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REEP1 mutation spectrum and genotype/phenotype correlation in hereditary spastic paraplegia type 31

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Abstract

Mutations in the receptor expression enhancing protein 1 (REEP1) have recently been reported to cause autosomal dominant hereditary spastic paraplegia (HSP) type SPG31. In a large collaborative effort, we screened a sample of 535 unrelated HSP patients for *REEP1* mutations and copy number variations. We identified 13 novel and 2 known *REEP1* mutations in 16 familial and sporadic patients by direct sequencing analysis. Twelve out of 16 mutations were small insertions, deletions or splice site mutations. These changes would result in shifts of the open-reading-frame followed by premature termination of translation and haploinsufficiency. Interestingly, we identified two disease associated variations in the 3′-UTR of *REEP1* that fell into highly conserved micro RNA binding sites. Copy number variation analysis in a subset of 133 HSP index patients revealed a large duplication of *REEP1* that involved exons 2–7 in an Irish family. Clinically most SPG31 patients present with a

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pure spastic paraplegia; rare complicating features were restricted to symptoms or signs of peripheral nerve involvement. Interestingly, the distribution of age at onset suggested a bimodal pattern with the appearance of initial symptoms of disease either before the age of 20 years or after the age of 30 years. The overall mutation rate in our clinically heterogeneous sample was 3.0%; however, in the sub-sample of pure HSP *REEP1* mutations accounted for 8.2% of all patients. These results firmly establish *REEP1* as a relatively frequent autosomal dominant HSP gene for which genetic testing is warranted. We also establish haploinsufficiency as the main molecular genetic mechanism in SPG31, which should initiate and guide functional studies on *REEP1* with a focus on loss-of-function mechanisms. Our results should be valid as a reference for mutation frequency, spectrum of *REEP1* mutations, and clinical phenotypes associated with SPG31.

Keywords

hereditary spastic paraplegia; SPG31; REEP1; haploinsufficiency; micro RNA

Introduction

Hereditary spastic paraplegia (HSP) comprises a clinically and genetically heterogeneous group of disorders for which progressive spasticity of the lower limbs is the major and unifying clinical feature. Pure and complicated HSP forms have been defined depending on the presence of additional signs or symptoms, such as mental retardation, peripheral neuropathy, optic atrophy or cerebellar ataxia. Variability with regard to age at onset, penetrance and clinical presentation can be present and is partially explained by the extensive genetic heterogeneity (Fink, 2002). More than 30 HSP loci and 15 genes are currently known including X-linked, autosomal recessive or autosomal dominant traits (Zuchner, 2007). The most common presentation of HSP is pure autosomal dominant HSP with onset in adulthood (Reid, 1999). Mutations in the two most frequent disease genes SPG3A and SPG4 account for ~10% and ~40% of all autosomal dominant HSP patients, respectively (Fonknechten *et al*., 2000; Namekawa *et al*., 2006). The remaining HSP genes appear to be relatively rare (Reid *et al*., 2002; Mannan *et al*., 2006; Hansen *et al*., 2007; Klebe *et al*., 2007). The recently identified SPG31 gene *REEP1* may be an exception (Zuchner *et al*., 2006*a*, *b*). The initial mutation screen on *REEP1* identified 6.5% SPG31 patients in a sample of 92 mainly pure HSP patients (Zuchner *et al*., 2006*b*). The broad spectrum of the molecular aberrations identified, which included two frameshift, one missense, one splice site and two 3′-UTR mutations (Zuchner *et al*., 2006*b*), hampered inference of a molecular genetic pathomechanism. Furthermore, large genomic deletions, indicative of haploinsufficiency, were recently shown to be frequent in SPG4 (Beetz *et al*., 2006) and would have been missed by a conventional PCR-based approach.

To address these open questions an international collaboration was set up to comprehensively screen 535 unrelated familial and sporadic HSP patients. We intended to derive more conclusive evidence for the molecular genetic mechanisms associated with SPG31 pathology from a increased number of identified *REEP1* mutations by applying a comprehensive screening strategy, which included direct sequencing, copy number variation analysis and 3′-UTR sequencing. We also aimed to give a comprehensive description of the clinical phenotypic spectrum of SPG31 in this mixed sample of pure and complicated HSP patients.

