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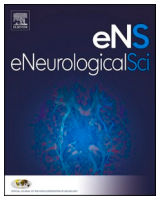

Case report

Phenotypic variability in a large kindred with spastic paraplegia associated with a novel *REEP1* variant

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ABSTRACT

Background and objectives: The aim of this study is to provide a comprehensive characterization of a large Estonian family spanning five generations with seventeen individuals affected by spastic paraplegia associated with a novel variant in the receptor expression-enhancing protein-1 (*REEP1*) gene.
Methods: Comprehensive clinical evaluation, neuroimaging, and neurophysiological studies were performed on six patients who provided oral and written consent. Whole-exome sequencing was performed on the index case. Targeted carrier testing was done in all other available affected and at-risk relatives.
Results: Four individuals presented with pure spastic paraplegia, with onset from early childhood to adult age. None had bladder or bowel dysfunction. Two subjectively asymptomatic mutation carriers displayed pyramidal signs on examination. Imaging of the neuroaxis was normal in three patients, three had MRI findings interpreted as unrelated. Motor evoked potential (MEP) was abnormal in five; the patient with the longest disease duration had additional somatosensory evoked potential (SSEP) abnormalities. The novel splice-site variant, c.32 + 1G > C in the *REEP1* gene, found in the index case, co-segregates with disease in the family. Expressivity in this family is variable.
Conclusion: Our findings are in keeping with previous descriptions of the SPG31 spectrum. The phenotype associated with splice variants is not necessarily more severe than other conventional *REEP1* variants. As for other forms of familial spastic paraplegias, the factors modulating variable expressivity in SPG31 are still unknown.

1. Introduction

Hereditary spastic paraplegia (HSP) is a large and heterogenous group of Mendelian disorders with different mechanisms of disease converging in damage to the corticospinal tracts [1,2]. Based on their phenotype, HSPs are classified as pure, or complex [3]. Currently, disease-causing variants in over 80 genes have been associated with the growing list of HSPs [4]. Spastic paraplegia type 31 (SPG31; OMIM #610250) is associated with pathogenic variants in the *REEP1* gene, located on chromosome 2p11.2. Since its delineation in 2006 [5], SPG31 has come to be the third most common form of autosomal dominant HSP, at least in Western populations [6,7]. Numerous pathogenic variants have been reported [8,9], the most common variants being truncating, which supports the notion that haploinsufficiency is a major mechanism of disease [6,9,10]. Gain-of-function *REEP1* gene mutations affect lower motor neurons, leading to distal hereditary motor neuropathy (dHMN),[e-1] whereas biallelic *REEP1* variants are associated with the early-onset and severe distal spinal muscular atrophy-6 (DSMA6).[e-2,e-3] A recent publication ascribes a microdeletion including the *REEP1* gene as causative in early-onset atypical parkinsonism.[e-4]

A proportion of patients harboring *REEP1* variants display an overlap between dHMN and spastic paraplegia.[e-5] SPG31 is characterized by wide phenotypic variability with a bimodal distribution in age of onset. The mean age of onset is 17 years, with the majority becoming symptomatic in the first two decades of life, and the rest at >30 years.[6,e-6,e-7] In most cases, SPG31 presents as pure spastic paraplegia, with reduced

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penetrance. [e-7]

Six types of *REEP* genes are known in the human genome (*REEP1–6*).[e-8,e-9] The gene product for *REEP1* plays an important role in the brain and spinal cord, in particular for the corticospinal tract with its axonal processes. The REEP1 protein is required for endoplasmic reticulum membrane shaping, interactions with the microtubule cytoskeleton and interactions between the endoplasmic reticulum and mitochondria.[e-10-e-13] Loss-of-*REEP1* expression has also been shown to negatively impact mitochondria, leading to decreased ATP production and increased susceptibility to oxidative stress.[e-14]

Here, we aimed to provide a multi-modal characterization of the phenotype of a large Estonian family with SPG31 associated with a novel pathogenic *REEP1* variant.

2. Materials and methods

2.1. Study approval

This study was approved by the Regional Ethics Board in Stockholm, Sweden, (approval number. 2016/2503–31/2) and it was conducted in accordance with the ethical standards of the Declaration of Helsinki. All participants, or their legal guardians, provided oral and written consent to participate.

2.2. Clinical assessment

The participants were part of a five-generation kindred originating from Estonia with seventeen people affected by spastic paraplegia, ten of them ascribed as symptomatic based on clinical history and six patients were evaluated at Karolinska University Hospital, Stockholm, Sweden. The evaluation included standardized scales, specifically the Spastic Paraplegia Rating Scale (SPRS), Inventory of Non-Ataxia Signs (INAS), Instituto de Pesquisa Clinica Evandro Chagas Scale (IPEC), and Scale for the Assessment and Rating of Ataxia (SARA). In two cases, charts from another pediatric clinic were revised.

