommends methodologic criteria for reporting of Delphi studies. *J Clin Epidemiol* 2014; 67: 401-409. 2014/03/04. DOI: 10.1016/j.jclinepi.2013.12.002.

24. MacDermid JC. ICF Linking and Cognitive Interviewing Are Complementary Methods for Optimizing Content Validity of Outcome Measures: An Integrated Methods Review. *Front Rehabil Sci* 2021; 2: 702596. 20211014. DOI: 10.3389/fresc.2021.702596.
25. Irwin DE, Varni JW, Yeatts K, et al. Cognitive interviewing methodology in the development of a pediatric item bank: a patient reported outcomes measurement information system (PROMIS) study. *Health Qual Life Outcomes* 2009; 7: 3. 20090123. DOI: 10.1186/1477-7525-7-3.
26. Gunatillake T, Shadbolt C, Gould D, et al. Embedding consumer and community involvement within an established research centre: moving from general recommendations to an actionable framework. *Res Involv Engagem* 2020; 6: 64. 20201027. DOI: 10.1186/s40900-020-00241-2.
27. National Health and Medical Research Council. Statement on Consumer and Community involvement in Health and Medical Research. 2016.
28. Wiering B, de Boer D and Delnoij D. Patient involvement in the development of patient-reported outcome measures: a scoping review. *Health Expect* 2017; 20: 11-23. 20160218. DOI: 10.1111/hex.12442.
29. Australian Bureau of Statistics. National Health Survey: SF-36 Population Norms. Australia: Australian Bureau of Statistics, 1995.

Chapter 5. Discussion and Conclusion

HSP is a rare disease with no known disease-modifying treatment despite the impact of this condition on patients' health and quality of life. There is an urgent need for standardised outcome measures to provide evidence of efficacy for novel HSP treatment options. However, challenges in rare disease research are well recognised including patient recruitment, poor understanding of natural history, clinical heterogeneity, and lack of sensitive outcome measures. [1] The overarching aim of my thesis is to identify outcome measures and biomarkers for HSP clinical trials to provide the evidence base for novel therapeutic agents targeted to this incurable disorder.

5.1 Knowledge Gain and Insights

In Chapter 1, I performed a scoping review demonstrating the heterogeneity and inconsistency of outcome measures used in HSP leading to lack of guidelines for HSP clinical trial design. [2] The scoping review provided an overview of the available evidence for pre-existing outcome measures and recommendations for future research. [2] This included (1) Development of a core outcome set to account for clinical complexity and variability by measuring multiple domains whilst providing consistency between different studies; (2) Identifying surrogate outcome measures, such as biomarkers targeted to underlying disease pathophysiology, that will be more sensitive to change compared to clinical measures that may not be sensitive in slowly progressive forms of HSP; (3) Development of an HSP-specific PROM to ensure the relevance of trial outcomes to the intended consumers and produce clinically-meaningful outcomes. Chapter 2 of my thesis provided further clinical evidence to inform the development of an HSP core outcome set. Chapter 3 explored a potential surrogate outcome measure for HSP-*SPAST* whilst Chapter 4 detailed the development of an HSP-specific PROM.

Based on the findings from the scoping review, a core outcome set should include a clinician rated outcome measure (CROM) such as the SPRS, PROM such as SF-36 or HSPQoL, and a biomarker (biochemical, imaging or neurophysiological). [2] The choice of biomarker may be dependent on the intended treatment effect, for example gait analysis would be best suited for

interventions to improve mobility; whilst neurofilament light chain levels would be suited for interventions that target axonal degeneration. The inclusion of standard CROM and PROM in every HSP-clinical trial ensures the clinical relevance of trial outcomes, i.e. whether a clinically meaningful biomarker outcome is reflected in the clinical outcomes; as well as allowing direct comparison of trial outcomes between different studies.

In Chapter 2, I evaluated the suitability of pre-existing outcome measures for HSP, identified in Chapter 1, for use in HSP clinical trials. A systematic review of motor evoked potentials (MEP) in HSP found that MEPs could be a useful diagnostic biomarker for HSP but there was insufficient evidence to demonstrate the ability of MEPs to measure disease severity. [3] This finding provided further clarity on the role of MEPs as previous studies report conflicting results on the ability of MEP to measure disease severity. [4, 5]

I evaluated a set of outcome measures in a cohort of patients with HSP. The results of this cohort study supported the use of SARA as a supplement to the SPRS in patients with HSP and prominent ataxia symptoms, such as HSP-*SPG7*. The use of a symptom-specific CROM is particularly important in clinical trials of interventions intended to improve ataxia and spasticity symptoms. I identified a need for an HSP-specific PROM. In keeping with the findings of our systematic review, I found that MEP measures did not correlate with disease severity as measured by the SPRS. This cross-sectional cohort study was a demonstration of the use of a potential core outcome set in a cohort of patients of HSP representative of potential future trial participants. I clearly identified the need for an objective biomarker that was able to measure treatment response and correlate with clinically relevant outcomes.

In Chapter 3, I investigated a potential biomarker for HSP-*SPAST* based on findings from *in vitro* studies that used HSP-*SPAST* patient-derived neuronal cell models to demonstrate microtubule instability and corresponding reduced acetylated α-tubulin levels. [6] I developed a flow cytometry assay to measure intracellular levels of acetylated α-tubulin in human PBMCs and demonstrated reduced levels of acetylated α-tubulin in HSP-*SPAST* patient cells compared to controls. [7] To my knowledge, this is the first study to use flow cytometry to measure acetylated α-tubulin levels in HSP-*SPAST* patient PMBCs. The methodology developed for this study can be applied to various nucleated cell types. [8] The findings of this study support further investigation of PBMC acetylated α-tubulin as a potential marker of response to tubulin-binding drugs, such as noscapine, which have demonstrated therapeutic effects in *in vitro* studies. [9]

In Chapter 4, I developed and validated an HSP-specific quality of life survey (HSPQoL) to capture the elements of the HSP patient experience that were missed by generic QoL measures. I identified key HSP issues that were important to patients and impacted their quality of life. These findings provide guidance for health professionals managing patients with HSP in identifying common HSP-associated problems that should be addressed in their health care.

To develop the HSPQoL, I supplemented the SF-36 with additional HSP-specific items derived through a literature review and consultation with key stakeholders including patients with HSP, their carers, and HSP-clinicians. I used established biostatistical analysis tools to demonstrate that the additional items enriched the pre-existing SF-36 tool whilst maintaining the validity of the PROM. Enrichment of the SF-36 with additional HSP-items is intended to improve the sensitivity of the PROM to QoL changes in response to interventions and better represent the HSP patient experience.

At the time of study conception, HSPQoL was the only HSP-specific PROM in development. However, the Treat-HSPQoL was published shortly before completion of our study. [10] This allowed comparison of our findings. Although several of the additional HSP-specific items in the HSPQoL were similar to the Treat-HSPQoL items, there were some differences. The Treat-HSPQoL included items on finances, employment and complex HSP symptoms including upper limb involvement, speech and memory, whilst the HSPQoL included an item on the genetics of HSP and items that were relevant to respondents regardless of employment status. Choice of HSPQoL or Treat-HSPQoL as preferred PROM in clinical trials would be informed by the target participants' age range, complexity of symptoms, and employment status.

5.2 Future Directions

A core outcome set for HSP is needed. The contents of Chapter 1 and 2 contributes to the knowledge base needed to establish the HSP core outcome set. Further research is needed to evaluate promising and relevant outcome measures identified through our scoping review for suitability for inclusion in the core outcome set. This is typically performed with a systematic review, as I have done for MEPs in HSP. The findings from the systematic review for each potential outcome measure are then presented to an expert panel, such as through a Delphi process to be ranked according to importance. Expert panel consensus will determine if an

outcome measure should be included or excluded. [11] The final core outcome set would be a key tool for improving the quality of evidence for HSP treatments.

In chapter 3, I presented acetylated α-tubulin as a potential biomarker for HSP-*SPAST*. Further studies are needed to establish the measurement properties of acetylated α-tubulin including accuracy (standard error of measurement, minimal detectable change), validity (area under the receiver operating curve, ability to differentiate patients vs controls) and reliability (intraclass coefficient for test-retest reliability). Longitudinal studies will assess change in acetylated α-tubulin levels over time. A larger sample size is needed to establish statistically significant findings and inclusion of patients with different neurodegenerative conditions will provide further information on specificity. The next stage of validating acetylated α-tubulin as a potential biomarker will require collaborative efforts for patient recruitment to ensure inclusion of patients across the age range, spectrum of disease severity, and diverse ethnicities. International collaborative efforts such as the European Reference Network for Rare Neurological Diseases (https://www.ern-rnd.eu/disease-knowledge-hub/ataxia/) and establishing rare disease registries such as NeuroConnect (https://kr.schn.health.nsw.gov.au/our-research/research-initiatives/neuroconnect) are key to improving equitable access and relevance of research findings to all patients with HSP.

In chapter 4, I developed and validated the HSPQoL, an HSP-specific PROM, to sensitively capture patient-centered outcomes in a clinical trial. Further validation of the HSPQoL with a larger sample size is needed to calculate factor loadings which are required to establish summary scores, such as the Mental Component Score and Physical Component Score for the SF-36. [12] In addition, validation in international cohorts and languages other than English will be required to establish the validity of the HSPQoL for use in multi-center international clinical trials. Longitudinal studies are required to evaluate the sensitivity of the HSPQoL to changes over time.

A common theme that has been recurrent through my thesis is the challenge of patient recruitment in HSP to ensure adequate representation of all patient groups and adequately powered studies. This remains a challenge in rare disease research and requires innovative approaches to overcome. Firstly, collaboration is key to inclusivity and generalisability of research findings. Innovative study designs such as adaptive platform trials, and extended trial durations, allow smaller sample sizes for clinical trials. Finally, dedicated core outcome

sets and specific biomarkers sensitive to treatment response will allow significant findings despite small sample sizes.

5.3 Conclusion

Challenges remain in identifying suitable outcome measures for HSP, a rare, clinically variable, and genotypically diverse condition. This thesis gathers evidence on pre-existing outcome measures and presents novel outcome measures for use in HSP. I collated and evaluated an extensive and diverse range of pre-existing outcome measures used in HSP and performed a systematic review of MEPs as an outcome measure for HSP. I evaluated a curated set of outcome measures in a cohort of patients with HSP to provide guidance for use of outcome measures in future clinical trials. I developed a novel assay for a potential blood-based biomarker for HSP-*SPAST*, that could be used in international, multi-center clinical trials. I recognised the importance of the patient voice in HSP research and developed and validated an HSP-specific PROM with extensive patient input. This thesis provides a solid foundation for outcome measure development in HSP, an essential step in the search for a cure. HSP remains a debilitating and devastating diagnosis for many but the chance of a cure is a beacon of hope for those who suffer the effects of HSP in their everyday lives.

5.4 References

1. Rath A, Salamon V, Peixoto S, et al. A systematic literature review of evidence-based clinical practice for rare diseases: what are the perceived and real barriers for improving the evidence and how can they be overcome? *Trials* 2017; 18: 556. DOI: 10.1186/s13063-017-2287-7.
2. Siow S-F, Yeow D, Rudaks LI, et al. Outcome Measures and Biomarkers for Clinical Trials in Hereditary Spastic Paraplegia: A Scoping Review. *Genes* 2023; 14: 1756. DOI: 10.3390/genes14091756.
3. Siow S-F, Smail RC, Ng K, et al. Motor Evoked Potentials in Hereditary Spastic Paraplegia—A Systematic Review. *Frontiers in neurology* 2019; 10: 967-967. DOI: 10.3389/fneur.2019.00967.

4. Karle KN, Schule R, Klebe S, et al. Electrophysiological characterisation of motor and sensory tracts in patients with hereditary spastic paraplegia (HSP). *Orphanet Journal of Rare Diseases* 2013; 8(1) (no pagination). DOI: https://dx.doi.org/10.1186/1750-1172-8-158.
5. Brighente SF, Vicuna P, Louzada ALR, et al. Evoked potentials as biomarkers of hereditary spastic paraplegias: A case-control study. *PLoS ONE* 2021; 16(11) (no pagination). DOI: https://dx.doi.org/10.1371/journal.pone.0259397.
6. Wali G, Sutharsan R, Fan Y, et al. Mechanism of impaired microtubule-dependent peroxisome trafficking and oxidative stress in SPAST-mutated cells from patients with Hereditary Spastic Paraplegia. *Scientific reports* 2016; 6: 27004-27004. DOI: 10.1038/srep27004.
7. Wali G, Siow SF, Liyanage E, et al. Reduced acetylated α-tubulin in SPAST hereditary spastic paraplegia patient PBMCs. *Front Neurosci* 2023; 17: 1073516. 20230328. DOI: 10.3389/fnins.2023.1073516.
8. Bonser LR, Koh KD, Johansson K, et al. Flow-Cytometric Analysis and Purification of Airway Epithelial-Cell Subsets. *Am J Respir Cell Mol Biol* 2021; 64: 308-317. DOI: 10.1165/rcmb.2020-0149MA.
9. Fan Y, Wali G, Sutharsan R, et al. Low dose tubulin-binding drugs rescue peroxisome trafficking deficit in patient-derived stem cells in Hereditary Spastic Paraplegia. *Biol Open* 2014; 3: 494-502. 20140523. DOI: 10.1242/bio.20147641.
10. Malina J, Huessler EM, Jöckel KH, et al. Development and validation of TreatHSP-QoL: a patient-reported outcome measure for health-related quality of life in hereditary spastic paraplegia. *Orphanet J Rare Dis* 2024; 19: 2. 20240102. DOI: 10.1186/s13023-023-03012-w.
11. Mitchell JW, Noble A, Baker G, et al. Protocol for the development of an international Core Outcome Set for treatment trials in adults with epilepsy: the EPilepsy outcome Set for Effectiveness Trials Project (EPSET). *Trials* 2022; 23: 943. DOI: 10.1186/s13063-022-06729-4.
12. Charles T, Karlsson J and Marianne S. Do SF-36 Summary Component Scores Accurately Summarize Subscale Scores? *Quality of life research* 2001; 10: 395-404. DOI: 10.1023/A:1012552211996.

Siow et al Scoping Review Supplementary Material 1

Study ID	Country	Aim of study	Study design	Longitudinal study	Number of participants with HSP	Particpant genotype	Control group	Age	Participant age (years)	Intervention	Type Outcome 1	Type Outcome 2	Type Outcome 3	Type Outcome 4	Type Outcome 5	Type Outcome 6	Type Outcome 7	Type Outcome 8	Type Outcome 9	Type Outcome 10	Type Outcome 11	Type Outcome 12
Adair 2016	Australia	Exploratory observational study to quantify gait kinematics at the trunk and pelvis of children with HSP	Cross sectional study	No	11	1 SPG3A, 1 SPG4, rest unknown	No (Normal values used)	11	median 11		Gait analysis MRI and DTI processing											
Aghakhanyan 2014	Italy	To investigate WM alterations across the whole brain in a group of patients with pHSP with SPG4, SPG5, SPG3a and SPG10 mutations, applying tract-based spatial statistics analysis with multiple DTI indices.	Cross sectional study	No	12	SPG4, SPG5, SPG3A, SPG10	Yes	49.2	mean 49.2 SD 8.1													
Antczak 2019	Poland	To investigate the therapeutic potential of bilateral, high-frequency rTMS over primary motor areas for muscles of lower extremities in various forms of HSP (pure, complicated, AMN). RCT, crossover design. First study using rTMS for therapeutic purposes in HSP.	Randomised controlled trial	No	14	1 SPG3A, 1 SPG7, 3 AMN, 3 AMN carrier, 7 unknown	No	33.8	Mean 44.8 SD 10.1	rTMS and sham rTMS(coil rotated posteriorly by 90 degrees which reduces magnetic field by 67-73%, making it devoid of biologic effect); 5 stimulating sessions, 1 a day, during consecutive working days. 1500 magnetic stimuli on each side (10Hz in trains lasting 7.5 secs, 40 trains per side per session). Daily rTMS for five consecutive working days. Magstim Rapid stimulator with 110mm double cone coil, 10 Hz in trains of 40 lasting 7.5s and separated by 56s over bilateral primary motor area for them muscles of lower extremities; stimulation at 90% of resting motor threshold, or in case of prominent spasticity, when patient could no maintain muscle relaxation, 90% of active motor threshold; 1500 magnetic stimuli per sessions fore each lower limb (3000 pulses per session)	10 metre walk test (10MWT)	MEP measures	Timed up and Go	Strength measured with microFET 2 hand-held dynanometer	Modified Ashworth Scale						
Ardolino 2021	Italy	Double-blind, randomised crossover and sham-controlled study to evaluate the effects of tsDCS on spasticity in patients with HSP	Randomised controlled trial	No	11	8 SPG4, 1 SPG3A, 1 SPG7, 1 SPG 15	No	37.3	mean 37.3 SD 8.1	tsDCS and sham tsDCS - Each session lasted 5 days a week (20min twice a day), 2.0mA, 20min, via programable stimulator HDCStim, one electrode on spinous process T10 and another above R shoulder. Every pt received both anodal and sham session in cross-over design (AS or SA assigned in alternating allocation). Each session at least 3 months apart. Sham current turned on for five seconds then turned off in ramp-shaped fashoun inducing skin sensations indistinguishable from anodal tsDCS. Patients blinded.	Ashworth Scale	5 Minutes Walking test (5MWT)	MEPs	H-reflex	F-waves	SPRS						
Bastani 2021	Iran	To study the effectiveness of rTMS as palliative measure for lower limb spasticity, function and gait improvement in patients with HSP	Randomised controlled trial	No	8	unknown	No	38.5	mean 38.5, SD 5.4	rTMS and sham rTMS - 5 sessions rTMS on 5 consecutive days in a week. Coil on vertex, 5 trains of stim lasting 1 min with freq 5 Hz. 2 minute break between trains. Amp of stim at 90%MT at hot spot. Sham coil placed perpendicular to scalp, otherwise same as treatment group	modified Ashworth scale	lower extremity subclass of Fugl-Meyer assessment. 5 subcategories for evaluation. higher scores indicate higher function.	10MWT	SF-36 (persian trnaslation)								
Bertolucci 2015	Italy	To test the effectiveness of a robotic-aided intensive program of gait training on balance, walking ability and quality of life in adult subjects affected by uncomplicated HSP.	Non-randomised experimental study (including interventional studies)	No	13	5 SPG4, 6 SPG7, 1 SPG11, 1 SPG5	No	46.3	mean 46.3 SD 8.9	Rehabilitative treatment (Lokomat - robotic gait orthosis): 3 weekly session for 6 weeks. Using maximum assistance of robot on both legs, max comfortable speed for patient and 40% body weight support. Then according to muscle tone and gait quality monitored by visual feedback by the machine, therapist gradually reduced guidance force on both legs, without dropping below 75%. Exercises modified in term of duration, distance and peed to increase workload and maximised efforts for patient to challenge adaptation to training.	Berg Balance Scale	Timed up and go test (TUG)	10-Metre Walking Test (10MWT)	Six-minute walk test (6MWT)	Physiological Cost Index (PCI)	SF36	HADS	Modified Ashworth Scale				
Bonnefoy-Mazure 2013	Switzerland	To compare full-body movements of HSP and SP groups and in particular the movement of upper limbs	Cross sectional study	No	10	unknown	Yes	16.7	mean 16.7 SD 5.8		Computer gait analysis											
Braschinsky 2009	Estonia	To characterise the spasticity and RoM in patients with HSP and to correlate these parameters with walking speed. Observational population based cohort study.	Cross sectional study	No	46	9 SPG4, the rest unknown	No	50.1	Mean 50.1		Active and passive RoM of hip flexion, hip abduction and foot dorsiflexion	Modified ashworth scale	10 metre walk test									
Braschinsky 2011	Estonia	To examine the relative effect of HSP on HrQoL	Cross sectional study	No	49	unknown	Yes		mean N/A		RAND 36-item Health Survey 1.0 in Estonian											
Brighente 2021	Brazil	To evaluate the role of evoked potentials (EPs) as disease biomarkers of HSPs	Case control study	No	18	12 SPG4, 3 SPG5, 1 SPG7, 1 SPG11, 1 CTX	Yes	39.7	Mean 39.7 SD 18.7		MEPs	SSEPs	SPRS									
Chrestian 2016	Canada	To describe the clinical, genetic and epidemiologic features of HSP in Canada, to determine which clinical, radiologic and genetic factors determine functional outcomes	Cross sectional study	No	150	72 SPG4, 24 ATL1, 12 SPG11, 10 SPG7, 8 KIAA0196, 5 PLP1, 5 SLC2A1, 4 CYP7B1, 3 VAMP1, 2 ALS2, 1 KIF5A, 1 SLC16A2, 1 ZFYVE26, 1 FA2H, 1 PNPLA6	No	39.2	Mean 39.2 SD 22.1		SPATAX-EUROSPA disability score	SPRS	MRI brain									
Coutelier 2015	France	Report of 7 families with ALDH18A1 mutations segregating in recessive or dominant inheritance associated with abnormal plasma amino acid levels	Case series	No	21	15 dominant SPG9, 6 recessive SPG9	No	43.84	Mean 43.84 - 50.87yo		Plasma amino acid profile											
Cubillos-Arcila 2022	Brazil	To investigate the progression of functional gait in individuals with HSP and to define sensitivity to change, minimal clinically important difference and validity of timed functional test of gait. Searching for best outcome measures for future clinical trials.	Cross-sectional and prospective cohort study	Yes	25	17 SPG4, 4 SPG5, 2 SPG7, 2 SPG3A	Yes	51	Mean 51		SPRS	10MWT	TUG	6MWT								
DelaCasa-Fages 2019	Spain	To characterise SPG7: genetic testing of patienst with suspected HSP	Case series	No	35	SPG7	Yes (for mtDNA only)	53	Mean 53 SD 11.3		SPRS, SARA, SPATAX-EUROSPA disability score	MRI brain	Neurophysiology	Muscle biopsy	mtDNA levels	Ioflupane SPECT						
deLima 2021	Brazil	To evaluate the efficacy and safety of BoNT-A in the treatment of lower limb spasticity in patients with HSP.	Randomised controlled trial	No	55	21 SPG4, 4 SPG8, 2 SPG3A, 2 SPG11, 2 SPG33, 3 SPG72, 1 SPG6, 1 SPG7, 1 SPG15, 1 SPG28, 1 SPG48, 1 PLA2G6, 1 X-linked adrenoleukodystrophy, 1 autosomal-recessive spastic ataxia of Charlevoix-Saguenay, 13 unknown	No (placebo group)	43	mean 43 SD13.4	BoNT-A IM, placebo IM (0.9% sodium chloride solution) - Single treatment with 8wk follow up. Crossover with 24-28 weeks washout. Total dose 400 units: 100 units in each adductor magnus and 100 units in each triceps surae (divided in 3 parts: 1/4 in the head of each gastrocnemius and 1/2 in the soleus)	10MWT	Gait at self-selected velocity with 10MWT performed once	SPRS	Adductors and triceps surae changes in muscle tone and strength (MAS, MRC)	Brief Pain Inventory	Modified Fatigue Impact Scale	Subjective perception of symptoms, or benefit, or gait improvement					
DuMontcel 2008	France	To evaluate quantitative tests of cerebellar function including assessment of their specificity; to find correlations with clinical signs; and to develop a composite functional score.	Cross sectional study	No	53	22 SPG4, 4 SPG3, 27 unknown	Yes	47.4	Mean 47.4 SD 13.4		SARA	Unified Huntington's Disease Rating Scale (UHDRS part IV)	Health-related quality of life (EQ-5D)	Patient health questionnaire (PHQ-9)	Writing test	Dysdiadochokinesis testing (Tapping test)	Nine-hole pegboard test	Click test	Lower limb score (time-restricted walking test)	Composite cerebellar functional severity (CCFS) score	
DuMontcel 2012	France	To evaluate disease progression and determine validity of clinical tools for therapeutic trials.	Cohort study	Yes	64	31 SPG4, 4 SPG3, 29 Unknown	No	45	mean 45-50 SD 14		CCFSw	SARA	AMBUS									
Ebrahimi-Fakhari 2021	USA	To validate a high-throughput ATG9A translocation assay in patient-derived fibroblasts as a diagnostic marker of AP-4-HSP.	Diagnostic test accuracy study	No	20	9 AP4B1, 4 AP4M1, 5 APS1, 2 AP4B1 VUS	Yes	8.65	Mean 8.65		ATG9A ratio	Clinical features	MRI features									
Erichsen 2009	Norway	To investigate whether SPG4-HSP leads to neuronal biochemical changes detectable on proton magnetic resonance spectroscopy (MRS).	Cross sectional study	No	18	SPG4	Yes	48.4	Mean 48.4 SD 7		MR spectroscopy	Cognitive assessment										
Faber 2018	Brazil	To characterise genetic and clinical features, including motor and cognitive manifestations, in a group of patients with SPG11, and to undertake neuroimaging studies in this group, to allow correlation of clinical findings with MRI/anatomic abnormalities.	Cohort study	Yes	25	SPG11	Yes	29	Mean 29		MRI	Addenbrooke's Cognitive Examination Revised (ACE-R)	Spastic Paraplegia Rating Scale (SPRS)	Neurophysiological assessment (NCS and EMG)		Functional gait assessment (3 tests undertaken: 10m walk test, Timed up and go test (TUG), 2 min walking test)						
Gassner 2021	Germany	To correlate functional gait measures with self-rating questionnaires for fear of falling and QoL, and against the SPRS.	Cross sectional study	No	22	11 SPG4, 2 SPG5, 1 SPG31, 8 Undetermined	Yes	52.2	Mean 52.2 SD 10		Spastic Paraplegia Rating Scale (SPRS)	Falls Efficacy Scale-International (FES-I)	ZUNG depression score	SF-12 (QoL score)	MoCA							
Geva-Dayan 2010	Israel	To report outcomes and experience with use of botulinum toxin type A injections to the lower limbs of children with HSP.	Case series (Retrospective review)	No	12	1 SPG4, 1 ALS2, 10 Unknown	No	4.8	Mean 4.8 SD 2.5	Botulinum toxin A. The mean interval between repeated injections was 13.2 +/- 11.0 months with the shortest duration being 3 months and the longest 3 years. The time between the first and last injections varied between patients with mean 2.8 +/- 1.8 years. Dysport was administered to 10 children and Botox to 2 children. Reevaluation of muscle tone (Ashworth Scale) and muscle function (Gross Motor Function Measure) was performed at routine visits scheduled 1 month after each injection. The need for re-injection was determined upon follow-up visits 3-4 months post-injection.	Modified Ashworth Scale	Gross Motor Function Measure (GMFM-88)									
Growdon 1991	USA	To assess the clinical effect of L-threonine in patients with HSP.	Randomised controlled trial	No	18	unknown	No	47.5	Mean 47.5	L-threonine vs placebo cross-over trial. Cross-over study with 2x treatment periods lasting 2 weeks each, an intervening 2-week washout period and a final testing period 2 weeks after completion of treatment period 2 (i.e. 8 weeks in total). Patients received L-threonine at either a dose of 1.5g TDS (total daily dose 4.5g) or 2g TDS (6g total dose). 7 patients received the 4.5g dose, 9 took the 6g dose and 2 patients took both doses (completed the study twice). For these latter two patients, only their scores from the 6g dose treatment period were used for analysis.	Motor function and spasticity scores	Amino acid analysis in blood and CSF									

