Evidence for Non-Mendelian Inheritance in Spastic Paraplegia 7

Mehrdad A. Estiar, MSc,1,2 Eric Yu, MSc,1,2 Ikhllass Haj Salem, PhD,3 Jay P. Ross, MSc,1,2 Kheireddin Multi, MSc,1,2 Fulya Akçimen, MSc,1,2 Etienne Leveille, MD,3 Dan Spiegelman, MSc,2 Jennifer A. Ruskey, MSc,2 Farnaz Asayesh, MSc,2 Alain Dagher, MD,2 Grace Yoon, MD,5 Mark Tarnopolsky, MD,6 Kym M. Boycott, MD,7 Nicolas Dupre, MD, MSc,8,9 Patrick A. Dion, PhD,1,2,10 Oksana Suchowersky, MD,11 Jean-Francois Trempe, PhD,12,13 Guy A. Rouleau, MD, PhD,1,2,10* and Ziv Gan-Or, MD, PhD1,2,10

1Department of Human Genetics, McGill University, Montréal, Québec, Canada
2The Neuro (Montreal Neurological Institute-Hospital), McGill University, Montréal, Québec, Canada
3Faculté de Médecine, Université Laval, Québec City, Québec, Canada
4Faculty of Medicine, McGill University, Montréal, Québec, Canada
5Divisions of Neurology and Clinical and Metabolic Genetics, Department of Paediatrics, University of Toronto, Toronto, Ontario, Canada
6Department of Pediatrics, McMaster University, Hamilton, Ontario, Canada
7Children’s Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, Ontario, Canada
8Neuroscience Axis, CHU de Québec, Université Laval, Québec City, Québec, Canada
9Department of Medicine, Faculty of Medicine, Université Laval, Québec City, Québec, Canada
10Department of Neurology and Neurosurgery, McGill University, Montréal, Québec, Canada
11Departments of Medicine (Neurology) and Medical Genetics, University of Alberta, Edmonton, Alberta, Canada
12Department of Pharmacology & Therapeutics, McGill University, Montréal, Québec, Canada
13Centre de Recherche en Biologie Structurale, McGill University, Montréal, Québec, Canada

ABSTRACT: Background: Although the typical inheritance of spastic paraplegia 7 is recessive, several reports have suggested that SPG7 variants may also cause autosomal dominant hereditary spastic paraplegia (HSP).

Objectives: We aimed to conduct an exome-wide genetic analysis on a large Canadian cohort of HSP patients and controls to examine the association of SPG7 and HSP.

Methods: We analyzed 585 HSP patients from 372 families and 1175 controls, including 580 unrelated individuals. Whole-exome sequencing was performed on 400 HSP patients (291 index cases) and all 1175 controls.

Results: The frequency of heterozygous pathogenic/likely pathogenic SPG7 variants (4.8%) among unrelated HSP patients was higher than among unrelated controls (1.7%; OR 2.88, 95% CI 1.24–6.66, P = 0.009). The heterozygous SPG7 p.(Ala510Val) variant was found in 3.7% of index patients versus 0.85% in unrelated controls (OR 3.65, 95% CI 1.49–13.07, P = 0.005). Similar results were obtained after including only genetically-undiagnosed patients. We identified four heterozygous SPG7 variant carriers with an additional pathogenic variant in known HSP genes, compared to zero in controls (OR 19.58, 95% CI 1.05–365.13, P = 0.0031), indicating potential digenic inheritance. We further identified four families with heterozygous variants in SPG7 and SPG7-interacting genes (CACNA1A, AFG3L2, and MORC2). Of these, there is especially compelling evidence for epistasis between SPG7 and AFG3L2. The p.(Ile705Thr) variant in AFG3L2 is located at the interface between hexamer subunits, in a hotspot of mutations associated with spinocerebellar ataxia type 28 that affect its proteolytic function.

Conclusions: Our results provide evidence for complex inheritance in SPG7-associated HSP, which may include recessive and possibly dominant and digenic/epistasis forms of inheritance. © 2021 International Parkinson and Movement Disorder Society

Key Words: spastic paraplegia; HSP; SPG7; oligogenic inheritance

*Correspondence to: Dr. Guy A. Rouleau, Director, Montreal Neurological Institute and Hospital, 3801, University Street, Office 636, Montréal, QC H3A 2B4, Canada; E-mail: guy.rouleau@mcgill.ca; or Dr. Ziv Gan-Or, Department of Neurology and Neurosurgery, McGill University, 1033 Pine Avenue, West, Ludmer Pavilion, Room 312, Montreal, QC H3A 1A1, Canada; E-mail: ziv.gan-or@mcgill.ca

Relevant conflicts of interest/financial disclosures: The authors report no competing interests.

Funding agency: This study was funded by CIHR Emerging Team Grant, in collaboration with the Canadian Organization for Rare Disorders (CORD), grant number RN127580 – 260005, and by a CIHR Foundation grant granted to G.A.R. This research was undertaken thanks in part to funding from the Canada First Research Excellence Fund, awarded to McGill University for the Healthy Brains for Healthy Lives initiative granted to M.A.E.

Received: 23 October 2020; Revised: 19 January 2021; Accepted: 20 January 2021

Published online 17 February 2021 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.28528
Introduction

Hereditary spastic paraplegia (HSP) is a group of rare neurodegenerative diseases considered to be inherited in a classical monogenic Mendelian manner. More than 80 loci/genes have been implicated in HSP, and some of these genes are also involved in diseases whose typical features differ from HSP.1 HSP patients may present with a wide spectrum of symptoms, from very subtle lower limb spasticity to severe neurological and non-neurological manifestations. These symptoms often overlap with other disorders, which may lead to incorrect diagnoses.2,3

Spastic paraplegia 7 (SPG7) is the first-identified autosomal recessive (AR) type of HSP, accounting for 5%–12% of AR-HSP.4 Pathogenic variants in SPG7 may also lead to spastic and/or cerebellar ataxia, peripheral neuropathy with no other neurological symptoms, primary progressive multiple sclerosis, amyotrophic lateral sclerosis, primary lateral sclerosis, parkinsonism, limb dystonia, and isolated dominant optic atrophy.5-13 Although the inheritance of SPG7 is considered to be AR, several reports suggested that SPG7 variants may also cause autosomal dominant (AD) HSP.5,14-16 None of these studies examined the possibility of digenic inheritance (carrying two mutations in different HSP genes), therefore the role of SPG7 in AD-HSP remains controversial.