2.3. Genetic analysis

Genomic DNA from blood from the index case was analyzed with exome sequencing (Blueprint Genetics, Espoo, Finland), and results were filtered through a spastic paraplegia gene panel, which at the time of assessment included 60 genes and 872 exons. All *REEP1* exons were sequenced and analyzed. Since the *REEP1* gene is not transcribed in blood, sequencing of messenger RNA (mRNA) was not feasible. Targeted analysis of the *REEP1* gene in relatives at risk was subsequently performed.

2.4. Neurophysiological tests

The test battery consisted of peripheral nerve conduction studies of both motor and sensory nerves, electromyography (EMG), SSEP, and MEP performed on Natus equipment, Viking EDX (Cephalon A/S; Denmark) by the same neurophysiologist.

2.5. Neuroimaging

All patients underwent brain and spinal cord MRI using an extensive standardized protocol on a Siemens MAGNETOM Prisma^{Fit} 3 Tesla scanner (Siemens Healthineers, Erlangen, Germany). Brain volumetry was performed on 3D T1-weighted imaging (MPRAGE) with 1 mm isotropic voxel size using cNeuro v. 2.2 (Combinostics, Tampere, Finland) All imaging was assessed by the same neuroradiologist.

2.6. Data availability statement

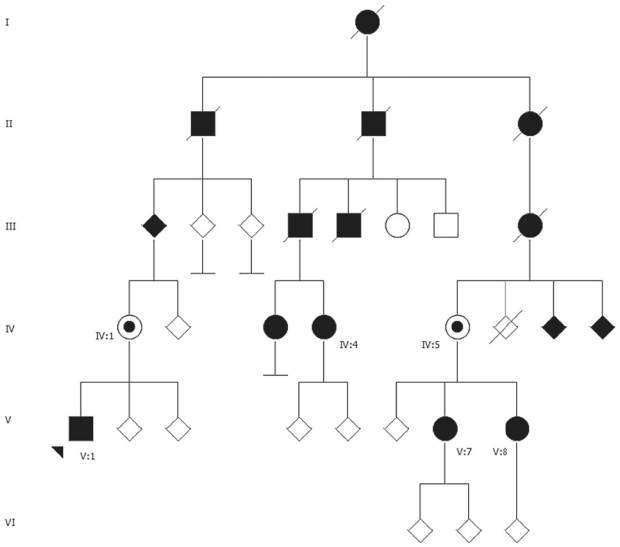
Anonymized data not published within this article will be made available by request from any qualified investigator.

3. Results

The kindred's pedigree is displayed in Fig. 1 and clinical data on family members evaluated at our center are summarized in Table 1. Briefly, four patients had slowly progressive spastic paraplegia and two were subjectively asymptomatic but displayed pyramidal signs (hyperreflexia, ankle clonus and an extensor plantar response) on examination. None had symptoms or signs suggestive of ataxia or any other movement disorder. Cognitive impairment and psychiatric symptoms were absent. The index case (V:1) is a 12-year-old boy with spastic paraplegia, referred to the pediatric neurology clinic at age 9 years with a four-year history of toe walking and frequent falls. Two additional cases also had childhood-onset paraplegia (V:7 and V:8) (supplementary data). Imaging of the neuroaxis was normal in three patients. Patient V:8 had three intracranial arachnoid cysts discovered in childhood; between the ages of 5–27 years she developed a low-grade brain tumor in the right temporal lobe (supplementary figure and supplementary data); patients IV:4 and IV:5 had white matter abnormalities (WMA, supplementary data). Brain volumetry results were normal. MEP showed delayed central motor conduction time to both legs in five patients, two of whom also had abnormal responses in both arms. Two others had abnormal motor NCS in the legs, but none have manifest neuropathy. One patient (V:7), the one with the longest disease duration, had pathological SSEP responses from the lower extremities (Table 2), corresponding to the tibial nerves on both sides, consistent with incipient sensory myelopathy. Sequencing analysis of the index case revealed the novel heterozygous splice-site variant c.32 + 1G > C, in the *REEP1* gene. This variant is predicted to abolish the 5́splice site finder site at the end of exon 1, likely to lead to an alternative splicing. It is absent in two large reference population cohorts (ClinVar and gnomAD, n > 120,000 exomes and > 15,000 genomes), and predicted to be pathogenic by different tools. No other variants in spastic paraplegia genes, including the *SPAST*, *ATL1*, and *HSPD1* genes, were identified in the index case. The *REEP1* variant cosegregates with disease in all the studied relatives (Fig. 1).

4. Conclusions

Overall, the phenotype reported here is compatible with a pure form



Patients V:1 (index case), IV:1, IV:4, IV:5, V:7, and V:8 were examined at our site. IV:1 and IV:5 are subjectively asymptomatic.

Fig. 1. The family's pedigree.
Patients V:1 (index case), IV:1, IV:4, IV:5, V:7, and V:8 were examined at our site. IV:1 and IV:5 are subjectively asymptomatic.

Table 1
Clinical and neuroradiologic features, as well as result of standardized scales in six individuals with a novel REEP1 variant.

