Received: 10 March 2021 | Revised: 18 May 2021 | Accepted: 2 August 2021

DOI: 10.1002/mgg3.1783

Molecular Genetics & Genomic Medicine Open Access WILEY

CLINICAL REPORT

HINT1 founder mutation causing axonal neuropathy with neuromyotonia in South America: A case report

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Funding information
S.A.B. is supported by a predoctoral fellowship and K.P. by a postdoctoral fellowship of the Fund for Scientific Research Flanders (FWO-Vlaanderen). This study was funded in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Brasil (CAPES, finance code 001) and the Fund for Scientific Research Flanders (FWO-Vlaanderen, grant #G049217N to A. J.).

Abstract

Background: Recessive loss-of-function mutations in *HINT1* are associated with predominantly motor axonal peripheral neuropathy with neuromyotonia. Twenty-four distinct pathogenic variants are reported all over the world, including four confirmed founder variations in Europe and Asia. The majority of patients carry the ancient Slavic founder variant c.110G>C (p.Arg37Pro) that shows a distribution gradient from east to west throughout Europe.

Methods: We report a case of *HINT1* neuropathy in South America, identified by massive parallel sequencing of a neuropathy gene panel. To investigate the origin of the variant, we performed haplotyping analysis.

Results: A Brazilian adolescent presented with recessive axonal motor neuropathy with asymmetric onset and fasciculations. Neuromyotonia was found on needle electromyography. His parents were not consanguineous and had no European ancestry. The patient carried biallelic pathogenic p.Arg37Pro alterations in the first exon of *HINT1*. Both alleles were identical by descent and originated from the same ancestral founder allele as reported in Europe.

Conclusion: Our findings expand the geographic distribution of *HINT1* neuropathy to South America, where we describe a recognized founder variant in a Brazilian adolescent with no apparent European ancestry. We confirm the association of the hallmark sign of neuromyotonia with the disease.

KEYWORDS
Charcot–Marie–Tooth disease, founder effect, HINT1, inherited peripheral neuropathy, neuromyotonia

Bianca de Aguiar Coelho Silva Madeiro and Kristien Peeters contributed equally to this study.

1 | INTRODUCTION

Inherited peripheral neuropathies (IPN) are a group of neurological disorders that, although genetically and clinically heterogeneous (Pisciotta & Shy, 2018), share common features, allowing their recognition. Charcot–Marie–Tooth disease (CMT) is the most frequent IPN, comprising a sensory-motor bilateral, symmetrical, and ascending neuropathy with an overall prevalence estimated at 10–28/100,000 (Pareyson et al., 2017). According to nerve conduction velocity (NCV) studies, CMT is classified as demyelinating (type 1), axonal (type 2) or an intermediate form combining features of both subtypes. More than 90 genes have been related to CMT and the numbers keep increasing (Pisciotta & Shy, 2018). Among these, loss-of-function mutations in the gene encoding the histidine-triad nucleotide binding protein 1 (*HINT1)* have been described in association with a distinct pure or predominantly motor, autosomal recessive axonal neuropathy with neuromyotonia (NMAN OMIM#137200), in individuals from Europe (Peeters et al., 2017; Zimoń et al., 2012), Russia (Shchagina et al., 2020), Asia (Meng et al., 2018; Wang et al., 2019), and North America (Peeters et al., 2017). Neuromyotonia is a syndrome characterized by delayed muscle relaxation after voluntary contraction, resulting from hyperexcitability of peripheral neurons. It is observed as a hallmark diagnostic feature in 70–80% of *HINT1* patients (Peeters et al., 2017; Zimoń et al., 2012).

Twenty-four different causal *HINT1* mutations have been described, out of which four are proven founder variants: three in Europe (p.Arg37Pro, p.Cys84Arg and p.His112Asn) (Shchagina et al., 2020; Zimoń et al., 2012) and one in China (p.Cys38Arg) (Meng et al., 2018). The p.Arg37Pro founder variant represents the most common by far, due to its high carrier frequency (1:67–182) (Laššuthová et al., 2015; Zimoń et al., 2012) in Central and South-Eastern Europe, Eastern-Asia and Turkey. In the Czech Republic and Russia, *HINT1* ranks among the most frequent causes of axonal neuropathy (Laššuthová et al., 2015; Shchagina et al., 2020) and cases of pseudodominant inheritance have been reported (Peeters et al., 2017).