SPG7 encodes the paraplegin/SPG7 protein, localized at the inner mitochondrial membrane. SPG7 is comprised of 17 exons with several mutational hotspots.17 The p.(Ala510Val) variant is a known hotspot in SPG7, which despite relatively high allele frequency (AF) in public databases (0.0027 in gnomAD controls) is considered pathogenic,5,16,18 likely with incomplete penetrance or variable expressivity. The high frequency of the p.(Ala510Val) variant, the wide clinical spectrum, and the possibility of dominant and recessive inheritance make diagnosis and genetic counseling in SPG7 challenging.

Herein, we performed a comprehensive genetic analysis in a large cohort of HSP patients. We examined whether heterozygous SPG7 variants are overrepresented in HSP patients, and whether digenic inheritance of SPG7 variants together with other HSP-related gene variants may occur in HSP.

Methods

Population

A total of 585 HSP patients from 372 families and 1175 control individuals (Tables S1 and S2, respectively) have been recruited across Canada, through the CanHSP consortium as previously reported,19 and were analyzed for the presence of SPG7 variants. Initially, 379 HSP patients were analyzed using different sequencing panels of known HSP genes, some included SPG7 and some did not. Of those, 194 were not genetically diagnosed, and went through whole-exome sequencing (WES). An additional 206 HSP patients and 1175 individuals who served as a control group were sequenced directly using WES. These 1175 controls were collected at the laboratory of Dr. Guy Rouleau, and included healthy individuals and individuals with non-movement disorders that do not have any known overlap with HSP (details of these controls are in Table S2). Of the 1175 controls, 580 were unrelated individuals. Of all the unrelated control individuals (n = 580) and HSP index cases (n = 372, one from each family), 70% and 88.4% were Europeans, respectively, according to the HapMap Project data.20 Principal component analysis (PCA, Fig. S1) shows overlapping main principal components. Since we study very rare variants in cases and controls, ethnicity is likely to have no or very minor effect. The diagnosis of HSP was based on previously published criteria.21 Standardized clinical assessments were applied and included demographic characteristics, family history, pedigree, developmental history, and clinical symptoms. For a subset of patients, brain and spinal cord magnetic resonance imaging (MRI) were performed and the Spastic Paraplegia Rating Scale (SPRS) score was assessed.22 All participants signed an informed consent form prior to enrollment, and the institutional review boards approved the study protocols.

Genetic and Data Analysis

DNA was extracted from peripheral blood using a standard salting-out procedure. In the HSP group, 379 patients were initially screened using panel sequencing of HSP-associated genes. In total, 1575 samples including 400 HSP and 1175 control samples went through WES. For exome target enrichment, SureSelect Human All Exon V4, V5, Nextera Rapid Capture Exomes, and TruSeq Exome (Illumina) kits were used, and the sequencing was performed on Illumina HiSeq 2000/2500 (San Diego, CA). The sequence reads were aligned to the human reference genome (GRCh37/hg19) using Burrows-Wheeler Aligner.23 Variant calling and annotation were done using Genome Analysis Toolkit and ANNOVAR, respectively.24,25 Missense and truncating variants with minor allele frequency less than 0.01 in gnomAD were included in the analysis.26 These variants were classified according to the American College of Medical Genetics and Genomics guidelines using VarSome.27 SPG7 variants classified as “Variants of Unknown Significance”, “Likely Benign”, and “Benign” were excluded, and only “Pathogenic” and “Likely Pathogenic” variants were included. Intronic splicing variants at ≥±3 position with uncertain significance higher were also excluded. Variant calls with less than 30× depth of coverage, a genotype quality of less than 97, and less than 25% genotyping
frequency were excluded from the analysis. All the detected variants were visually inspected with the Integrated Genomics Viewer and suspicious variants were validated using Sanger Sequencing. To check for relatedness in the control group and in HSP families with complex inheritance of SPG7, we used somalier. To examine whether carriers of SPG7 variants may carry other variants in other HSP-associated genes, or in genes linked to similar neurogenetic disorders, we screened for variants in 787 genes (Table S3) in HSP patients who carried at least one SPG7 allele.

Gene Ontology (GO) enrichment analysis was carried out using g:Profiler, with Benjamini–Hochberg adjusted P values for statistical significance set at <0.05. For protein–protein interaction/network analysis, STRING and GeneMANIA were used. In order to predict the presence of important domains and sites of the corresponding protein, as well as multiple protein sequence alignment, we applied InterPro and Clustal Omega tools, respectively. A three-dimensional atomic model of tubulin cofactor E (TBCE) (a.a. 97–443) was built using the automated server I-TASSER. The model was derived from the coordinates of different leucine-rich repeat (LRR) domains sharing 12%–20% identity. A second model of the TBCE LRR domain (a.a. 97–347) was also built with SWISS-MODEL with the structure of the plant receptor BRI1 (pdb 3rj0, 33% sequence identity for this segment). The atomic coordinates of the TBC CAP-Gly domain (pdb 4b6m), TBCE Ubl domain (pdb 4icu), and human AFG3L2 (pdb 6nyy) were downloaded from the Protein Data Bank. The steric clashes induced by each mutation were evaluated using the “mutagenesis” toolbox in PyMol v. 2.3.5.

To study genotype–phenotype correlations in SPG7, we removed carriers of other pathogenic/likely pathogenic variants in HSP-related genes to avoid bias by other, non-SPG7 variants. We compared the following groups of patients: (1) carriers of homozygous variants; (2) carriers of compound heterozygous variants; and (3) carriers of heterozygous variants. Since it was previously suggested that HSP patients with the p.(Ala510Val) variant have a milder phenotype, we also compared carriers of this variant to other SPG7 patients. In addition, due to the report of differences in clinical presentations between SPG7 patients with loss-of-function (LoF) and missense variants, patients were classified and compared based on the variant type including patients with: (1) one missense; (2) one LoF; (3) one missense and one LoF; (4) two missense; and (5) two LoF variants.