Henderson 2019	UK	To evaluate efficacy of gait corrective surgery for paediatric patients with HSP.	Case series	No		9	unknown	No		N/A	Gait correction surgery	Gait evaluation	Functional questionnaire score									
Jordan 2021	USA	To establish a metric for health-related quality of life (HRQoL) in patients with AP-4-HSP, and investigate correlations with major symptoms and disease severity.	Cross sectional study	No		64	31 SPG47 (AP4B1), 21 SPG50 (AP4M1), 2 SPG51 (AP4E1), 10 SPG52 (AP4S1)	No	12.45	mean 12.45 SD 10.53		Caregiver Priorities and Child Health Index of Life with Disabilities (CPCHILD) questionnaire	Spastic Para-plegia Rating Scale (SPRS) total and spasticity subscores	4-stage functional mobility scale (4FMS)								
Joussain 2019	France	To characterise clinical and urodynamic aspects of LUTS encountered in HSP, to describe urological complications and therapeutic strategies.	Cohort study	Yes		33	unknown	No	62	Mean 62 SD 14		Lower urinary tract symptoms (LUTS), bowel or sexual dysfunction	Urodynamic assessment									
Kai 2014	China	To assess the effect of selective dorsal rhizotomy (SDR) as a treatment for patients with severe pure HSP.	Non-randomised experimental study (including interventional studies)	Yes		4	unknown	No	31.25	mean 31.25	Selective dorsal rhizotomy (SDR) -Operation completed in May-October 2009 with follow up range 25-30 months. A T12-L1 laminectomy was performed. Rootlets from L2-S2 dorsal roots were were sectioned based on pre-defined lesioning criteria, evaluated by stimulation-evoked responses (with intraoperative electromyography). Nerve rootlets were considered to be involved in the spastic process if the response spread to muscle groups in the lower limbs, outside the distribution of the nerve rootlets being stimulated, or to contralateral lower limb muscles. The goal of the procedure was to reduce spasticity while preserving as many sensory rootlets as possible. A decision was made to save more of the L4 nerve rootlets in an attempt to decrease the degree of quadriceps muscle weakness that had been noted in the first 6 weeks postoperatively in other studies. S2 rootlets with positive sphincter responses were preserved. Two weeks after the operation, patients were transferred for a 4-week rehabilitation program.	Ashworth score	Spasm frequency scale									
Karle 2013	Germany	To evaluate clinical, and motor and sensory electrophysiological features in patients with HSP.	Cross sectional study	No		128	1 SPG3, 35 SPG4, 3 SPG5, 3 SPG7, 1 SPG8, 1 SPG10, 6 SPG11, 4 SPG15, 74 unknown	No (Normal values used)	47.5	Mean 47.5 SD 14.9		SPRS	Clinical diagnosis of motor or sensory peripheral neuropathy	MEPs	Nerve conduction studies	SSEPs						
Kessler 2021	Germany	To evaluate CSF neurofilament light chain (cNfL) levels in patients with HSP and provide information on the influence of demographic factors.	Cross sectional study	No		59	17 SPG5, 10 SPG4, 1 SPG3, 1 SPG6, 1 SPG11, 1 SPG30, 1 SPG35, 1 SPG46	Yes	43.8	Mean 43.8		CSF neurofilament light chain (cNfL)										
Kessler 2022	Germany	To investigate serum neurofilament light chain (sNfL) as a potential therapy response, diagnostic, monitoring, and prognostic biomarker in SPG4.	Cohort study	Yes		93	SPG4	Yes	50.9	Mean 50.9		sNfL	cNfL	SPRS	SPATAX disability scale							
Klebe 2012	France	Studied 134 patients with HSP with SPG7 analysis to define the clinical and mutation spectrum of SPG7 and to propose guidelines for when to test for SPG7 in patients with HSP and autosomal dominant optic neuropathy	Cross sectional study	No		28	SPG7	Yes	42.5	Mean 42.5, SD 18.3		Clinical features in HSP-SPG7 patients	Ophthalmologic assessment including OCT	Clinical features in AD ON family (carrying the SPG7 Asp411Ala variant)								
Klimpe 2012	Germany	To assess the correlation of HRQoL with disease severity and clinical features in patients with HSP.	Cross sectional study	No		143	47 SPG4, rest unknown	No	47.8	Mean 47.8 SD 13		Short-Form 36 (SF-36)	Spastic Paraplegia Rating Scale (SPRS)	Walking ability (landmarks of disability)								
Kloth 2020	Germany	To report clinical/genetic/imaging findings in 2 siblings with SPG46	Case series	No		2	SPG46	Yes	44.5	mean 44.5		Glycosylsphingosine (lyso-Gb1)	Glycosylceramide profile									
Lassmann 2022	Germany	To characterise prodromal SPG4-related gait changes in prodromal and mild-to-moderate manifest SPG4 patients	Cross sectional study	No	17 SPG4 (30 prodromal SPG4)		SPG4	Yes	48.2	mean 48.2, SD 8.2		Instrumented gait analysis using an infrared-camera-based motion capture system (VICON FX)	SPRS	Serum neurofilament light chain	CSF neurofilament light chain	Motor-evoked potentials of leg						
Lin 2021	China	To identify potential clinical, CSF or MRI biomarkers that could be used to assess therapeutic efficacy in SPG5.	Cross sectional study	No		34	SPG5	Yes	30	Median 30		SPRS	Spinal cord atrophy on 3T MRI	Brain atrophy on 3T MRI	CSF 27-hydroxycholesterol	CSF neurofilament light	MMSE					
Lith 2019	Netherlands	To investigate the functional effects of bilateral botulinum toxin A treatment and subsequent stretching of spastic hip adductors on gait and reactive lateral stepping responses in patients with pure hereditary spastic paraplegia	Non-randomised experimental study (including interventional studies)	No		25	12 SPG4, 2 SPG10, 1 SPG17, 2 SPG31, 8 unconfirmed dominant HSP	No	53.5	Mean 53.5	Botulinum toxin followed by 16 weeks of stretching exercises. Botulinum toxin type A (Xeomin) into hip adductors (gracilis, adductor magnus, adductor longus) - 100 units diluted in 5mL of 0.9% saline - dose depended on MAS: 150 units for MAS 1, 200 units for MAS >= 2 - distribution across gracilis/adductor magnus/adductor longus predefined (see Table II in pdf) Followed by stretching exercises of the hip adductors (with the hips both flexed and extended) for approximately 10 min, 3 times per day 16 - patients performed exercises independently but performance logged in exercise diary	Instrumented gait analysis	Instrumented dynamic balance assessment	Muscle strength (MRC grading) of hip adductors and abductors	Modified Ashworth (MAS)	Passive ROM of hip abduction using goniometer	Berg Balance Scale	Timed up and go (TUG)	6MWT	Activities-specific Balance Confidence (ABC) scale		
Loureiro 2013	Portugal	To describe the clinical, genetic, and epidemiological features of Portuguese AD-HSP families	Cross sectional study	No		239	27 families with SPG4, 5 families with SPG3, 1 family with SPG31, 9 unknown	No	50.7	Mean 50.7, SD 17.9		Motor severity and rate of disease progression	Modified Ashworth Scale	Muscle strength using MRC								
Marelli 2018	France	1) To validate plasma 25-OHC,27-OHC and their ratio to total cholesterol as diagnostic biomarkers for SPG5 2) To test whether candidate molecules (atorvastatin, CDCA, resveratrol) can lower plasma oxysterols and improve serum bile acids profile	Non-randomised experimental study (including interventional studies)	Yes		21	SPG5	Yes	49.5	mean 49.5, SD 14	Atorvastatin 20mg BD CDCA 500mg BD (intervention 2) Resveratrol 40mg BD (intervention 3) Three-period of 2 months, three-treatment in random order, crossover (each separated by 4-month washout) = 6 sequences (with each sequence administered to 2 patients)	Fasting (12 hours) 25-OHC, 27-OHC and 24S-OHC levels + bile acid levels (in 3 patients measured at 1 yearly intervals)	MRI (historical)									
Marelli 2020	France	To describe the phenotype of a three-generation family of SPG9	Case series	No		4	SPG9	No		N/A		Citrulline										
Margetis 2014	Greece	To study the effectiveness and safety of intrathecal baclofen therapy for the treatment of spasticity and gait improvement in patients suffering from hereditary spastic paraplegia	Non-randomised experimental study (including interventional studies)	Yes		16	unknown	No	43	Mean 43	Intrathecal baclofen pump, Initially given at 50ug over 24 hours; then slowly uptitratde in 5-10ug increments; 2 of the 6 female patients had a negative baclofen IT bolus test dose trial so did not proceed to pump; Average follow up was 25.8 months	Modified Ashworth scale	Modified version of functional walking scale of the Gillette Functional Assessment Questionnaire									
Martinuzzi 2016	Italy	Full clinical and paraclinical characterisation of large population of Italian patients with molecularly diagnosed HSP, detail how various assessment tools measure CST impairment	Cross sectional study	No		70	32 SPG4, 11 SPG11, 7 SPG3A, 7 SPG5, 4 SPG7, 3 SPG10, 3 SPG15, 1 SPG31, 2 SPG35	Yes (for DTI and MRS only)	42.97	Mean 42.97 SD 18.58		SPRS	Modified Ashworth Scale	6MWT	LL Deep Tendon Reflexes, LL Medical Research Council	WAIS-R	Functional Independence Measure (FIM)	EMG and NCS	Standard MRI	DTI and MRS	SSEP	Motor evoked responses
Milenkovic 2019	Austria	To investigate oculomotor and vestibular dysfunction in five patients with genetically verified SPG7	Case series	No		5	SPG7	Yes	58.4	mean age 58.4		Video-oculography and rotational chair testing to assess: spontaneous nystagmus, smooth pursuit, horizontal & vertical saccades, VOR										
Mishra 2015	USA	To test the effectiveness of TSFA in clinical research	Cross sectional study	No		29	unknown	Yes	8.9	mean 8.9 SD 2.1		Diffusion Tensor Imaging - MRI	Gross Motor Function Classification System (GMFCS) measurement									
Montanaro 2020	Italy	To investigate the role of a multimodal advanced MRI approach (DTI, VBM, MRS) in defining disease-specific alterations in HSP, in groups of HSP patients and Healthy controls, to evaluate longitudinal brain changes and identify specific anatomic involvement and types of brain modifications.	Cohort study	Yes		31	12 SPG4, 4 SPG5, 3 SPG30, 3 SPG3, 2 SPG8, 2 SPG11, 1 SPG7, SPG72, SPG10, SPG31, unknown	Yes	43.5	mean 43.5, SD 12		SPRS	MRI	Voxel based morphometry	H1-MR spectroscopy (MRS)	DTI						

Musacchio 2018	Germany	To establish biological markers for the natural history and course of SPG11	Cohort study	Yes		13	SPG11	No		N/A		SPRS	NCS/EMG/MEP	Video-supported posturography	Cognitive testing	OCT							
Navas-Sanchez 2021	Spain	To detect potential alterations in the volume and shape of the thalamus and various basal ganglia structures in SPG4	Cross sectional study	No		24	SPG4	Yes	46	Mean 46, SD 13		MRI with automated segmentation of the subcortical structures (thalamus, hippocampus, caudate nucleus, globus pallidus, and putamen) in native space and shape analysis of the structures	SPRS	MMSE									
Olchik 2021	Brazil	To evaluate the frequency and clinical characterization of dysarthria in different types of HSP	Cross sectional study	No		66	31 SPG4, 4 SPG11, 4 SPG5, 4 CTX, 3 SPG7, 2 SPG3A	Yes		N/A		Protocol for the Evaluation of Acquired Speech Disorders (PADAF)											
Orsucci 2014	Italy	To clinically and molecularly characterise a big cohort of patients with HSP in Tuscany	Retrospective cohort study	No		45	13 SPG4, 5 SPG7, 1 SPG10, 1 SPG11, 1 SPG17	No	51.2	Mean 51.2 SD 12.8		Four-stage functional scale of motor disability (4SMD)	SF-36	SPRS	MRI								
Paparella 2020	Italy	To evaluate the effect of the combined BoNT-A and intensive PT in patients with HSP	Cohort study	No		18	5 SPG4, 1 SPG5, 1 SPG7, 2 SPG8, 1 SPG11, 1 SPG72, 7 unknown	No	53.9	Mean 53.9 SD 12.2	Botulinum toxin (Xeomin, Dysport and/or Botox) + physiotherapy, EMG guided toxin; dose determined for individual patients	SPRS	Walking Handicap Scale	Modified Ashworth Scale	10MWT and 2MWT	Timed up and go (TUG)	Visual analogical scale (for QOL)	Numeric rating scale (for pain)					
Pointon 2022	UK	Evaluate the use of Intrathecal baclofen (ITB) on 5 ambulant children with HSP	Case series	No		5	1 SPG30, 4 unknown	No	7.2	mean 7.2	Intrathecal baclofen. Flexible dosing mode with lower rates at night (typically 25-30% lower than day rate). Several cases needed gradual increases in dose over time potentially due to the progressive nature of the disease	Gross motor function measure-66 item test (GMFM-66)	Gross motor function classification score (GMFCS) equivalent	Modified Ashworth Score (MAS)	6MWT	(Modified) Goal Attainment Scale (mGAS)	Cerebral palsy quality of life questionaire (CPQoL)	Laboratory gait analysis					
Prestsaeter 2020	Norway	To characterize Norwegian patients with SPG5, including clinical examinations, genetic analyses, measurements of hydroxycholesterols, electrophysiological investigations and brain imaging	Cross sectional study	No		5	SPG5	No	49.6	Mean 49.6		Serum 25-OHC and 27-OHC	SPRS	MRI brain	OCT	NCS/EMG/SEP/BAEP/VEP							
Rattay 2019	Germany	Determine the phenotypic spectrum of FAHN/SPG35, define the disease course and identify clinical and imaging biomarkers	Cohort study	No		19	SPG35	No	14	Mean 14		Neurophysiology: MEP, SEP, VEP, NCS	MRI brain including midbrain & pons atrophy on mid-sagittal section	Scanning electron microscopy of hair shafts									
Rattay 2019	Germany	To evaluate non-motor symptoms in SPG4 and their relevance with regard to health-related quality of life.	Cross sectional study	No		118	SPG4	Yes	50.4	Mean 50.4 SD 9.4		EQ-5D	2014 Revised IRLSSG Diagnostic Criteria for RLS	Becks Depression Inventory (BDI-V)	Modified Fatigue Impact Scale (MFI)	Brief Pain Inventory (BPI)	Montreal cognitive assessment (MoCA)	CANTAB Cognitive Assessment Software					
Rattay 2022	Germany	To characterise the prodromal phase of hereditary spastic paraplegia type 4 (SPG4) by biomarkers and clinical signs and symptoms that develop before manifest gait abnormalities.	Cohort study	No		30	SPG4	Yes	33.7	mean 33.7 SD 12		SPRS	Brief pain inventory	Beck's depression inventory	Multidimensional fatigue inventory	Montreal Cognitive Assessment	EQ-5D	3 minute walk test and pulse rates	Neurophysiology: NCS & MEP	CSF neurofilament light	Other CSF markers (Abeta1-42, total-tau, phospho-tau)	Serum neurofilament light	Brain MRI volumetry with neural network analysis
Regensburger 2022	Germany	To investigate gait cyclicity parameters by application of a mobile gait analysis system in a cross-sectional cohort of patients with HSP and a longitudinal fast progressing subcohort	Cross sectional study	Yes		112	34% SPG4, 34% SPG7, 13% SPG11, 17% no genetic diagnosis	Yes	47.7	mean 47.7 SD 15.2		Mobile gait analysis	SPRS	Timed up and go (TUG)	Falls efficacy scale international questionnaire (FES I)	Short form 12 (SF-12)	Montreal cognitive assessment (MoCA)						
Rezende 2015	Brazil	To identify regions of CNS (cerebral and spinal cord) damaged in pateints with SPG4-HSP using a multi-modal neuroimaging approach (GM and WM, Spinal cord) and to identify possible clinical correlates of damage.	Cross sectional study	No		11	SPG4	Yes	46	mean 46 SD 15		SPRS	MRI brain - cortical thickness analyses	DTI - TBSS, tractography	MRI - spinal cord morphometry								
Sarret 2016	France	To analyze the natural timecourse of hypomyelinating PLP1-related disorders (Pelizaeus-Merzbacher disease [PMD] and spastic paraplegia type 2) using brain magnetic resonance imaging (MRI) motor development score (MDS) correlations.	Cohort study	Yes		35	PLP1	No	11.7	mean 11.7 SD 7.8		MRI											
Scheuer 2007	Denmark	To investigate the effects of gabapentin on clinical disability and motor cortical excitability in SPG4-linked hereditary spastic paraplegia patients.	Randomised controlled trial	No		10	SPG4	No	45.4	mean 45.4	2 months gabapentin + 10 days washout + then 1 month placebo - Gabapentin started at 2400mg daily -> 4000mg daily titrated over 10 days	Visual analogue score (VAS)	Disability score (DIS)	Ambulatory score (AMB)	Self-reported severity of sphincter disturbances (urinary symptoms)	Combinations of MS Impairment Scale and SPATAX diagnostic form - paresis (hip, knee, ankle), tendon reflexes, tone	paired transcranial magnetic stimulation	Blood measurements of S-gabapentin					
Schneider 2019	Germany	To characterize lower urinary tract/urological symptoms and patient satisfaction in genetic and sporadic HSP patients.	Cross sectional study	No		71	34 SPG4, rest unknown	No	55.4	mean 55.4 SD 13.9		ICIQ-SF	SCOPA-AUT	ICIQ-LUTSqol									
Schols 2017	Belgium, Greece, Italy, Germany, Italy, Brazil	To perform a detailed clinical and biochemical analysis in 34 genetically confirmed SPG5 cases from 28 families, study dose-dependent neurotoxicity of oxysterols in human cortical neurons and perform a randomized placebo-controlled double blind interventional trial targeting oxysterol accumulation in serum of SPG5 patients.	Cross sectional analysis and Randomised controlled trial	Yes	34 (14 in RCT)		SPG5	Yes	40	mean 40	Atorvastatin 40mg/day 9 weeks, Placebo 9weeks. Adult dose 40mg, Child dose under 18yo: 20mg/day.	SPRS,	Serum 27-OHC	Change of 27-OHC in CSF, reduction in 24-OHC, 25-OHC and 3b-CA in serum and CSF	3-min endurance walk	physical cost index							
Schuurs-Hoeijmakers 2012	Netherlands	To report on four families affected by a clinical presentation of complex hereditary spastic paraplegia (HSP) due to recessive mutations in DDHD2.	Case series	No		12	SPG54	No	11.8	mean 11.8		Thin corpus callosum	Periventricular white-matter hyperintensities	Magnetic resonance spetroscopy - Lipid peak									
Simonini 2021	Italy	To assess whether phosphorylated neurofilament heavy chain (pNfH) can discriminate different upper motor neuron (UMN) syndromes, namely, ALS, UMN-predominant ALS, primary lateral sclerosis (PLS) and hereditary spastic paraparesis (hSP) and to test the prognostic value of pNfH in UMN diseases.	Cohort study	Yes		13	6 unknown	No	51.6	mean 51.60 SD 18.84		Serum pNfH	CSF pNfH	ALS functional rating scale Revised	Brain MRI	Motor evoked potentials							
Unrath 2010	Germany	To investigate three different rare MNDs (PLS, HSP, X-linked Spinobulbar Muscular Atrophy), by applying DTI to brain MRI data of patients with MNDs to analyse and quantify WM tract integrity by a whole brain-based approach and fiber tracking with the aim of producing a DTI-based definition of disease-associated patterns of microstructural WM alterations that might correlate with clinical presentation.	Cross sectional study	No		24	6 SPG4, rest unknown	Yes	50	mean 50.0 SD 9.3		Diffusion tensor imaging (DTI)											
Uygunoglu 2016	Turkey	To investigate the effects of dalfampridine on hereditary spastic paraparesis.	Non-randomised experimental study (including interventional studies)	No		5	3 SPG4, 1 probable SPG4, 1 SPG15	No	35.2	mean 35.2 SD 10.8	Dalfampridine-SR 10mg BD	Modified Ashworth Scale (MAS)	10m walk time	20m walk time	9-hole peg test (NHPT)								
Vavla 2019	Italy	To measure RNFL thickness with OCT in patients with HSP including longitudinal F/U in subgroup, to compare changes and correlate to disease progression. Studied as a potential biomarker.	Cohort study	Yes		23	8 SPG4, 6 SPG3a, 3 SPG72, 2 SPG5, 2 SPG7, 2 SPG8	Yes	44.6	Mean 44.6 SD 17.7		SD-OCT Exam: Retinal nerve fiber layer (RNFL) thickness	SPRS										
Vaz 2019	Netherlands	To investigate the effect of PCYT2 variants on phenotype and lipid profile in human cell and mouse models.	Non-randomised experimental study (including interventional studies)	No		5	SPG82	Yes	11	mean 11yo		Lipidomics in fibroblasts and plasma											
Wali 2021	Australia	To quantify cell morphological features using cell morphometry from patient fibroblasts 15 people with SPAST and SPG7 HSP and matched controls using machine learning.	Cross-sectional study	No		15	10 SPG4, 5 SPG7	Yes	51.8	mean 51.8		Cell Morphometry											
Wilke 2018	Germany	To provide first proof-of-concept that serum Nfl serves as biomarker of neuronal damage and disease progression in HSP. Measured serum levels of Nfl in HSP patients, ALS patients and age-matched healthy controls with established Simo assay.	Cohort study	No		96	35 SPG4, 11 SPG7, 5 SPG11, 4 SPG5, 3 SPG3, 2 SPG15, 1 SPG10, 1 ABCD1, 1 GAN, rest unknown	Yes	51.4	Mean 51.4		Serum neurofilament light chain (NfL) level	SPRS	Cross-sectional disease progression									
Zhang 2014	New Zealand	To evaluate effect of hydrotherapy treatment in individuals with late onset HSP by quantifying gait parameters in adults with HSP. Biomechanics analysis, 10-week hydrotherapy, final biomechanics. First study to report joint angles and moments in three planes for patients with HSP pre and post therapy	Non-randomised experimental study (including interventional studies)	No		9	unknown	Yes		N/A	Hydrotherapy - 10-week programme. 1 group, 5 weeks individual, group, 5 weeks individual. 45-min sessions twice per week.	Modified Ashworth Scale	Gait analysis	Cadence, step length, step time, single support (%)	Joint kinematics (range of motion)	Joint kinetics							

Zucchi 2018	Italy	To test whether phosphorylated neurofilament heavy chain (pNfH) may discriminate different UMN syndromes at diagnosis and to test their prognostic role among these diseases.	Case control study	Yes	9	unknown	Yes	48	Mean 48 SD 14.77	CSF and serum pNfH	Modified Rankin Scale	ALS rating scale revised (ALSFRS-R)	Ashworth Scale
							SD	14.8614					
							Mean	39.915					

