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ORIGINAL ARTICLE

VPS53 gene is associated with a new phenotype of complicated hereditary spastic paraparesis

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Abstract

Hereditary spastic paraparesis (HSP) is a progressive neurodegenerative disorder, characterized by progressive lower limb weakness and spasticity. Multiple genes are associated with both the pure and complicated HSP types. Our study is aimed at seeking for novel genetic basis of HSP in a family with two affected siblings. Genetic analysis using whole exome sequencing was conducted in a family quartet with two female siblings, who presented with complicated HSP featuring slowly progressive paraparesis, mild-moderate intellectual disability, normal head circumference (HC), and normal magnetic resonance imaging (MRI). A homozygous pathogenic variant was identified in both siblings in the *VPS53* gene (c.2084A>G: c.2084A>G, p.Gln695Arg). This gene acts as a component of the Golgi-associated retrograde protein (GARP) complex that is involved, among others, in intracellular cholesterol transport and sphingolipid homeostasis in lysosomes and was previously associated with progressive cerebello-cerebral atrophy (PCCA) type 2. This is the first description of the *VPS53* gene as a cause of autosomal recessive complicated HSP. Lysosomal dysfunction as a result of impaired cholesterol trafficking can explain the neurodegenerative processes responsible for the HSP. Our finding expands the phenotype of VPS53-related disease and warrants the addition of VPS53 analysis to the genetic investigation in patients with autosomal recessive HSP. The exact role of GARP complex in neurodegenerative processes should be further elucidated.

Keywords Hereditary spastic paraparesis · Hereditary spastic paraplegia · *VPS53* · PCCA type 2

Abbreviations

HSP	Hereditary spastic paraparesis
PCCA	Progressive cerebello-cerebral atrophy
GARP	Golgi-associated retrograde protein
HC	Head circumference
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
ID	Intellectual disability
ADHD	Attention deficit hyperactivity disorder
CMA	Chromosomal microarray analysis
WES	Whole exome sequencing

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Introduction

Hereditary spastic paraparesis (HSP) is a heterogeneous group of neurodegenerative genetic disorders with an estimated prevalence ranging from 1.3:100,000 [1] to 9.6:100,000 [2] in which the prominent clinical feature is progressive lower limb weakness and spasticity, which may be more prominent than the paresis [3–5]. Other possible symptoms are diminished vibration sense and bladder dysfunction. HSP is classified as "pure," when there are no additional features apart from the abovementioned spasticity, disturbed vibration sense, and bladder dysfunction, and "complex" when it is accompanied by other neurological signs such as ataxia, intellectual disability (ID), movement disorder, epilepsy, polyneuropathy, ophthalmologic manifestations, and extraneurologic signs such as hearing loss and ichthyosis, among others [4, 6, 7].

Brain MRI can show involvement of the cerebellum, basal ganglia, cerebral cortex, white matter, and corpus callosum. Inheritance pattern may be autosomal dominant, autosomal recessive, or X-linked. Due to the unique features and the high consanguinity rate seen in some ethnic groups in Israel, the autosomal recessive inherited forms of HSP are more common in this country. Clinical characterization and determination of the inheritance mode according to familial history can guide the diagnostic process. In addition, neuroimaging clues can point to more specific diagnoses, such as a thin corpus callosum and an axial fluid attenuation inversion recovery (FLAIR) image showing the "ear-of-the-lynx" in SPG11 or SPG15, and white matter T2 hyperintensities in SPG5, SPG21, and SPG35 [8]. However, in most cases, particularly in the autosomal recessive forms, the exact diagnosis cannot be inferred from clinical, laboratory, or imaging data, and therefore, genetic testing is needed for a final diagnosis and appropriate family counseling.

To date, 84 HSP loci and 67 HSP-related genes have been identified [9–17] in both the pure and complicated types of HSP. Nevertheless, only a fraction of cases receive a genetic diagnosis. For this reason, the absence of a genetic etiology for HSP does not exclude the diagnosis of HSP.

Methods

This is part of a broader genetic study which was conducted in order to explore the genetic basis of HSP in familial cases using whole exome sequencing (WES). A family with two female siblings of Moroccan-Jewish ancestry with complicated HSP, who had been on follow-up at our spasticity clinic, underwent a WES. Informed consent was obtained from siblings' parents prior to genetic testing. All study protocols were reviewed and approved by the Institutional Review Board at the Tel Aviv Sourasky Medical Center.