### Statistical Analysis

For binary variables, chi-square and Fisher’s exact test were used, and for continuous variables Mann–Whitney U and Kruskal–Wallis tests were used, as required. SPSS was used to perform all statistical analyses. For the genotype–phenotype analysis of symptoms, Bonferroni correction for multiple comparisons was applied and corrected P value threshold was set to <0.0005.

### Results

#### Bi-Allelic and Monoallelic SPG7 Variant Carriers

Of 585 HSP patients, potentially pathogenic rare SPG7 variants were identified through either panel sequencing or WES in 38 patients (6.5%) with homozygous or compound heterozygous variants and in 21 patients (3.6%) with heterozygous variants. In order to further compare frequencies of SPG7 variants between patients and controls, we only included samples that went through WES, since controls did not go through panel sequencing, and since different sequencing panels were used, some of which did not include SPG7. In index (unrelated) cases of HSP who underwent WES (n = 291), 48 pathogenic/likely pathogenic SPG7 alleles were detected compared to 10 in unrelated controls (n = 580, OR 6.57, 95% CI 2.12–20.39, P = 0.0013). After excluding biallelic SPG7 mutation carriers, 13 (of n = 270, 4.81%) heterozygous pathogenic/likely pathogenic SPG7 variant carriers were identified versus 10 (1.72%) carriers which were identified among 580 unrelated controls (OR 2.88, 95% CI 1.24–6.66, P = 0.009). The heterozygous SPG7 p.(Ala510Val) variant was found in 10 (3.70%) of the index cases versus 5 (0.85%) in unrelated controls (OR 4.42, 95% CI 1.49–13.07, P = 0.005). We further examined the role of heterozygous pathogenic/likely pathogenic SPG7 variants only in genetically undiagnosed HSP patients. Of 148 index cases with genetically undiagnosed HSP patients, 10 (6.76%) carried an SPG7 allele.

Evidence of Potential Digenic Inheritance and Modifier Effects in SPG7-Associated HSP

We further hypothesized that the overrepresentation of heterozygous SPG7 variants in HSP patients versus controls could be due to digenic inheritance, namely that patients with heterozygous SPG7 variants may also carry variants in other HSP or similar neurogenetic disorders-associated genes (Table S3). We found four
index cases with heterozygous SPG7 variant who also carried pathogenic variants in other genes that are associated with spastic paraplegia/ataxia (Table 1) compared to zero in controls (OR 19.58, 95% CI 1.05–365.13, \( P = 0.0031 \); Fisher’s exact test after Haldane–Anscombe correction). Of note, the penetrance of HSP-related mutations is not necessarily complete,\(^{39}\) therefore there could be controls with such mutations. One of these four families (01-013) had two affected members who carried a pathogenic variant in BSCL2. One of the affected members of this family carried an SPG7 p.(Ala510Val) variant while the other one was a non-carrier. In addition to the core symptoms, the SPG7 variant carrier also presented with swallowing difficulties, upper extremity weakness, hyperreflexia, and lower motor neuron features. One patient with heterozygous SPG7 variant also carried two variants in TBCE (Table 1), which is known to cause encephalopathy with spasticity. One of the two TBCE variants was annotated as pathogenic, while the other was annotated as likely benign. This patient presented with clinical features that fit both SPG7 and TBCE (Table 1). In addition, this patient presented with nephropathy and focal segmental glomerulosclerosis, which have not been previously reported in SPG7 and are a very rare clinical finding in HSP.\(^{40}\) We could not find a pathogenic variant in a gene related to nephropathy in this patient. Two other patients carried SPAST mutations (Table 1). The average age at onset (AAO) of these four potentially digenic patients was 2.7 ± 2.1 years compared to 28.0 ± 17.9 years in all other patients with SPG7 variants (\( P = 0.060 \), Mann–Whitney \( U \) test). Upper extremity weakness (2/2 vs. 3/38, \( P = 0.013 \)) and hyperreflexia (3/3 vs. 11/39, \( P = 0.032 \)) were more common among the potentially digenic patients with available data. None of these differences was statistically significant after correction for multiple comparisons.

To examine whether SPAST, BSCL2, and TBCE (the genes in which we found additional mutations in patients who also carry SPG7 heterozygous mutation, Table 1) are involved in specific cellular pathways in which SPG7 is also involved, we performed pathway enrichment analysis. We found enrichment mainly in axonal transport and cellular organization (adjusted \( P < 0.05 \), Table S5), with SPAST and SPG7 having the highest enrichment. Both proteins play a role in axonal transport and involved in biosynthesis, assembly, and arrangement of macromolecules and cellular membrane and other components (Table S5). Spastin (SPG4) and paraplegin (SPG7) contain the same ATPase domain (IPR003959, IPR003593).

To identify additional patients who potentially have HSP due to digenic inheritance involving SPG7 we examined very rare variants (AF < 0.001) in genes that produce proteins that interact with SPG7 (Table S6). We found very rare heterozygous variants with uncertain significance in three genes (CACNA1A, AFG3L2, and MORC2) in heterozygous carriers of SPG7 (Table 2). Among them, AFG3L2 had the closest interaction with SPG7 and both share similar features, including protein structure and domains, interacting proteins, and biological functions and pathways (Table S7). Except for one homozygous AFG3L2 variant, c.122G > A;[p.[Arg41Gln]) with gnomAD AF 0.0000048, which was identified in one control (who did not carry an SPG7 variant) out of 1175, no rare variants were detected in CACNA1A, AFG3L2, and