Siow et al Scoping Review Supplementary Material 2

Outcome measure/ Biomarker	Study	Classify patients vs controls		Indicate disease progression		Response to intervention		Correlation with other biomarker/outcome measure	
		Controls included	Outcome	Longitudinal study	Outcome	Intervention	Outcome	Biomarker	Significant correlation
CROMs – HSP specific									
SPRS	Ardolino 2021	No	N/A	No	N/A	Yes	No	No	N/A
	Brighente 2021	Yes	N/A	No	N/A	No	N/A	MEP amp or CMCT	No
	Chrestian 2016	No	N/A	No	N/A	No	N/A	Brain MRI	Yes
	Cubillos-Arcila 2022	Yes	N/A	Yes	Yes (but only in SPG4 group)	No	N/A	10MWT 6MWT and TUG	Yes No
	deLima 2021	No	N/A	No	N/A	Yes	No	No	N/A
	DelaCasa-Fages 2019	No	N/A	No	No – descriptive paper	No	N/A	No	N/A
	Faber 2018	Yes	N/A	Yes	Yes	No	N/A	Disease duration MRI measures	Yes
	Gassner 2021	No	N/A	No	N/A	No	N/A	SF-12 PCS only, 10MWT, 2MWT, TUG.	Yes.
	Jordan 2021	No	N/A	No	N/A	No	N/A	CPCHILD	Yes
	Karle 2013	No	N/A	No	N/A	No	N/A	CMCT to the legs and arms.	Yes

	Kessler 2022	Yes	N/A	Yes	Yes (3/31 patients)	No	N/A	Serum Nfl	No
	Klimpe 2012	No	N/A	No	N/A	No	N/A	SF-36	Yes
	Lassman 2022	Yes	Yes	No	N/A	No	N/A	Gait analysis	Yes
	Lin 2021	Yes	N/A	No	N/A	No	N/A	CSF 27-OHC CSF NfL	No
	Martinuzzi 2016	No	N/A	No	N/A	No	N/A	Age at onset and disease duration. ENB2. FIM DTI - FA and MD.	Yes
	Montanaro 2020	Yes	N/A	Yes	No	No	N/A	VBM and DTI indices	No
	Musacchio 2018	No	N/A	Yes	Yes	No	N/A	No	N/A
	Navas-Sanchez 2021	Yes	N/A	No	N/A	No	N/A	Age B/L thalamus volume	Yes
	Orsucci 2014	No	N/A	No	N/A	No	N/A	Age of onset. Age at last evaluation. SF36 score.	No (age) Yes (SF-36)
	Paparella 2020	No	N/A	No	N/A	Yes	Yes	No	N/A
	Prestsaeter 2020	No	N/A	No	N/A	No	N/A	No	N/A
	Rattay 2022	Yes	Yes	No	N/A	No	N/A	CSF NfL	Yes
	Regensburger 2022	Yes	N/A	Yes	Yes (but only in patients with cHSP)	No	N/A	Mobile gait analysis parameters	Yes (weak)

	Rezende 2015	Yes	N/A	No	N/A	No	N/A	DTI indices	Yes (for TBSS but not tractography)
	Schols 2017	No	N/A	Yes	Yes	Yes	No	10MWT Time to climb 10 steps Serum 27-OHC	Yes
	Vavla 2019	No	N/A	Yes	N/A	No	N/A	RNFL thickness	No
	Wilke 2018	Yes	N/A	No	N/A	No	N/A	Serum Nfl	Yes
SPATAX-EUROSPA disability score	Chrestian 2016	No	N/A	No	No	No	N/A	No	N/A
	Dela-Casa Fages 2019	No	N/A	No	N/A	No	N/A	Variant type mtDNA	Yes No
	Kessler 2022	Yes	N/A	Yes	Yes (3/31 patients)	No	N/A	Serum Nfl	No
	Scheuer 2007	No	N/A	No	N/A	Yes	No	No	N/A
CROMs – other conditions									
SARA	Dela Casa Fages 2019	No	N/A	No	N/A	No	N/A	No	N/A
	Du Montcel 2008	Yes	Yes	No	N/A	No	N/A	No	N/A
	Du Montcel 2012	No	N/A	Yes	Yes	No	N/A	AMBUS	Yes
ALS rating scale revised (ALSFRS-R)	Simonini 2021	No	N/A	Yes	No	No	N/A	Serum and CSF crt-pNfH (for whole group including ALS)	Yes

	Zucchi 2018	Yes	N/A	Yes	Yes	No	N/A	CSF and Serum pNfH	Yes (with progression rate and total score)
Unified Huntington's Disease Rating Scale Part IV	Du Montcel 2008	Yes	N/A	No	N/A	No	N/A	CCFS	Yes
MS Impairment Scale	Scheuer 2007	No	N/A	No	N/A	Yes	No	No	N/A
CROMs – Generic functional									
Functional questionnaire score	Henderson 2019	No	N/A	No	N/A	Yes	No	No	N/A
Modified Rankin Scale	Zucchi 2018	Yes	N/A	Yes	N/A	No	N/A	Serum and CSF pNfH (whole group including ALS)	Yes (at last observation but not at diagnosis)
Disability Score (DIS)	Scheuer 2007	No	N/A	No	N/A	Yes	No	No	N/A
Functional Independence Measure (FIM)	Martinuzzi 2016	No	N/A	No	N/A	No	N/A	SPRS	Yes
PerfOMs – motor function									
Modified Ashworth Scale	Antczak 2019,	No	N/A	No	N/A	Yes	Yes (proximal not distal)	No	N/A
	Ardolino 2021	No	N/A	No	N/A	Yes	Yes	No	N/A
	Bastani 2021	No	N/A	No	N/A	Yes	Yes	No	N/A

	Bertolucci 2015	No	N/A	No	N/A	Yes	No	No	N/A
	Braschinsky 2009,	No	N/A	No	N/A	No	N/A	Active ROM (goniometer) Walking speed (10MWT)	Yes
	deLima 2021	No	N/A	No	N/A	Yes	Yes (hip adductors) No (triceps surae)	No	N/A
	Geva-Dayan 2010	No	N/A	No	N/A	Yes	Yes	No	N/A
	Kai 2014	No	N/A	Yes	Yes (immediately after treatment <12 months but not long term 12-24 months)	Yes	Yes	No	N/A
	Lith 2019	No	N/A	No	N/A	Yes	Yes	No	N/A
	Loureiro 2013	No	N/A	No	N/A	No	N/A	No	N/A
	Margetis 2014	No	N/A	No	N/A	Yes	Yes	No	N/A
	Martinuzzi 2016	No	N/A	No	N/A	No	N/A	No	N/A
	Paparella 2020	No	N/A	No	N/A	Yes	Yes	No	N/A
	Pointon 2022	No	N/A	No	N/A	Yes	Yes	No	N/A
	Simonini 2021	No	N/A	Yes	N/A	No	N/A	No	N/A
	Uygunoglo 2016	No	N/A	No	N/A	Yes	Yes	No	N/A

	Zhang 2014	Yes	N/A	No	N/A	Yes	N/A (not used as outcome measure)	No	N/A
	Zucchi 2018,	Yes	N/A	Yes	No	No	N/A	Serum and CSF pNfH	Yes
10m walk time (10MWT), 6 MWT, 2 min WT, 5 min WT, 3-min endurance walk, 20m walk time	Antczak 2019,	No	N/A	No	N/A	Yes	No	No	N/A
	Ardolino 2021	No	N/A	No	N/A	Yes	No	No	N/A
	Bastani 2021	No	N/A	No	N/A	Yes	No	No	N/A
	Bertolucci 2015	No	N/A	No	N/A	Yes	Yes	No	N/A
	Braschinsky 2009	No	N/A	No	N/A	No	N/A	Active ROM as measured with Goniometer (hip abduction) MAS	Yes
	Cubillos-Arcila 2022	Yes	Yes	Yes	Yes (for max but not self-selected speed)	No	N/A	SPRS	Yes for 10MWT but no for 6MWT
	deLima 2021	No	N/A	No	N/A	Yes	No	No	N/A
	Gassner 2021	Yes	Yes	No	N/A	No	N/A	FES-I, SPRS, TUG, SF-12 (PCS only)	Yes
	Lith 2019	No	N/A	No	N/A	Yes	No	No	N/A
	Martinuzzi 2016	No	N/A	No	N/A	No	N/A	No	N/A
	Paparella 2020	No	N/A	No	N/A	Yes	Yes	No	N/A
	Pointon 2022	No	N/A	No	N/A	Yes	No	No	N/A
	Rattay 2022	Yes	No	No	N/A	No	N/A	No	N/A

	Uygunoglu 2016	No	N/A	No	N/A	Yes	Yes	No	N/A
Timed Up and Go test (TUG)	Antczak 2019	No	N/A	No	N/A	Yes	No	No	N/A
	Bertolucci 2015	No	N/A	No	N/A	Yes	No (close to significance)	No	N/A
	Cubillos-Arcila 2022	Yes	Yes	Yes	Yes for max but not self selected speed	No	N/A	SPRS	No
	Gassner 2021	Yes	Yes	No	N/A	No	N/A	FES-I SPRS SF-12	Yes Yes No
	Lith 2019	No	N/A	No	N/A	Yes	No	No	N/A
	Paparella 2020	No	N/A	No	N/A	Yes	Yes	No	N/A
	Regensburger 2022	Yes	N/A	Yes	Yes (for cHSP)	No	N/A	No	N/A
MRC muscle strength	deLima 2021	No	N/A	No	N/A	Yes	No	No	N/A
	Lith 2019	No	N/A	No	N/A	Yes	Yes (Hip adductors but not abductors)	No	N/A
	Loureiro 2013	No	N/A	No	N/A	No	N/A	No	N/A
	Martinuzzi 2016	No	N/A	No	N/A	No	N/A	No	N/A
Gross motor function measure (GMFM-66 or GMFM-88)	Geva-Dayan 2010	No	N/A	No	N/A	Yes	Yes	No	N/A
	Pointon 2022	No	N/A	No	N/A	Yes	No	No	N/A
Gross motor function classification	Mishra 2015	Yes	N/A	No	N/A	No	N/A	TSFA (DTI) in corticospinal tract	Yes

score (GMFCS)	Pointon 2022	No	N/A	No	N/A	Yes	No	No	N/A
Falls Efficacy Scale-International (FES-I)	Gassner 2021	Yes	Yes	No	N/A	No	N/A	SPRS 10MWT, 2MWT, TUG SF-12	Yes. No for total SF-12 (but yes for PCS)
	Regensburger 2022	Yes	N/A	Yes	N/A	No	N/A	Gait parameters (stride and stance time)	Yes
Berg balance scale	Bertolucci 2015	No	N/A	No	N/A	Yes	Yes	No	N/A
	Lith 2019	No	N/A	No	N/A	Yes	No	No	N/A
Composite cerebellar functional severity score (CCFSw)	Du Montcel 2008	Yes	Yes	No	N/A	No	N/A	UHDRS-IV VAS (EQ5D)	Yes
	Du Montcel 2012	No	N/A	Yes	No	No	N/A	AMBUS	Yes
Nine hole pegboard test, click test, writing test, tapping test	DuMontcel 2008	Yes	No	No	N/A	No	N/A	No	N/A
	Uygunoglu 2016	No	N/A	No	N/A	Yes	Yes (left hand) No (right hand)	No	N/A
Physiological Cost Index (increase in HR per metre walked, estimate of oxygen	Bertolucci 2015	No	N/A	No	N/A	Yes	No	No	N/A
	Schols 2017	Yes	N/A	Yes	N/A	Yes	No	No	N/A

consumption)									
Four-stage functional scale of motor disability (4SMD or 4FMS)	Orsucci 2014	No	N/A	No	N/A	No	N/A	SPRS, SF-36	Yes
	Jordan 2021	No	N/A	No	N/A	No	N/A	CPCHILD	Yes
Activities specific Balance Confidence scale (ABC)	Lith 2019	No	N/A	No	N/A	Yes	No	No	N/A
Modified version of Gillette Functional Assessment Questionnaire	Margetis 2014	No	N/A	No	N/A	Yes	Yes	No	N/A
Walking Handicap scale	Paparella 2020	No	N/A	No	N/A	Yes	Yes	No	N/A
Lower extremity subclass of Fugl-Meyer assessment	Bastani 2021	No	N/A	No	N/A	Yes	No	No	N/A
Ambulatory score (AMB)	Scheuer 2007	No	N/A	No	N/A	Yes	No	No	N/A
AMBUS (distance in	DuMontcel 2012	No	N/A	Yes	N/A	No	N/A	SARA CCFSw	Yes

metres walked in 5 sec without help)									
Spasm frequency Scale	Kai 2014	No	N/A	Yes	Yes (post Rx)	Yes	Yes	No	N/A
Strength with microFET 2 hand-held dynanometer	Antczak 2019	No	N/A	No	N/A	Yes	Yes	No	N/A
Walking ability (landmarks of disability)	Klimpe 2012	No	N/A	No	N/A	No	N/A	SF-36	Yes
PerfOMs – Cognition									
Montreal Cognitive Assessment (MoCA)	Gassner 2021	Yes	N/A	No	N/A	No	N/A	No	N/A
	Rattay 2022	Yes	No	No	N/A	No	N/A	No	N/A
	Rattay 2019	Yes	No	No	N/A	No	N/A	SPRS	No
	Regensburger 2022	Yes	N/A	Yes	N/A	No	N/A	Gait stride parameters	Yes for coefficient of variation but not absolute values.
WAIS-R	Erichsen 2009	Yes	No	No	N/A	No	N/A	CVLT-T (multiple neuropsych tests performed)	Yes
	Musacchio 2018	No	N/A	Yes	Yes	No	N/A	No	N/A

	Martinuzzi 2016	No	N/A	No	N/A	No	N/A	No	N/A
MMSE	Navas-Sanchez 2021	Yes	N/A	No	N/A	No	N/A	No	N/A
	Lin 2021	Yes	N/A	No	N/A	No	N/A	CSF 27OHC, MMSE	No
Addenbrooke's Cognitive Examination Revised (ACE-R)	Faber 2018	Yes	N/A	Yes	N/A	No	N/A	SPRS	Yes
CANTAB Cognitive Assessment Software	Rattay 2019	Yes	No	No	N/A	No	N/A	No	N/A
VLMT, FWIT, TMT A/B, FAB, d2-R, RWT	Musacchio 2018	No	N/A	Yes	Yes	No	N/A	No	N/A
PROMs – Quality of life									
SF-36, SF-12, RAND 36-Item Health Survey	Bastani 2021	No	N/A	No	N/A	Yes	No	No	N/A
	Bertolucci 2015	No	N/A	No	N/A	Yes	Yes	No	N/A
	Braschinsky 2011	Yes (previously published values)	Yes	No	N/A	No	N/A	No	N/A
	Gassner 2021	Yes	Yes	No	N/A	No	N/A	FES-I, SPRS TUG, 10MWT, MWT	Yes Yes for PCS but not total

	Klimpe 2012	No	N/A	No	N/A	No	N/A	SPRS, Landmarks of disability	Yes
	Orsucci 2014	No	N/A	No	N/A	No	N/A	4SMD, SPRS	Yes
	Regensburger 2022	Yes	N/A	Yes	N/A	No	N/A	Gait parameters	Yes for PCS
EQ-5D	DuMontcel 2008	Yes	N/A	No	N/A	No	N/A	CCFS	Yes
	Rattay 2019	Yes	Yes	No	N/A	No	N/A	Maximum gait distance BPI BDI-V	Yes No (RLS)
	Rattay 2022	Yes	No (pre SPG4)	No	N/A	No	N/A	No	N/A
Becks Depression Inventory (BDI-V)	Rattay 2022	Yes	No (pre SPG4)	No	N/A	No	N/A	No	N/A
	Rattay 2019	Yes	Yes	No	N/A	No	N/A	EQ5D	Yes
Visual analogue score (for QoL)	Paparella 2020	No	N/A	No	N/A	Yes	Yes	No	N/A
	Scheuer 2007	No	N/A	No	N/A	Yes	No	No	N/A
Patient Health Questionnaire (PHQ-9)	DuMontcel 2008	Yes	N/A	No	N/A	No	N/A	No	N/A
Modified Goal Attainment Scale (mGAS)	Pointon 2022	No	N/A	No	N/A	Yes	Yes	No	N/A
ICIQ-LUTSqol	Schneider 2019	No	N/A	No	N/A	No	N/A	ICIQ-SF, SCOPA-AUT	Yes

Cerebral Palsy QoL questionnaire (CPQoL)	Pointon 2022	No	N/A	No	N/A	Yes	Improved in 2/5 participants	No	N/A
CPCHILD questionnaire	Jordan 2021	No	N/A	No	N/A	No	N/A	4FMS	Yes
ZUNG depression score	Gassner 2021	Yes	Yes	No	N/A	No	N/A	No	N/A
ICIQ-SF	Schneider 2019	No	N/A	No	N/A	No	N/A	ICIQ-LUTSqoL	Yes
Hospital Anxiety and Depression Scale (HADS)	Bertolucci 2015	No	N/A	No	N/A	Yes	Yes	No	N/A
PROMs – Other									
Brief pain inventory	deLima 2021	No	N/A	No	N/A	Yes	No	No	N/A
	Rattay 2019	Yes	Yes	No	N/A	No	N/A	EQ5D	Yes
	Rattay 2022	Yes	No	No	N/A	No	N/A	No	N/A
Modified Fatigue Impact Scale (MFI)	deLima 2021	No	N/A	No	N/A	Yes	No	No	N/A
	Rattay 2019	Yes	Yes	No	N/A	No	N/A	EQ5D	Yes
Multidimensional fatigue inventory	Rattay 2022	Yes	No	No	N/A	No	N/A	No	N/A
SCOPA-AUT	Schneider 2019	No	N/A	No	N/A	No	N/A	ICIQ-LUTSQoL	Yes
Numeric rating scale for pain	Paparella 2020	No	N/A	No	N/A	Yes	Yes	No	N/A
Biochemical/Lab studies									

Serum and CSF neurofilament light chain	Kessler 2021	Yes	Yes (CSF NfL)	No	N/A	No	N/A	Age Gender	Yes
	Kessler 2022	Yes	Yes (Serum NfL)	Yes	No	No	N/A	Age SPRS	Yes No
	Lassmann 2022	Yes	Yes	No	N/A	No	N/A	Gait parameter (foot angle and RoM)	Yes (serum NfL) No (CSF NfL)
	Lin 2021	No (historical controls)	N/A (Yes compared to historical controls)	No	N/A	No	N/A	Spinal cord area, SPRS, MMSE CSF 27-OHC	No Yes (27-OHC)
	Rattay 2022	Yes	Serum - Yes for symptomatic SPG4, No for presymptomatic SPG4 CSF- yes for both	No	N/A	No	N/A	SPRS	Yes (CSF NfL)
	Wilke 2018	Yes	Yes	No	N/A	No	N/A	Disease progression (SPRS/disease duration)	Yes
Serum and CSF 24-OHC, 25-OHC and 27-OHC and 3b-CA	Lin 2021	No (previously reported healthy controls)	N/A (Yes compared to prev HC)	No	N/A	No	N/A	Spinal cord area, SPRS, MMSE CSF NfL	No Yes (Nfl)
	Prestsaeter 2020	No (reference range used)	N/A (Yes compared to reference range)	No	N/A	No	N/A	No	N/A

	Schols 2017	Yes	Yes for all except 24-OHC	Yes	N/A	Yes	Yes for serum 27-OHC, 25-OHC and 24 OHC (No for CSF or 3b-CA)	SPRS	Yes for 27-OHC only
	Marelli 2018	Yes	Yes (plasma 27-OHC and 25-OHC) No (24S-OHC)	Yes	No	Yes	Yes (plasma 27-OHC and 24S-OHC) No (25-OHC)	No	N/A
Blood(plasma) and CSF amino acids	Coutelier 2015	Yes (historical controls)	Yes (ornithine, citrulline, arginine, proline)	No	N/A	No	N/A	No	N/A
	Growdon 1991	No	N/A	No	N/A	Yes	No (plasma and CSF glycine)	No	N/A
	Marelli 2020	No	N/A Low citrulline compared to reference value	No	N/A	No	N/A	No	N/A
Lipidomics: fibroblast and plasma	Vaz 2019	Yes	Yes (plasma PC etherphospholipids)	No	N/A	No	N/A	No	N/A
Glycosylceramide profile	Kloth 2020	No	Increased compared to reference values	No	N/A	No	N/A	No	N/A

Neurofilament heavy chain: CSF and serum	Simonini 2021	No (but differentiated from ALS and UMNp)	N/A	No	N/A	No	N/A	Progression rate and total ALSFRS-R score (for whole group including ALS)	Yes
	Zucchi 2018	Yes	Yes	Yes	N/A	No	N/A	ALSFRS-R total score and progression rate, Ashworth scale (for whole group including ALS)	Yes
ATG9A ratio	Ebrahimi-Fakhari 2021	Yes	Yes	No	N/A	No	N/A	No	N/A
CSF A beta 1-42, total tau, phosho tau	Rattay 2022	Yes	No	No	N/A	No	N/A	No	N/A
mtDNA levels; Muscle biopsy	DelaCasa-Fages 2019	Yes	Yes	No	N/A	No	N/A	Age of onset, degree of spasticity, disability scale	No
Cell morphometry	Wali 2021	Yes	Yes	No	N/A	Yes	Yes (SPAST only)	No	N/A
Scanning electron	Rattay 2019	Yes	Yes	No	N/A	No	N/A	No	N/A

microscopy of hair shafts									
Imaging									
MRI brain and spine, volumetry with neural network analysis, automated segmentation of subcortical structures	Chrestian 2016,	No	N/A	No	N/A	No	N/A	SPRS	Yes
	DelaCasa-Fages 2019	No	N/A	No	N/A	No	N/A	No	N/A
	Ebrahimi-Fakhari 2021	Yes	N/A	No	N/A	No	N/A		
	Faber 2018	Yes	Yes (cortical thickness, deep gray matter volume, spinal cord area)	Yes	N/A	No	N/A	ACE-R, SPRS, disease duration	Yes
	Lin 2021	Yes	Yes (spinal cord area) No (cerebral atrophy)	No	N/A	No	N/A	CSF Nf, 27-OHC	No
	Marelli 2018	Yes	N/A	Yes	N/A	Yes	N/A	No	N/A
	Martinuzzi 2016	No	N/A	No	N/A	No	N/A	No	N/A
	Montanaro 2020	Yes	Yes (right prefrontal cortx & B/L thalami vol)	Yes	No	No	N/A	SPRS, disease duration	No
	Navas-Sanchez 2021	Yes	Yes (thalamic atrophy)	No	N/A	No	N/A	SPRS Disease duration	Yes
	Orsucci 2014	No	N/A	No	N/A	No	N/A	No	N/A
	Prestsaeter 2020	No	N/A	No	N/A	No	N/A	No	N/A

	Rattay 2019	No	N/A	No	N/A	No	N/A	No	N/A
	Rattay 2022	Yes	Yes	No	N/A	No	N/A	No	N/A
	Rezende 2015	Yes	No (cortical thickness) Yes (spinal cord atrophy)	No	N/A	No	N/A	DTI (FA R CST, spinal cord area)	Yes
	Sarret 2016	No	N/A	Yes	Yes (atrophy score, cerebellar volume)	No	N/A	No	N/A
	Schuurs-Hoeijmakers 2012	No	N/A	No	N/A	No	N/A	No	N/A
	Simonini 2021	No	N/A	Yes	N/A	No	N/A	No	N/A
DTI	Aghakhanyan 2014	Yes	Yes(FA, MD, RD)	No	N/A	No	N/A	No	N/A
	Faber 2018	Yes	Yes (FA, MD, AD, RD)	Yes	N/A	No	N/A	ACE-R	Yes
	Martinuzzi 2016	Yes	Yes	No	N/A	No	N/A	SPRS	Yes
	Mishra 2015	Yes	Yes (lower FA)	No	N/A	No	N/A	GMFCS	Yes for DTFA in 1 section of CST
	Montanaro 2020	Yes	Yes	Yes	Yes	No	N/A	SPRS	No
	Rezende 2015	Yes	Yes	No	N/A	No	N/A	SPRS (TBSS but not tractography) Spinal cord area	Yes
	Unrath 2010	Yes	Yes	No	N/A	No	N/A	No	N/A

MR spectroscopy	Erichsen 2009	Yes	Yes (Cho/Cr ratio)	No	N/A	No	N/A	CVLT	Yes (Cho/Cr ratio)
	Martinuzzi 2016	Yes	No	No	N/A	No	N/A	No	N/A
	Montanaro 2020	Yes	Yes	Yes	No	No	N/A	No	N/A
	Schuurs-Hoeijmakers 2012	No	N/A (abnormal lipid peak in basal ganglia and thalamus in 5/5 patients)	No	N/A	No	N/A	No	N/A
Ioflupane SPECT	DelaCasa-Fages 2019	No	N/A (abnormal in 2/3 patients)	No	N/A	No	N/A	No	N/A
Neurophysiology									
MEPs	Antczak 2019	No	N/A	No	N/A	Yes	No	Strength post-rTMS	Lower MT correlated with bigger improvement in strength with rTMS
	Ardolino 2021	No	N/A	No	N/A	Yes	No	No	N/A
	Brighente 2021	Yes	Yes	No	N/A	No	N/A	Age, disease duration, SPRS	No
	Karle 2013	No (used normal values)	N/A	No	N/A	No	N/A	SPRS	Yes (UL and LL CMCT)
	Lassmann 2022	Yes	No	No	N/A	No	N/A	Gait parameters	Yes