HINT1 is a globular 13.7 kDa protein that is ubiquitously expressed and has an evolutionary conserved function (Peeters et al., 2017). The HINT1 enzyme acts as a homodimer that binds purine nucleosides and nucleotides through clefts containing a conserved sequence His-X-His-X-His-XX, where X is a hydrophobic residue. Although its endogenous substrate(s) remain unknown, *in vitro*, HINT1 is a promiscuous enzyme, hydrolyzing purine nucleotide substrates containing different phosphate linkages, such as phosphoramidates, mixed anhydrides, phosphorofluoridates and phosphorothioates (Peeters et al., 2017). HINT1 plays a role in multiple transcriptional and signaling pathways, like tumor suppression and apoptosis (Genovese et al., 2012; Weiske & Huber, 2006). Loss of HINT1 increases susceptibility to carcinogenesis in mice (Li et al., 2006) and causes behaviors related to anxiety, depression and aggression (Garzón-Niño et al., 2017). Intriguingly, these mice do not manifest clinical signs of peripheral neuropathy (Seburn et al., 2014).

We report the first case of *HINT1* mutations associated with NMAN in South America and confirm a shared haplotype with the original founder mutation.

2 | CASE REPORT

A 16-year-old adolescent visited the neurology clinic complaining of weakness and cramps in all four limbs. He was the second child of a healthy non-consanguineous couple, had a healthy 20-year-old sister and no family history of neurological disease. He was born from an uneventful pregnancy and delivery and had normal developmental milestones in his infancy and early childhood. By the age of 8, he noticed left foot drop that slowly progressed to bilateral weakness in lower and, later, upper limbs with difficulty in walking and handling objects. Muscle wasting was also noticed. He denied any sensory complaints.

His clinical examination found: normal cognition and cranial nerve function; distal weakness in the four limbs (Medical Research Council Scale 2/5 in hands and feet) with associated distal atrophy and deformity (claw hands and pes cavus; Figure 1a,b); diminished tendon reflexes in upper limbs and abolished reflexes in lower ones; absent plantar responses; bilateral thigh fasciculations and steppage gait; no sensory abnormality and no myotonia.

The patient's creatine kinase level was 987 (33–145 U/L). Hematological, biochemical, and cerebral spinal fluid results were normal. His electroneuromyography (ENMG) revealed a pure motor axonal polyneuropathy with neuromyotonia, fasciculations, denervation and chronic reinnervation (Figure 1c); his upper limb motor nerve conduction velocities ranged from 50.1 to 56.5 m/s (Table 1).

We performed a molecular investigation of 47 CMT causing genes using next generation sequencing (*AARS*, *AIFM1*, *BSCL2*, *DNAJB2*, *DNM2*, *DYNC1H1*, *EGR2*, *FGD4*, *FIG4*, *GARS*, *GDAP1*, *GJB1*, *GNB4*, *HARS*, *HINT1*, *HSPB1*, *HSPB8*, *IGHMBP2*, *INF2*, *LITAF*, *LMNA*, *LRSAM1*, *MARS*, *MED25*, *MFN2*, *MORC2*, *MPZ*, *MTMR2*, *NDRG1*, *NEFL*, *PDK3*, *PLEKHG5*, *PMP22*, *PRPS1*, *PRX*, *RAB7A*, *SBF2*, *SH3TC2*, *SLC25A46*, *SPG11*, *SURF1*, *TFG*, *TRIM2*, *TRPV4*, *YARS*) and multiplex ligation-dependent probe amplification (*PMP22*, *COX10*, *TEKT3*, *MPZ*, *GJB1*). A homozygous missense variant was found in the first exon of the