Patients and Methods

Patients

A total of 535 DNA samples of unrelated HSP patients were collected by different centres: University of Antwerp (*n* = 166), University of Dublin (*n* = 11), German Network for Hereditary Movement Disorders (GeNeMove) (*n* = 122) and Athena Diagnostics Inc. (*n* =

236). The samples from Athena Diagnostics will be referred to as the 'non-academic diagnostic sample'. Although neither detailed clinical information on the index patient nor additional family members for segregation analysis were available, we included this non-academic diagnostic cohort in the present study to provide realistic information on the typically mixed population tested by a large internationally operating testing laboratory. The remaining samples, collectively making up the 'academic' collection ($n = 299$) were composed as follows: 133 patients reported a family history consistent with autosomal dominant inheritance, 119 patients presented as apparently sporadic and for 47 patients information regarding family history was lacking. The HSP phenotype was pure in 135 and complicated in 97 patients; it was unknown for 67 of the samples. SPG4 and SPG3A mutations had been excluded in the academic sample by sequencing in 297 (>99%) and 261 (87%) cases, respectively. For the non-academic cohort, this figure was 94% for both genes. Samples were not selected for age at onset (range 1–91 years). A control cohort of 366 unrelated subjects (732 chromosomes) of European descent was tested for the occurrence of newly detected sequence variations. Institutional review boards of all collaborators approved the study and informed consent was obtained from all individuals analysed.

Sequencing analysis

DNA was isolated from peripheral blood by standard methods. DNA samples were sequenced at the Center for Human Genetics, Duke University or at Athena Diagnostics Inc. All seven exons including at least 30 bp of the flanking intronic sequence and 120 bp of the 3′-UTR were amplified by PCR employing a touch-down thermocycling protocol. Oligonucleotide sequences will be provided upon request. PCR products were visualized on 1.5% agarose gels, quantified and purified over Sephadex columns. Products were directly sequenced using the BigDye chemistry and an ABI3730 sequencer. Sequencing traces were analysed using the Sequencher software (Ann Arbour, USA). Sequence aberrations were confirmed by re-PCR and re-sequencing in both directions. When available, DNA from other family members was analysed to test for co-segregation of mutation and phenotype.

Screening for genomic copy number variations

In order to screen for copy number variations at the SPG31 locus, we developed a *REEP1*-specific multiplex ligation-dependent probe amplification (MLPA) assay. This method allows for the identification of large copy number variations not detectable by PCR-based approaches, namely deletions and duplications (Schouten *et al*., 2002). *REEP1*-directed MLPA probes targeted the coding sequence of each exon as well as 5′-UTR, 3′-UTR and intronic sequences. MLPA reactions were performed as recommended by the manufacturer (MRC-Holland, The Netherlands) and reaction products were separated on an automated sequencer (LI-COR; Lincoln, NE). The intensity of each amplicon was measured densitometrically. The signal of each REEP1-directed probe was set in relation to the total signal obtained for the four nearest control probes. The resulting value was compared to the corresponding mean from six control samples. Deviations of >25% were regarded aberrant, i.e. to be caused by a change in the copy number of the target sequence. After completion of this study, the assay has been made commercially available by MRC-Holland.

Imaging and electrophysiological studies

Seven unrelated SPG31 patients of the academic sample had brain imaging. MRI studies typically included T1, T2 and flair imaging sequences. CT was performed with or without contrast enhancement. Electrophysiological studies were performed in six index patients and comprised peripheral nerve conduction studies and central motor evoked potentials.

In silico analysis

In order to estimate evolutionary conservation, *REEP1* sequences of different vertebrate species were downloaded from the UCSC genome browser (http://genome.ucsc.edu/) and manually aligned. Potential transmembrane domains and N-terminal cleavage signals in the *REEP1* protein were identified using TMpred (http://www.ch.embnet.org/software/TMPRED_form.html), TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0/) and TargetP (http://www.cbs.dtu.dk/services/TargetP/). Effects of *REEP1* alterations on splicing were analysed with NNSplice0.9 (www.fruitfly.org/seq-tools/splice.html) and Automated Splice Site Analysis (https://splice.cmh.edu/). The miRBase database (http://microrna.sanger.ac.uk/) was browsed to see if 3′-UTR variants affect micro RNA-binding sites.

Results

Direct sequencing of *REEP1*

All coding exons of *REEP1* were sequenced in 535 unrelated HSP patients, including flanking intronic regions and the proximal 3′-UTR. We identified 16 index patients (3.0%) carrying a potentially pathogenic *REEP1* variant that was absent in 366 healthy controls (Table 1, Fig. 1). For seven index cases additional relatives were available for segregation analysis (Fig. 2). The 16 mutations identified in the current study include 14 novel mutations and 2 mutations (c.59C>A and c.606 + 43G>T) that have been previously described (Zuchner *et al*., 2006*b*).