								(leg RoM, foot angle)	
	Martinuzzi 2016	No	N/A	No	N/A	No	N/A	No	N/A
	Musacchio 2018	No	N/A	Yes	N/A	No	N/A	No	N/A
	Rattay 2019	No	N/A	No	N/A	No	N/A	No	N/A
	Scheuer 2007	No	N/A	No	N/A	Yes	No	No	N/A
	Simonini 2021	No	N/A	Yes	N/A	No	N/A	No	N/A
NCS/EMG	Ardolino 2021 (H-reflex, F-waves)	No	N/A	No	N/A	Yes	No	No	N/A
	DelaCasa-Fages 2019	No	N/A	No	N/A	No	N/A	No	N/A
	Faber 2018	Yes	N/A	Yes	N/A	No	N/A	No	N/A
	Karle 2013	No	N/A	No	N/A	No	N/A	CMCT	Yes (NCV and F wave latency)
	Martinuzzi 2016	No	N/A	No	N/A	No	N/A	No	N/A
	Musacchio 2018	No	N/A	Yes	N/A	No	N/A	No	N/A
	Prestsaeter 2020	No	N/A	No	N/A	No	N/A	No	N/A
	Rattay 2019	No	N/A	No	N/A	No	N/A	No	N/A
	Rattay 2022	Yes	No	No	N/A	No	N/A	No	N/A
SSEP	Brighente 2021	Yes	Yes (LL only)	No	N/A	No	N/A	Age, disease duration SPRS	Yes No
	Karle 2013	No	N/A	No	N/A	No	N/A	No	N/A
	Martinuzzi 2016	No	N/A	No	N/A	No	N/A	No	N/A

	Prestsaeter 2020	No	N/A	No	N/A	No	N/A	No	N/A
	Rattay 2019	No	N/A	No	N/A	No	N/A	No	N/A
VEP	Prestsaeter 2020	No	N/A	No	N/A	No	N/A	No	N/A
	Rattay 2019	No	N/A	No	N/A	No	N/A	No	N/A
BAEP	Prestsaeter 2020	No	N/A	No	N/A	No	N/A	No	N/A
Other biomarkers									
Laboratory and mobile gait analysis	Adair 2016	No (normal values)	Yes (abnormal Gait Progile Score and Gait variable scores)	No	N/A	No	N/A	No	N/A
	Bonnefoy-Mazure 2013	Yes	Yes (normalized gait velocity, lower limb, thorax, pelvis and spine kinematics)	No	N/A	No	N/A	No	N/A
	Henderson 2019	No	N/A	No	N/A	Yes	No	No	N/A
	Lith 2019	No	N/A	No	N/A	Yes	Yes (comfortable gait speed) No (Gait width, maximal gait speed)	No	N/A

	Lassman 2022	Yes	Yes	No	N/A	No	N/A	SPRS, serum NfL, MEP CMCT	Yes No (CSF NfL)
	Pointon 2022	No	N/A	No	N/A	Yes	No	No	N/A
	Regensburger 2022	Yes	Yes	Yes	Yes for stride time, stance time and swing duration in cHSP subgroup.	No	N/A	SPRS, SF-12 PCS, FES-I	Yes
	Zhang 2014	Yes (historical)	Generally no but yes for some parameters (e.g. abnormal hip extension, increased hip int rotation)	No	N/A	Yes	Yes for walking speed, hip rot, knee rot, but no for cadence, step length/time, hip, knee, ankle F/E, Abd/Ad	No	N/A
SD-OCT Retinal nerve fibre layer	Klebe 2012	No	N/A (optic atrophy present in 10/10 patients examined)	No	N/A	No	N/A	No	N/A
	Musacchio 2018	No	N/A	Yes	N/A	No	N/A	No	N/A
	Prestsaeter 2020	No	N/A	No	N/A	No	N/A	No	N/A

	Vavla 2019	No (historical normative values)	Statistically significant reduction of RNFL thickness in SUP, INF, NAS)	Yes (subgroup of patients)	No (avg F/U 10.7 months)	No	N/A	SPRS	No
Video-oculography and rotational chair testing	Milenkovic 2019	Yes	Yes (mean peak velocity vertical saccades)	No	N/A	No	N/A	No	N/A
RoM using goniometer	Braschinsky 2009	No	N/A	No	N/A	No	N/A	10MWT MAS	Yes
	Lith 2019	No	N/A	No	N/A	Yes	Yes	No	N/A
Instrumented dynamic balance assessment	Lith 2019	No	N/A	No	N/A	Yes	Yes (known direction) No (unknown direction)	No	N/A
Urodynamic assessment	Joussain 2019	No	N/A (majority had neurogenic detrusor overactivity)	No	N/A	No	N/A	No	N/A
Video supported posturography	Musacchio 2018	No	N/A	Yes	N/A	No	N/A	No	N/A
Protocol for Evaluation of Acquired Speech	Olchik 2021	Yes	Yes (abnormal resonance, phonation,	No	N/A	No	N/A	No	No

Disorders (PADAF)			breathing, articulation)						

Siow et al Scoping Review Supplementary Material 3

Table 3A: Comparison of HSP Randomized Controlled Trials

	Growdon 1991	Scheuer 2007	Schols 2017	Antczak 2019	Ardolino 2021	Bastani 2021	deLima 2021	Van de Venis 2023
Aim	To assess the clinical effect of L-threonine in patients with HSP.	To investigate the effects of gabapentin on clinical disability and motor cortical excitability in SPG4-linked hereditary spastic paraplegia patients.	To perform a detailed clinical and biochemical analysis in 34 genetically confirmed SPG5 cases from 28 families, study dose-dependent neurotoxicity of oxysterols in human cortical neurons and perform a randomized placebo-controlled double blind interventional trial targeting oxysterol accumulation in serum of SPG5 patients. 2 phase: cross sectional study and randomized controlled trial	To investigate the therapeutic potential of bilateral, high-frequency repetitive TMS over primary motor areas for muscles of lower extremities in various forms of HSP (pure, complicated, AMN). RCT, crossover design. First study using rTMS for therapeutic purposes in HSP.	Double-blind, randomised crossover and sham-controlled study to evaluate the effects of tsDCS on spasticity in patients with HSP	To study the effectiveness of rTMS as palliative measure for lower limb spasticity, function and gait improvement in patients with HSP	To evaluate the efficacy and safety of BoNT-A in the treatment of lower limb spasticity in patients with HSP.	To assess efficacy of 5-week gait adaptability training in people with pure HSP
Number of participants	18 (8 declined lumbar puncture)	10	14	15 recruited (1 drop out)	11	8	55 enrolled (6 dropped out)	36
Genotype	Not specified	SPG4	SPG5	1 SPG3A 1 SPG7 3 AMN 3 AMN carrier 7 unknown (5 pure, 2 complex)	8 SPG4 1 SPG3A 1 SPG7 1 SPG15	Not specified	21 SPG4 4 SPG8 2 SPG3A 2 SPG11 2 SPG33 3 SPG72 1 SPG6 1 SPG7 1 SPG15 1 SPG28 1 SPG48	Unknown

							1 PLA2G6 1 X-linked adrenoleukodystrophy 1 autosomal-recessive spastic ataxia of Charlevoix-Saguenay 13 unknown	
Inclusion Criteria	Clinical diagnosis of HSP	Clinically affected. Genetic linkage to SPG4. Age 18-68yo.	Clinical diagnosis of HSP. Genetically confirmed SPG5. >10 years old.	Age >18 years old. Diagnosis of HSP and AMN confirmed by genetic testing or family history or by exclusion. Able to walk 10 meters with or without crutches.	Diagnosis of HSP	Fink criteria (definitely or probably affected with HSP). Other possible causes ruled out with CNS MRI, HTLV1 Ab titre, VDRL, vitamin B12, plasma VLCFAs, EMG, NCS.	Diagnosis of HSP based on either genetic testing or family history along with clinical features. 18-80 years of age. Able to walk at least 14m without stopping (assistive devices permitted).	Pure HSP diagnosed by movement disorder specialist, 18-70 years old, able to walk barefoot on level ground without a walking aid (orthopedic devices allowed)
Intervention	L-threonine: Patients received L-threonine at 1.5g TDS (n=7) or 2g TDS (n=11) for 2 weeks, 2-week washout period, then 2 week placebo. 2 groups receiving L-threonine>placebo or placebo>L-threonine.	Gabapentin commenced at 2400mg daily, gradually titrated to 4000mg over 10 days. 2 months treatment with gabapentin, 10 days washout, then 1 month drug free interval, 2 months placebo. Cross over design: 2 groups of placebo>gabapentin and gabapentin>placebo.	Atorvastatin 40mg/day for 9 weeks (n=7) or placebo for 9 weeks (n=7).	Repetitive transcranial magnetic stimulation (rTMS): 5 stimulating sessions, 1 a day during consecutive working days or sham rTMS. Crossover design, 8 patients received active treatment first.	Spinal direct current stimulation (tsDCS): 20 minute session twice a day 5 days a week with programmable stimulator or sham tsDCS. At least 3 months apart. Cross over design: 2 groups of tsDCS>sham and sham>tsDCS.	Repetitive transcranial magnetic stimulation (rTMS): 5 sessions rTMS on 5 consecutive days in a week (n=4) and sham rTMS (n=4).	Botulinum toxin type A (BoNT-A): Single treatment with BoNT-A or placebo(0.9% sodium chloride solution) with 8wk follow up. Crossover with 24-28 weeks washout. Total BoNT-A dose 400 units (100 units in each adductor magnus and 100 units in each triceps surae).	Gait adaptability training on treadmill with augmented reality: 60 minutes twice a week for 5 weeks (Total 10 hours). Cross-over design: Participants randomized to intervention group (n=18) or waiting list control group (n=18). Following assessment after first 5 week period, the intervention group enters follow-up period and control groups starts 5-week of gait adaptability training.
Duration of intervention	2 weeks	2 months	9 weeks	5 days	5 days	5 days	8 weeks	5 weeks

Duration of intervention	2 weeks	2 months	9 weeks	5 days	5 days	5 days	8 weeks	5 weeks
Primary outcome measure	Disability score based on: Leg strength, leg tone, leg deep tendon reflexes, clonus, walking, hopping on right and left foot, running. Physician overall clinical assessment. Patient self-evaluation of response.	Primary vs Secondary not defined. Clinical measures: MS Impairment Scale, SPATAX diagnostic form, lower limb patellar reflexes, muscle strength, muscle tone, disability score, ambulatory scoring (number of meters travelled in 5 sec without aid). Self-reported parameters: severity of sphincter disturbances, Visual Analogue Scale score of quality of life. Paired-transcranial magnetic stimulation (P-TMS). Blood gabapentin levels.	Change of 27-OHC in serum after 9 weeks.	10-meter walk test (10MWT)	Primary vs Secondary not defined. Ashworth scale, 5-minute walking test (n=9), motor evoked potentials (resting motor threshold, onset latency, AUC of motor response), H-reflex tibial nerve, F-waves abductor hallucis, SPRS (n=9)	Modified Ashworth Scale.	10-meter walk test (10MWT) to calculate maximal gait velocity.	Gait adaptability assessed with obstacle subtask of E-FAP
Secondary outcome measures	Amino acid analysis in blood and CSF.		Change of 27-OHC in CSF. Reduction of 24-OHC, 25-OHC and 3β-CA levels in serum and CSF. Serum bile acids. SPRS, 3-min endurance walk, physical cost index.	MEP amplitude, CMCT, CSP. Timed up and go test (TUG), strength measured with microFET 2 handheld dynamometer, modified Ashworth scale		Lower extremity subclass of Fugl-Meyer assessment. 10 meter walk test. SF-36 Persian translation.	SPRS Change in modified Ashworth scale and MRC for adductor and triceps surae. Brief pain inventory. Modified Fatigue Impact Scale. Subjective perception of symptoms, or benefit, or gait improvement	Mini Balance Evaluation Test, Activities-specific Balance Confidence scale, Walking Adaptability Ladder Test (WALT), 10 meter Walk Test (10MWT), 3-D gait analysis (Vicon Motion Systems)

Results	**Statistically significant improvement in disability scores** with L-threonine compared to placebo. Physician's overall clinical assessment and patients' self-evaluation of response demonstrated no significant difference between threonine and placebo. L-threonine increased the mean blood and CSF threonine levels, no change in mean glycine levels in plasma or CSF with L-threonine vs placebo. Overall, L-threonine did not produce clinically valuable improvement in motor unction although there was improvement in mean spasticity rating scores.	No statistical differences between gabapentin and placebo groups in median scores of self-reported and clinical ratings. Blood showed increase in S-gabapentin in all patients during gabapentin treatment. No evidence of altered intracortical excitability (compared average amplitude ratios) with P-TMS in both groups.	**Atorvastatin significantly reduced serum 27-OHC compared to placebo.** Serum cholesterol lowered by 40% in atorvastatin but not placebo group. Atorvastatin but not placebo reduced serum 24S-OHC and 25-OHC. 3β-CA levels showed large variability in both groups with no significant difference between groups. No significant difference in changes of CSF 24S-OHC, 27-OHC or 3β-CA in both groups. No effect of atorvastatin on SPRS or 3-min endurance walk or physical cost index.	No effect of rTMS in 10MWT and TUG. Strength of proximal and distal muscles of lower extremities increased, and spasticity of proximal muscles decreased after rTMS. Big range of motor threshold values, change of strength showed inverse correlation with mean MT, change in spasticity showed no significant correlation.	**Ashworth scale for lower limbs improved in anodal compared to sham group**, in particular up to 2 months following end of stimulation. Significant improvement in hip flexion and knee extension. No significant change in 5MWT, MEP variables, H-reflex, F-waves and SPRS between groups.	**rTMS had lower MAS scores at end of rTMS and end of follow up compared with sham group**. No difference between both groups in Lower extremity subclass of Fugl-Meyer assessment, 10MWT and SF-36.	No change in maximal gait velocity (10MWT), SPRS, MRC(strength), BPI, MFIS or subjective perception between treatment and placebo groups. Reduction in adductor muscle tone (MAS) in treatment compared to placebo but no significant change in triceps surae muscle.	Time required to perform E-FAP obstacle subtask did not decrease more in intervention compared to control group. Non-significant results found for secondary outcomes except for single run of WALT. 5-weeks of gait adaptability training does not lead to greater improvement of gait adaptability compared to usual care.

Siow et al Systematic Review Supplementary Material

1 Appendix 1 – Search Strategies

Searches performed on 13 March 2019

1.1 Database: PubMed

Search strategy:

((((hereditary spastic paraplegia) OR spastic paraparesis) OR HSP)) AND ((((((motor evoked potential) OR MEP) OR transcranial magnetic stimulation) OR TMS) OR central motor conduction time) OR CMCT)

Results: 121

1.2 Database: Scopus

ALL (("hereditary spastic parap*" OR "spastic parap*" OR "HSP") AND ("motor evoked potential*" OR "MEP" OR "transcranial magnetic stimulation" OR "TMS" OR "central motor conduction*" OR "CMCT"))

Limited to journals, English, article

Results: 547

1.3 Database: Embase

Search Strategy:

[("hereditary motor sensory neuropathy/" OR "spastic paraplegia/" OR "spastic paraparesis.mp." OR "spastic para*.mp." OR "HSP.mp.") AND ("motor evoked potential/" OR "motor evoked potential*.mp." OR "MEP.mp." OR "transcranial magnetic stimulation/" OR "transcranial magnetic stimulation*.mp." OR "TMS.mp." OR "central motor conduction time*.mp." OR "CMCT.mp.")]

Limit to (human and english language)

Results: 146

1.4 Database: Ovid MEDLINE(R)

Search Strategy:

[(“Spastic Paraplegia, Hereditary/” OR “Paraparesis, Spastic/” OR “spastic paraparesis.mp.” OR “spastic para*.mp.” OR “HSP.mp.”) AND (“Evoked Potentials, Motor/” OR “motor evoked potential*.mp.” OR “MEP.mp.” OR “Transcranial Magnetic Stimulation/” OR “transcranial magnetic stimulation*.mp.” OR “TMS.mp.” OR “central motor conduction time*.mp.” OR “CMCT.mp.”)]

Limit to (english language and humans)

Results: 68

2 Appendix 2 - Data Collection Form

2.1 Study Characteristics

- Study ID
- Study Author
- Year of publication
- Reviewer
- Study type
- Patient number
- Genotype known
- Genotype unknown
- Males
- Females
- Mean age
- Age SD or Range
- Number of controls
- Other patient details
- Clinical scales used

2.2 Neurophysiological Techniques

- Stimulator
- Coil
- Stimulation intensity
- Number of stimuli
- Muscles studied
- Other parameters studied
- CMCT calculation method

2.3 Results

- Results - UL CMCT
- Results - LL CMCT
- Results – Amplitude
- Results – resting MT
- Results - Correlation
- Results – Other

3 Appendix 3

3.1 NIH Study Quality Assessment Tool (* items not included as not relevant to current study)

3.1.1 Quality Assessment of Case-Control Studies

1. Was the research question or objective in this paper clearly stated and appropriate?
2. Was the study population clearly specified and defined?
3. Did the authors include a sample size justification?
4. Were controls selected or recruited from the same or similar population that gave rise to the cases (including the same timeframe)?
5. Were the, inclusion and exclusion criteria, algorithms or processes used to identify or select cases and controls valid, reliable, and implemented consistently across all study participants?
6. Were the cases clearly defined and differentiated from controls?
7. If less than 100 percent of eligible cases and/or controls were selected for the study, were the cases and/or controls randomly selected from those eligible?*
8. Was there use of concurrent controls?
9. Were the investigators able to confirm that the exposure/risk occurred prior to the development of the condition or event that defined a participant as a case?*
10. Were the measures of exposure/risk clearly defined, valid, reliable, and implemented consistently (including the same time period) across all study participants?*
11. Were the assessors of exposure/risk blinded to the case or control status of participants?
12. Were key potential confounding variables measured and adjusted statistically in the analyses? If matching was used, did the investigators account for matching during study analysis definitions?

Author	Year	1	2	3	4	5	6	8	11	12	TOTAL (9)
Thompson	1987	1	1	0	0	1	1	1	0	0	5
Claus	1990	1	1	0	0	1	1	0	0	0	4
Schady	1991	1	1	0	1	1	1	1	0	0	6
Sue	1997	0	1	0	0	0	1	1	0	0	3
Di Lazarro	1999	1	1	0	1	1	1	unclear	0	1	6
Bonsch	2003	1	1	0	1	1	1	unclear	0	1	6
Sartucci	2007	1	1	0	1	1	1	1	0	0	6
Fisher	2013	1	1	0	1	1	1	unclear	0	0	5
Oguz	2013	1	1	0	1	1	1	1	0	0	6
Giananneschi	2014	1	1	0	1	1	1	unclear	0	1	6
Geevasinga	2015	1	1	0	0	1	1	unclear	0	1	5
Denton	2016	1	1	0	0	1	1	1	0	1	6
Martinuzzi	2016	1	1	0	0	1	1	0	0	0	4

Table 3.1.1 Scores for quality assessment of case-control studies

3.1.2 Quality Assessment Tool for Case Series Studies

1. Was the study question or objective clearly stated?
2. Was the study population clearly and fully described, including a case definition?
3. Were the cases consecutive?
4. Were the subjects comparable?
5. Was the intervention clearly described?
6. Were the outcome measures clearly defined, valid, reliable, and implemented consistently across all study participants?
7. Was the length of follow-up adequate?*
8. Were the statistical methods well-described?
9. Were the results well-described?

Author	Year	1	2	3	4	5	6	8	9	TOTAL (8)
Pelosi	1991	1	1	0	1	1	1	0	1	6
Polo	1993	1	1	0	1	1	1	0	1	6
Nielsen	1998	1	1	0	1	1	1	1	1	7
Cruz	1999	0	1	1	1	1	1	0	1	6
Nardone	2003	1	1	0	1	1	1	1	1	7
Schulte	2003	1	1	0	1	0	0	0	0	3
Klebe	2004	1	1	0	1	1	1	1	1	7
Orlacchio	2005	1	1	1	1	0	0	0	1	5
Winner	2006	1	1	0	0	0	0	0	0	2
Liu	2008	1	1	1	1	0	1	0	0	5
Serranova	2008	1	1	1	1	1	1	1	1	8
Orlacchio	2008	0	0	0	0	0	0	0	0	6
Liu	2009	1	1	1	1	0	1	0	1	6
Schule	2009	1	1	1	1	0	0	0	0	4
Battini	2011	1	1	0	1	0	1	1	1	6
Manganelli	2011	1	1	1	1	1	1	1	1	8
Karle	2013	1	1	0	0	1	0	1	1	5
Roos	2014	1	1	1	1	0	0	0	0	4
Rinaldi	2015	1	1	0	0	0	0	0	0	2

Table 3.1.2 Scores for quality assessment of case series studies

3.2 Chipchase TMS checklist **(* items not included as not relevant to current study)**

Were the following participant factors reported/controlled?

1. Age of subjects
2. Gender of subjects
3. Handedness of subjects
4. Subjects prescribed medication
5. Use of CNS active drugs
6. Presence of neurological/psychiatric disorders when studying healthy subjects *
7. Any medical conditions
8. History of specific repetitive motor activity *

Were the following methodological factors reported/controlled?

9. Position and contact of EMG electrodes
10. Amount of relaxation/contraction of target muscles
11. Prior motor activity of the muscle to be tested
12. Level of relaxation of muscles other than those being tested
13. Coil type (size and geometry)
14. Coil orientation
15. Direction of induced current in the brain
16. Coil location and stability (with or without a neuronavigation system)
17. Type of stimulator used (e.g. brand)
18. Stimulation intensity
19. Pulse shape (monophasic or biphasic)
20. Determination of optimal hotspot
21. The time between MEP trials
22. Time between days of testing *
23. Subject attention (level of arousal) during testing
24. Method for determining threshold (active/resting)
25. Number of MEP measures made
26. Paired pulse only: Intensity of test pulse *
27. Paired pulse only: Intensity of conditioning pulse *
28. Paired pulse only: Inter-stimulus interval *

Were the following analytical factors reported/controlled?

