

# Hereditary spastic paraplegia due to a novel mutation of the *REEP1* gene

## Case report and literature review

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### Abstract

**Rationale:** Hereditary spastic paraplegia (HSP) is a heterogeneous group of diseases little known in clinical practice due to its low prevalence, slow progression, and difficult diagnosis. This results in an underestimation of HSP leading to belated diagnosis and management. In depth diagnosis is based on clinical presentation and identification of genomic mutations. We describe the clinical presentation and pathogeny of HSP through a report of a case due to a novel mutation of the *REEP1* gene (SPG31).

**Patient concerns:** A 64-year-old woman presented gait disturbances due to spasticity of the lower limbs progressing since her third decade. Previous investigations failed to find any cause.

**Interventions:** DNA analysis was performed to search for HSP causing mutations.

**Diagnoses:** A novel heterozygote mutation (c.595 + 1G>A) of the *REEP1* gene, within the splice site of intron 6, was discovered. This nucleotide change causes exon 6 skipping leading to frame shift and a truncated transcript identified by complementary DNA sequencing of reverse transcription polymerase chain reaction products.

**Outcomes:** REEP1 is a known protein predominantly located in the upper motor neurons. Mutation of REEP1 primary affects the longest axons explaining predominance of pyramidal syndrome on lower limbs.

**Lessons:** Slow progressive pyramidal syndrome of the lower limbs should elicit a diagnosis of HSP. We describe a novel mutation of the *REEP1* gene causing HSP. Pathogeny is based on resulting abnormal REEP1 protein which is involved in the development of longest axons constituting the corticospinal tracts.

**Abbreviations:** cDNA = complementary DNA, ER = endoplasmic reticulum, HSP = hereditary spastic paraplegia, MLPA = multiplex ligation-dependent probe amplification, MRI = magnetic resonance imaging, mRNA = messenger RNA, PCR = polymerase chain reaction, RT = reverse transcription.

**Keywords:** corticospinal tract, gait disorders, hereditary spastic paraplegia, progressive paraparesis, REEP1, REEP1 mutation, SPG31

## 1. Introduction

Hereditary spastic paraplegia (HSP) is a group of genetic diseases commonly characterized by slow progressive pyramidal syndrome of the lower limbs. Adolf von Strümpell and Sigmund Freud were among the first to describe the condition towards the

end of the 19th century.<sup>[1]</sup> Prevalence is low at about 3 to 10/100,000.<sup>[2]</sup> Most patients consult following gait disturbances due to spasticity but often many years after onset. Clinical and paraclinical examinations are unspecific and diagnosis requires confirmation by DNA analysis. Because of these issues, clinicians have little knowledge about HSP and diagnosis is often belated. Improving knowledge of HSP would lead to a better estimation of incidence, result in earlier diagnosis and rehabilitation, isolate genetic causes, and develop therapeutic strategies. We describe the case of a patient who was diagnosed with HSP more than 30 years after onset. The case is all the more original as she presented HSP due to a novel mutation of the *REEP1* gene. Through this report, we describe clinical and paraclinical features of HSP. We discuss theories referring to pathogeny found in literature to better understand the origins of the diseases, and in particular for mutations of the *REEP1* gene.

## 2. Consent

The patient has given written consent for description of the case including clinical examination on video file. All data have been anonymized.

## 3. Case report

A 64-year-old woman with a medical history of malignant thyroid nodules requiring daily substitutive therapy, a uterine fibroma and

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past smoking, consulted a neurologist because of gait disturbance dating from many years. She was being managed for her symptoms in a physical rehabilitation department with undetermined diagnosis. The first symptoms began during her twenties when she noticed that she was walking more slowly. She explained that her relatives had only noticed the disorder when she was in her thirties. During the medical interview, she remembered that her mother had also suffered from gait disturbances, but she was unable to describe them. Her mother died at the age of 57 years of unknown causes. The patient has 5 brothers (including one who is deceased and another with whom she has lost contact), a 37-year-old son and a 9-year-old granddaughter neither of whom apparently present the same disorder.