The most common type of *REEP1* alteration was small frameshift mutations. We identified three deletions of a single nucleotide (c.193delT, c.222delC and c.282delC), one deletion and one insertion of two nucleotides each (c.104_105delAT, c.181-1_181insCT), and one deletion of four nucleotides (c.537_540delCGGC) (Fig. 1, Table 1). All of them lead to pre-terminal stop codons. A null-allele is being created by a deletion of three nucleotides overlapping the start codon (c.-1_2delCAT) because the next available downstream ATG (c.9_11) is out of frame. Another class of mutations that usually results in frame-shifts and pre-terminal stops is the destruction of canonical splicing motives. We identified a mutation that disrupts the conserved splice donor site of intron 5 (c.417 + 1G>T) in two apparently unrelated families, 02-1764 and 05-3263 (Fig. 1, Table 1). Correct splicing will also be impaired by substitution of the last bases in exon 4 (c.303G>A) and exon 6 (c.595G>C) (Fig. 1, Table 1). Formally, these changes of highly conserved nucleotides (Supplementary Fig. 3) represent silent (p.K101K) and missense alterations (p.G199R), respectively. However, *in silico* analysis strongly suggests a deleterious effect on the neighbouring splice acceptors (Table 1). Unfortunately, since *REEP1* is not expressed in peripheral blood or skin biopsies we were unable to test for missplicing of transcripts (Zuchner *et al*., 2006*b*). Only two changes, c.56C>G and c.59C>A, represent classical missense mutations that alter adjacent N-terminal amino acids, p.P19R and p.A20E (Fig. 1,Table 1). Both affected residues are part of the first transmembrane domain of REEP1 (Fig. 1). Since micro-RNA target site alterations have been reported previously (Zuchner *et al*., 2006*b*), we sequenced the proximal 3′-UTR of *REEP1* in all index patients. We identified two substitutions, c.606 + 14C>T (family ATH-04) and c.606 + 43G>T (family BO1-0009), which were predicted to change such target sites and were not present in 1000 controls (2000 chromosomes). The latter of these variations has been reported previously and alters a conserved binding site for mirR-140 (Zuchner *et al*., 2006*b*). Similarly, the new variation c.606 + 14C>T changes a conserved nucleotide in the binding site of mirR-691 (Supplementary Fig. 1). We finally identified three synonymous variations in HSP patients that were not present in 366 controls: c.57T>C (p.P19P), c.381C>T (N127N) and c. 408T>A (p.A36A). Two of them affect evolutionary conserved sites, c.57T>C and c.408T>A.

Copy number variations in *REEP1*

The above results implied haploinsufficiency induced by pre-terminal stops as a major molecular genetic mechanism in SPG31. We therefore screened 133 HSP index patients from the GeNeMove collection and the University of Dublin for copy number variations applying a newly designed MLPA assay. We identified a large multi-exonic *REEP1* duplication in one index patient (c.33-?_606+?dup) (Fig. 1). The 5′-start of the duplication can be narrowed to a 54.5 kb region defined by MLPA probes at g.863 (intron 1, not duplicated) and g.55453 (exon 2, duplicated) (Supplementary Fig. 2). The extent of the 3′-end of the duplicated element could not be determined with our MLPA assay, because the most distal probes were still involved in the duplicated segment. If the duplication occurs in tandem, our data imply an expansion of intron 1 by >70 kb, which is highly likely to interfere with splicing of the adjacent exons 1 and 2 of *REEP1*. Since the duplicated segment does not contain the full *REEP1* gene it will not be transcribed either. We therefore predict that this partial duplication could in fact result in haploinsufficiency of the affected chromosome. This duplication was not observed in 132 DNA samples measured with the same assay.

Clinical findings

Clinical data were available from eleven SPG31 index patients and their relatives. These families originated from Belgium, Germany, Ireland, Spain and The Netherlands. The identified SPG31 patients presented with a pure phenotype. Five families reported mild impairment of vibration sense. Urge incontinence was present in three families. Two patients in addition had distal amyotrophy and/or weakness of the distal upper limbs (ANT28, M1516) and one patient had early involvement of the upper extremities (I1018). The age of onset across all tested patients suggested a bi-modal distribution: age at onset was in the first and second decade of life in the majority of patients (71%), with the remaining patients showing ages at onset >30 years (range 31–91 years) (Fig. 3). A wide range of onset ages, encompassing early and late onset, was also observed within single families. We identified two mutation carriers, who were clinically unaffected at age 56 and 76, respectively (B01-0009-II.2, ANT25-II.2). The patient ANT 40-III.4, who was examined at the age of 5 years, might still be too young to display a clinical phenotype.