29. Method for determining MEP size during analysis
30. Size of unconditioned MEP

Author	Year	1	2	3	4	5	7	9	10	11	12	13	14
Thompson	1987	1	1	0	0	0	0	1	1	0	1	1	0
Claus	1990	1	1	0	0	0	1	1	1	0	0	1	1
Pelosi	1991	1	1	0	0	0	0	1	1	0	0	1	1
Schady	1991	1	1	0	0	0	1	1	1	0	1	1	1
Polo	1993	1	0	0	0	0	0	1	0	0	0	1	0
Sue	1997	1	1	0	0	0	0	1	1	0	1	1	0
Nielsen	1998	0	0	0	0	0	0	1	1	0	1	0	0
Cruz	1999	1	1	0	0	0	0	1	1	0	0	0	0
Di Lazarro	1999	0	0	0	0	0	0	1	1	0	0	1	1
Bonsch	2003	1	1	0	0	0	0	1	0	0	0	1	0
Nardone	2003	0	1	0	0	0	0	1	1	0	0	1	0
Schulte	2003	1	1	0	0	0	0	1	0	0	0	0	0
Klebe	2004	1	1	0	0	0	0	1	1	0	0	1	1
Orlacchio	2005	1	1	0	0	0	0	1	0	0	0	0	0
Winner	2006	1	1	0	1	0	0	0	0	0	0	0	0
Sartucci	2007	1	1	0	0	0	0	1	1	0	1	1	1
Orlacchio	2008	0	0	0	0	0	0	1	0	0	0	0	0
Serranova	2008	1	1	0	0	0	1	1	1	0	1	1	0
Liu	2008	1	0	0	0	0	0	1	0	0	0	0	0
Liu	2009	1	1	0	0	0	0	1	0	0	0	0	0
Schule	2009	0	0	0	0	0	0	0	0	0	0	0	0
Battini	2011	1	1	0	0	0	0	0	0	0	0	0	0
Manganelli	2011	1	1	0	0	0	0	1	0	0	0	0	0
Fisher	2013	1	1	0	0	0	0	1	1	0	0	1	1
Karle	2013	1	1	0	0	0	0	1	1	0	0	0	0
Oguz	2013	1	1	0	0	0	0	1	0	0	0	0	0
Giananneschi	2014	1	1	0	0	0	0	1	1	0	0	1	1
Roos	2014	1	1	0	0	0	0	0	0	0	0	0	0
Geevasinga	2015	1	1	0	0	0	0	1	1	0	0	1	0
Rinaldi	2015	1	1	0	0	0	0	0	0	0	0	0	0
Denton	2016	1	1	0	0	0	0	1	1	1	0	1	0
Martinuzzi	2016	0	0	0	0	0	0	0	0	0	0	0	0

Author	Year	15	16	17	18	19	20	21	23	24	25	29	30	TOTAL (24)
Thompson	1987	0	1	1	1	1	0	0	0	0	0	1	1	12
Claus	1990	0	1	1	1	0	0	0	0	0	1	0	0	11
Pelosi	1991	1	1	1	1	0	1	0	0	0	0	1	1	13
Schady	1991	0	1	1	1	0	0	0	9	0	1	0	1	22
Polo	1993	0	0	1	1	0	0	0	0	0	1	0	0	6
Sue	1997	0	1	1	1	0	1	0	0	0	1	0	1	12
Nielsen	1998	0	1	0	1	0	1	0	0	0	0	0	0	6
Cruz	1999	0	1	1	1	0	0	0	0	0	1	0	0	8
Di Lazarro	1999	1	1	1	1	0	0	0	0	0	0	0	0	8
Bonsch	2003	0	0	1	1	0	0	0	0	0	1	1	0	8
Nardone	2003	0	0	1	1	0	0	1	1	1	1	1	0	11
Schulte	2003	0	0	0	0	0	0	0	0	0	0	0	0	3
Klebe	2004	0	0	1	1	0	0	0	0	0	1	0	0	9
Orlacchio	2005	0	0	0	0	0	0	0	0	0	0	0	0	3
Winner	2006	0	0	0	0	0	0	0	0	0	0	0	1	4
Sartucci	2007	1	1	1	1	1	1	0	0	1	1	1	1	17
Orlacchio	2008	0	0	0	0	0	0	0	0	0	0	0	0	1
Serranova	2008	0	0	1	1	0	0	0	0	1	0	1	1	12
Liu	2008	0	0	0	0	0	0	0	0	0	0	0	0	2
Liu	2009	0	0	0	0	0	0	0	0	0	0	0	0	3
Schule	2009	0	0	0	0	0	0	0	0	0	0	0	0	0
Battini	2011	0	0	0	0	0	0	0	0	0	0	0	0	2
Manganelli	2011	0	0	0	0	0	0	0	0	0	0	0	0	3
Fisher	2013	1	1	1	1	0	1	0	0	1	1	1	0	14
Karle	2013	0	0	0	1	0	0	0	0	1	0	0	0	6
Oguz	2013	0	0	0	0	0	0	0	0	0	0	0	0	3
Giananneschi	2014	1	1	1	1	1	1	0	0	1	1	1	1	16
Roos	2014	0	0	0	0	0	0	0	0	0	0	0	0	2
Geevasinga	2015	0	1	1	1	1	1	0	0	1	1	0	0	12
Rinaldi	2015	0	0	0	0	0	0	0	0	0	0	0	0	2
Denton	2016	1	0	1	1	0	0	0	0	1	1	0	0	11
Martinuzzi	2016	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 3.2 Scores for Chipchase TMS checklist

4 Appendix 4

4.1 Studies with abnormal UL CMCT results

Study	Genotype	Results - prolonged	Results - absent	Results – total abnormal
Claus 1990	Unknown, pure HSP	2/10 (20%)		2/10 (20%)
Pelosi 1991	Unknown, pure HSP		2/10 (20%)	2/10 (20%)
Schady 1991	Unknown, ADPSP	11/25		11/25 (44%)
Nardone 2003	SPG4, SPG7	1/7 (14%) SPG4		1/7 (14%)
Schulte 2003	SPG4, Non-SPG4	Prolonged in 2/10 (20%) Non-SPG4 patients Normal in 8/8 SPG4 patients		2/18 (11%)
Orlacchio 2005	SPG4	6/11 (55%)		6/11 (55%)
Liu 2008	SPG6	6/6 (100%)		6/6 (100%)
Orlacchio 2008	SPG38	NS	NS	1/19 (5%)
Serranova 2008	Pure HSP	2/11 (18%)		2/11 (18%)
Manganelli 2011	SPG5	3/4 (75%)	1/4 (25%)	4/4 (100%)
Fisher 2013	SPG31	1/2 (50%)		1/2 (50%)
Karle 2013	Mixed genotype	36/128 (28%)	5/128 (4%)	41/128 (32%)
Martinuzzi 2016	Mixed genotype	NS	NS	14/31 (45%)
TOTAL				93/282 (33%)

Table 4.1 Studies with abnormal upper limb central motor conduction time. SPG31 (HSP-*REEP1*), SPG6 (HSP-*NIPA1*), SPG5 (HSP-*CYP7B1*), SPG4 (HSP-*SPAST*), SPG7 (HSP-*paraplegin*), SPG38 (HSP-*SPG38*).

4.2 Studies with normal UL CMCT results

Study	**Genotype**	**Number of patients**
Thompson 1987	Unknown	2
Polo 1993	Unknown	8
Nielsen 1998	SPG4	16
Cruz 1999	Unknown	2
Bonsch 2003	SPG4	10
Sartucci 2007	SPG4	12
Liu 2009	SPG31	2
Giananneschi 2014	SPG4	12
Geevasinga 2015	SPG4	13
TOTAL		77

Table 4.2 Studies with normal upper limb CMCT in all patients studied. SPG4 (HSP-*SPAST*), SPG31 (HSP-*REEP1*).

4.3 Studies with abnormal LL CMCT results

Study	**Genotype**	**Results – prolonged CMCT**	**Results – Absent MEP**	**Results – Total abnormal**
Thompson 1987	Unknown	2/2 (100%)		2/2 (100%)

Pelosi 1991	Unknown	4/10	6/10	10/10 (100%)
Schady 1991	Unknown	12/25	9/25	21/25 (84%)
Polo 1993	Unknown	5/8 (63%)		5/8 (63%)
Bonsch 2003	SPG4	5/10 (50%)		5/10 (50%)
Nardone 2003	SPG4, SPG7	3/7 (43%)		3/7 (43%)
Schulte 2003	Mixed	9/18	1/18	10/18 (56%) SPG4 12.5% Non-SPG4 90%
Klebe 2004	SPG4	12/22 (55%)		12/22 (55%)
Orlacchio 2005	SPG4	11/11 (100%)		11/11 (100%)
Sartucci 2007	SPG4	11/12	1/12	12/12 (100%)
Orlacchio 2008	SPG38	19/19 (100%)		19/19 (100%)
Serranova 2008	Unknown	9/11	2/11	11/11 (100%)
Liu 2008	SPG 6	2/6	4/6	6/6 (100%)
Liu 2009	SPG 31	2/2 (100%)		2/2 (100%)
Battini 2011	SPG4, SPG31	14/14 (100%)		14/14 (100%)
Manganelli 2011	SPG 5	1/3	2/3	3/3 patients studied (100%)
Fisher 2013	SPG 31	2/2 (100%)		2/2 (100%)
Karle 2013	Mixed	48/128 (37%)	46/128	94/128 (73%)
Oguz 2013	SPG11	3/3 (100%)		3/3 (100%)
Rinaldi 2015	SPG10	1/1 (100%)		1/1 (100%)

Denton 2016	Unknown	14/14 (100%)		14/14 (100%)
Martinuzzi 2016	Mixed	NS	NS	48/49 (98%)
TOTAL				308/377 (82%)

Table 4.3 Studies with abnormal lower limb central motor conduction time results. SPG4 (HSP-*SPAST*), SPG31 (HSP-*REEP1*), SPG6 (HSP-*NIPA1*), SPG5 (HSP-*CYP7B1*), SPG7 (HSP-*paraplegin*), SPG11(HSP-*spatacsin*), SPG38 (HSP-*SPG38*), SPG10 (HSP-*KIF5A*).

4.4 Studies that did not distinguish between UL and LL CMCT

Study	**Genotype**	**Results**
Sue 1997	Unknown (5 patients)	4/5 had B/L increased CMCT, 1/5 had absent MEP
Di Lazarro 1999	Unknown (31 patients)	CMCT had 0.8 sensitivity, 0.16 false negativity compared to clinical exam (1.0 sensitivity)
Schule 2009	SPG5(3 patients)	Increased CMCT, reduced amplitude for all 3 patients
Roos 2014	SPG5A (3 patients)	Prolonged CCT for all 3

Table 4.4 Studies that did not distinguish between UL and LL CMCT. SPG5A/5 (HSP-*CYP7B1*).

Siow et al Outcome Measures Study (Section 2.3) Supplementary Material

One way ANOVA for between group comparisons of CROMs

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	SPG_type	Statistic	df	Sig.	Statistic	df	Sig.
SPRS	0	.254	7	.191	.820	7	.064
	4	.191	10	.200*	.888	10	.162
	7	.297	4	.	.834	4	.180
SARA	0	.239	7	.200*	.917	7	.445
	4	.218	10	.195	.917	10	.329
	7	.326	4	.	.802	4	.106

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
SPRS	Based on Mean	.943	2	18	.408
	Based on Median	.498	2	18	.616
	Based on Median and with adjusted df	.498	2	17.559	.616
	Based on trimmed mean	.869	2	18	.436
SARA	Based on Mean	.274	2	18	.763
	Based on Median	.243	2	18	.787
	Based on Median and with adjusted df	.243	2	17.036	.787

	Based on trimmed mean	.289	2	18	.753

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SPRS	Between Groups	173.588	2	86.794	1.689	.213
	Within Groups	925.079	18	51.393		
	Total	1098.667	20			
SARA	Between Groups	238.035	2	119.017	8.109	.003
	Within Groups	264.204	18	14.678		
	Total	502.238	20			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) SPG_type	(J) SPG_type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
SPRS	0	4	-.386	3.533	.993	-9.40	8.63
		7	-7.536	4.493	.241	-19.00	3.93
	4	0	.386	3.533	.993	-8.63	9.40
		7	-7.150	4.241	.238	-17.97	3.67
	7	0	7.536	4.493	.241	-3.93	19.00
		4	7.150	4.241	.238	-3.67	17.97
SARA	0	4	.064	1.888	.999	-4.75	4.88
		7	-8.536*	2.401	.006	-14.66	-2.41
	4	0	-.064	1.888	.999	-4.88	4.75

		7	-8.600*	2.267	.004	-14.38	-2.82
	7	0	8.536*	2.401	.006	2.41	14.66
		4	8.600*	2.267	.004	2.82	14.38

*. The mean difference is significant at the 0.05 level.

SPRS

Tukey HSD[a,b]

SPG_type	N	Subset for alpha = 0.05
		1
0	7	17.71
4	10	18.10
7	4	25.25
Sig.		.187

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.087.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

SARA

Tukey HSD[a,b]

		Subset for alpha = 0.05	
SPG_type	N	1	2

4	10	6.65	
0	7	6.71	
7	4		15.25
Sig.		1.000	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 6.087.

b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

One way ANOVA for between group comparisons of PROM

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	SPG_type	Statistic	df	Sig.	Statistic	df	Sig.
SF36_phyfn	0	.228	7	.200*	.947	7	.703
	4	.176	10	.200*	.917	10	.336
	7	.298	4	.	.849	4	.224
SF36_phylim	0	.354	7	.008	.729	7	.008
	4	.259	10	.055	.808	10	.018
	7	.250	4	.	.945	4	.683
SF36_emolim	0	.275	7	.117	.800	7	.041
	4	.360	10	<.001	.670	10	<.001
	7	.151	4	.	.993	4	.972
SF36_energy	0	.227	7	.200*	.815	7	.057

	4	.183	10	.200*	.896	10	.196
	7	.162	4	.	.989	4	.952
SF36_emot	0	.255	7	.188	.822	7	.067
	4	.184	10	.200*	.880	10	.130
	7	.306	4	.	.912	4	.492
SF36_social	0	.160	7	.200*	.935	7	.591
	4	.212	10	.200*	.879	10	.126
	7	.155	4	.	.998	4	.995
SF36_pain	0	.179	7	.200*	.982	7	.967
	4	.233	10	.133	.863	10	.082
	7	.198	4	.	.977	4	.882
SF36_genhealth	0	.199	7	.200*	.903	7	.352
	4	.185	10	.200*	.893	10	.185
	7	.192	4	.	.971	4	.850
MCS	0	.258	7	.174	.871	7	.189

	4	.284	10	.022	.852	10	.062
	7	.218	4	.	.981	4	.905
PCS	0	.245	7	.200*	.863	7	.162
	4	.212	10	.200*	.896	10	.199
	7	.264	4	.	.908	4	.472

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Tests of Homogeneity of Variances

		Levene Statistic	df1	df2	Sig.
SF36_phyfn	Based on Mean	.794	2	18	.467
	Based on Median	.689	2	18	.515

	Based on Median and with adjusted df	.689	2	15.318	.517
	Based on trimmed mean	.709	2	18	.505
SF36_phylim	Based on Mean	3.226	2	18	.063
	Based on Median	.596	2	18	.561
	Based on Median and with adjusted df	.596	2	15.640	.563
	Based on trimmed mean	3.048	2	18	.072
SF36_emolim	Based on Mean	.225	2	18	.801
	Based on Median	.036	2	18	.965
	Based on Median and with adjusted df	.036	2	14.138	.965
	Based on trimmed mean	.207	2	18	.815
SF36_energy	Based on Mean	1.981	2	18	.167
	Based on Median	.967	2	18	.399

	Based on Median and with adjusted df	.967	2	15.763	.402
	Based on trimmed mean	2.004	2	18	.164
SF36_emot	Based on Mean	.214	2	18	.810
	Based on Median	.103	2	18	.903
	Based on Median and with adjusted df	.103	2	15.609	.903
	Based on trimmed mean	.196	2	18	.824
SF36_social	Based on Mean	.349	2	18	.710
	Based on Median	.268	2	18	.768
	Based on Median and with adjusted df	.268	2	15.320	.769
	Based on trimmed mean	.288	2	18	.753
SF36_pain	Based on Mean	2.424	2	18	.117
	Based on Median	1.017	2	18	.382

	Based on Median and with adjusted df	1.017	2	15.030	.385
	Based on trimmed mean	2.202	2	18	.139
SF36_genhealth	Based on Mean	.360	2	18	.702
	Based on Median	.238	2	18	.791
	Based on Median and with adjusted df	.238	2	15.953	.791
	Based on trimmed mean	.334	2	18	.720
MCS	Based on Mean	.319	2	18	.731
	Based on Median	.064	2	18	.939
	Based on Median and with adjusted df	.064	2	16.998	.939
	Based on trimmed mean	.262	2	18	.773
PCS	Based on Mean	6.293	2	18	.008
	Based on Median	2.852	2	18	.084

	Based on Median and with adjusted df	2.852	2	13.068	.094
	Based on trimmed mean	5.902	2	18	.011

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SF36_phyfn	Between Groups	1817.738	2	908.869	1.544	.241
	Within Groups	10598.929	18	588.829		
	Total	12416.667	20			
SF36_phylim	Between Groups	1247.024	2	623.512	.438	.652
	Within Groups	25598.214	18	1422.123		
	Total	26845.238	20			

SF36_emolim	Between Groups	1218.831	2	609.416	.284	.756
	Within Groups	38575.194	18	2143.066		
	Total	39794.025	20			
SF36_energy	Between Groups	523.988	2	261.994	.645	.536
	Within Groups	7306.964	18	405.942		
	Total	7830.952	20			
SF36_emot	Between Groups	435.810	2	217.905	.467	.635
	Within Groups	8406.857	18	467.048		
	Total	8842.667	20			
SF36_social	Between Groups	2599.702	2	1299.851	2.201	.140
	Within Groups	10629.464	18	590.526		

	Total	13229.167	20			
SF36_pain	Between Groups	383.214	2	191.607	.304	.742
	Within Groups	11357.857	18	630.992		
	Total	11741.071	20			
SF36_genhealth	Between Groups	445.357	2	222.679	.877	.433
	Within Groups	4568.929	18	253.829		
	Total	5014.286	20			
MCS	Between Groups	201.994	2	100.997	.419	.664
	Within Groups	4336.951	18	240.942		
	Total	4538.946	20			
PCS	Between Groups	40.664	2	20.332	.397	.678

	Within Groups	920.790	18	51.155		
	Total	961.454	20			

One way ANOVA for between group comparisons of MEP measures

Tests of Normality

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	SPG_type	Statistic	df	Sig.	Statistic	df	Sig.
TA_CMCT	0	.275	4	.	.942	4	.669
	4	.238	7	.200*	.814	7	.056
	7	.349	3	.	.831	3	.191
AH_CMCT	0	.266	4	.	.945	4	.685
	4	.219	7	.200*	.863	7	.160
	7	.236	3	.	.977	3	.712
ADM_CMCT	0	.196	4	.	.976	4	.876
	4	.170	7	.200*	.950	7	.726
	7	.371	3	.	.784	3	.076
TA_Amp	0	.251	4	.	.956	4	.756

	4	.243	7	.200*	.881	7	.231
	7	.230	3	.	.981	3	.737
AH_Amp	0	.356	4	.	.798	4	.098
	4	.331	7	.019	.662	7	.001
	7	.178	3	.	.999	3	.956
ADM_Amp	0	.236	4	.	.904	4	.453
	4	.166	7	.200*	.966	7	.865
	7	.176	3	.	1.000	3	.976

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Tests of Homogeneity of Variances

	Levene Statistic	df1	df2	Sig.

TA_CMCT	Based on Mean	2.088	2	18	.153
	Based on Median	2.078	2	18	.154
	Based on Median and with adjusted df	2.078	2	15.105	.160
	Based on trimmed mean	2.192	2	18	.141
AH_CMCT	Based on Mean	.244	2	12	.788
	Based on Median	.176	2	12	.840
	Based on Median and with adjusted df	.176	2	9.543	.841
	Based on trimmed mean	.223	2	12	.803
ADM_CMCT	Based on Mean	.510	2	16	.610
	Based on Median	.251	2	16	.781
	Based on Median and with adjusted df	.251	2	10.415	.782
	Based on trimmed mean	.485	2	16	.624

TA_Amp	Based on Mean	4.613	2	18	.024
	Based on Median	3.532	2	18	.051
	Based on Median and with adjusted df	3.532	2	17.420	.052
	Based on trimmed mean	4.631	2	18	.024
AH_Amp	Based on Mean	.509	2	12	.614
	Based on Median	.124	2	12	.885
	Based on Median and with adjusted df	.124	2	9.719	.885
	Based on trimmed mean	.390	2	12	.685
ADM_Amp	Based on Mean	.077	2	16	.926
	Based on Median	.078	2	16	.925
	Based on Median and with adjusted df	.078	2	15.208	.925
	Based on trimmed mean	.077	2	16	.926

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
TA_CMCT	Between Groups	13.700	2	6.850	.642	.538
	Within Groups	192.102	18	10.672		
	Total	205.802	20			
AH_CMCT	Between Groups	3.400	2	1.700	.215	.810
	Within Groups	95.054	12	7.921		
	Total	98.454	14			
ADM_CMCT	Between Groups	1.074	2	.537	.210	.813
	Within Groups	40.870	16	2.554		
	Total	41.944	18			

TA_Amp	Between Groups	.007	2	.003	.055	.947
	Within Groups	1.100	18	.061		
	Total	1.106	20			
AH_Amp	Between Groups	.024	2	.012	.510	.613
	Within Groups	.288	12	.024		
	Total	.313	14			
ADM_Amp	Between Groups	.183	2	.092	5.742	.013
	Within Groups	.255	16	.016		
	Total	.439	18			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) SPG_type	(J) SPG_type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
TA_CMCT	0	4	.93929	1.60992	.831	-3.1695	5.0481
		7	-1.21071	2.04761	.826	-6.4366	4.0151
	4	0	-.93929	1.60992	.831	-5.0481	3.1695
		7	-2.15000	1.93270	.519	-7.0826	2.7826
	7	0	1.21071	2.04761	.826	-4.0151	6.4366
		4	2.15000	1.93270	.519	-2.7826	7.0826
AH_CMCT	0	4	-1.04286	1.64798	.805	-5.4394	3.3537
		7	-.91667	2.05539	.897	-6.4002	4.5668
	4	0	1.04286	1.64798	.805	-3.3537	5.4394
		7	.12619	1.94216	.998	-5.0552	5.3076
	7	0	.91667	2.05539	.897	-4.5668	6.4002

		4	-.12619	1.94216	.998	-5.3076	5.0552
ADM_CMCT	0	4	-.47833	.82533	.833	-2.6079	1.6513
		7	-.59167	1.13012	.861	-3.5078	2.3244
	4	0	.47833	.82533	.833	-1.6513	2.6079
		7	-.11333	1.05209	.994	-2.8281	2.6014
	7	0	.59167	1.13012	.861	-2.3244	3.5078
		4	.11333	1.05209	.994	-2.6014	2.8281
TA_Amp	0	4	.00028	.12181	1.000	-.3106	.3112
		7	.04568	.15493	.953	-.3497	.4411
	4	0	-.00028	.12181	1.000	-.3112	.3106
		7	.04540	.14623	.948	-.3278	.4186
	7	0	-.04568	.15493	.953	-.4411	.3497
		4	-.04540	.14623	.948	-.4186	.3278
AH_Amp	0	4	.08094	.09073	.655	-.1611	.3230
		7	.09449	.11316	.689	-.2074	.3964

	4	0	-.08094	.09073	.655	-.3230	.1611
		7	.01355	.10692	.991	-.2717	.2988
	7	0	-.09449	.11316	.689	-.3964	.2074
		4	-.01355	.10692	.991	-.2988	.2717
ADM_Amp	0	4	.22104*	.06523	.010	.0527	.3894
		7	.14133	.08932	.281	-.0891	.3718
	4	0	-.22104*	.06523	.010	-.3894	-.0527
		7	-.07971	.08315	.612	-.2943	.1349
	7	0	-.14133	.08932	.281	-.3718	.0891
		4	.07971	.08315	.612	-.1349	.2943

*. The mean difference is significant at the 0.05 level.

Correlation between MEP measures, clinician rated outcome measures and disease duration

		SPRS	SARA	Disease_duration
TA_CMCT	Pearson Correlation	.239	.345	.311
	Sig. (2-tailed)	.296	.126	.170
	N	21	21	21
AH_CMCT	Pearson Correlation	-.056	-.025	-.173
	Sig. (2-tailed)	.843	.931	.537
	N	15	15	15
ADM_CMCT	Pearson Correlation	.101	.132	-.127
	Sig. (2-tailed)	.680	.591	.604
	N	19	19	19
TA_Amp	Pearson Correlation	-.238	-.344	-.224
	Sig. (2-tailed)	.298	.127	.329

	N	21	21	21
AH_Amp	Pearson Correlation	-.385	-.283	.057
	Sig. (2-tailed)	.157	.307	.841
	N	15	15	15
ADM_Amp	Pearson Correlation	-.378	-.331	-.023
	Sig. (2-tailed)	.111	.166	.927
	N	19	19	19

Independent t-test comparing TA CMCT means between patients with mild and moderate SPRS scores

Group Statistics

	SPRS_severity	N	Mean	Std. Deviation	Std. Error Mean
TA_CMCT	mild	9	15.5556	3.27914	1.09305
	moderate	12	17.6417	2.97565	.85900
AH_CMCT	mild	8	14.6625	1.91363	.67657
	moderate	7	16.3929	3.20526	1.21147

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means					
						Significance			Std.
		F	Sig.	t	df	One-Sided p	Two-Sided p	Mean Difference	Differ
TA_CMCT	Equal variances assumed	.046	.833	-1.523	19	.072	.144	-2.08611	
	Equal variances not assumed			-1.501	16.387	.076	.152	-2.08611	
AH_CMCT	Equal variances assumed	4.340	.058	-1.290	13	.110	.219	-1.73036	
	Equal variances not assumed			-1.247	9.532	.121	.242	-1.73036	

Independent Samples Effect Sizes

				95% Confidence Interval	
		Standardizer[a]	Point Estimate	Lower	Upper
TA_CMCT	Cohen's d	3.10705	-.671	-1.553	.227
	Hedges' correction	3.23681	-.644	-1.491	.218
	Glass's delta	2.97565	-.701	-1.598	.225
AH_CMCT	Cohen's d	2.59105	-.668	-1.702	.390
	Hedges' correction	2.75356	-.628	-1.601	.367
	Glass's delta	3.20526	-.540	-1.577	.538

a. The denominator used in estimating the effect sizes.

Cohen's d uses the pooled standard deviation.

Hedges' correction uses the pooled standard deviation, plus a correction factor.

Glass's delta uses the sample standard deviation of the control group.

Correlation between MEP measures and SF36 scores

		TA_CMCT	AH_CMCT	ADM_CMCT	TA_Amp	AH_Amp	ADM_Amp
SF36_phyfn	Pearson Correlation	-.247	-.046	-.085	.041	.529*	.327
	Sig. (2-tailed)	.281	.872	.730	.859	.043	.171
	N	21	15	19	21	15	19
SF36_phylim	Pearson Correlation	-.087	.440	.150	-.221	-.153	-.248
	Sig. (2-tailed)	.708	.100	.540	.336	.587	.307
	N	21	15	19	21	15	19
SF36_emolim	Pearson Correlation	.483*	.442	-.124	.023	-.263	-.122
	Sig. (2-tailed)	.027	.099	.612	.920	.343	.619
	N	21	15	19	21	15	19

SF36_energy	Pearson Correlation	.445*	.449	-.197	.107	-.238	-.139
	Sig. (2-tailed)	.043	.093	.419	.644	.393	.570
	N	21	15	19	21	15	19
SF36_emot	Pearson Correlation	.260	.288	-.218	-.024	-.082	-.202
	Sig. (2-tailed)	.255	.298	.369	.918	.771	.408
	N	21	15	19	21	15	19
SF36_social	Pearson Correlation	.193	.259	-.158	.076	-.158	-.126
	Sig. (2-tailed)	.403	.352	.519	.742	.574	.607
	N	21	15	19	21	15	19
SF36_pain	Pearson Correlation	.227	.264	-.208	-.127	.120	-.226
	Sig. (2-tailed)	.323	.341	.393	.585	.671	.351

	N	21	15	19	21	15	19
SF36_genhealth	Pearson Correlation	.044	-.259	-.306	-.355	.407	-.127
	Sig. (2-tailed)	.851	.350	.202	.114	.132	.605
	N	21	15	19	21	15	19
MCS	Pearson Correlation	.411	.419	-.153	.017	-.299	-.230
	Sig. (2-tailed)	.064	.120	.532	.941	.278	.343
	N	21	15	19	21	15	19
PCS	Pearson Correlation	-.375	.159	.134	-.040	.354	.133
	Sig. (2-tailed)	.094	.571	.584	.865	.196	.588
	N	21	15	19	21	15	19

Correlation between MEP measures, clinician rated outcome measures and disease duration for HSP-SPAST subgroup.