Clinical examination revealed an abnormal gait with tiptoe walking due to spastic hypertonia of the lower limbs (see Video, Supplemental Video, <http://links.lww.com/MD/B521> which shows patient medical interview and clinical examination). Spasticity was confirmed in the hamstrings, quadriceps, adductors, gastrocnemius and soleus, as well as proximal weakness of the lower limbs (quadriceps and gluteal muscles). Other manifestations of pyramidal syndrome—hyperreflexia, clonus, and Babinski sign—were also present but only in the lower limbs. The patient did not report any sphincter dysfunction. In-depth sensitivity examination, including pallesthesia, was normal. There were no signs of cerebellar dysfunction. Further somatic examination revealed a reduced right vocal cord mobility after the patient complained about a hoarse voice. Neuropsychological assessment found only mild depressive symptoms and divided-attention disorder. Explorations of intellectual, phasic, executive, and memory capacities were normal.

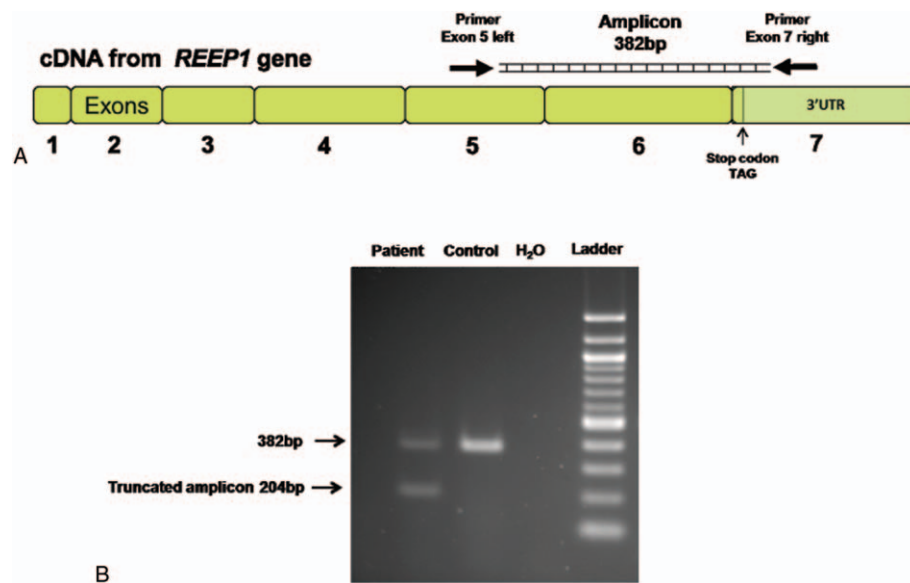
Laboratory tests did not reveal any abnormalities in blood count, electrolyte values, coagulation test, or renal, hepatic or thyroid functions. No inflammatory syndrome was observed with a C-reactive protein level under 5 mg/L. Vitamin B12 and erythrocyte folate levels were normal, respectively at 342 pg/mL (normal range 116–782 pg/mL) and 762 nmol/L (normal range

572–1840 nmol/L). Serology results were negative for HIV, syphilis, HTLV-1, Lyme disease as was investigation for *Tropheryma whipplei* DNA. There were no biological signs of autoimmunity with absence of anticoagulant, antiphospholipid, and anti-DNA antibodies. The patient's angiotensin-converting enzyme level was normal, but tests for very long-chain fatty acids were not conducted. Cerebrospinal fluid analysis showed a normal protein level (40 mg/dL) and electrophoresis, no cells and was negative for *Borrelia burgdorferi* DNA. Spinal and brain magnetic resonance imaging (MRI) did not find any cause for the pyramidal syndrome. There was no cerebral atrophy with normal trophism of the cortex, corpus callosum, cerebellum, and the whole spinal cord.