Patient	Age at onset (y)	Age at inclusion (y)	Presenting symptoms	Pyramidal signs	SPRS	INAS	IPEC	SARA	Brain MRI	Spine MRI
V:1	5	11	Gait instability, balance problems	Hyperreflexia LE. Bilateral ankle clonus. + Babinski sign	4	3	2	3	NAD	NAD
IV:1	–	40	Subjectively asymptomatic	Hyperreflexia LE. Bilateral ankle clonus. + Babinski sign	0	0	1	0	NAD	NAD
IV:4	45	50	Balance problems	Hyperreflexia LE. Bilateral ankle clonus. + Babinski sign	0	3	2	0	Cavum septum pellucidum et vergae, mild white matter changes	NAD
V:7	6	33	Gait instability	Hyperreflexia LE. Bilateral ankle clonus. + Babinski sign	5	4	3	1	NAD	NAD
V:8	5	25	Gait instability	Hyperreflexia LE. Bilateral ankle clonus. + Babinski sign	11	6	6	3	Arachnoid cysts, non-contrast-enhancing tumor in the right temporal lobe (se supplementary figure)	NAD
IV:5	–	61	Subjectively asymptomatic	Clonus left ankle, otherwise no objective findings	1	0	1	0.5	White matter changes, and mild atrophy of parietal cortical area and cerebellar vermis	NAD

Abbreviations: NAD = No abnormality detected; SPRS = Spastic Paraplegia Rating Scale; INAS = Inventory of Non-Ataxia Signs; IPEC = Instituto de Pesquisa Clinica Evandro Chagas Scale; SARA = Scale for the assessment and rating of ataxia; LE = Lower Extremities; + = positive.

Table 2
Electrodiagnostic features in six individuals with a novel REEP1 variant.

Patient	ENoG	EMG	SSEP	MEP
V:1	NAD	NAD	NAD	Abnormal, upper and lower extremities
IV:1	NAD	NAD	NAD	NAD
IV:4	NAD	NAD	NAD	Abnormal, prolonged transmission time to lower extremities (tibialis anterior)
V:7	NAD	NAD	Abnormal tibial nerves bilaterally, normal upper extremities	Abnormal, lower extremities
V:8	Abnormal motor NCS in lower extremities. No signs of diffuse polyneuropathy.	NAD	NAD	Abnormal, both upper and lower extremities, more pronounced in the latter
IV:5	Discretely abnormal motor NCS in lower extremities. No signs of diffuse polyneuropathy.	Mild focal neurogenic changes corresponding to L4 bilaterally and S1 left.	NAD	Abnormal, lower extremities

Abbreviations: NAD = no abnormality detected; NCS = nerve conduction study; ENoG = electroneurography; EMG = electromyography; SSEP = somatosensory evoked potential; MEP = motor evoked potential.

of spastic paraplegia with slow progression which is in keeping with previous descriptions for SPG31.[9,e-7,e-15] Neuroimaging findings are non-specific for SPG31, therefore the MRI abnormalities found in three of our patients could be interpreted as incidental findings. Novel here are the ethnic background of this family, Estonian, and the splice variant in *REEP1*. Splice variants in *REEP1* have been reported previously, although genotype-phenotype correlations are difficult to establish.[e-7] *REEP1* knockout mice exhibit a progressive spastic paraplegia phenotype as well as significant lipodystrophy.[10,e-16] Our patients were not assessed for lipodystrophy specifically, which may constitute a limitation of our work. Three of the affected family members live in Estonia and were not available for examination, impacting our sample size.

The factors that underlie phenotypic variability and age of onset even within families in familial spastic paraplegia are still unknown.[e-17] Sequence variants in two spastic paraplegia genes, *SPAST* and *HSPD1*, have been reported as modulators.[e-18,e-19] Both genes were analyzed in the index case of the reported family. A recent publication describes three asymptomatic female carriers, as well as a tendency towards earlier age of onset in males.[e-5] Our cohort is too small to draw firm conclusions.

Future studies on larger samples could possibly reveal neuroimaging findings that are more likely to be associated with SPG31. Finally, biomarkers and large-scale genetic studies are required to evaluate the variable expressivity and reduced penetrance of SPG31.

Author's contributions

Authors name:	Location:	Role:
Helgi Thor Hjartarson	Karolinska University Hospital, Stockholm, Sweden	Patient care, study concept and design, analysis, and interpretation of data. Wrote the first manuscript version.
Martin Paucar	Karolinska University Hospital and Karolinska Institute, Stockholm, Sweden	Patient care, study concept and design, supervision, analysis, and interpretation of data. Revision of the manuscript.
Humberto Scott	Karolinska University Hospital, Stockholm, Sweden	Responsible for neurophysiological testing. Revision of the manuscript.
Tobias Granberg	Karolinska University Hospital and Karolinska Institute, Stockholm, Sweden	Responsible for neuroimaging. Revision of the manuscript.

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CRediT authorship contribution statement

Helgi Thor Hjartarson: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Humberto Skott:** Writing – review & editing, Methodology, Investigation. **Tobias Granberg:** Writing – review & editing, Methodology, Investigation. **Martin Paucar:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ensci.2024.100497.

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