		Disease_duration	SPRS	SARA
TA_CMCT	Pearson Correlation	.841**	.153	.420
	Sig. (2-tailed)	.002	.673	.227
	N	10	10	10
AH_CMCT	Pearson Correlation	.930**	-.137	.193
	Sig. (2-tailed)	.002	.770	.678
	N	7	7	7
ADM_CMCT	Pearson Correlation	.198	-.149	.008
	Sig. (2-tailed)	.583	.681	.982
	N	10	10	10
TA_Amp	Pearson Correlation	-.275	.251	-.055
	Sig. (2-tailed)	.442	.483	.880
	N	10	10	10

AH_Amp	Pearson Correlation	-.412	-.390	-.399
	Sig. (2-tailed)	.359	.387	.375
	N	7	7	7
ADM_Amp	Pearson Correlation	.142	-.225	-.335
	Sig. (2-tailed)	.695	.532	.345
	N	10	10	10

Correlation between MEP measures and SF36 scores for HSP-SPAST subgroup

		TA_CMCT	AH_CMCT	ADM_CMCT	TA_Amp	AH_Amp	ADM_Amp
SF36_phyfn	Pearson Correlation	-.004	.313	.168	-.344	.406	.293
	Sig. (2-tailed)	.992	.494	.642	.331	.366	.411
	N	10	7	10	10	7	10
SF36_phylim	Pearson Correlation	-.002	.728	.371	-.262	-.462	-.190
	Sig. (2-tailed)	.997	.064	.291	.465	.296	.598
	N	10	7	10	10	7	10
SF36_emolim	Pearson Correlation	.701*	.585	.547	-.258	-.531	.176
	Sig. (2-tailed)	.024	.168	.102	.473	.220	.626
	N	10	7	10	10	7	10

SF36_energy	Pearson Correlation	.514	.371	.296	-.114	-.364	-.096
	Sig. (2-tailed)	.128	.413	.406	.753	.422	.793
	N	10	7	10	10	7	10
SF36_emot	Pearson Correlation	.658*	.464	.096	.183	-.672	-.117
	Sig. (2-tailed)	.038	.294	.792	.613	.098	.747
	N	10	7	10	10	7	10
SF36_social	Pearson Correlation	.528	.605	.402	-.068	-.761*	-.139
	Sig. (2-tailed)	.116	.150	.250	.852	.047	.703
	N	10	7	10	10	7	10
SF36_pain	Pearson Correlation	.181	.172	.114	-.295	.380	.062
	Sig. (2-tailed)	.617	.713	.754	.409	.400	.865

	N	10	7	10	10	7	10
SF36_genhealth	Pearson Correlation	.156	-.273	-.374	.090	.440	-.192
	Sig. (2-tailed)	.666	.553	.288	.804	.324	.595
	N	10	7	10	10	7	10
MCS	Pearson Correlation	.664*	.540	.363	-.011	-.751	-.086
	Sig. (2-tailed)	.036	.210	.303	.975	.052	.813
	N	10	7	10	10	7	10
PCS	Pearson Correlation	-.333	.370	.190	-.415	.513	.110
	Sig. (2-tailed)	.348	.414	.600	.233	.239	.763
	N	10	7	10	10	7	10

Journal of the Neurological Sciences 462 (2024) 123100

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Letter to the Editor

Outcome measures for hereditary spastic paraplegia clinical trials: Learnings from an Australian HSP center

ARTICLE INFO

Keywords
Hereditary spastic paraplegia
Outcome measures
Biomarker
Rating scales
Motor evoked potentials

Dear editor,

Hereditary spastic paraplegia (HSP) is a progressive neurodegenerative condition with no disease modifying therapy. HSP clinical trials to date are heterogeneous in terms of study design and outcome measures used as primary endpoints. [1–3] There are few studies that compare outcome measures providing little guidance on choice of endpoints for clinical trials. Based on a scoping review of the literature, we recommended a set of outcome measures to determine disease severity including an HSP-specific clinician rated outcome measure, the Spastic Paraplegia Rating Scale (SPRS), a generic patient reported outcome measure, Short Form 36 (SF36), and an objective biomarker, such as a biochemical or neuroimaging biomarker. [3] In preparation for clinical trials, we evaluated baseline outcome measures for patients with HSP from the Neurogenetics Clinic, Royal North Shore Hospital, Sydney, Australia. We evaluated SPRS, Scale for the Assessment and Rating of Ataxia (SARA), SF36 and motor evoked potentials. This study was approved by the local Institutional Ethics Committee (Reference number HREC/17/HAWKE/21).

Twenty-one patients with HSP were recruited; ten HSP-*SPAST*, four HSP-*SPG7*, five unknown genotype, and one each HSP-*ATL1* and HSP-*KIF1A (Supplementary Table 1)*. SPRS, an HSP-specific clinical rating scale, scores ranged from mild (2/52) to moderate (30/52) with no significant differences between HSP-*SPAST*, HSP-*SPG7*, and other/unknown genotype subgroups. SARA, an ataxia clinical rating scale, showed significantly higher mean scores for the HSP-*SPG7* subgroup compared to HSP-*SPAST* and other/unknown genotypes.

Mean SF36 scores for HSP patients showed significantly worse quality of life compared to the general Australian population (unpaired *t*-test two-tailed *p* value 0.0002 for Physical Component Score, 0.0229 for Mental Health Component Score).

There was significant correlation between SPRS and SARA scores, SPRS Scores and SF36 physical function subscale, and SARA scores and SF36 physical function subscale (Fig. 1). The SPRS and SARA scores also correlated with the SF36 Physical component summary score. There was no significant correlation between the SPRS and SARA with the other SF36 subscales. Although lower limb central motor conduction time was abnormal in 15/21 (71%) of patients, there was no correlation with SPRS, SARA or SF-36 scores.

We found that SPRS was able to differentiate patients across a spectrum of severities with SPRS scores ranging from 2 to 30 (mild to moderate) in our patient cohort. Overall, the authors found that the SPRS was a comprehensive clinical disease severity measure encompassing relevant symptomatic features of HSP.

SARA scores were significantly higher for patients with HSP-*SPG7* compared to other genotypes. This suggests that SARA may be more sensitive to severity of ataxic symptoms involving upper limb and bulbar function, [4] that can be seen in patients with complex HSP but are not assessed in SPRS. Therefore, we recommend use of SARA in addition to SPRS for HSP studies which include HSP genotypes with ataxic symptoms, such as HSP-*SPG7*.

Rate of yearly change of SPRS (total SPRS score/disease duration) ranged from 0.07 points per year to 3 points per year whilst rate of yearly change of SARA scores (total SARA score/disease duration) ranged from 0 to 1.25 points per year (Supplementary Table 1). This wide range of degree of disease progression reflects the clinical variability of HSP.

Despite SF-36 scores being significantly lower in our patient cohort compared to the general population, there was no correlation with overall disease severity as measured with the SPRS. Although SF-36 and SPRS measure different domains, it is reasonable to expect some degree of correlation between clinical disease severity and quality of life. The lack of any correlation could be attributed to the lack of HSP-specific items in the SF-36 such as bladder and bowel symptoms, spasticity and spasms, and frequency of falls. [5] We suggest use of an HSP-specific quality of life scale over generic quality of life scales in an HSP patient cohort.

We found a significant correlation between the SF36 physical function subscore with SPRS ($r = -0.870$, $p < 0.001$) and SARA scores ($r = -0.665$, $p < 0.001$) (Fig. 1) demonstrating concordance of patient-reported disease severity with clinician-rated measures. SPRS items measuring walking distance, gait quality and speed, stair climbing, arising from chair, and bladder and bowel function strongly correlated with SF-36 physical function subscore (items 1–6 and 13, $r = -0.641$–0738, $p < 0.001$) whilst items measuring spasticity (items 7 and 8, $r = -0.462$–0.583, $p < 0.05$) showed moderate correlation (Supplementary Table 2). SARA items measuring gait and stance showed

https://doi.org/10.1016/j.jns.2024.123100
Received 15 April 2024; Received in revised form 4 June 2024; Accepted 19 June 2024
Available online 20 June 2024

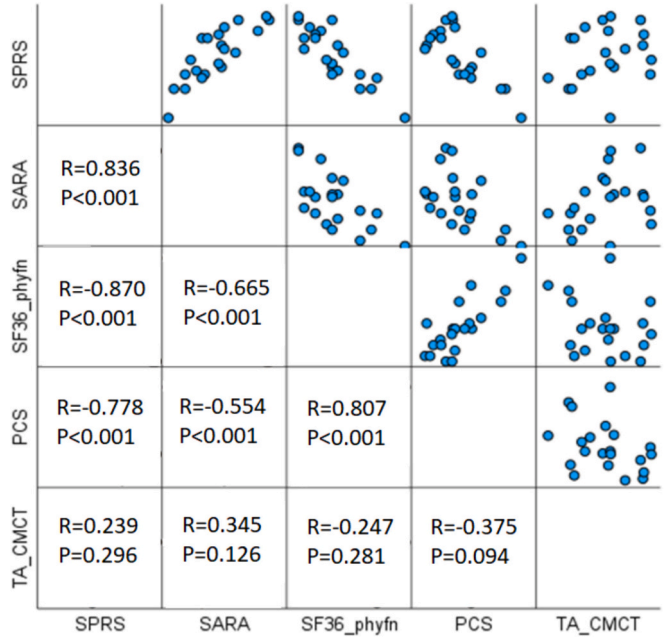


Fig. 1. Scatterplot Matrix of correlations between SPRS, SARA, SF36 physical function subscore (SF36_phyfn), SF36 Physical health Component Score (PCS), and lower limb central motor conduction time (TA_CMCT). R = Pearson correlation coefficient, P = p values.

moderate to strong correlation with SF-36 physical function subscore (items 1 and 2, $r = -0.577$–0.679, $p < 0.001$) whilst the other items did not correlate with the SF-36 physical function subscore (Supplementary Table 2). We propose use of the SF36 physical function subscore as a surrogate measure of clinical disease severity. Incorporation of a patient-reported measure of clinical disease severity will increase accessibility of clinical trials, particularly for patients who are unable to travel to trials centers for clinician assessment.

There was no correlation between motor evoked potential measures and clinical disease severity or quality of life (Fig. 1). This could be attributed to difference in domains measured by each outcome measure. Evidence to support use of motor evoked potentials as a biomarker in HSP has been weak to date and the findings of this study is in line with previous reports. [6–8] We found that lower limb central motor conduction time values did not differentiate mild from moderate clinical severity as measured with the SPRS.

There were several limitations in our study. We had a small patient cohort due to the rarity of the condition and low population density in Australia. However, we were able to include a range of HSP genotypes and disease severities. Inclusion of various HSP genotypes may be seen as a limitation as clinical presentations are variable between genotypes. However, it is well reported that phenotypic variability exists even within genotypes [9] and this is an unavoidable challenge in HSP studies. As we have investigated the relationship between the various outcome measures, these findings remain relevant to the greater HSP community. Larger studies will help validate our findings.

Due to time and resource limitations, we were unable to collect longitudinal data and therefore were unable to determine sensitivity of the studied outcome measures to change over time. Further longitudinal studies are crucial to determine optimal trial duration, a challenge in a slowly progressive disorder like HSP.

We acknowledge that there are several more promising biomarkers than the motor evoked potentials that were used in our study, such as gait analysis, diffusion tensor imaging, and serum neurofilament light chain. Due to resource limitations, we were unable to include these biomarkers in our study.

In this study, we compared clinician and patient reported outcome measures, and an objective biomarker to identify suitable outcome measures for use in HSP studies. Our study highlights the utility of the SPRS and SARA scales (both correlate with SF36 physical function), that SARA may be particularly relevant for forms of HSP complicated by ataxia, and the need for further development of a disease-specific patient reported outcome measure.

Funding

S-F S is the recipient of the National Health and Medical Research Council and Australian and New Zealand Association of Neurologists Postgraduate Scholarships APP113610.

Ethics approval

This study was approved by the Northern Sydney Local Health District Human Research Ethics Committee (Reference number HREC/17/HAWKE/21).

CRediT authorship contribution statement

Sue Faye Siow: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Amy Waters:** Resources, Methodology, Investigation. **Sharon Coward:** Resources, Methodology, Investigation. **Gautam Wali:** Writing – review & editing, Visualization, Validation, Supervision, Formal analysis. **Karl Ng:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Carolyn M. Sue:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Kishore R. Kumar:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

All authors declare there is no conflict of interest.

Data statement

Data is available upon request from the corresponding author.

Acknowledgements

The authors would like to thank the patients, their families, and the HSP research foundation for their support of this study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2024.123100.

References

[1] M. Bellofatto, G. De Michele, A. Iovino, et al., Management of hereditary spastic paraplegia: a systematic review of the literature, Front. Neurol. 10 (2019) 3, https://doi.org/10.3389/fneur.2019.00003.
[2] B. Trummer, D. Haubenberger, C. Blackstone, Clinical trial designs and measures in hereditary spastic paraplegias, Front. Neurol. 9 (2018) 1017, https://doi.org/10.3389/fneur.2018.01017.
[3] S.-F. Siow, D. Yeow, L.I. Rudaks, et al., Outcome measures and biomarkers for clinical trials in hereditary spastic paraplegia: a scoping review, Genes 14 (2023) 1756, https://doi.org/10.3390/genes14091756.

[4] T. Schmitz-HÜBsch, S. Tezenas Du Montcel, B. Kremer, et al., Scale for the assessment and rating of ataxia: development of a new clinical scale, Neurology 66 (2006) 1717–1720, https://doi.org/10.1212/01.wnl.0000219042.60538.92.
[5] H.C.J.W. Kerstens, Lith BJHV, M.J. Nijkrake, et al., Healthcare needs, expectations, utilization, and experienced treatment effects in patients with hereditary spastic paraplegia: a web-based survey in the Netherlands, Orphanet J. Rare Dis. 16 (2021) 1–283, https://doi.org/10.1186/s13023-021-01915-0.
[6] S.-F. Siow, R.C. Smail, K. Ng, et al., Motor evoked potentials in hereditary spastic paraplegia—a systematic review, Front. Neurol. 10 (2019) 967, https://doi.org/10.3389/fneur.2019.00967.
[7] S.F. Brighente, P. Vicuna, A.L.R. Louzada, et al., Evoked potentials as biomarkers of hereditary spastic paraplegias: a case-control study, PLoS One 16 (11) (2021), https://doi.org/10.1371/journal.pone.0259397 (no pagination).
[8] A. Martinuzzi, D. Montanaro, M. Vavla, et al., Clinical and Paraclinical indicators of motor system impairment in hereditary spastic paraplegia: a pilot study, PLoS One 11 (2016) e0153283, https://doi.org/10.1371/journal.pone.0153283.
[9] F.M. Santorelli, C. Patrono, D. Fortini, et al., Intrafamilial variability in hereditary spastic paraplegia associated with an SPG4 gene mutation, Neurology 55 (2000) 702–705, https://doi.org/10.1212/wnl.55.5.702.

Sue Faye Siow[a,b,c,*], Amy Waters[d], Sharon Coward[d], Gautam Wali[b,c,g], Karl Ng[b,d], Carolyn M. Sue[c,g,h], Kishore R. Kumar[b,e,f]

[a] *Department of Clinical Genetics, Royal North Shore Hospital, NSW, Australia*
[b] *Sydney Medical School, The University of Sydney, NSW, Australia*
[c] *Department of Neurogenetics, Royal North Shore Hospital, NSW, Australia*
[d] *Department of Neurology and Clinical Neurophysiology, Royal North Shore Hospital, NSW, Australia*
[e] *Translational Neurogenomics Group, Genomics and Inherited Disease Program, Garvan Institute of Medical Research, NSW, Australia*
[f] *Molecular Medicine Laboratory and Department of Neurology, Concord Repatriation General Hospital, NSW, Australia*
[g] *Neuroscience Research Australia, University of New South Wales, NSW, Australia*
[h] *Faculty of Medicine and Health, University of New South Wales Sydney, NSW, Australia*

[*] Corresponding author at: Department of Clinical Genetics, Royal North Shore Hospital, NSW, Australia.
E-mail address: Suefaye.siow@health.nsw.gov.au (S.F. Siow).

Siow et al Letter to the Editor Supplementary Material

Supplementary Table 1. Patient demographics and rate of yearly change for Spastic Paraplegia Rating Scale (SARA) and Scale for the Assessment and Rating of Ataxia (SARA) scores.

Study ID	Age	Gender	Disease Duration	Genotype	Phenotype	SPRS	Rate of yearly change of SPRS	SARA	Rate of yearly change of SARA
1	51	F	50	SPG3A	pure	29	0.58	12.5	0.25
2	64	M	24	SPG7	complex	26	1.083333	16	0.666667
3	53	F	12	SPAST	pure	10	0.833333	3	0.25
4	80	M	42	SPAST	pure	22	0.52381	9.5	0.22619
5	35	F	30	SPAST	pure	2	0.066667	0	0
6	53	F	18	SPG7	complex	16	0.888889	9.5	0.527778
7	57	M	42	SPAST	pure	20	0.47619	12	0.285714
8	46	M	15	SPAST	pure	10	0.666667	1	0.066667
9	71	M	20	SPAST	pure	17	0.85	9	0.45
10	60	F	22	SPG7	complex	29	1.318182	18	0.818182
11	71	M	46	unknown	pure	21	0.456522	10	0.217391
12	61	M	35	SPAST	pure	25	0.714286	9	0.257143
13	57	F	14	SPAST	pure	24	1.714286	6	0.428571
14	60	F	19	SPAST	pure	24	1.263158	7	0.368421
15	58	M	39	unknown	complex	14	0.358974	6.5	0.166667
16	63	M	14	SPG7	complex	30	2.142857	17.5	1.25
17	29	F	26	SPG30	complex	15	0.576923	5	0.192308
18	52	F	20	SPAST	complex	27	1.35	10	0.5
19	29	M	6	Unknown	complex	18	3	4	0.666667
20	32	F	32	Unknown	complex	13	0.40625	6	0.1875
21	61	F	6	Unknown	pure	14	2.333333	3	0.5

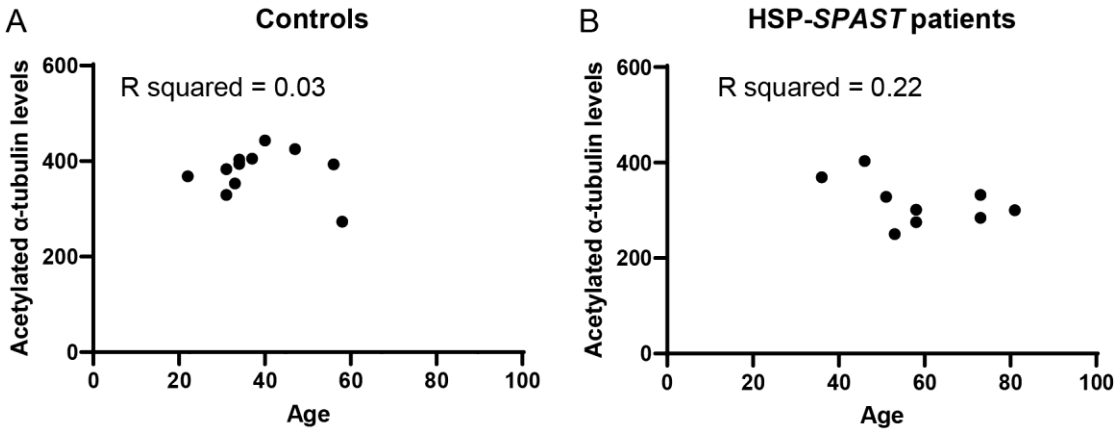
Supplementary Table 2. Correlation between individual items from Spastic Paraplegia Rating Scale (SARA) and Scale for the Assessment and Rating of Ataxia (SARA) scores with Short Form-36 Physical Function Subscore.

SPRS and SARA Items	**Pearson Correlation with SF-36 Physical Function Subscore**
SPRS item 1: Walking distance without pause	-.641**
SPRS item 2: Gait quality	-.650**
SPRS item 3: Maximum gait speed	-.738**
SPRS item 4: Climbing stairs	-.717**
SPRS item 5: Speed of stair climbing	-.656**
SPRS item 6: Arising from chair	-.672**
SPRS item 7: Spasticity – hip adductor muscles	-.583**
SPRS item 8: Spasticity – knee flexion	-.462*
SPRS item 9: Weakness – hip abduction	-.353
SPRS item 10: Weakness – foot dorsiflexion	-.249
SPRS item 11: Contractures of lower limbs	-.125
SPRS item 12: Pain due to SP related symptoms	-.317
SPRS item 13: Bladder and bowel function	-.680**
Total SPRS score	-.870**
SARA item 1: Gait	-.679**
SARA item 2: Stance	-.577**
SARA item 3: Sitting	.031
SARA item 4: Speech disturbance	-.152
SARA item 5: Finger chase	-.359
SARA item 6: Nose-finger test	-.309
SARA item 7: Fast alternating hand movements	-.321

SARA item 8: Heel-shin slide	-.222
Total SARA score	-.665**
**. Correlation is significant at the 0.01 level (2-tailed).	
*. Correlation is significant at the 0.05 level (2-tailed).	

Wali, Siow et al Supplementary Material

Image 1



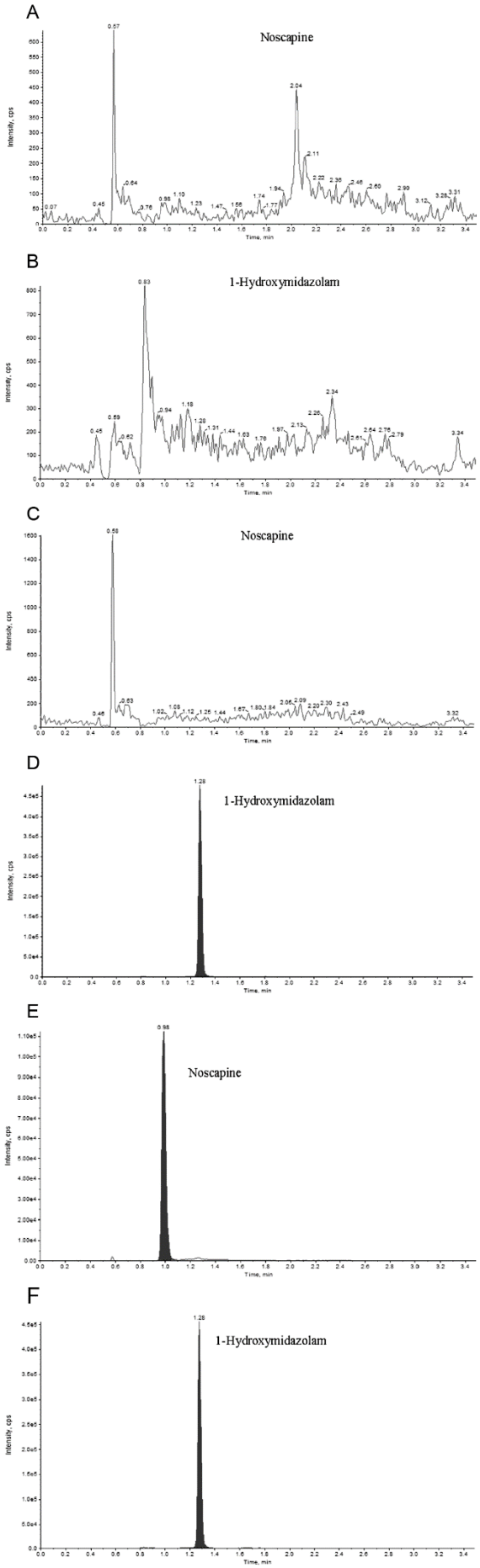

A
Noscapine
Intensity, cps
Time, min
B
1-Hydroxymidazolam
Intensity, cps
Time, min
C
Noscapine
Intensity, cps
Time, min
D
1-Hydroxymidazolam
Intensity, cps
Time, min
E
Noscapine
Intensity, cps
Time, min
F
1-Hydroxymidazolam
Intensity, cps
Time, min

Image 2

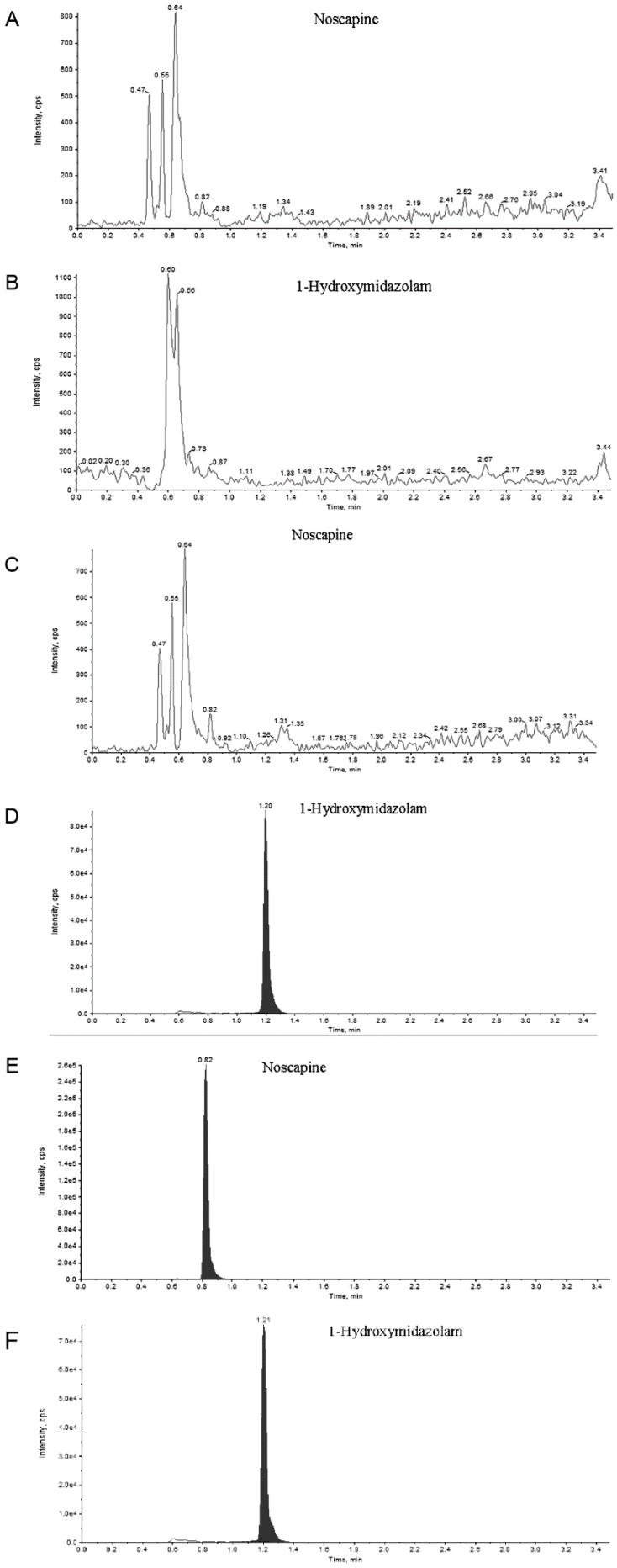

A
Noscapine
Intensity, cps
Time, min
B
1-Hydroxymidazolam
Intensity, cps
Time, min
C
Noscapine
Intensity, cps
Time, min
D
1-Hydroxymidazolam
Intensity, cps
Time, min
E
Noscapine
Intensity, cps
Time, min
F
1-Hydroxymidazolam
Intensity, cps
Time, min

Image 3

Siow et al HSPQoL Supplementary Material 1

List of items presented to modified Delphi panel in Round 1.