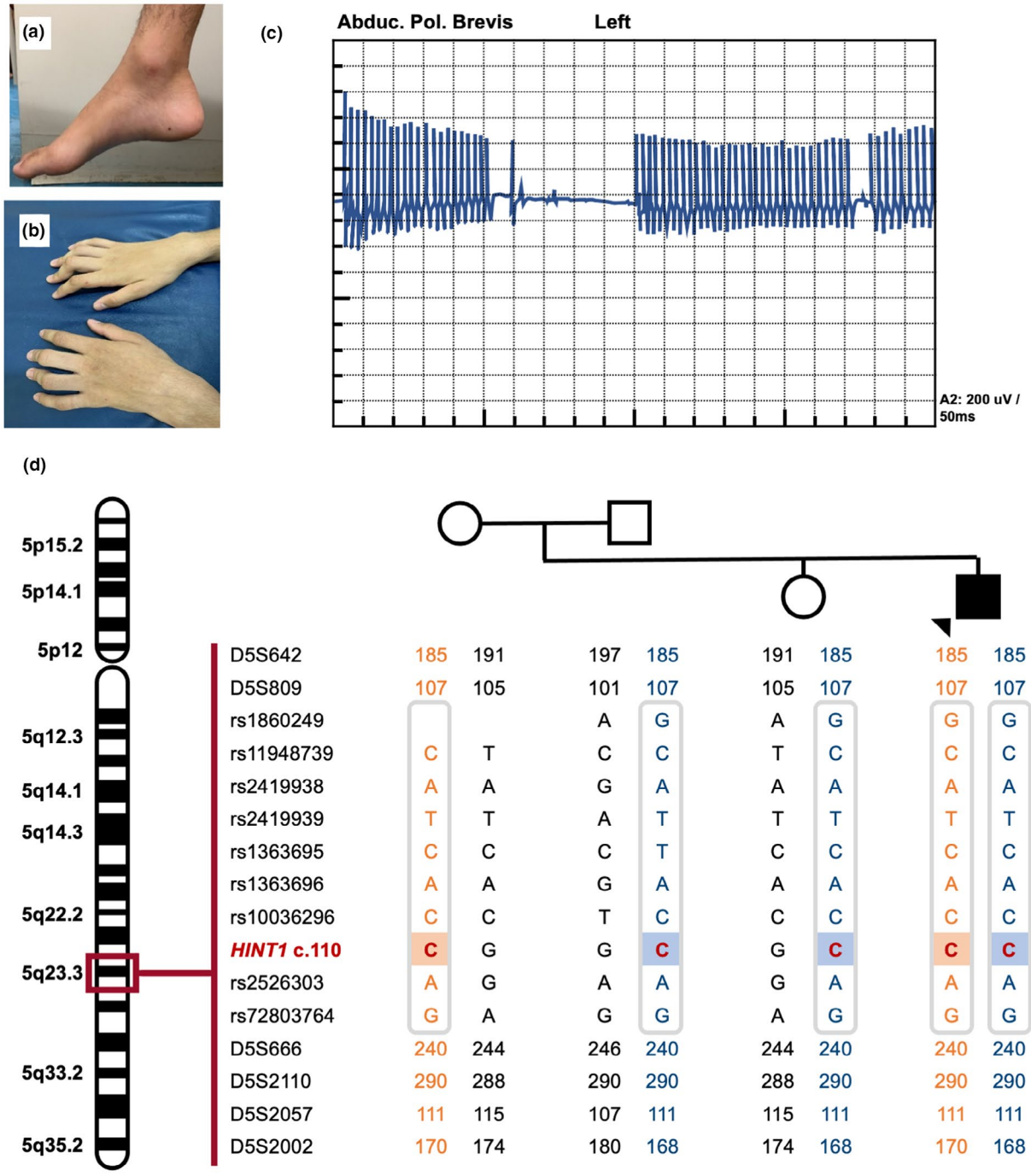

FIGURE 1 Patient pictures, EMG and genetic results. (a and b) Limb deformities: *pes cavus* and claw hands. (c) Neuromyotonic discharge on needle EMG of left abductor policis brevis muscle performed at age 16y. (d) Haplotype analysis of the patient's nuclear family demonstrating in the patient a homozygous region surrounding the c.110G>C *HINT1* variants on chromosome 5, which shares the minimal founder haplotype identified in European *HINT1* patients(Zimoń et al., 2012) (grey box). The parents and unaffected sister are all heterozygous carriers of the pathogenic mutation. Squares represent males and circles females; arrow indicates the index patient. Permission was granted by the patient in order to publish the pictures of his extremities, electromyography and genetic results