![FIG. 1. Schematic representation of the locations of SPG7 variants in hereditary spastic paraplegia (HSP) patients and controls. InterPro predicted four domains for spastic paraplegia 7 (SPG7) including Pept_M41, AAA + ATPase, AAA_lid_3, and Peptidase_M41 domains. Missense variants are indicated in red while loss of function variants are in black. The numbers below represent the number of individuals carrying the specific variant. [Color figure can be viewed at wileyonlinelibrary.com]](attachment:file.png)
TABLE 1. Genetic and clinical characteristics of hereditary spastic paraplegia (HSP) patients who carried at least one SPG7 allele along with pathogenic non-spastic paraplegia 7 (SPG7) variant(s) that could explain the disease

| CanHSP | Symptoms | OMIM | Identified gene variant (inheritance mode) | Novelty | VarSome | CADD | Identified SPG7 variants (inheritance mode) | VarSome/pathogenicity of SPG7 variant in previous studies |
|--------|----------|------|------------------------------------------|---------|---------|------|------------------------------------------|---------------------------------------------------------------|
| FSP 01-013 | LEW, LES, LEH, extensor plantar responses, abnormal bladder function, ankle clonus, swelling difficulties, upper extremity weakness, upper extremity hyperreflexia, amytotrophy or lower motor neuron features, abnormal EMG results, AAO: 5 | LEW, LES, LEH, hyperreflexia, extensor plantar responses, pes cavus, decreased vibratory sense, distal limb muscle weakness and atrophy, AAO: 8–40 | BSCL2 NM_032667 c.269C > T; (p.[Ser90Leu]) (Het) | Reported Pathogenic | 34 | 0.5876 (disease causing) | c.1523C > T; (p.[Ala510Val]) (Het) | Pathogenic |
| FSP 01-171 | LES, LEH, abnormal bladder function, ankle clonus, upper extremity hyperreflexia, mild vibratory loss, AAO: 2 | LEW, LES, LEH, extensor plantar responses, pyramidal signs, cognitive decline, decreased vibratory sense, deficits in language expression, AAO: variable | SPAST NM_014946 c.1529C > T; (p.[Ala510Val]) (Het) | Novel Pathogenic | – | – | c.1523C > T; (p.[Ala510Val]) (Het) | Pathogenic |
| FSP 01-208 | NA | – | SPAST deletion of exons 5–7 (Het) | – | – | – | 2293G > A; (p.[Asp765Asn]) (Het) | Likely pathogenic/– |
| FSP 04-012 | LEW, LES, LEH, extensor plantar responses, ankle clonus, speech delay or abnormality, learning disability, progressive cognitive deficits, deafness, dysarthria, upper extremity weakness, upper extremity hyperreflexia, nephropathy, focal segmental glomerulosclerosis, SPRS: 37, AAO: 1 | Distal amyotrophy, encephalopathy, delayed psychomotor development, motor regression, spinal muscular atrophy, intellectual disability, ataxia, spastic tetraplegia, dysarthria, absence of speech, cerebellar atrophy, thin corpus callosum, axonal peripheral neuropathy, AAO: infantile | TBOE NM_003193 c.661G > C; (p.[Val221Leu]) & c.1537C > T; (p.[Gln513Ter]) (Comp Het) | Novel & novel Likely benign & pathogenic | 15.44 & 41 | 0.5876 (disease causing) & 0.81 (disease causing) | c.1796G > T; (p.[Arg599Leu]) (Het) | Likely pathogenic/– |

Abbreviations: OMIM, Online Mendelian Inheritance in Man; CADD, Combined Annotation-Dependent Depletion; LEW, lower extremity weakness; LES, lower extremity spasticity; LEH, lower extremity hyperreflexia; Het, heterozygous; Comp Het, compound heterozygous; AAO, age at onset (years); EMG, electromyography; NA, not available; SPRS, Spastic Paraplegia Rating Scale.
MORC2 in our controls. Yet, to confirm their potential pathogenicity in digenic HSP, additional genetic and functional studies are needed. Of note, we have also identified three controls with rare variants in the genes ATP13A2 (p.[Val803Ile]), FARS2 (p.[Ala302Ser]), and MTUS2 (p.[Arg1091Leu]), which may also interact with SPG7.

**Family History in Carriers of Heterozygous and Potential Digenic SPG7 Variants**

Families with heterozygous SPG7 variants, with or without variants in other genes, showed different modes of inheritance, further supporting potential complex and/or non-Mendelian inheritance associated with SPG7 (Fig. S2). For example, family 03-019 had a dominant model of inheritance, and three individuals from three different generations carried the SPG7 p.(Ala510Val) variant, and no other variants that can explain this inheritance. In families 01-090 and 02-048, the index cases also carried the same variant, and in these families too the inheritance seems to be dominant. In family 01-190, the index case carried the SPG7 p.(Ala510Val) variant and the CACNA1A p.(Arg1661Cys), and while there are patients in each of the three generations documented in this pedigree, the inheritance could only be dominant with partial penetrance, as the mother of the index case (indicated with an arrow) was not reported to have the disease at the time the data were collected. Other families, such as 01-009, 01-013, and 01-052, may suggest incomplete inheritance, digenic inheritance, or other forms of complex inheritance (Fig. S2).

**Structural Analysis of TBCE and AFG3L2 Variants**

Among the non-HSP genes that may contribute to oligogenic inheritance or epistasis, AFG3L2 and TBCE were the strongest candidates. To further examine whether the two TBCE variants identified in family 04-012 (Table 1) may be pathogenic, we performed structural analysis of their potential effects on TBCE structure and function. The human tubulin cofactor E (TBCE) consists of an N-terminal CAP-Gly domain, followed by an LRR domain and a C-terminal ubiquitin-like (Ubl) domain (Fig. 2A). The structures of TBCE alone and in complex with α-tubulin and tubulin folding cofactor B (TBCB) were determined at low resolution by electron microscopy.46 The complex structure shows that α-tubulin binds to the CAP-Gly domain as well as the concave surface of the LRR domain. This interaction enables the TBCE−TBCB complex to dissociate the α/β tubulin heterodimers into monomers that can be degraded by the ubiquitin−proteasome system.47 To investigate the impact of the potential HSP-related variant p.(Val221Leu) on TBCE’s structure and function, we performed in silico mutagenesis of the residue in
two homology models of TBCE. Val221 is located on the “exterior” side of the LRR domain, opposite to the side of interaction with tubulin (Fig. 2A). In the homology model generated with I-TASSER, the side chain of Val221 points towards the solvent. The variant Val to Leu results in a clash with a helix in an adjacent repeat. In the homology model generated with SWISS-MODEL using the homologous LRR domain of the plant steroid hormone receptor BRI1 (pdb 3rj0), Val221 is located in the hydrophobic core, and the p.(Val221Leu) variant also leads to steric clashes (Fig. 2C). In both models, our prediction is that this variant destabilizes the domain and might inactivate it. The mutation p.(Gln513Ter) would result in a 15 amino acid deletion in the Ubl domain of TBCE (Fig. 2A). The crystal structure of the Ubl (pdb 4icu) shows that this segment comprises a helix and the C-terminal β strand, which is typically involved in protein–protein interactions. Deletion of this segment would therefore completely unfold the Ubl and disrupt its function. The Ubl domain might be involved in shuttling tubulin to the proteasome, given that the proteasome subunit Rpn10 binds to ubiquitin and Ubl domains.