SECTION 1.
The aim of this section is to assess the implications of the genetic nature of HSP on quality of life.

Rationale for section: Hereditary spastic paraplegia is an inherited condition. Individuals with a genetic condition can experience concerns about passing on the condition to their children. This can affect their reproductive choices. In this section, we explore the worry, guilt and uncertainty associated with the inherited nature of HSP.

Question 1.1 explores the concerns that individuals with HSP have for their genetic relatives due to the inherited nature of the condition.
Question 1.1
How much do you worry that your siblings could have HSP?
• Not at all
• A little bit
• Moderately
• Quite a bit
• Extremely
• Not applicable (I don't have siblings)

Question 1.2 explores the worries associated with reproductive decision making for individuals with a genetic condition.

Conditional question for Question 1.2
Did/does your diagnosis of HSP influence your reproductive decisions (such as having/not having children, IVF, adoption, etc.)?
• Yes
• No
• Not applicable (I was diagnosed with HSP after I decided to/not to have children)

Question 1.2
If yes, how much worry did/do your reproductive decisions cause you?
• Not at all
• A little bit
• Moderately
• Quite a bit
• Extremely

Questions 1.3 to 1.5 address the worry and uncertainty experienced by parents associated with the inheritance risk of HSP. This risk is variable depending on which type of HSP an individual has and can be uncertain in individuals with a clinical diagnosis but no genetic diagnosis.

Conditional question for Questions 1.3 to 1.6
Do you have children?
• Yes, Please answer questions 1.3 to 1.6
• No, Please proceed to Section 2

Question 1.3
How much do you worry that your children could have HSP?
• Not at all
• A little bit
• Moderately
• Quite a bit
• Extremely

Question 1.4
If your children have HSP, how much do you worry about the impact of HSP on their future relationships?
• Not at all
• A little bit
• Moderately
• Quite a bit
• Extremely
• Not applicable (my children don't have HSP)

Question 1.5
If your children have HSP, how much do you worry about the impact of HSP on their future employment?
• Not at all
• A little bit
• Moderately
• Quite a bit
• Extremely
• Not applicable (my children don't have HSP)

Question 1.6 explores the guilt experienced by parents with HSP. Studies have identified that worries of an individual with a genetic condition passing the condition on to their children are very important to them.
Question 1.6
How much do you worry about passing on HSP to your children?
• Not at all
• A little bit
• Moderately
• Quite a bit
• Extremely
• Not applicable (I am not planning to have children)

SECTION 2.

The aim of this section is to assess the impact of symptoms specific to HSP on quality of life.

Rationale for section:
Individuals with HSP have specific symptoms that are unique to the condition. Generic QoL scales do

not measure the impact of these symptoms. This section addresses areas unique to HSP identified in quality of life studies to have a substantial impact on individuals' quality of life.

Questions 2.1 to 2.6 address the most common symptoms (pain and stiffness, gait and balance, and bladder/bowel dysfunction) experienced by individuals with HSP.

Question 2.1
During the past 4 weeks, how much have you stumbled?
• Not at all
• A little bit
• Moderately
• Quite a bit
• Extremely

Question 2.2
During the past 4 weeks, how much have you fallen?
• Not at all
• A little bit
• Moderately
• Quite a bit
• Extremely

Question 2.3
During the past 4 weeks, how much of the time have you experienced leg spasms?
• All of the time
• Most of the time
• Some of the time
• A little of the time
• None of the time

Question 2.4
During the past 4 weeks, how much did leg stiffness interfere with your normal activities?
• Not at all
• A little bit
• Moderately
• Quite a bit
• Extremely

Question 2.5
During the past 4 weeks, how much of the time have your legs given way?
• All of the time
• Most of the time
• Some of the time
• A little of the time
• None of the time

Question 2.6
During the past 4 weeks, how much of the time have your bladder and/or bowel symptoms interfered with your normal social activities?
• All of the time
• Most of the time
• Some of the time
• A little of the time
• None of the time

Question 2.7 addresses the impact of HSP symptoms on sleep quality. Poor sleep quality impacts individuals' quality of life.

Question 2.7

During the past 4 weeks, how much have your symptoms (e.g. pain, spasms) interrupted your sleep?

- Not at all
- A little bit
- Moderately
- Quite a bit
- Extremely

Question 2.8 to 2.9 addresses the fluctuating nature of HSP symptoms. Individuals with HSP have reported difficulty planning their day to day activities due to the unpredictability of their condition.

Question 2.8

How much do your symptoms fluctuate?

- Not at all
- A little bit
- Moderately
- Quite a bit
- Extremely

Question 2.9

How much do fluctuations in your symptoms affect your day to day activities?

- Not at all
- A little bit
- Moderately
- Quite a bit
- Extremely
- Not applicable

SECTION 3.

The aim of this section is to assess the visibility of HSP and its impact on quality of life.

Rationale for section:

Individuals with HSP experience symptoms that can be visible to the general public, such as leg stiffness, unsteady gait and incontinence. Individuals with HSP have described difficulty explaining their symptoms in social settings. In this section, we explore the emotional impact of the visibility of HSP on individuals' quality of life.

Questions 3.1 to 3.3 explore feelings of shame and being judged based on the visibility of HSP symptoms.

Question 3.1

Over the last 4 weeks, how much of the time have you felt that you had to conceal your symptoms of HSP?

- All of the time
- Most of the time
- Some of the time
- A little of the time
- None of the time

Question 3.2
Over the last 4 weeks, how much of the time have you felt ashamed of your symptoms of HSP?
• All of the time
• Most of the time
• Some of the time
• A little of the time
• None of the time

Question 3.3
Over the last 4 weeks, how much of the time have you felt judged based on your symptoms of HSP?
• All of the time
• Most of the time
• Some of the time
• A little of the time
• None of the time

SECTION 4.

The aim of this section is the impact of the progressive nature of HSP on quality of life.

Rationale: Individuals with HSP experience progression of their symptoms that can lead to gradual restriction of their activities. This often leads to emotional distress that impacts individuals' quality of life. In this section, we explore the physical and emotional impact of the progressive nature of HSP and the associated activity limitations.

Questions 4.1 to 4.2 assess the impact of HSP on individuals' activity limitations.

Question 4.1
I am able to continue doing the things I enjoy despite my HSP symptoms.
(e.g. leisure or work activities).
• Definitely true
• Mostly true
• Don't know
• Mostly false
• Definitely false

Question 4.2
I have had to quit the activities I enjoy due to the progression of my HSP symptoms (e.g. leisure or work activities).
• Definitely true
• Mostly true
• Don't know
• Mostly false
• Definitely false

Question 4.3 assesses feelings of fear and anxiety experienced by individuals' with HSP due to the progressive nature of the condition and uncertainty about their future.
Question 4.3
I fear for my future due to the progression of my HSP symptoms.
• Definitely true
• Mostly true

• Don't know
• Mostly false
• Definitely false

SECTION 5.

The aim of this section is to assess individuals' access to adequate healthcare and the impact of this on their quality of life.

Rationale: It is a common experience amongst individuals with HSP that there is a lack of awareness and expertise amongst healthcare providers. In this section, we explore the degree of support individuals' with HSP have from their health professionals.

Question 5.1
Do you feel you have adequate support from health professionals for the management of your HSP?
• Strongly Agree
• Mostly agree
• Neutral
• Mostly disagree
• Strongly Disagree

Question 5.2
How much does difficulty accessing health care for your HSP impact on your quality of life?
• Not at all
• A little bit
• Moderately
• Quite a bit
• Extremely

Score calculation methods for modified Delphi Round 1

Participants were asked:

Do you think this question is relevant to the aim of this section?

- Strongly Agree
- Mostly agree
- Neutral
- Mostly disagree
- Strongly Disagree

Do you think this question is clear?

- Strongly Agree
- Mostly agree
- Neutral
- Mostly disagree
- Strongly Disagree

Participant responses were assigned points according to the table below.

Participant response	**Points**
Strongly Agree	+2
Mostly Agree	+1
Neutral	0
Mostly disagree	-1
Strongly Disagree	-2

Points were added for each item and divided by maximum number of points (2*number of respondents) multiplied by 100 to produce a % score.

e.g. 4 participants “strongly agree”, 2 participants “mostly agree”, 1 participant “neutral”, 3 participants “mostly disagree”, 2 participants “strongly agree” = (4*2 + 2*1 + 1*0 + 3*-1 + 2*-2)/24*100 = 12.5%

List of items presented in modified Delphi round 2 (Highlighted items were included following this round)

Additional Demographic questions (to be included as matrix with yes/no options)

1. Do you have a clinical diagnosis of HSP?

2. Has your HSP been confirmed by a genetic test?

3. Do you have a partner?

4. Do you have children?

5. Do you have brothers or sisters to the same parents as you?

6. Do you have extended family (aunts, uncles, cousins, grandparents) who have a diagnosis of HSP?

7. Are you able to walk more than 10 steps without another person's help?

8. Where do you live? City - Y (Nearest hospital < 50km), Rural – N (Nearest Hospital >50km)

9. Do you have access to a specialised HSP clinic?

Original question	**Modified question**
SECTION 2 - Symptoms specific to HSP Question 2.1 to 2.3 are only for ambulant respondents Question 2.1 During the past 4 weeks, how much have you stumbled? • Not at all • A little bit • Moderately • Quite a bit • Extremely Relevance score: 79.2% Clarity score: 12.5% Question 2.2 During the past 4 weeks, how much have you fallen? • Not at all • A little bit • Moderately • Quite a bit • Extremely Relevance score: 79.2% Clarity score: 33.3% Question 2.3	Q2.1 Proposed modification: Questions 2.1 to 2.4 are combined in to one question. Participants will be able to respond to each part of the question separately. Options (a-c) for ambulant participants only. Over the past 4 weeks, how many times have you (a) lost your balance (stumbled or tripped)? (b) fallen? (c) experienced leg weakness? (d) felt leg spasms, stiffness, spasticity or cramps? • Not at all • 0-4 times a week • >4 times a week • 1-4 times a day • >4 times a day

During the past 4 weeks, how much of the time have your legs given way? • All of the time • Most of the time • Some of the time • A little of the time • None of the time Relevance score: 70.8% Clarity score: 37.5% Question 2.4 During the past 4 weeks, how much of the time have you experienced leg spasms? • All of the time • Most of the time • Some of the time • A little of the time • None of the time Relevance score: 83.3% Clarity score: 54.2%	
Additional question for section 2	Over the past year, how often have you had the following injuries from falls? (a) Cuts, bruises or sprains. (b) Fractures (broken bones) or dislocations. (c) Major injuries requiring surgery. • 0-10 times over the past year. • 11-20 times over the past year. • 21-30 times over the past year. • 31-40 times over the past year • >40 times over the past year
Question 2.5 During the past 4 weeks, how much did leg stiffness interfere with your normal activities? • Not at all • A little bit • Moderately • Quite a bit • Extremely Relevance score: 91.7% Clarity score: 79.2%	Q2.5 Proposed modification: During the past 4 weeks, how much did leg spasms, spasticity, stiffness or cramps affect your walking or limit your activities? • Not at all • A little bit • Moderately • Quite a bit • Extremely • Not applicable
Question 2.6 During the past 4 weeks, how much of the time have your bladder and/or bowel symptoms interfered with your normal social activities? • All of the time • Most of the time • Some of the time • A little of the time • None of the time Relevance score: 87.5% Clarity score: 58.3%	Q2.6 Proposed modification: During the past 4 weeks, have your bladder and/or bowel symptoms interfered with your life, (i.e. cannot get to the bathroom on time, have accidents, etc.) affecting (a) social activities? (b) ability to work? • Not at all • A little bit • Moderately • Quite a bit

	• Extremely
Question 2.7 During the past 4 weeks, how much have your symptoms (e.g. pain, spasms) interrupted your sleep? • Not at all • A little bit • Moderately • Quite a bit • Extremely Relevance score: 79.2% Clarity score: 54.2%	Q2.7 Proposed modification: During the past 4 weeks, how much have the following symptoms interrupted your sleep? (a) pain (b) spasms and stiffness (c) restless legs (d) bladder/bowel urgency • Not at all • A little bit • Moderately • Quite a bit • Extremely
Question 2.8 How much do your symptoms fluctuate? • Not at all • A little bit • Moderately • Quite a bit • Extremely Relevance: 66.7% Clarity score: 37.5%	Q2.8 Proposed modification: How much do your HSP symptoms (pain, spasms, stiffness, restless legs, bladder/bowel urgency) change during the day? • Not at all • A little bit • Moderately • Quite a bit • Extremely
Question 2.9 How much do fluctuations in your symptoms affect your day to day activities? • Not at all • A little bit • Moderately • Quite a bit • Extremely • Not applicable Relevance score: 58.3% Clarity score: 58.3%	Q2.9 Proposed modification: Q2.9 only to appear if participants experience symptom fluctuations in Q2.8. How much do changes in your HSP symptoms affect your ability to plan your day to day activities? • Not at all • A little bit • Moderately • Quite a bit • Extremely
SECTION 3 - Progressive Nature of HSP Question 3.1 I am able to continue doing the things I enjoy despite my HSP symptoms. (e.g. leisure or work activities). • Definitely true • Mostly true • Don't know • Mostly false • Definitely false Relevance score: 87.5% Clarity score: 79.2%	Q3.1 Proposed modification: I am able to continue enjoying leisure activities despite my HSP symptoms. • Definitely true • Mostly true • Don't know • Mostly False • Definitely false
Question 3.2 I have had to quit the activities I enjoy due to the progression of my HSP symptoms (e.g. leisure or work activities). • Definitely true • Mostly true • Don't know • Mostly false	Q3.2 Proposed modification: I have had to quit the type of work I enjoy due to progression of my HSP symptoms. • Definitely true • Mostly true • Don't know • Mostly False • Definitely false

• Definitely false Relevance score: 66.7% Clarity score: 54.2%	• Not applicable (I do not work)
Question 3.3 I fear for my future due to the progression of my HSP symptoms. • Definitely true • Mostly true • Don't know • Mostly false • Definitely false Relevance score: 79.2% Clarity score: 75%	Q3.3 Proposed modification: I remain hopeful for my future despite the progression of my HSP symptoms. • Definitely true • Mostly true • Don't know • Mostly False • Definitely false
Section 4 - Visibility of HSP Question 4.1 Over the last 4 weeks, how much of the time have you felt that you had to conceal your symptoms of HSP? • All of the time • Most of the time • Some of the time • A little of the time • None of the time Relevance score: 66.7% Clarity score: 58.3%	Q4.1 Proposed modification: Over the last 4 weeks, how much of the time have you felt that you wanted to hide your symptoms of HSP (e.g. avoiding situations where you have to walk or stand)? • Not at all • A little bit • Moderately • Quite a bit • Extremely
Question 4.2 Over the last 4 weeks, how much of the time have you felt ashamed of your symptoms of HSP? • All of the time • Most of the time • Some of the time • A little of the time • None of the time Relevance score: 54.2% Clarity score: 50%	Q4.2 Proposed modification: Over the last 4 weeks, how much of the time have you felt embarrassed by your symptoms of HSP? • All of the time • Most of the time • Some of the time • A little of the time • None of the time
Question 4.3 Over the last 4 weeks, how much of the time have you felt judged based on your symptoms of HSP? • All of the time • Most of the time • Some of the time • A little of the time • None of the time Relevance score: 75% Clarity score: 75%	Q4.3 Proposed modification: Over the last 4 weeks, how much of the time have you felt people acted differently around you because of your symptoms of HSP? • All of the time • Most of the time • Some of the time • A little of the time • None of the time
SECTION 5 - Genetic Nature of HSP Question 5.1 How much do you worry about passing on HSP to your children? • Not at all • A little bit	Q5.1 Proposed modification: Q5.1 only for participants who have children. How much does the chance of passing on HSP to your children concern you? • Not at all

• Moderately • Quite a bit • Extremely • Not applicable (I am not planning to have children) Relevance score: 83.3% Clarity score: 87.5%	• A little bit • Moderately • Quite a bit • Extremely
Question 5.2 Conditional question Did/does your diagnosis of HSP influence your reproductive decisions (such as having/not having children, IVF, adoption, etc.)? • Yes • No • Not applicable (I was diagnosed with HSP after I decided to/not to have children) If yes, how much worry did/do your reproductive decisions cause you? • Not at all • A little bit • Moderately • Quite a bit • Extremely Relevance score: 91.7% Clarity score: 75%	Q5.2 Proposed modification: Did/will your diagnosis of HSP influence your and your partner's decision about reproductive decisions (such as having/not having children, IVF, adoption, etc.)? • Yes • No If yes, how much concern did/will your decision to have/not have children cause (a) you? (b) your partner? • Not at all • A little bit • Moderately • Quite a bit • Extremely
Question 5.3 How much do you worry that your children could have HSP? • Not at all • A little bit • Moderately • Quite a bit • Extremely Relevance score: 83.3% Clarity score: 79.2%	Q5.3 Proposed modification: Combined with question 5.6. See proposed modification for Q5.6.
Question 5.4 If your children have HSP, how much do you worry about the impact of HSP on their future relationships? • Not at all • A little bit • Moderately • Quite a bit • Extremely • Not applicable (my children don't have HSP) Relevance score: 75% Clarity score: 50%	Q5.4 Proposed modification: If your children have HSP, how concerned are you about the impact of HSP on their: (a) future relationships (partner/spouse, family, friends)? (b) reproductive decisions (e.g. having/not having children, IVF, adoption, etc.) ? • Not at all • A little bit • Moderately • Quite a bit • Extremely • Not applicable
Question 5.5 If your children have HSP, how much do you worry about the impact of HSP on their future employment? • Not at all	Q5.5 Proposed modification: If your children have HSP, how concerned are you about the impact of HSP on (a) their opportunities for work?

• A little bit • Moderately • Quite a bit • Extremely • Not applicable (my children don't have HSP) Relevance score: 79.2% Clarity score: 87.5%	(b) their need for early retirement or going on the disability pension? • Not at all • A little bit • Moderately • Quite a bit • Extremely • Not applicable
Question 5.6 How much do you worry that your siblings could have HSP? • Not at all • A little bit • Moderately • Quite a bit • Extremely • Not applicable (I don't have siblings) Relevance score: 62.5% Clarity score: 62.5%	Q5.6 Proposed modification: How concerned are you that your blood relatives could have HSP? (a) Children (b) Brothers and sisters (b) Extended family (cousins, etc.) • Not at all • A little bit • Moderately • Quite a bit • Extremely • Not applicable
SECTION 6 - Accessibility of Healthcare Question 6.1 Do you feel you have adequate support from health professionals for the management of your HSP? • Strongly Agree • Mostly agree • Neutral • Mostly disagree • Strongly Disagree Relevance score: 83.3% Clarity score: 83.3%	Q6.1 Proposed modification: Do you feel you have enough support (treatments, counselling, information) from health professionals for management of your HSP? • Strongly agree • Mostly agree • Neutral • Mostly disagree • Strongly disagree
Question 6.2 How much does difficulty accessing health care for your HSP impact on your quality of life? • Not at all • A little bit • Moderately • Quite a bit • Extremely Relevance: 91.7% Clarity: 87.5%	Q6.2 Proposed modification: How much does difficulty getting health care to manage your HSP impact on your quality of life (day to day activities, social life, work life balance)? • Not at all • A little bit • Moderately • Quite a bit • Extremely

Interview Transcript

Standard opening at start of each interview:

"Thank you for coming to help us test out our survey questions. We are not collecting information about you. We are trying out questions on a few people so we can improve them.

I will read you the questions and I'd like to hear about what you're thinking when you answer them. Everything that comes to mind, whether it seems important or not.

I'll be asking you about how you come up with your answers and how you interpret the questions. We will record the interview with your consent and take lots of notes. If any question is unclear, hard to answer or does not make sense, please let me know. We will take our time within the 30 minutes. Do you have any questions before we start.

Practice think aloud: Try to visualize the place where you live, and think about how many windows there are in that place. As you count the windows, tell me what you are seeing and thinking about"

Examples of questions asked:

Was the question easy to understand?

How would you change the question to make it easier to understand?

Can you tell me in your own words what the question is asking?

Are you able to recall the time period asked? Is the time period appropriate?

Is this question appropriate to ask in a quality of life survey for HSP?

Overall, do you have any feedback for this question?

Was there anything else that you think should have been included in this questionnaire?

Siow et al HSPQoL Supplementary Material 2

Table 1. Key Themes of Quality of Life in HSP identified from literature review[1-10]

1. **HSP specific symptoms**
    - Pain
    - Spasticity, stiffness, cramps
    - Mobility
    - Fatigue
2. **Visibility of HSP and social and emotional impact**
    - Gait and Balance
    - Bladder and/or Bowel Dysfunction
    - Substantial fluctuation in symptoms
    - Sleep problems
    - Feeling ashamed and judged
    - Fear and frustration
    - Mood: Depression and anxiety
3. **Progressive nature of HSP and associated activity limitations**
    - Activity Limitations and quitting activities
4. **Access to specialized health care for HSP**
    - Access to reliable and practical information
    - Access to healthcare professionals with understanding of HSP
5. **Genetic nature of HSP**

Footnote. Five additional relevant articles were published since the literature review and were found to contain similar themes to the first ten articles[11-15].

Table 2. Relevance and clarity scores from Round 1 of modified Delphi. Calculation methods and list of items in Supplementary Material File 2.

Item	Relevance (%)	Clarity (%)
Section 1: Genetic nature of HSP		
1.1	62.5	62.5
1.2	91.7	75
1.3	83.3	79.2
1.4	75	50
1.5	79.2	87.5
1.6	83.3	87.5
Section 2: Symptoms specific to HSP		
2.1	79.2	12.5
2.2	79.2	33.3
2.3	83.3	54.2
2.4	91. 7	79.2
2.5	70.8	37.5
2.6	87.5	58.3
2.7	79.2	54.2
2.8	66.7	37.5
2.9	58.3	58.3
Section 3: Visibility of HSP		
3.1	66.7	58.3
3.2	54.2	50

3.3	75	75
Section 4: Progressive nature of HSP		
4.1	87.5	79.2
4.2	66.7	54.2
4.3	79.2	75
Section 5: Access to specialized healthcare		
5.1	83.3	83.3
5.2	91.7	87.5

Table 3. Results from Round 2 of modified Delphi process. List of items available in Supplementary Material File 2.