At this point of the investigation we concluded a slowly progressive pyramidal syndrome exclusively involving lower limbs without any obvious biological or radiological cause suggesting the possibility of HSP. A first molecular DNA analysis of the *SPG4* gene (Sanger and multiplex ligation-dependent probe amplification (MLPA) method) did not reveal any mutation or genomic modification. We then explored the *REEP1* gene (*SPG31* NM\_022912.2) through sequencing of coding sequences including flanking intronic sequences using MLPA for copy number variant detection as previously described,<sup>[3]</sup> and found a heterozygote mutation c.595+1G>A within the donor splice site of intron 6. This anomaly was confirmed by a second blood withdrawal and DNA sequencing. In order to test how the mutation affected the splicing event of the *REEP1* gene, fibroblasts were cultured from a skin biopsy of the patient to extract messenger RNA (mRNA). Reverse transcription (RT) was performed for synthesis of complementary DNA (cDNA). The ALAMUT v2.0 software (Interactive Biosoftware, Rouen, France) was used for in silico predicted scores of splicing and showed a marked decrease in donor splice site usage suggestive of exon 6 skipping (Fig. 1). To validate this hypothesis, primers corresponding to exon 5 and 7 were used for cDNA amplification by polymerase chain reaction (PCR) of the implicated area.



**Figure 1.** Almut 2.0 documentation, splicing predictor: predictions window around the *REEP1* c.595+1G>A variant. The top box represents the wild-type sequence, with a G at position c.595+1, and the bottom box represents the mutant sequences, with an A at c.595+1. Probabilities for the use of the 5'-splice sites are indicated for 5 different algorithms.



**Figure 2.** Amplification of the muted area from complementary DNA (A), and agarose gel electrophoresis showing truncated amplicon (B). bp=base pairs.

Agarose gel electrophoresis highlighted a truncated amplicon not found in a control sample (Fig. 2). Direct sequencing of the PCR products confirmed complete deletion of the exon 6 at the mRNA level leading to a frame shift (Fig. 3), inferring modification of the C-terminal portion of the resulting protein (p.G140Afs\*22). Diagnosis of HSP *SPG31* due to mutation of *REEP1* was confirmed. We found no previous reports of this mutation in the databases HGMD (<http://www.hgmd.cf.ac.uk/ac/index.php>) and ClinVar (<http://www.ncbi.nlm.nih.gov/clinvar/intro/>).

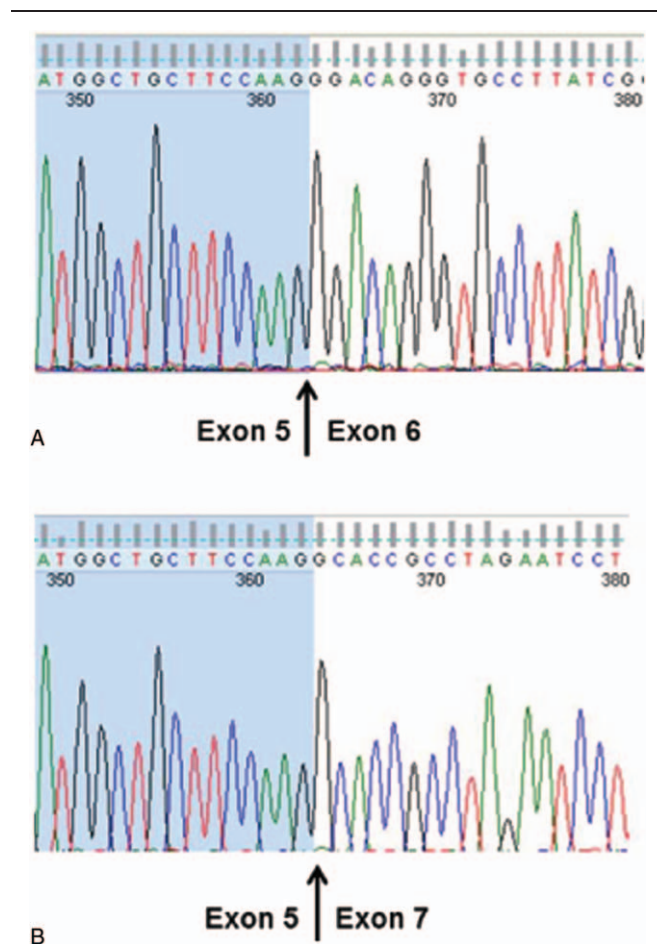
None of the patient's family members was willing to undergo the diagnostic procedure. Oral antispastic drugs failed to improve the patient's clinical state, only intramuscular botulinum toxin injections offered relief.