Nerve conduction studies were performed in seven index patients. They were normal in six cases. Only one patient (ANT28-II.1), who also showed asymmetric distal amyotrophy of the first interosseus muscle had abnormal sensory and motor nerve conduction velocities limited to the upper limbs: motor distal latency of median nerve was 4.6 ms on the right side and 4.7 ms on the left side (normal less than 3.9 ms); sensory nerve conduction velocity (NCV) were slightly reduced for the right median nerve (38.3 m/s) and the left median nerve (37.8 m/s). Normal sensory NCV in the upper limbs are >46 m/s. No conduction block was detected at the elbow for the ulnar nerve. Central motor evoked potentials were obtained in three index patients. They were abnormal in two cases; one patient showed signs of subclinical corticospinal tract involvement to the upper extremities (TU2-0077-II.2). No specific discriminating features were noted in available cerebral and spinal MRI. Abnormal findings included a cerebellar venous angioma in one individual (ANT25-III.2) and unspecific white matter lesions in two patients (ANT28-II.1, TU2-0077-II.2) (Table 2).

Discussion

Medical genetics considerations

In a large mutation screen of *REEP1* in 535 HSP patients, we identified 16 individuals carrying 14 different pathogenic sequence variants and one partial gene duplication. These changes were absent in healthy controls and co-segregated with the phenotype in all pedigrees available for analysis. Together with a previous report (Zuchner *et al*., 2006*b*), this brings the number of

presumably causative distinct mutations to 19, with three occurring in two unrelated families each (Fig. 1, Table 1). The overall detection frequency of 3.0% in a clinically mixed sample of pure and complicated HSP families and patients establishes *REEP1* as a comparatively frequently mutated HSP gene. When considering pure HSP patients only (n = 134), in whom SPG3A and SPG4 were ruled out, *REEP1* accounts for 8.2% of the samples. In patients with a family history of autosomal dominant HSP REEP1 accounted for 7.5%, whereas 1.7% of apparently sporadic patients carried a mutation. In a previous study we reported a mutation rate of 6.5% in an unscreened sample of 92 mainly pure HSP cases (Zuchner *et al*., 2006*b*). These data suggest that SPG31 represents the third most common cause for autosomal dominant HSP.

The analysed HSP families and isolated patients consisted of an academic collection (n = 299) and a non-academic diagnostic sample (n = 236). The inclusion of a large non-academic diagnostic sample allowed for some interesting additional conclusions. Although we do not know the clinical criteria that were used by physicians to initiate testing at Athena Diagnostic Inc., it might be fair to assume that these criteria were less stringent than the ascertainment protocols of the academic centres. The non-academic diagnostic sample represented patients from a large number of referring physicians, not all of them necessarily being neurologists. Thus, the reported numbers should be meaningful especially for clinical practitioners outside large hospitals, who probably see the majority of HSP patients and their families. The mutation detection rate in the non-academic sample (5 in 236 or 2.1%) was lower than in the academic one (11 in 299 or 3.7%). Given that the technical differences between both sequencing centres were probably small and SPG3A and SPG4 mutations were nearly completely ruled out in both sample sets, it appears that the DNA samples contributed by academic centres underwent stricter clinical selection. We suspect that inclusion criteria such as ethnicity, family history and clinical phenotype explain the differences between the academic and the non-academic diagnostic sample. In support of this notion, there has been also considerable inconsistency between reports for other major HSP genes like SPG3A (Wilkinson *et al*., 2003; Sauter *et al*., 2004), SPG4 (Hentati *et al*., 2000; Patrono *et al*., 2005) and SPG6 (Chen *et al*., 2005; Klebe *et al*., 2007). Our results also suggest that commercial testing is efficient in detecting the underlying mutations in *REEP1* and referring physicians appear to be well placed in initiating the genetic tests. Even in the absence of clinical data, making the mutations identified in the Athena Diagnostics laboratories available to the scientific community will benefit the understanding of the mutational spectrum in *REEP1*. Taken together, these observations emphasize the need for large multi-centre studies for deriving meaningful prevalence rates for the various genetic forms of HSP.

Molecular genetics considerations

Nucleotide changes in *REEP1* were identified in six of the seven exons, the exception being exon 3.

In agreement with the initial report on *REEP1* mutations in SPG31 (Zuchner *et al*., 2006*b*), we found a heterogeneous mutation spectrum, including small insertions and deletions, splice site mutations, missense mutations and a large duplication. Nine of the alterations identified to date directly alter the open-reading-frame (Table 1). An additional five mutations affect splice motives and are likely to result in frameshifts (Table 1). The primary consequence in such instances is the creation of pre-terminal stop codons and degradation of the affected mRNA transcripts by non-sense mediated decay (Frischmeyer and Dietz, 1999). The detection of a deletion that eliminates the first methionine in family ANT25 could be considered as a proof of concept for the null allele/haploinsufficiency hypothesis as the underlying molecular genetic mechanism for REEP1 in SPG31.