HINT1 gene: NM_005340.6: c.110G>C (p.Arg37Pro). This variant was previously reported as pathogenic in more than 80 families world-wide (Peeters et al., 2017; Scarpini et al., 2019; Shchagina et al., 2020; Zimoń et al., 2012). The variant was validated by Sanger sequencing (ABI3730xl DNA Analyzer, Applied Biosystems) on a newly extracted DNA sample. Segregation analysis confirmed that the patient's unaffected parents and sister were all heterozygous carriers of the c.110G>C substitution (Figure 1d).

Although the proband's parents denied any relatedness, haplotyping analysis using short tandem repeat and SNP markers (Zimoń et al., 2012) demonstrated in the patient

TABLE 1 Nerve conduction studies of the index patient at age 16 y

Motor nerve conduction			
	Latency (ms)	**Amplitude (mV)**	**NCV (m/s)**
Median left			
Wrist	5.5	7.8	
Elbow	9.5	7.0	50.1
Median right			
Wrist	4.5	4.3	
Elbow	8.8	4.7	51.6
Ulnar left			
Wrist	4.0	3.6	
Elbow	7.7	3.8	52.3
Above elbow	9.5	3.5	54.6
Ulnar right			
Wrist	4.1	1.7	
Elbow	7.9	1.5	56.5
Above elbow	9.8	1.6	50.5
Tibial left			
Ankle	5.1	2.7	
Popliteal fossa	14.4	2.1	41.7
Tibial right			
Ankle	5.3	1.7	
Popliteal fossa	15.8	1.1	39.0
Fibular left			
Ankle	0.0		
Knee			
Fibular right			
Ankle	0.0		
Knee			
Fibular (TA) left			
Fibula head (TA)	4.9	4.4	
Popliteal fossa	6.4	4.2	52.3
Fibular (TA) right			
Fibula head (TA)	4.9	5.2	
Popliteal fossa	6.6	5.0	56.5

Sensory nerve conduction				
	Latency 1 (ms)	**Latency 2 (ms)**	**Amplitude (uV)**	**NCV (m/s)**
Median left				
Finger 2	2.7	3.4	39.3	52.2
Median right				
Finger 2	2.4	3.1	33.8	57.4
Ulnar left				
Finger 5	2.3	3.3	27.3	51.3
Ulnar right				
Finger 5	2.1	2.8	27.7	53.4

(Continues)

TABLE 1 (Continued)

Sensory nerve conduction				
	Latency 1 (ms)	**Latency 2 (ms)**	**Amplitude (uV)**	**NCV (m/s)**
Radial left				
Dorsal	1.9	2.5	38.8	52.1
Radial right				
Dorsal	1.9	2.4	38.5	53.8
Sural left				
Ankle	3.2	3.9	11.6	44.3
Sural right				
Ankle	3.2	4.2	11.3	43.5
Fibular left				
Dorsal foot	3.4	4.2	8.0	41.2
Fibular right				
Dorsal foot	3.3	4.4	11.0	42.2

a homozygous region surrounding both c.110G>C alleles, implying they are identical by descent. Moreover, the disease region contained the minimal haplotype shared by European carriers of the c.110G>C founder variant (Zimoń et al., 2012) confirming that all alleles originate from a single mutational event (Figure 1d).

3 | DISCUSSION

We report the first case of *HINT1* neuropathy in South America. Recessive *HINT1* mutations are associated with an axonal pure motor or motor-greater-than-sensory neuropathy that has the distinctive feature of neuromyotonia (NMAN) (Peeters et al., 2017). In genetically heterogeneous patient cohorts, *HINT1* mutations account for about 2% of all CMT cases and about 10–12% of recessive CMT (Dohrn et al., 2017; Peeters et al., 2017), yet this frequency rises to about 80% when considering axonal CMT patients with the hallmark sign of neuromyotonia (Zimoń et al., 2012). Neuromyotonia consists of spontaneous and continuous muscle activity of peripheral nerve origin – muscle twitching at rest, cramps triggered by involuntary or induced muscle contraction, and impaired muscle relaxation. However, because it is a very rare condition, neuromyotonia is not always easily identified (Peeters et al., 2017). Needle electromyography may help disclose neuromyotonia and distinguish NMAN from myotonic dystrophy and channelopathies causing non-dystrophic myotonia (Jerath et al., 2015; Peeters et al., 2017; Wang et al., 2019) and thus narrow the molecular diagnosis.