The variant with phenotype description has been submitted and registered in the public database Clin Var (RCV000210477).

#### 4. Discussion

HSP diseases preferentially affect the corticospinal tract causing degeneration of the longest axons which leads to pyramidal syndrome mainly in the lower limbs. The low prevalence, insidious progression, and wide variety of symptoms make it difficult for physicians to recognize and diagnose cases of HSP. As a result, most patients begin rehabilitation only after many years of gait deterioration. Age of symptom onset spans from childhood to old age. Many disease courses are possible from stabilization permitting gait improvement to progressive deterioration until handicap. Most of all, HSP diseases are characterized by very long-term progression resulting in spasticity and gait disorder. As in the case we describe here, HSP is diagnosed after the main causes of pyramidal syndrome have been ruled out. Most of the usual paraclinical examinations are negative. MRI can reveal cortex, corpus callosum or cerebellar atrophy, white matter lesions, thinning cervical and thoracic cords but without any specificity.<sup>[1,2]</sup> Diagnosis is based on clinical presentation and identification of the genetic mutation causing the disease.

Our patient presented typical spasticity with hyperreflexia, clonus and the resulting gait disorder found in the "pure" form.



**Figure 3.** Complementary DNA sequencing with Sanger method of reverse transcription polymerase chain reaction products showing truncation of exon 6 in messenger RNA. (A) Wild-type allele, (B) mutant allele.

Bladder dysfunction, pes cavus and decreased distal pallesthesia due to posterior column degeneration containing fasciculus gracilis fibers can also be part of the clinical presentation of HSP.<sup>[4]</sup> Many other signs such as involvement of the upper limbs, optic atrophy, retinal depigmentation, cataracts, cerebellar dysfunction, cognitive impairment, peripheral neuropathy, epilepsy, deafness, and ichthyosis can be encountered and define “complicated” forms.<sup>[2,4,5]</sup> We did not know how to integrate the right vocal cord deficit in our patient. We suggest that it is a possible sign of degeneration of the corticonuclear tracts even if, to date, the patient does not present any other form of bulbar palsy.

Due to the little information we obtained about our patient’s family, it is difficult to determine the mode of inheritance. The patient’s mother exhibited gait disorders leading us to suspect an autosomal dominant HSP which accounts for about 70% of cases. However, we were unable to confirm this hypothesis due to the poorly described family history and the fact that the other family members declined to undergo testing. HSP can also be autosomal recessive, X-linked recessive or due to a sporadic mutation.<sup>[6]</sup> Furthermore, incomplete penetrance is possible and may explain the few positive phenotypes in a family. Mutations including deletion, insertion, duplication, missense, nonsense, and splice site mutations in more than 30 genes and 70 loci have already been described.<sup>[7]</sup> The direct consequence is the rapid destruction of the resulting mRNA or production of a dysfunctional mutant protein.

The most commonly involved genes are the *SPG4* and *SPG3A* (respectively also called *SPAST* and *ATL1*) accounting for about 50% of mutations. They respectively encode for the proteins spastin and atlastin. Thus, *SPG4* and *SPG3A* are the first targets to explore in cases of suspected HSP. Mutations in the *REEP1* gene, of which the corresponding protein is the receptor expression enhancing protein 1 (REEP1), are associated with autosomal-dominant HSP *SPG31*, which is the third cause of HSP.<sup>[8]</sup> Strategy for exploration of genes of interest has to be guided by clinical presentation, mode of inheritance, and age of symptom onset. A complete algorithm has been proposed by Finsterer et al<sup>[9]</sup> and Coignon et al.<sup>[10]</sup> In our case, the pure clinical form, suspected autosomal dominant pattern, and age of onset more than 20 years led us to explore firstly *SPG4* before *REEP1* gene. Patients with HSP due to *SPG3A* mutations classically present first symptoms younger.