Patient 1

A 14-year-old female (the proband) is the third-born offspring of a Moroccan-Jew nonconsanguineous couple. She had been followed in our spasticity clinic since the age of 4 years due to slowly progressive motor and cognitive difficulties. She was born following normal pregnancy and delivery, with normal HC at birth (50th percentile). Both parents as well as two older siblings were healthy, whereas her younger sister had progressive motor and cognitive difficulties (patient 2) (Fig. 1). She had developmental delay, with achievement of independent walking at the age of 21 months. Her gait was unstable with tendency for recurrent falls in both directions. She had mild to moderate global developmental delay with expressive and receptive speech delay, and normal visual and auditory functions. At the age of 5 years, she was diagnosed with mild ID and since then she has attended a special education program. Attention deficit hyperactivity disorder (ADHD) was diagnosed at school age and she was treated with methylphenidate. She had no history of clinical seizures, but her EEG showed paroxysmal multifocal epileptic activity with left frontocentral as well as right central interictal spike and sharp wave epileptiform discharges. Neurological examination found no dysmorphic features, the HC was normal (25th percentile), as well as the cranial nerves including normal visual acuity and visual field. There was no evidence of ophthalmoplegia or nystagmus. Normal horizontal and vertical saccades were noted, with normal smooth pursuit. Mild dysarthria was evident, without dysphonia or dysphagia and with normal gag reflex and palate movement and normal tongue mobility and strength. She had normal tone, muscle strength, and deep tendon reflexes in the upper limbs, with increased tone (Modified Ashworth Scale 1+) in the lower limbs (knee flexion and extension, ankle dorsiflexion) and mild muscle weakness of 4/5 in both proximal (hip flexion and extension, hip abductors, knee flexion and extension) and distal (ankle dorsiflexion), with normal range of motion in all joints. Pyramidal signs manifested as hyperreflexia and sustained clonus were noted with flexor plantar response. There was no evidence of ataxia or other cerebellar dysfunctions. Anal tone was normal. Sensory function was within normal. She was able to run awkwardly and to alternately climb stairs as well as to hop on one foot.

The patient underwent an extensive evaluation that included repeat brain MRI with magnetic resonance spectroscopy (MRS) at age 5, 8, and 12 years, spine MRI at age 14 (Fig. 2), metabolic workup, ophthalmological examination, echocardiography, abdominal ultrasound, electromyography (EMG), and nerve conduction studies. The findings were all within normal. Chromosomal microarray analysis (CMA) detected a 436-kb microdeletion at 2q31.2 (chr2:180,535,146-180,972,463) (HG19 version), which was inherited from her healthy mother and which was also found in her affected sister (patient 2). This deletion spans 3 known genes including MIR1258, ZNF385B, and CWC22 genes and was not previously reported in the healthy population or identified as pathogenic.

Over the subsequent years, the patient's balance gradually worsened, with increased tendency to recurrent falls at both directions. At the age of 14 years, she was still ambulatory, but presented with urge urine incontinence, episodes of uncontrollable flatulence, and inability to hold back stools, without soiling or urinary retention. There has been no further deterioration in cognitive abilities. On physical examination at age 14, her weight was 60 kg and height 146 cm (−2SD), with BMI of 28.1 (95th percentile for age). On neurological examination, she had a normal HC (25th percentile) with noticeable brisk deep tendon reflexes in the upper limbs without spasticity or apparent weakness. Muscle tone in the lower limbs was