The disease presentation of our patient was similar to other reports of *HINT1* neuropathy (Jerath et al., 2015; Rauchenzauner et al., 2016; Veltsista & Chroni, 2016). The onset was in the first decade of life with asymmetric weakness (left foot drop). By the time of evaluation at age 16, he had neither clinical nor electrophysiological sensory impairment and showed no neuromyotonia on neurological examination. He presented fasciculations in lower limbs and a high creatine kinase level. His electroneuromyography was compatible with pure motor axonal neuropathy with neuromyotonia. Only symptomatic treatment is available for *HINT1* neuropathies. The patients may benefit from physical therapy, ankle-foot orthoses and orthopedic corrections to maintain ambulation (Peeters et al., 2017). Disabling myotonia may be treated with sodium-channel blockers. This has not been necessary for our patient since he did not experience overt myotonia.

The c.110G>C variant is the most common reported cause of *HINT1*-associated neuropathy, due to the high frequency of this founder variation in Central and South-Eastern Europe. A recent haplotyping study in the Russian population (Shchagina et al., 2020) demonstrated that this pathogenic variant most likely arose in Slavic ethnicities and then gradually spread throughout Europe resulting in an unequal gradient of distribution (Peeters et al., 2017). So far, there was a single report of the c.100G>C variant on the North American continent in a US citizen with European ancestry (Slovenian and Greek) (Jerath et al., 2015).

Our patient's parents are seemingly unrelated and have no known European ancestry, yet both are heterozygous carriers of the c.110G>C variation and we demonstrated that both share a common disease haplotype with the European founder allele (Zimoń et al., 2012). These results indicate that the c.110G>C founder variant has spread to South America. In addition, in the Genome Aggregation Database (Karczewski et al., 2019)

(GnomAD v2.1.1) the c.110G>C variation was seen in heterozygous state in one individual of Latin American origin.

Our findings expand the geographic distribution of *HINT1* neuropathy to South America, where we report a recognized founder mutation in a Brazilian CMT patient with no apparent European ancestry. Physicians and researchers should be aware of this genotype when dealing with an NMAN phenotype patient in that continent. We confirm the association of the hallmark sign of neuromyotonia with the disease.

ACKNOWLEDGEMENTS

We are grateful to the patient and his family for their cooperation. We thank the Neuromics Support Facility of the Center of Molecular Neurology, VIB-UAntwerp, Belgium for assistance with Sanger sequencing and haplotyping.

CONFLICTS OF INTEREST

The authors have no conflict of interest to report.

AUTHOR CONTRIBUTIONS

BACSM, KP, AJ, MTCM, CCC: conception and design of the study; BACSM, ELSL, SAB, EDV: acquisition and analysis of data; BACSM, KP, AJ, MTCM, CCC: drafting the text. All authors read and approved the final manuscript.

ETHICS AND CONSENT

This study was approved by Oswaldo Cruz University Hospital Ethics Committee – Recife – Brazil. CAAE 23656819.0.0000.5192. An informed consent form was obtained from the patient (he is nineteen years old now) to report his case and publish the pictures of his extremities, electromyography and genetic results.

DATA AVAILABILITY STATEMENT

Data available from the authors upon request.

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How to cite this article: de Aguiar Coelho Silva Madeiro, B., Peeters, K., Santos de Lima, E. L., Amor-Barris, S., De Vriendt, E., Jordanova, A., Cartaxo Muniz, M. T., & da Cunha Correia, C. (2021). *HINT1* founder mutation causing axonal neuropathy with neuromyotonia in South America: A case report. *Molecular Genetics & Genomic Medicine*, 9, e1783. https://doi.org/10.1002/mgg3.1783