*REEP1* is located on chromosome 2p12 and comprises seven exons.<sup>[11]</sup> It is thought to represent 3% of all cases of HSP and about 8% of negative *SPG3A* and *SPG4* cases overall,<sup>[12]</sup> but seems to be less common in cases of HSP in Asian populations.<sup>[13–15]</sup> The clinical presentation is more often “pure” HSP but some unusual symptoms can accompany pyramidal syndrome.<sup>[16]</sup> Studies isolating patients with HSP due to an *SPG31* mutation observed a bimodal distribution of age of symptom onset: during the 2 first decades and then in the third decade.<sup>[3,12,17]</sup> However some patients have been reported to present their first symptoms after 90 years.<sup>[12]</sup> The disease course of HSP *SPG31* disorders is unspecific.<sup>[3]</sup>

All the cited proteins play a role in the development and the maintenance of axons from the cortical neurons. Long tracts are the most exposed to degeneration explaining why pyramidal syndrome almost always affects the lower limbs in HSP. REEP1 is highly predominant in upper motor neurons though it is also present in smaller amounts in the spinal cord, sympathetic ganglions, olfactory epithelium, and tongue.<sup>[18,19]</sup> REEP1 is inserted in the cellular membranes through 2 hydrophobic

regions. The first theory to explain the relevance of REEP1 in HSP was based on its involvement in mitochondrial functions to maintain the structures of long axons. Alteration of the mitochondrial network and fusion/fission leading to energy production has been demonstrated in patients with mutations of the *REEP1* gene.<sup>[3]</sup> It has been suggested that REEP1 has a chaperone-like activity in maintaining protein folding in mitochondria after oxidative stress.<sup>[20]</sup> Following works demonstrated that the REEP1 protein is present in the membranes of the endoplasmic reticulum (ER) and has several essential binding functions. It shapes the ER by contributing to its development and architecture.<sup>[21]</sup> In synergy with the 2 other proteins, spastin and atlastin, it promotes exchanges between the ER and cytoskeleton which constitutes the axon structure, and facilitates protein trafficking along the microtubules.<sup>[8,21,22]</sup> Finally both theories appear complementary: REEP1 (with spastin and atlastin) improves contact between mitochondria and ER through shaping, and promotes exchanges with cytoskeleton. Transfer of Ca<sup>2+</sup>, protein and lipids are essential between organelles for energy production and homeostasis.<sup>[19]</sup> In our work, we describe a novel mutation c.595 + 1G>A in the *REEP1* gene. We hypothesized that this mutation abolished the splice donor site, and the RT-PCR experiment demonstrated skipping of exon 6. The mutant transcript was consequently predicted to generate a shorter protein p.G140Afs\*22. The C terminal part affected by this mutation, as in our patient, is different and would be expected to play a determinant role for function loss or interference with the wild-type REEP1, giving a dominant negative effect and leading to disease development. From our knowledge, we never observed this mutation in more than 150 patients with HSP *SPG31* explored in our diagnosis laboratory. More it has never been reported in the exhaustive databases searched. However, we found four other splice mutations in our cohort suggesting *REEP1* gene is prone to this mutational mechanism.<sup>[3]</sup>

In conclusion, any patient presenting long-term progressive pyramidal syndrome of the lower limbs should elicit a diagnosis of HSP after exclusion of obvious causes. Earlier diagnosis would lead to earlier rehabilitation measures to preserve patient mobility. Final diagnosis is based on the identification of a genomic mutation causing degeneration of the corticospinal tract. We describe a novel heterozygote mutation of the *REEP1* gene with truncation of the resulting REEP1 protein which plays a crucial role in the development and maintenance of axons from upper motor neurons.

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