This consideration prompted us to screen for copy number variations too large to be detected by conventional PCR. The significance of this mutational class has recently been shown in SPG4 where large deletions and duplications account for 25–40% of all patients (Beetz *et al*., 2006; Depienne *et al.*, 2007; Beetz *et al.*, 2007). We identified one partial REEP1 duplication, which likely interferes with expression from the affected allele. Overall, however, copy number variations in REEP1 appear to be rare.

Two families in this study have been identified with unique 3′-UTR changes, which were predicted to alter highly conserved micro RNA target sites. These variants are unlikely to represent polymorphisms as they were absent from an extended set of 1000 controls (2000 chromosomes). Micro RNAs represent small non-translated molecules, which bind to specific 7–8 nt target sites in 3′-UTRs of mRNAs and inhibit translation and alter transcript stability (Bartel, 2004). Genetic variation in micro RNA target sites has previously been associated with disease (Abelson *et al*., 2005; Clop *et al*., 2006). Together with our previous results (Zuchner *et al*., 2006*b*) our present data further suggest that this kind of aberration may also contribute to the mutational spectrum of SPG31. Based on the relatively frequent involvement of *REEP1* 3′-UTR changes in SPG31 HSP (4 out of 22 mutations), inclusion of this region in diagnostic and research mutation screens is recommended. Further studies are needed to reveal if these variants are indeed causative for the disease.

We also identified two classic missense mutations, c.56C>G (p.P19R) and c.59C>A (p.A20E). Interestingly, the affected adjacent residues lie in an N-terminal motif predicted to be involved in protein localization. Disruption of such a sequence can be expected to hamper proper sub-cellular targeting and reduced availability of protein at the site of function. Taken together, both mRNA depletion and reduced protein-availability could have similar consequences, i.e. a loss of function. However, the relevance of additional molecular genetic mechanism, including a gain of function, cannot be excluded at present. More detailed investigations into the effects of the 3′-UTR and missense changes are clearly needed.

Clinical considerations

SPG31 is almost exclusively associated with a pure spastic paraplegia phenotype. The frequency of bladder disturbances and impaired vibration sense seems to be comparable to that reported in a large SPG4 sample (McDermott *et al*., 2006) and thus to be higher than in SPG3A (Durr *et al*., 2004). The only complicating feature observed was a peripheral neuropathy in one patient. Clinical peripheral nerve involvement, as observed in other forms of HSP, seems to be rare in SPG31. However, more extensive electrophysiologal studies will have to be performed to reach firm conclusions.

SPG31 is the fourth autosomal dominant form of HSP reported with age of onset predominantly in the first decade of life. However, adult onset patients are found and penetrance is incomplete even at high age (Fig. 3, Table 2). Strong variability regarding age at onset is observed for other autosomal dominant HSP forms as well (Fonknechten *et al*., 2000;Sauter *et al*., 2004;Blair *et al*., 2006). A striking feature of SPG31 in this study is the bi-modal distribution of age at onset (Fig. 2). This phenomenon is not explained by the presence of specific *REEP1* mutations since both early and late onset patients can be found within single families (Table 2). Further work is needed to substantiate this observation and to clarify what environmental and/or genetic factors contribute to the different courses of SPG31. However, our description of a broad overlap in age at onset with the two other major forms of autosomal dominant HSP (Fig. 2) and a virtually indistinguishable phenotype (Table 2) substantiate inclusion of SPG31 screening in any diagnostic setting.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

HSP	hereditary spastic paraplegia
MLPA	multiplex ligation-dependent probe amplification
NCV	nerve conduction velocity
SPG	spastic paraplegia gene
REEP1	receptor expression enhancing protein 1