However, the Ubl domain of TBCE has only 21% sequence identity with ubiquitin, and comparison of the TBCE Ubl with the complex of Rpn10 and the Ubl of UBQLN2 reveals that most residues interacting with Rpn10 are not conserved between UBQLN2 and TBCE. Therefore, the TBCE Ubl is unlikely to bind to the proteasome and its function remains unknown.

AFG3L2 is a subunit of the m-AAA protease complex, which cleaves proteins in the mitochondrial inner membrane. It consists of an N-terminal segment anchored to the membrane, followed by an ATPase and protease domains that assemble into a hexamer. The structure of the soluble domains of human AFG3L2 occurred in the peptidase M41 domain (IPR000642) which belongs to metallopeptidase family.

Sequence alignment of AFG3L2 orthologs, showing conservation of Ile705. The AFG3L2 variant occurred in an amino acid that is highly conserved among species. [Color figure can be viewed at wileyonlinelibrary.com]
results in no steric clash, but would make the interface less hydrophobic. Intriguingly, Ile705 is located in a "hotspot" of SCA28-associated mutations such as p.(Thr654Ile), p.(Met666Arg/Val/Thr), p.(Pro688Thr), p.(Tyr689His/Asn), p.(Glu691Lys), p.(Glu700Lys), and p.(Arg702Gln) (Fig. 2E). All of these mutations strongly reduce the ATPase and degradation rates of AFG3L2, and the mutations p.(Met666Arg), p.(Pro688Thr), and p.(Glu691Lys) prevent formation of hexamers.52 Thus, p.(Ile705Thr) may also affect the stability of the AFG3L2 hexamer and may compromise the functional coupling between the protease subunits.

**Genotype–Phenotype Correlations Among SPG7 Mutation Carriers**

Clinical data were available for 12 heterozygous carriers, nine homozygous carriers (seven of whom are homozygous carriers of the p.(Ala510Val) variant), and 22 compound heterozygous carriers. Table 3 details the

| TABLE 3. Genotype–phenotype correlation analysis in hereditary spastic paraplegia (HSP) patients according to SPG7 variants status |
| --- |
| **Variable** | **Heterozygous (n = 17)** | **Homozygous (n = 12)** | **Compound heterozygous (n = 26)** | **Het vs. Homo** | **Het vs. Comp Het** | **Homo vs. Comp Het** |
| Age at onset ± SD (y) | 16.5 ± 19.4 (n = 12) | 37.1 ± 17.4 (n = 9) | 30.5 ± 14.5 (n = 22) | 0.009 | 0.074 | 0.174 |
| Male/female ratio | 0.83 (n = 11) | 0.37 (n = 11) | 4.25 (n = 21) | 0.659 | 0.056 | 0.006 |
| SPRS score | 25.8 ± 11.4 (n = 5) | 14.50 ± 6.30 (n = 8) | 17.3 ± 6.9 (n = 14) | 0.093 | 0.156 | 0.482 |
| Lower extremity weakness | 8/12 | 8/9 | 15/21 | 0.338 | 1.00 | 0.393 |
| Lower extremity spasticity | 11/12 | 9/9 | 20/21 | 0.486 | 0.538 | 1.00 |
| Lower extremity hyperreflexia | 10/12 | 9/9 | 20/21 | 1.00 | 1.00 | 1.00 |
| Abnormal bladder function | 8/11 | 3/9 | 8/21 | 0.175 | 0.135 | 1.00 |
| Ankle clonus | 5/10 | 7/9 | 15/20 | 0.350 | 0.231 | 1.00 |
| Motor delay | 2/8 | 0/9 | 0/21 | 0.206 | 0.069 | 1.00 |
| Learning disability | 0/7 | 0/9 | 0/21 | 1.00 | 1.00 | 1.00 |
| Progressive cognitive deficits | 1/9 | 2/9 | 0/21 | 1.00 | 0.30 | 0.083 |
| Retinopathy or optic atrophy | 0/9 | 0/9 | 1/20 | 1.00 | 1.00 | 1.00 |
| Ocular movement abnormalities | 0/9 | 1/9 | 7/21 | 1.00 | 0.071 | 0.374 |
| Deafness | 0/9 | 1/9 | 0/20 | 1.00 | 0.310 | 1.00 |
| Swallowing difficulties | 1/9 | 0/9 | 2/20 | 1.00 | 1.00 | 1.00 |
| Dysarthria | 1/9 | 3/9 | 9/20 | 0.576 | 0.107 | 0.694 |
| Upper extremity weakness | 0/9 | 1/9 | 2/20 | 1.00 | 1.00 | 1.00 |
| Upper extremity hyperreflexia | 3/9 | 3/9 | 5/21 | 1.00 | 0.666 | 0.666 |
| Amyotrophy or lower motor neuron features | 5/9 | 3/9 | 3/19 | 1.00 | 1.00 | 1.00 |
| Sensory abnormalities | 2/9 | 3/9 | 6/21 | 1.00 | 1.00 | 1.00 |
| Peripheral neuropathy | 3/8 | 1/8 | 4/19 | 0.569 | 0.633 | 1.00 |
| Pes cavus | 4/9 | 3/9 | 4/20 | 1.00 | 0.209 | 0.642 |
| Ataxic gait | 1/9 | 4/9 | 9/20 | 0.294 | 0.107 | 1.00 |
| Upper extremity ataxia | 0/9 | 3/9 | 8/20 | 0.206 | 0.033 | 1.00 |
| Upper extremity intent tremor | 0/9 | 2/9 | 7/21 | 0.471 | 0.071 | 0.681 |
| Lower extremity ataxia | 1/9 | 3/9 | 10/20 | 0.576 | 0.096 | 0.454 |
| Lower extremity intent tremor | 1/9 | 3/9 | 7/20 | 0.576 | 0.371 | 1.00 |
| Seizures | 0/9 | 0/9 | 1/20 | 1.00 | 1.00 | 1.00 |
| Skeletal abnormalities | 1/9 | 0/9 | 1/20 | 1.00 | 0.310 | 1.00 |
| Myoclonus | 1/9 | 0/9 | 0/19 | 1.00 | 0.321 | 1.00 |
| Abnormal brain MRI | 1/6 | 3/5 | 4/17 | 0.242 | 1.00 | 0.274 |
| Abnormal spine MRI | 1/6 | 0/6 | 0/15 | 1.00 | 0.286 | 1.00 |