Item	**Include question (% respondents)**	**Include with modification (% respondents)**
Section 1: Symptoms specific to HSP		
1.1	50	100
1.2	75	91.67
1.3	66.67	83.33
1.4	58.33	91.67
1.5	75	75
1.6	75	83.33
Section 2: Progressive nature of HSP		
2.1 to 2.4 combined into one modified question	58.33	91.67
2.5	91.67	100
2.6	66.67	100

2.7	83.33	83.33
2.8	50	66.67
2.9	50	75
Section 3: Visibility of HSP		
3.1	58.33	91.67
3.2	58.33	75
3.3	75	66.67
Section 4: Genetic nature of HSP		
4.1	75	91.67
4.2	58.33	75
4.3	66.67	83.33
Section 5: Access to specialized healthcare for HSP		
5.1	75	58.33
5.2	91.67	83.33

Footnote: *Item 4.3 was not included as 5/12 respondents commented that it was vague and responses would be heavily influenced by whether it was phrased positively or negatively. The original question was phrased positively and the modified question was phrased negatively, therefore the authors felt that the nature of the question will influence an individual's response whichever way it was phrased.

Table 4. Cognitive interview results

Original Item	**Reason for revision**	**Revised Item**
Question 1 Over the past 4 weeks, how many times have you (a) Lost your balance (stumbled or tripped)? (b) Fallen? (c) Experienced leg weakness? (d) Felt leg spasms, stiffness, spasticity or cramps? • Not at all • 0-4 times a week • >4 times a weeks • 1-4 times a day • >4 times a day • I am non-ambulant	[Question: 2 respondents did not realise the question was referring to "the past 4 weeks" but were able to respond once prompted. [d] and [e]: 4 respondents found they had a different response for "stiffness and spasticity" symptoms to "spasms and cramps". Suggested separating the symptoms. Likert scale: 3 respondents found the response options difficult to choose from, they found the prescribed frequencies too specific and 1 respondent found their symptoms varied and another found their symptoms difficult to quantify.	Modified question 1 Over the past 4 weeks, how **often** have you (a) Lost your balance (stumbled or tripped)? (b) Fallen? (c) Experienced leg weakness? **(d) Felt leg stiffness or spasticity?** **(e) Felt spasms or cramps?** • Not at all • **Rarely (Once or twice a week)** • **Sometimes (Most days)** • **Often (Once or twice a day)**

	2 respondents found "non-ambulant" was difficult to understand.	• **All the time (More than twice a day)** **I do not walk**
<u>Question 2</u> Over the past 4 weeks, how much did leg spasms, spasticity, stiffness or cramps affect your walking or limit your activities? • Not at all • A little bit • Moderately • Quite a bit • Extremely • Not applicable	1 respondent felt that "affect your walking" and "limit your activities" were different concepts 1 respondent felt the question was similar to question 1 2 respondents mentioned that their symptoms varied but felt that they were able to choose a suitable answer.	Include as is
<u>Question 3</u> During the past 4 weeks, have your bladder and/or bowel symptoms interfered with your life, (i.e. cannot get to the bathroom on time, have accidents, etc.) affecting your (a) Social activities	[b]: 3 respondents did not work and felt that the second half of the question was not applicable to them. [c]: 2 respondents have made significant modifications to their lifestyle to manage their symptoms and felt the	<u>Modified question 3</u> During the past 4 weeks, (a) have your bladder and/or bowel symptoms interfered with your life (e.g. cannot get to the bathroom on time, have accidents)

(b) Ability to work • Not at all • A little bit • Moderately • Quite a bit • Extremely	question did not capture these changes. 1 respondent said that their social activities were limited by HSP symptoms other than bladder/bowel and therefore was unable to differentiate the various reasons for lifestyle limitations.	• Not at all • A little bit • Moderately • Quite a bit • Extremely **Additional questions** **(b) Have you had to stop working due to your bladder and/or bowel symptoms?** • **Yes** • **No** **(c) Do you make lifestyle modifications (e.g. drink less, frequent visits to the toilet) to manage your symptoms?** • **Yes** • **No**

Question 4 During the past 4 weeks, how much have the following symptoms interrupted your sleep? (a) Pain (b) Spasms and stiffness (c) Restless legs (d) Bladder/bowel urgency • Not at all • A little bit • Moderately • Quite a bit • Extremely	1 respondent was unsure of the meaning of "restless legs" 1 respondent said they did not have any symptoms as they were on baclofen 4 respondents felt it was an easy question to answer and appropriate to leave as is.	Include as is
Question 5 I am able to continue enjoying leisure activities despite my HSP symptoms. • Definitely true • Mostly true • Don't know • Mostly false • Definitely false	1 respondent described variability in symptoms depending on weather, and physical and mental limitations associated with HSP but was able to answer the question appropriately.	Include as is

	All respondents found the question easy to understand and answer.	
<u>Question 6</u> Over the last 4 weeks, how much of the time have you felt that you wanted to hide your symptoms of HSP (e.g. avoiding situations where you have to walk or stand)? • Not at all • A little bit • Moderately • Quite a bit • Extremely • Not applicable	1 respondent thought the question was unclear, "what else would you be doing if you're not walking or standing?". Not something she thought of before, suggested having more explanation on the aim of the question, e.g. changed what you do to avoid being judged. Felt it was OK to include but should be better worded, perhaps with the word "self-conscious" 1 respondent needed the question repeated and took some time to understand the purpose of the question.	<u>Modified question 6</u> **Symptoms of HSP can be visible to others and people may act differently around you because of your symptoms.** Over the last 4 weeks, how much of the time have you felt that you wanted to hide your symptoms of HSP (e.g. avoiding situations where you have to walk or stand)? • Not at all • A little bit • Moderately • Quite a bit Extremely
<u>Question 7</u> Did/will your diagnosis of HSP influence your and your	3 respondents felt the question was not applicable to them as they had already	<u>Modified question 7</u> **How much concern does the chance of passing on**

partner's reproductive decisions (such as having or not having children, IVF, adoption, etc.)? • Yes • No If yes, how much concern did/will your decision to have/not have children cause (a) You? (b) Your partner? • Not at all • A little bit • Moderately • Quite a bit • Extremely	completed their family before receiving their HSP diagnosis. 1 respondent already knew from an early age they weren't going to have children but felt the second part of the question was not applicable as the respondent had already made their decision before meeting their partner. 1 respondent suggested clarifying the partner's carrier status (e.g. for autosomal recessive HSP). (Referring back to the Delphi process, one of the participants very clearly articulated these complexities and proposed the above question which is more inclusive to those with and without children, as well as those who were	**HSP to your children or the reality of having passed on HSP to your children cause you?** • Not at all • A little bit • Moderately • Quite a bit • Extremely I do not have and am not planning to have children

	diagnosed before and after they had children. The proposed question wasn't used initially as there was a high concordance from the Delphi process for including the question in its original form but further feedback from these interviews have highlighted the issues with that question.)	
<u>Question 8</u> If your children have HSP, how concerned are you about the impact of HSP on their: (a) Future relationships (partner/spouse, family, friends)? (b) Reproductive decisions (e.g. having/not having children IVF, adoption, etc.)? • Not at all	3 respondents found the question unclear and difficult to answer. 2 respondents thought the questions was hypothetical as they did not have children or their children did not have HSP. 1 respondent thought the question may be too confronting for parents and that a clinician should be	Not included as questions was unclear and some respondents answered it as a hypothetical question. There was also concern that the question could be confronting. Modified question 7 addresses a similar concept.

• A little bit • Moderately • Quite a bit • Extremely • Not applicable	present when the question was being asked. 1 respondent found the first part of the question "future relationships" not applicable as their children were already in established relationships.	
<u>Question 9</u> How concerned are you that your blood relatives could have HSP? (a) Children? (b) Brothers and sisters? (c) Extended family (cousins, etc.)? • Not at all • A little bit • Moderately • Quite a bit • Extremely • Not applicable	4 respondents found the question difficult to answer. There were too many complexities with regards to multiple relatives who were affected or not affected. 2 respondents found it confrontational and 1 preferred not to answer the question as it was a sensitive topic. 1 suggested this concern should be discussed in a consultation with a clinician rather than in a survey.	Not included as concept is too complex to capture in a survey and modified question 7 addresses the concerns regarding heredity of the condition.

	1 respondent thought it was a hypothetical question. 1 respondent found it difficult to quantify their worry. 1 respondent found it easy to answer as it was applicable to their particular family situation.	
<u>Question 10</u> How much does difficulty accessing health care for your HSP impact on your quality of life? • Not at all • A little bit • Moderately • Quite a bit • Extremely	4 out of 5 respondents interpreted the question as referring to the physical logistics of attending a doctor's appointment. 1 respondent felt the question was unclear.	<u>Modified question 10</u> **HSP is a rare condition, and it may be difficult to access health care providers with an understanding of HSP.** Do you have difficulty accessing health care for your HSP? • Yes • No If yes, how much does this impact your quality of life? • Not at all • A little bit • Moderately

		• Quite a bit • Extremely

Table 5. Spearman Correlation of SF-36 Subscores with additional HSP-Specific Items (* and ** indicates statistical significance p<0.05 and p<0.01)

SF-36 Subscore/item numbers		37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
Physical functioning	Correlation Coefficient	.603**	.555**	.582**	.456**	.502**	.389**	.520**	.294*	.079	.364**	.343**	.193	.458**	.287*	.229	.067	.019	.127
	Sig. (2-tailed)	<.001	<.001	<.001	<.001	<.001	.005	<.001	.022	.545	.004	.007	.136	<.001	.025	.076	.608	.886	.495
Role functioning/physical	Correlation Coefficient	.312*	.401**	.408**	.413**	.253*	.361**	.299*	.176	.119	.223	.245	.147	.341**	.151	-.025	.060	.211	.316
	Sig. (2-tailed)	.014	.001	.001	<.001	.049	.009	.019	.174	.362	.084	.057	.257	.007	.245	.846	.645	.102	.083

	Role functioning/emotional	Correlation Coefficient	.285**	.267**	.276**	.402**	.337**	.546**	.412**	.189	.280*	.270*	.217	.186	.401**	.304*	-.270*	.190	.203	.517**
		Sig. (2-tailed)	.026	.038	.032	.001	.008	<.001	<.001	.144	.029	.035	.093	.151	.001	.017	.035	.143	.117	.003
	Energy/fatigue (vitality)	Correlation Coefficient	.294*	.331**	.397**	.501**	.389**	.485**	.388**	.268*	.144	.368**	.441**	.371**	.364**	.444**	-.090	.251	.063	.315
		Sig. (2-tailed)	.021	.009	.002	<.001	.002	<.001	.002	.037	.270	.004	<.001	.003	.004	<.001	.491	.052	.632	.084
	Emotional well-being (mental	Correlation Coe	.217	.323*	.306*	.418**	.326*	.441**	.362**	.310*	.093	.336**	.343**	.258*	.312*	.605**	-.396**	.319*	.276*	.359*

	health)	fficient																	
		Sig. (2-tailed)	.093	.011	.017	<.001	.010	.001	.004	.015	.478	.008	.007	.044	.014	<.001	.002	.012	.032	.047
	Social functioning	Correlation Coefficient	.2911*	.3044*	.4811**	.5277**	.4711**	.5488**	.4755**	.2006	.1005	.4117**	.4411**	.4666**	.4566**	.5532**	-.3332**	.1866	.2155	.2822
		Sig. (2-tailed)	.023	.017	<.001	<.001	<.001	<.001	<.001	.111	.420	<.001	<.001	<.001	<.001	<.001	.009	.151	.096	.125
	Pain	Correlation Coefficient	.166	.2544*	.245	.2966*	.3533**	.4588**	.5033**	.168	.126	.7711**	.4906**	.3846**	.4666**	.3766**	-.062	.3622**	-.031	.4399*
		Sig. (2	.2	.0	.0	.0	.0	<.0	<.0	.1	.3	<.0	<.0	.0	<.0	.0	.6	.0	.8	.0

		-tailed)	02	48	57	20	05	01	01	97	33	01	01	02	01	03	35	04	13	14
	General Health	Correlation Coefficient	.342**	.399**	.559**	.503**	.412**	.370**	.230	.171	.028	.260*	.419**	.158	.269*	.251	.027	.040	.027	.398*
		Sig. (2-tailed)	.007	.001	<.001	<.001	<.001	.008	.075	.187	.830	.043	<.001	.223	.036	.051	.835	.758	.836	.027

Table 6. Exploratory Factor Analysis results (* Items 51 and 52 loaded on the second component however were retained in this subscore as theoretically was the best fit and showed moderate-strong correlation)

SF36 subscore	Original Items	Additional Items	% variance explained by first component without additional items	% variance explained by first component with additional items	% variance explained by second component with additional items	Minimum factor loading
Physical Functioning	3-12	37-41	66.18%	54.95%	13.95%	0.565
Social Functioning	20, 32	42-43	79.47%	62.76%	19.77%	0.588
Pain	21,22	46	91.01%	83.38%	10.74%	0.884
Energy/Fatigue (vitality)	23, 27, 29, 31	47-49	64.33%	48.62%	18.05%	0.633
Emotional well-being (mental health)	24, 25, 26, 28, 30	50-52	58.82%	45.27%	13.41%	0.652*
General Health	1, 33, 34, 35, 36	54	51.87%	54.01%	19.23%	0.466
Role limitations due to physical health	13-16	Nil				
Role limitations due to emotional problems	17-19	Nil				
Health change	2	Nil				

References

1. Kerstens H, Satink T, Nijkrake MJ, et al. Stumbling, struggling, and shame due to spasticity: a qualitative study of adult persons with hereditary spastic paraplegia. *Disabil Rehabil* 2020; 42: 3744-3751. 20190513. DOI: 10.1080/09638288.2019.1610084.
2. Schneider SA, Beckinger VE, Moller B, et al. Urinary symptoms, quality of life, and patient satisfaction in genetic and sporadic hereditary spastic paraplegia. *J Neurol* 2019; 266: 207-211. 20181122. DOI: 10.1007/s00415-018-9129-8.
3. Sartori RDG, Marelli M, D'Angelo MG, et al. Autonomy level and quality of everyday experience of people with Hereditary Spastic Paraplegia. *Health Soc Care Community* 2019; 27: e850-e860. 20190717. DOI: 10.1111/hsc.12812.
4. Servelhere KR, Faber I, Saute JA, et al. Non-motor symptoms in patients with hereditary spastic paraplegia caused by SPG4 mutations. *Eur J Neurol* 2016; 23: 408-411. 2016/01/26. DOI: 10.1111/ene.12839.
5. Fjermestad KW, Kanavin OJ, Naess EE, et al. Health survey of adults with hereditary spastic paraparesis compared to population study controls. *Orphanet J Rare Dis* 2016; 11: 98. 20160713. DOI: 10.1186/s13023-016-0469-0.
6. Orsucci D, Petrucci L, Ienco EC, et al. Hereditary spastic paraparesis in adults. A clinical and genetic perspective from Tuscany. *Clin Neurol Neurosurg* 2014; 120: 14-19. 20140217. DOI: 10.1016/j.clineuro.2014.02.002.
7. Andrews JA, Paganoni S, Braastad C, et al. Disease burden in upper motor neuron syndromes: a survey of patient perspectives. *J Clin Neuromuscul Dis* 2014; 16: 104-105. DOI: 10.1097/cnd.0000000000000051.
8. Grose J, Freeman J and Marsden J. Service delivery for people with hereditary spastic paraparesis living in the South West of England. *Disabil Rehabil* 2014; 36: 907-913. 20130814. DOI: 10.3109/09638288.2013.824032.
9. Klimpe S, Schule R, Kassubek J, et al. Disease severity affects quality of life of hereditary spastic paraplegia patients. *Eur J Neurol* 2012; 19: 168-171. 20110601. DOI: 10.1111/j.1468-1331.2011.03443.x.
10. Braschinsky M, Rannikmae K, Krikmann U, et al. Health-related quality of life in patients with hereditary spastic paraplegia in Estonia. *Spinal Cord* 2011; 49: 175-181. 20100525. DOI: 10.1038/sc.2010.61.
11. Amprosi M, Indelicato E, Eigentler A, et al. Toward the Definition of Patient-Reported Outcome Measurements in Hereditary Spastic Paraplegia. *Neurol Genet* 2023; 9: e200052. 20230110. DOI: 10.1212/NXG.0000000000200052.
12. Jordan C, Geisel G, Alecu JE, et al. Disease Severity and Motor Impairment Correlate With Health-Related Quality of Life in AP-4-Associated Hereditary Spastic Paraplegia. *Neurol Genet* 2021; 7: e605. 20210720. DOI: 10.1212/NXG.0000000000000605.
13. Gassner H, List J, Martindale CF, et al. Functional gait measures correlate to fear of falling, and quality of life in patients with Hereditary Spastic Paraplegia: A cross-sectional study. *Clin Neurol Neurosurg* 2021; 209: 106888. 20210817. DOI: 10.1016/j.clineuro.2021.106888.
14. Rattay TW, Boldt A, Volker M, et al. Non-motor symptoms are relevant and possibly treatable in hereditary spastic paraplegia type 4 (SPG4). *J Neurol* 2020; 267: 369-379. 20191023. DOI: 10.1007/s00415-019-09573-w.
15. van Lith BJH, Kerstens H, van den Bemd LAC, et al. Experienced complaints, activity limitations and loss of motor capacities in patients with pure hereditary spastic paraplegia: a web-based survey in the Netherlands. *Orphanet J Rare Dis* 2020; 15: 64. 20200304. DOI: 10.1186/s13023-020-1338-4.

Siow et al HSPQoL Survey

Demographic questions

What is your age?
How do you describe your gender identity?
Do you have a clinical diagnosis of Hereditary Spastic Paraplegia (HSP)?
Has your HSP been confirmed by a genetic test?
Do you have a partner?
Do you have children?
Do your children have a diagnosis of HSP?
Do you have relatives who have a diagnosis of HSP?
Are you able to walk more than 10 steps without another person's help?
Where do you live?
Do you have access to a specialised HSP clinic?
Do you have other health issues not related to HSP?
Do your other health issues not related to HSP impact your quality of life?

HSPQoL (*items 44, 45 and 53 highlighted in grey were removed following the final validation step; additional HSP-specific items highlighted in blue)

1	In general, would you say your health is	1, Excellent 2, Very good 3, Good 4, Fair 5, Poor
2	Compared to one year ago, how would you rate your health in general now?	1, Much better now than one year ago 2, Somewhat better now than one year ago 3, About the same 4, Somewhat worse now than one year ago 5, Much worse now than one year ago
The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?		
3	Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	1, Yes, limited a lot 2, Yes, limited a little 3, No, not limited at all
4	Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	1, Yes, limited a lot 2, Yes, limited a little 3, No, not limited at all
5	Lifting or carrying groceries	1, Yes, limited a lot 2, Yes, limited a little

		3, No, not limited at all
6	Climbing several flights of stairs	1, Yes, limited a lot 2, Yes, limited a little 3, No, not limited at all
7	Climbing one flight of stairs	1, Yes, limited a lot 2, Yes, limited a little 3, No, not limited at all
8	Bending, kneeling, or stooping	1, Yes, limited a lot 2, Yes, limited a little 3, No, not limited at all
9	Walking more than one kilometre	1, Yes, limited a lot 2, Yes, limited a little 3, No, not limited at all
10	Walking half a kilometre	1, Yes, limited a lot 2, Yes, limited a little 3, No, not limited at all
11	Walking 100 metres	1, Yes, limited a lot 2, Yes, limited a little 3, No, not limited at all
12	Bathing or dressing yourself	1, Yes, limited a lot 2, Yes, limited a little 3, No, not limited at all
During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?		
13	Cut down the amount of time you spent on work or other activities	1, Yes 2, No
14	Accomplished less than you would like	1, Yes 2, No
15	Were limited in the kind of work or other activities	1, Yes 2, No
16	Had difficulty performing the work or other activities (for example, it took extra effort)	1, Yes 2, No
	During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?	
17	Cut down the amount of time you spent on work or other activities	1, Yes 2, No
18	Accomplished less than you would like	1, Yes 2, No

19	Didn't do work or other activities as carefully as usual	1, Yes 2, No
20	During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?	1, Not at all 2, Slightly 3, Moderately 4, Quite a bit 5, Extremely
21	How much bodily pain have you had during the past 4 weeks?	1, None 2, Very mild 3, Mild 4, Moderate 5, Severe 6, Very severe
22	During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?	1, Not at all 2, A little bit 3, Moderately 4, Quite a bit 5, Extremely
These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks...		
23	Did you feel full of life?	1, All of the time 2, Most of the time 3, A good bit of the time 4, Some of the time 5, A little of the time 6, None of the time
24	Have you been a very nervous person?	1, All of the time 2, Most of the time 3, A good bit of the time 4, Some of the time 5, A little of the time 6, None of the time
25	Have you felt so down in the dumps that nothing could cheer you up?	1, All of the time 2, Most of the time 3, A good bit of the time 4, Some of the time 5, A little of the time 6, None of the time
26	Have you felt calm and peaceful?	1, All of the time 2, Most of the time 3, A good bit of the time 4, Some of the time 5, A little of the time 6, None of the time

27	Did you have a lot of energy?	1, All of the time 2, Most of the time 3, A good bit of the time 4, Some of the time 5, A little of the time 6, None of the time
28	Have you felt down?	1, All of the time 2, Most of the time 3, A good bit of the time 4, Some of the time 5, A little of the time 6, None of the time
29	Did you feel worn out?	1, All of the time 2, Most of the time 3, A good bit of the time 4, Some of the time 5, A little of the time 6, None of the time
30	Have you been a happy person?	1, All of the time 2, Most of the time 3, A good bit of the time 4, Some of the time 5, A little of the time 6, None of the time
31	Did you feel tired?	1, All of the time 2, Most of the time 3, A good bit of the time 4, Some of the time 5, A little of the time 6, None of the time
32	During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc.)?	1, All of the time 2, Most of the time 3, Some of the time 4, A little of the time 5, None of the time
How TRUE or FALSE is each of the following statements for you?		
33	I seem to get sick a lot easier than other people	1, Definitely true 2, Mostly true 3, Don't know 4, Mostly false 5, Definitely false
34	I am as healthy as anybody I know	1, Definitely true 2, Mostly true

		3, Don't know 4, Mostly false 5, Definitely false
35	I expect my health to get worse	1, Definitely true 2, Mostly true 3, Don't know 4, Mostly false 5, Definitely false
36	My health is excellent	1, Definitely true 2, Mostly true 3, Don't know 4, Mostly false 5, Definitely false
Over the past 4 weeks, how often have you..		
37	Lost your balance (stumbled or tripped)	1, Not at all 2, Rarely (Once or twice a week) 3, Sometimes (Most days) 4, Often (Once or twice a day) 5, All the time (More than twice a day) 6, I do not walk
38	Fallen	1, Not at all 2, Rarely (Once or twice a week) 3, Sometimes (Most days) 4, Often (Once or twice a day) 5, All the time (More than twice a day) 6, I do not walk
39	Experienced leg weakness	1, Not at all 2, Rarely (Once or twice a week) 3, Sometimes (Most days) 4, Often (Once or twice a day) 5, All the time (More than twice a day) 6, I do not walk
40	Felt leg stiffness or spasticity	1, Not at all 2, Rarely (Once or twice a week) 3, Sometimes (Most days) 4, Often (Once or twice a day)

		5, All the time (More than twice a day) 6, I do not walk
41	Felt spasms or cramps	1, Not at all 2, Rarely (Once or twice a week) 3, Sometimes (Most days) 4, Often (Once or twice a day) 5, All the time (More than twice a day) 6, I do not walk
42	Over the past 4 weeks, how much did leg spasms, spasticity, stiffness, or cramps affect your walking or limit your activities?	1, Not at all 2, A little bit 3, Moderately 4, Quite a bit 5, Extremely 6, Not applicable
43	During the past 4 weeks, have your bladder or bowel symptoms interfered with your life (for example cannot get to the bathroom on time, have accidents)?	1, Not at all 2, A little bit 3, Moderately 4, Quite a bit 5, Extremely
44*	Have you had to stop working due to your bladder or bowel symptoms?	Yes, No
45*	Do you make lifestyle modifications (for example drink less, frequent visits to the toilet) to manage your symptoms?	Yes, No
During the past 4 weeks, how much have the following symptoms interrupted your sleep?		
46	Pain	1, Not at all 2, A little bit 3, Moderately 4, Quite a bit 5, Extremely
47	Spasms and stiffness	1, Not at all 2, A little bit 3, Moderately 4, Quite a bit 5, Extremely
48	Restless legs	1, Not at all 2, A little bit 3, Moderately 4, Quite a bit 5, Extremely
49	Bladder or bowel urgency	1, Not at all 2, A little bit 3, Moderately 4, Quite a bit 5, Extremely
50	I am able to continue enjoying leisure activities despite my HSP symptoms.	1, Definitely true 2, Mostly true 3, Don't know

		4, Mostly false 5, Definitely false
51	Symptoms of HSP can be visible to others and people may act differently around you because of your symptoms. Over the last 4 weeks, how much of the time have you felt that you wanted to hide your symptoms of HSP (for example avoiding situations where you have to walk or stand)?	1, All of the time 2, Most of the time 3, Some of the time 4, A little of the time 5, None of the time
52	Do you have any concern about the potential for passing on HSP to your children? (Please answer, regardless of whether or not you are planning to have children or have completed your family prior to your HSP diagnosis)	1, Not at all 2, A little bit 3, Moderately 4, Quite a bit 5, Extremely
53*	HSP is a rare condition, and it may be difficult to access health care providers with an understanding of HSP. Do you have difficulty accessing health care for your HSP?	Yes, No
54	How much does difficulty accessing health care for your HSP impact your quality of life?	1, Not at all 2, A little bit 3, Moderately 4, Quite a bit 5, Extremely
	Any additional comments?	Free-text