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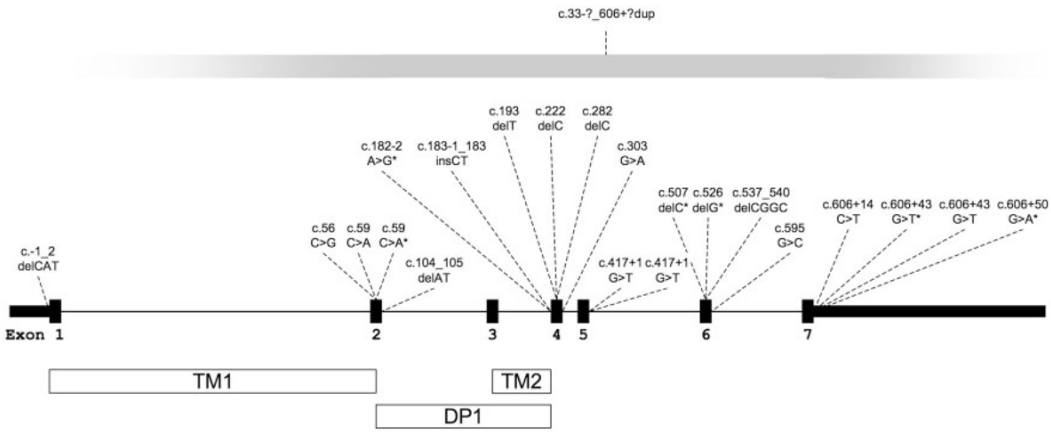

Fig. 1.

REEP1 mutations identified to date. Mutations are distributed over the entire gene except exon 3. The grey bar indicates a partial duplication in family I1018, which starts in intron 1 and reaches beyond the 3′-end of the coding sequence. TM: transmembrane domain, DP1: domain with similarity to the human DP1 gene. *mutation reported previously (Zuchner *et al.*, 2006*b*).

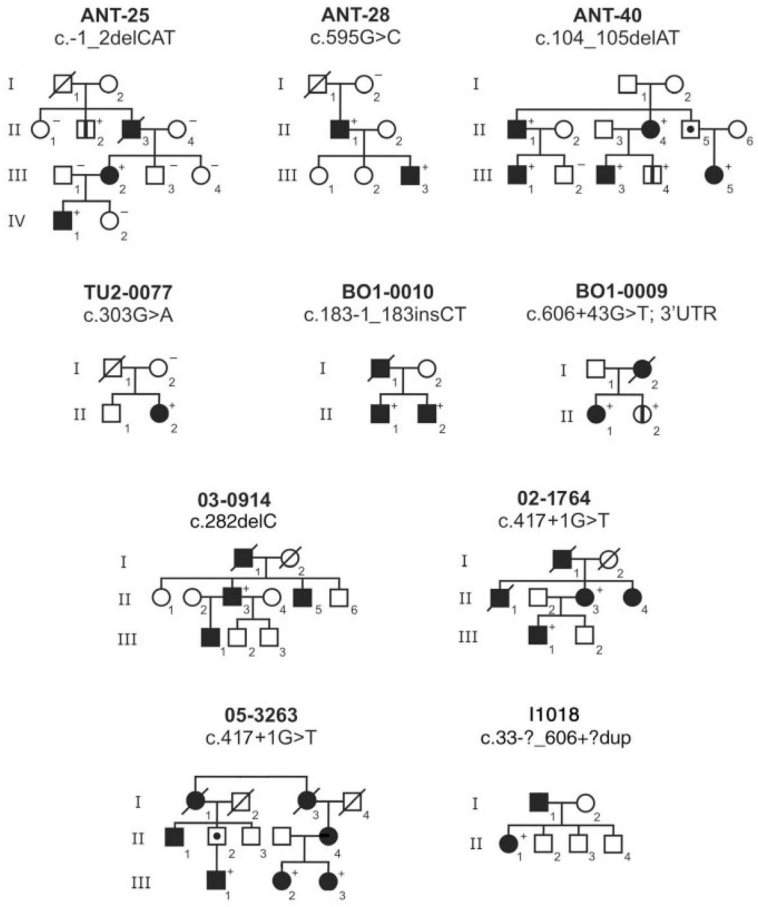

Fig. 2.

Pedigree of SPG31 families identified in the present study. Shaded symbols denote affected individuals, open symbols indicate unaffected individuals; unaffected carriers are marked by a vertical slash, obligate carriers of unknown clinical status by a dot and deceased family members by a diagonal slash.'+' tested mutation carriers.