The corrected P value threshold was P < 0.0005. None of the clinical features significance passes Bonferroni correction. Abbreviations: SD, standard deviation; SPRS, Spastic Paraplegia Rating Scale; Het, heterozygous; Homo, homozygous; Comp Het, compound heterozygous; MRI, magnetic resonance imaging.
clinical data for each of these groups, each time comparing one group to the other two. After correction for multiple comparisons, there were no statistically significant differences between the groups. However, some differences between heterozygous carriers and biallelic variant carriers are notable. While upper extremity ataxia (37.9%) and intent tremor (30%) were relatively common in biallelic carriers of SPG7 variants, none of the heterozygous carriers showed these symptoms. Two heterozygous carriers presented with motor developmental delay, which was not found in any biallelic patients. Patients with heterozygous SPG7 variants had younger AAO compared to biallelic patients (16.5 vs. 33.8 years, \( P = 0.021 \)). Cerebellar atrophy was the most common imaging finding among biallelic patients (22.7%).

We further examined whether there are differences between subgroups of patients, based on the type of variants that they carried (missense, LoF) and the presence of the most common variant, p.(Ala510Val), which was carried by 34 (53.9%) patients with at least one allele. No statistically significant differences were identified after correction for multiple comparisons (Table 3, Table S8), likely due to the small number of patients in each of these groups.

**Discussion**

The current study summarizes genetic and clinical data on SPG7 from a large Canadian cohort of 585 patients, of which 6.5% carried biallelic SPG7 variants. Our findings show that the number of pathogenic/likely pathogenic SPG7 alleles in HSP patients is higher than in controls, even when considering only heterozygous carriers in index HSP patients versus unrelated controls, suggesting potential dominant or digenic inheritance in some cases. We also found that some of the heterozygous carriers of SPG7 pathogenic variants with HSP carried other potentially pathogenic variants in other genes, a phenomenon which was not observed in controls. These findings, which require replication, may suggest digenic inheritance in HSP associated with SPG7. Of note, we cannot rule out that in some of the heterozygous carriers of SPG7 variants an additional SPG7 variant exists that was not detected through WES.

Our results therefore suggest that non-Mendelian inheritance may have a role in SPG7-associated HSP, and it should be considered in HSP in general. In classic Mendelian inheritance, a single gene is associated with a single trait. As shown here, it is possible that more than one gene is involved in SPG7-associated HSP. The simplest form of non-Mendelian inheritance is digenic inheritance, in which a combination of two genes may lead to a disease.\(^{53}\) The patients with mutations in both SPG7 and other genes as detailed below could be examples of such inheritance. More complex forms of non-Mendelian inheritance may also exist,\(^{54}\) and their potential role in HSP should be further investigated.

The four patients with heterozygous SPG7 variants who also carried other variants in genes related to HSP had younger AAO (2.7 vs. 28 years) and higher average SPRS score (37 vs. 18.1), indicating that their disease may be more severe than those who did not carry variants in two genes. We further examined the possibility of genetic interaction/modification by performing biological pathway enrichment analysis. SPAST and SPG7, whose variants co-occurred in three HSP patients, share a similar ATPase domain and closely interact in multiple pathways. In the control cohort, none of the participants carried pathogenic variants in the genes which were identified in the HSP patients (SPAST, BSCL2, and TBCE). Overall, these findings may suggest either digenic inheritance, or epistasis between heterozygous SPG7 variants and other HSP-related genes, as previously reported in SPG4.\(^{55-57}\) Digenic inheritance has been suggested in spinocerebellar ataxia and Charcot-Marie-Tooth (CMT), diseases initially considered as a simple Mendelian monogenic disorder.\(^{58,59}\) Recently it was demonstrated that the EXOC4 gene may be involved in complex inheritance in axonopathies, including HSP and CMT.\(^{60}\)

By combining interaction and genetic analyses, we identified heterozygous carriers of SPG7 who also carried variants in genes potentially interacting with SPG7, including AFG3L2, CACNA1A, and MORC2. It is possible that the co-occurrence of the heterozygous variants of these genes with SPG7 heterozygous variants may lead to HSP. These results require replication in additional cohorts and additional functional evidence. The pathway enrichment, domain prediction (Fig. 2F), protein network, and protein conservational analysis (Fig. 2G) showed that AFG3L2 is the strongest potential candidate interacting with SPG7. This is further supported by functional studies, demonstrating the potential interaction of these two proteins within the mitochondria.\(^{41,61}\) SPG7 and AFG3L2 exert overlapping substrate specificities, hence the expression level of AFG3L2 and SPG7 might be important in cell-type specificity in disorder. Dominant AFG3L2 mutations cause spinocerebellar ataxia type 28, whereas bi-allelic mutations may affect the interaction of SPG7 and AFG3L2 and cause spastic ataxia 5, a disease whose phenotype includes features of both SCA28 and SPG7. A recent study reported a patient with heterozygous variants in both genes with syndromic parkinsonism and optic atrophy.\(^{42}\) SPG7 and AFG3L2 are components of mitochondrial m-AAA proteases and they can assemble hetero-oligomeric proteolytic complexes with SPG7.\(^{41}\) Together with the current study, these data suggest that SPG7-AFG3L2 digenic variants may be a...
cause of HSP and similar disorders, and that individuals with heterozygous SPG7 variants with neurodegeneration should be specifically screened for AFG3L2 variants.

We also report several rare clinical features of SPG7, including dopa-responsive parkinsonism, dysphagia, dysmetria, jaw jerk, ptosis, ophthalmoplegia, optic atrophy, and hands dystonia in biallelic SPG7 patients. A few studies previously reported some of these symptoms in patients with SPG7 mutations. Optic atrophy and dopa-responsive parkinsonism were also reported in a patient with concurrent AFG3L2 and SPG7 heterozygous variants.42 Similar to other genes involved in HSP, SPG7 may be involved in a continuum of spastic neurogenetic disorders, and SPG7 variants should not be ruled out only because of the presence of rare clinical manifestations.

Our study has several limitations. Despite being one of the world’s largest HSP cohorts, the total number of SPG7 patients is still relatively small, especially for genotype–phenotype studies. In addition, not all our HSP cohort went through WES, which prevented the participation of all patients in some of the analyses. One limitation of WES is that it cannot properly detect genetic variants such as large copy number variants (CNVs). While we did analyze the data with ExomeDepth, a computational tool for CNV detection using WES data, and did not identify CNVs, we cannot rule out that some of our supposedly heterozygous carriers of SPG7 variants carry an additional undetected SPG7 variant. For example, a recent study demonstrated that a deep intronic SPG7 variant that led to inclusion of pseudoexon and early termination of the SPG7 protein could not be detected through WES and caused HSP.67 An additional limitation of our study is the lack of age- and sex-matched controls. The male:female ratio in patients is 0.83 and in controls it is 1.08, both relatively close to 1 but with opposite direction. The average age at onset of patients was 22.1 years, and the age of controls was mostly average. Yet this should still be considered as a limitation of the current study, as accurate data are not available.

To conclude, our results suggest that the inheritance of SPG7 may be complex, and also include dominant or digenic inheritance. One of the most intriguing findings, which requires replication, is the potential digenic inheritance with AFG3L2 variants. The relatively high allele frequency of some of the pathogenic SPG7 variants in the general population, the results of the genotype–phenotype correlation analysis, and a recent functional study support this possibility. Future studies will benefit from whole-genome sequencing, which will allow for identifying CNVs, and deep intronic variants that can lead to aberrant splicing, as well as for comprehensive investigation of complex inheritance in SPG7 and other forms of HSP.

Acknowledgments: We thank the patients and their families for participating in this study. M.A.E. and F.A. are funded by the Fonds de Recherche du Québec–Santé (FRQS). K.M.B. holds a Canada Research Chair (Tier 1) in Rare Disease Precision Health. J.F.T. holds a Canada Research Chair (Tier 2) in Structural Pharmacology. G.A.R. holds a Canada Research Chair (Tier 1) in Genetics of the Nervous System and the Windsor Pan Field Chair in Neuroscience. Z.C.O. is supported by the Fonds de recherche du Québec–Santé Chercheur-Boursier award and is a Parkinson Canada New Investigator awardee. We thank D. Rochefort, H. Catheo, and V. Zaharieva for their assistance.

References
1. Synofzik M, Gonzalez MA, Lourenco CM, et al. PNPLA6 mutations cause Boucher-Neuhäuser and Gordon Holmes syndromes as part of a broad neurodegenerative spectrum. Brain 2014;137(1):69–77.
2. Diomedi M, Gan-Or Z, Placidi F, et al. A 23 years follow-up study identifies GLUT1 deficiency syndrome initially diagnosed as complicated hereditary spastic paraplegia. Eur J Med Genet 2016;59(11):564–568.
3. Leveille E, Gonorazky HD, Rioux MF, et al. Triple A syndrome presenting as complicated hereditary spastic paraplegia. Mol Genet Genomic Med 2018;6(6):1134–1139.
4. Brugman F, Scheffer H, Wokke J, et al. Paraplegin mutations in sporadic adult-onset upper motor neuron syndromes. Neurology 2008;71(19):1500–1505.
5. Klebe S, Depienne C, Gerber S, et al. Spastic paraplegia gene 7 in patients with spasticity and/or optic neuropathy. Brain 2012;135(10):2980–2993.
6. De la Casa-Fages B, Fernández-Eulate G, Gamez J, et al. Parkinsonism and spastic paraplegia type 7: expanding the spectrum of mitochondrial parkinsonism. Mov Disord 2019;34(10):1547–1561.
7. Kruger S, Battke F, Sprecher A, et al. Rare variants in neurodegeneration associated genes revealed by targeted panel sequencing in a German ALS cohort. Front Mol Neurosci 2016;9:92.
8. Mitsumoto H, Nagy PL, Gennings C, et al. Phenotypic and molecular analyses of primary lateral sclerosis. Neurrol Genet 2015;1(1):e3.
9. Belliniva A, Pastò L, Nicolai C, et al. A new paraplegin mutation in a patient with primary progressive multiple sclerosis. Mult Scler Relat Disord 2020;44. https://doi.org/10.1016/j.msard.2020.102302.
10. Osmanovic A, Widjaja M, Förster A, et al. SPG7 mutations in amyotrophic lateral sclerosis: a genetic link to hereditary spastic paraplegia. J Neurol 2020;267:2732–2743.
11. Liu Y, Xu J, Tao W, et al. Exome sequencing identifies a mutation (Y740C) in spastic paraplegia 7 gene associated with adult-onset primary lateral sclerosis in a Chinese family. Eur Neurol 2019;81(1–2):87–93.
12. Schaefer SM, Szekely AM, Moeller JJ, Tinaz S. Hereditary spastic paraplegia presenting as limb dystonia with a rare SPG7 mutation. Neurol Clin Pract 2018;8(6):e49–e50.
13. Charif M, Chevrierolle A, Gueguen N, et al. Mutations in the m-AAA proteases AFG3L2 and SPG7 are causing isolated dominant optic atrophy. Neurrol Genet 2020;6(3). https://doi.org/10.1212/NXG.0000000000000428.
14. Arnoldi A, Tonelli A, Crippa F, et al. A clinical, genetic, and biochemical characterization of SPG7 mutations in a large cohort of patients with hereditary spastic paraplegia. Hum Mutat 2008;29(4):522–531.
15. McDermott CJ, Dayaratne R, Tomkins J, et al. Paraplegin gene analysis in hereditary spastic paraparesis (HSP) pedigrees in Northeast England. Neurology 2001;56(4):467–471.
16. Sánchez-Ferrero E, Coto E, Beetz C, et al. SPG7 mutational screening in spastic paraplegia patients supports a dominant effect for some mutations and a pathogenic role for p. A510V. Clin Genet 2013;83(3):257–262.