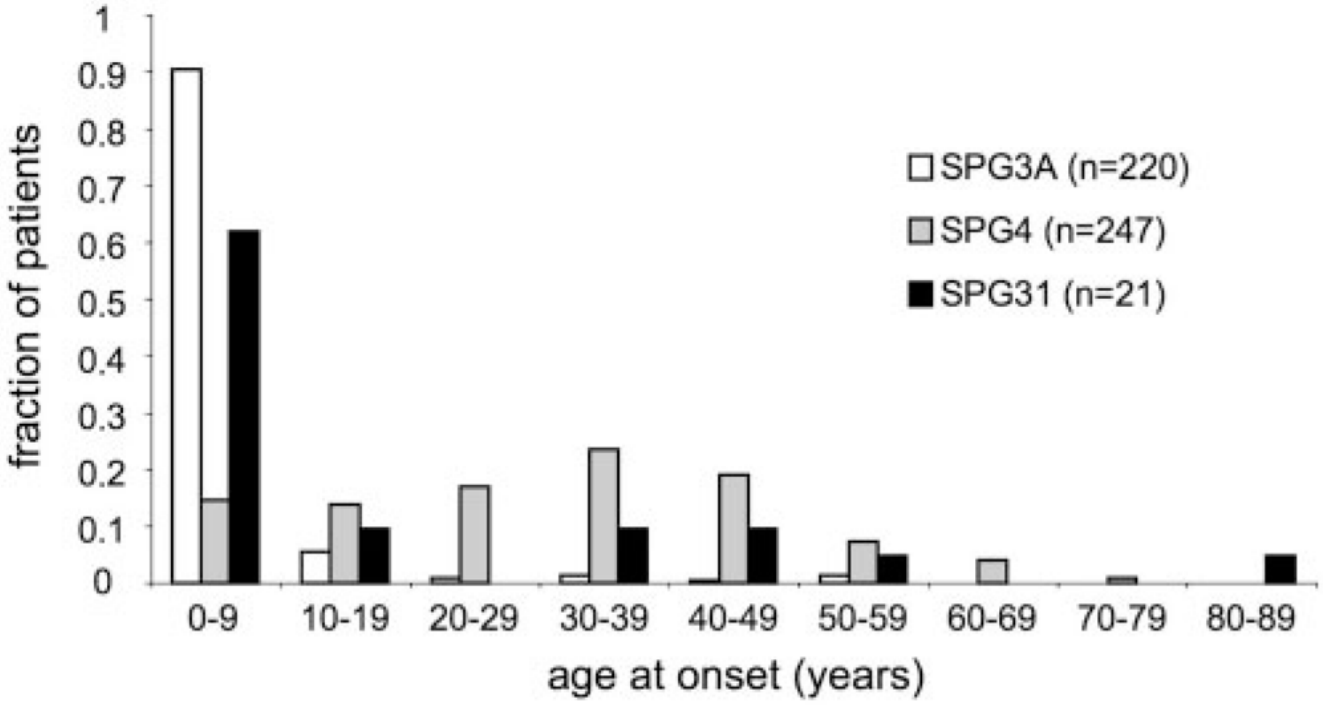

Fig. 3.

Age at onset (AAO) in SPG3A, SPG4 and SPG31 HSP plotted against frequency. The majority of SPG3A patients have an AAO before age 10 years, whereas SPG4 may start much later. SPG31 has an early onset, but about 15% of patients have an AAO after 30 suggesting a bi-modal distribution.

Table 1

Newly identified and previously reported *REEP1* mutations

Number	Sequence variation	Family	Inheritance	Exon	Predicted effect on splicing[a]	Effect on protein	Creation of alternative stop codon
1	c.-1_2delCAT	ANT25	Familial	1	None	p.M1fs	Yes
2	c.56C>G	ATH01	Unknown	2	None	p.P19R	No
3	c.59C>A	ATH05	Unknown	2	None	p.A20E	No
4	c.59C>A	DUK2189	Familial	2	None	p.A20E	No
5	c.104_105delAT	ANT40	Familial	2	None	p.Y35fs	Yes
6	c.182-2A>G	DUK2036	Familial	4	Abolishment SA intron 3	p.W61fs	Yes
7	c.183-1_183insCT	BO1-0010	Familial	4	None	p.W61fs	Yes
8	c.193delT	ATH02	Unknown	4	None	p.Y65Ifs	Yes
9	c.222delC	ATH03	Unknown	4	None	p.W75fs	Yes
10	c.282delC	03-0914	Unknown	4	None	p.T95fs	Yes
11	c.303G>A	TU2-0077	Sporadic	4	Abolishment SD intron 4	p.K101fs	Yes
12	c.417 + 1G>T	02-1764	Familial	5	Abolishment SD intron 5	p.K139fs	Yes
13	c.417 + 1G>T	05-3263	Familial	5	Abolishment SD intron 5	p.K139fs	Yes
14	c.507delC	DUK2299	Familial	6	None	p.P170fs	Yes
15	c.526delG	DUK2369	Familial	6	None	p.G176fs	Yes
16	c.537_540delCGGC	M1516	Familial	6	None	p.S179fs	Yes
17	c.595G>C	ANT28	Familial	6	Serious weakening SD intron 6	p.G199R p.G199fs	Yes
18	c.606 + 14C>T	ATH04	Unknown	7	None	miR-691	No
19	c.606 + 43G>T	BO1-0009	Familial	7	None	miR-140	No
20	c.606 + 43G>T	DUK2354	Unknown	7	None	miR-140	No
21	c.606 + 50G>A	DUK1959	Familial	7	None	miR-140	No
22	c.33-?_606+?dup	I1018	Familial	2–7	SD and SA intron 1 detached	p.V11fs	Yes