17. Coarelli G, Schule R, Van de Warrenburg BP, et al. Loss of paraplegin drives spasticity rather than ataxia in a cohort of 241 patients with SPG7. Neurology 2019;92(23):e2679—e2690.

18. Bonn F, Pantakani K, Shoukier M, Langer T, Mannan AU. Functional evaluation of paraplegin mutations by a yeast complementation assay. Hum Mutat 2010;31(5):617–621.

19. Christan N, Dupre N, Gan-Or Z, et al. Clinical and genetic study of hereditary spastic paraplegia in Canada. Neurol Genet 2017;3(1):e122.

20. Nature HCJ. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449(7164):851.

21. Gasser T, Finsterer J, Baets J, et al. EFNS guidelines on the molecular diagnosis of ataxias and spastic paraplegias. Eur J Neurolog 2010;17(2):179–188.

22. Schule 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(3):430–434.

23. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009;25(14):1754–1760.

24. McKenna A, Hanna M, Banks E, et al. The genome analysis toolkit: a MapReduce framework for analysing next-generation DNA sequencing data. Genome Res 2010;20(9):1297–1303.

25. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010;38(16):e164—e164.

26. Karczewski KJ, Francolici LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. Nature 2020;581(7809):434–443.

27. Kopanos C, Tsiolkas V, Kouris A, et al. VarSome: the human genomic variant search engine. Bioinformatics 2019;35(11):1978.

28. Pedersen BS, Bhetariya PJ, Marth G, et al. Somalier: rapid relatedness estimation for cancer and germline studies using efficient variant search engine. Bioinformatics 2019;35(11):1978.

29. Mi L, Gan N, Cheema A, et al. Cancer preventive isoychianates induce selective degradation of cellular α- and β-tubulins by proteasomes. J Biol Chem 2009;284(25):17039–17051.

30. Trempe JF. Reading the ubiquitin postal code. Curr Opin Struct Biol 2011;21(6):792–801.

31. Riedinger C, Boehringer J, Trempe JF, et al. Structure of Rpn10αβ tandem subunit of proteasome. J Mol Biol 2019;431(5):939–955.

32. Koppen M, Langer T. Protein degradation within mitochondria: versatile activities of AAA proteases and other peptidases. Crit Rev Biochem Mol Biol 2007;42(3):211–242.

33. Pachoud C, Ding B, Song A, Wiseman RL, Lander GC, Glynn SE. Unique structural features of the mitochondrial AAA+ protease AFG3L2 reveal the molecular basis for activity in health and disease. Mol Cell 2017;75(5):1073–1085.

34. Schäffer AA. Digenic inheritance in medical genetics. Eur J Med Genet 2013;56(10):354–356.

35. Newton T, Allison R, Edgar JR, et al. Mechanistic basis of an epistatic interaction reducing age at onset in hereditary spastic paraplegia. Brain. 2017;140(6):1579–1594.

36. Serna M, Carranza G, Martin-Benito J, et al. The structure of the complex between β-tubulin, TBCE, and TBCB reveals a tubulin dimer dissociation mechanism. J Cell Sci 2015;128(9):1824–1834.

37. Parodi L, Fenu S, Barbier M, et al. Spastic paraplegia mutations associated with spasticity and dysarthria. Brain 2014;137(8):2200–2209.

38. Cho HJ, Sung D-H, Ki C-S. Identification of de novo BSCL2 mutation in a Korean family with Silver syndrome and distal hereditary motor neuropathy. Muscle Nerve 2007;36(3):384–386.
60. Bis-Brewer DM, Gan-Or Z, Sleiman P, et al. Assessing non-Mendelian inheritance in inherited axonopathies. Genet Med 2020; 12:2114–2119.

61. Martinelli P, La Mattina V, Bernacchia A, et al. Genetic interaction between the m-AAA protease isoenzymes reveals novel roles in cerebellar degeneration. Hum Mol Genet 2009;18(11):2001–2013.

62. Pedroso JL, Vale TC, Bueno FL, et al. SPG7 with parkinsonism responsive to levodopa and dopaminergic deficit. Parkinsonism Relat Disord 2018;47:88–90.

63. Pfeffer G, Gorman GS, Griffin H, et al. Mutations in the SPG7 gene cause chronic progressive external ophthalmoplegia through disordered mitochondrial DNA maintenance. Brain 2014;137(5): 1323–1336.

64. Van Gassen KL, van der Heiden CD, de Bot ST, et al. Genotype–phenotype correlations in spastic paraplegia type 7: a study in a large Dutch cohort. Brain 2012;135(10):2994–3004.

65. Jacinto-Scudeiro LA, Machado GD, Ayres A, et al. Prevalence of oropharyngeal dysphagia in hereditary spastic paraplegias. Arq Neuropsiquiatr 2019;77(12):843–847.

66. Plagnol V, Curtis J, Epstein M, et al. A robust model for read count data in exome sequencing experiments and implications for copy number variant calling. Bioinformatics 2012;28(21):2747–2754.

67. Verdura E, Schluter A, Fernandez-Eulate G, et al. A deep intronic splice variant advises reexamination of presumably dominant SPG7 cases. Ann Clin Tranl Neurol 2020;7(1):105–111.

**Supporting Data**

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.