Shaded rows denote previously reported mutations (Zuchner *et al.*, 2006*b*); SA = splice acceptor; SD = splice donor; fs = frameshift.

[a] splice site analysis at https://splice.cmh.edu and at www.fruitfly.org/seq-tools/splice.html.

Table 2

REEP1 mutations and clinical characteristics

Family	ANT25	ANT28	ANT40	BO1-0009	BO1-0010	TU2-0077	03-0914	02-1764	05-3263	M1516	I1018
Mutation	c.-1_2delCAT	c.595G>C	c.104_105delAT	c.606 + 43G>T; miR140	c.183-1 _183insCT	c.303G>A	c.282delC	c.417 + 1G>T	c.417 + 1G>T	c.537_540delCGGC	c.33-?_606+ ?dup
No of individuals genotyped (affected)	9(2)	3(2)	8(5)	2(1)	2(2)	1(1)	1(1)	2(2)	2(2)	39(21)	1(1)
No of individuals clinically examined	3	2	3	1	2	1	1	2	3	14	2
Origin	Belgium	Belgium	Spain	Germany	Germany	Germany	The Netherlands	The Netherlands	The Netherlands	The Netherlands	Ireland
Mode of inheritance	AD	AD	AD	AD	AD	S	S	AD	AD	AD	AD
Phenotype	Pure	Pure	Pure	Pure	Pure	Pure	Pure	Pure	Pure	Pure	Pure
age at onset in years	4; 32[a]	4; 5	<10 (3x)	50[c]	3; 3	42	<10	2; 38	8; 11; 14	range 1–75	42; 90
Mean disease duration in years [range]	18 [17–19]	16.5 [3–30]	2–5 decades/unknown	9	21	12	> 4 decades	unknown	25 [19–31]	0.5–1	5
Severity	Mild– moderate	Mild–moderate	Mild– moderate	Mild	Mild	Mild	Severe	Mild	Moderate – severe	Mild–moderate – severe	Mild–moderate
Lower limb spasticity	+	+	+	+	+	+	+	+	+	+	+
Lower limb weakness	Distal	Variable	–	+	+	+	++	Distal	Variable	Variable	+
Muscle wasting	–	Variable	–	–	+	Distal	–	–	Variably distal	Variable	
Hyperreflexia	+	+	+	+	+	+	–	+	+	+	
Plantar reflexes	Extensor	Extensor	Variably extensor	Extensor	Extensor	Extensor	Extensor	Extensor	Variably extensor	Extensor	Extensor
Vibration sense (ankles)	Normal	Variably decreased	Normal	Decreased	Normal	Normal	Decreased	Variably decreased	Variably decreased	Normal	Decreased
Bladder disturbances	–	Variably urgency	–	Urgency	–	–	–	–	Urgency	–	Urgency
Additional features	–	Distal amyotrophy of upper limbs, scoliosis[b]	–	–	–	–	–	–	–	Muscle weakness of hands[b]	Early upper limb involvement
MRI	Normal (III.2: right cerebellar venous angioma)	II.1: small unspecific white matter lesions in frontal lobe	III.1 normal	n.d.	n.d.	white matter lesion above the trigonum	n.d.	normal	CCT normal	normal	normal
Neurophysiology	IV.1: normal	II.1: sensory-motor per- ipheral neuropathy in upper limbs	III.1 normal	No MEP; NCV normal	MEP normal, NCV normal	CMT increased latency; NCV normal	n.d.	n.d.	normal	n.d.	n.d.

n.d. = not determined; No MEP = no motor-evoked potential after cortical stimulation; CMT = central motor conduction time; NCV = nerve conduction velocity; AD = autosomal dominant; S = sporadic; CCT = cranial computer tomography.

[a]One mutation carrier (ANT 25-II.2 in Fig. 2) did not show any signs of spasticity upon examination at age 76.

[b] Additional features only found in older affected.

[c] Sibbling of index patient (BO1-0009-II.2 in Fig. 2) is subjectively asymptomatic at age 56 but has not consented to a neurological examination.