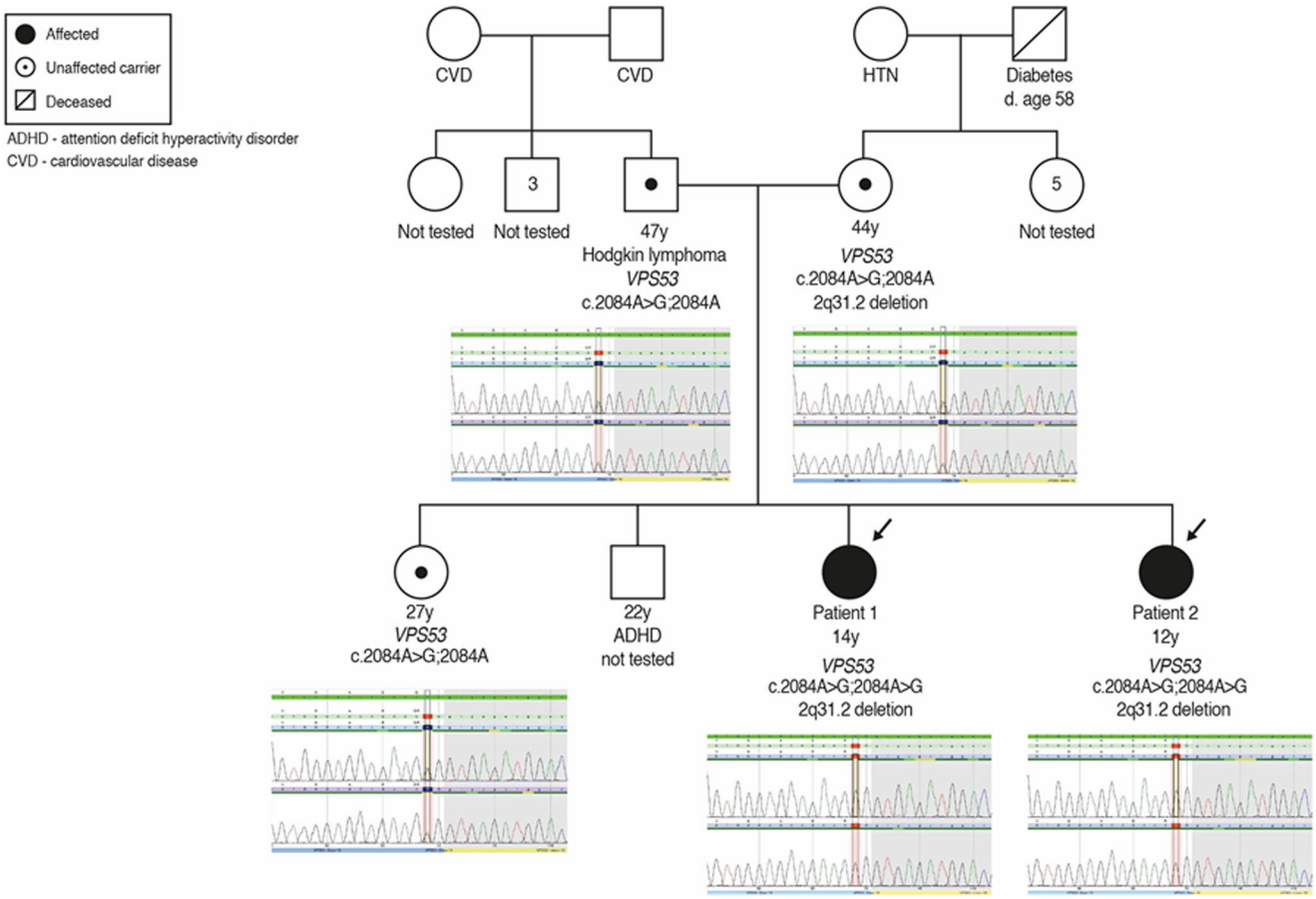

Fig. 1 Family pedigree and Sanger sequence chromatograms of the detected *VPS53* homozygous pathogenic variant. The arrows show the affected probands. ADHD—Attention-Deficit/Hyperactivity Disorder; CVD—cardiovascular disease

markedly increased with no deterioration of muscle weakness. Sensory function, including proprioception, light touch, pain, and vibration, was still within normal range. Her gait was more spastic, but she was still able to climb stairs alternately and to hop on one leg.

Patient 2

Proband's younger sister, 12 years old at present, had been examined by us for the first time at the age of 9 years due to progressive gait difficulties and epilepsy since the age of 3.5 years. She had an unremarkable perinatal history and early motor development, with achievement of independent walking at 13 months. She had a moderate global developmental delay starting at early infancy with delay in the acquisition of expressive and receptive language skills as well as cognitive difficulties, with normal communication skills. Visual and auditory functions were normal. Progressive gait abnormality had manifested at the age of 3.5 years, with unsteadiness and tendency for recurrent falls in both directions. She had no regression in other developmental milestones. The patient had attended a special early education program and received speech therapy with gaining of speech at the age of 3 years. She was later diagnosed with mild ID. At the age of 4 years, generalized epilepsy appeared, with mostly generalized nocturnal tonic seizures and myoclonic jerks. Her EEG at that time showed frequent generalized epileptic activity. She became seizure-free with levetiracetam (50 mg/kg/day) and clobazam (0.6 mg/kg/day) treatment, with a follow-up EEG showing left frontotemporal as well as right parietotemporal interictal epileptic activity. Extensive evaluation, including brain MRI, was performed at the age of 4 years which was normal. Metabolic workup and repeat MRI and MRS at age 6.7 years were normal (Fig. 3). CMA revealed the same 436-kb microdeletion at 2q31.2 noted in patient 1, which was inherited from their healthy mother and which was not reported previously as pathogenic.

At 9 years of age, the patient was ambulatory and was able to jump and climb stairs reciprocally. On neurological examination, she had no dysmorphic features, normal HC, and normal cranial nerves including normal visual acuity and visual field. There was no evidence of ophthalmoplegia or nystagmus. Normal horizontal and vertical saccades were noted, with normal smooth pursuit. Speech was normal with no apparent dysarthria, dysphonia, or dysphagia. She had normal tone, muscle strength, and deep tendon reflexes in the upper

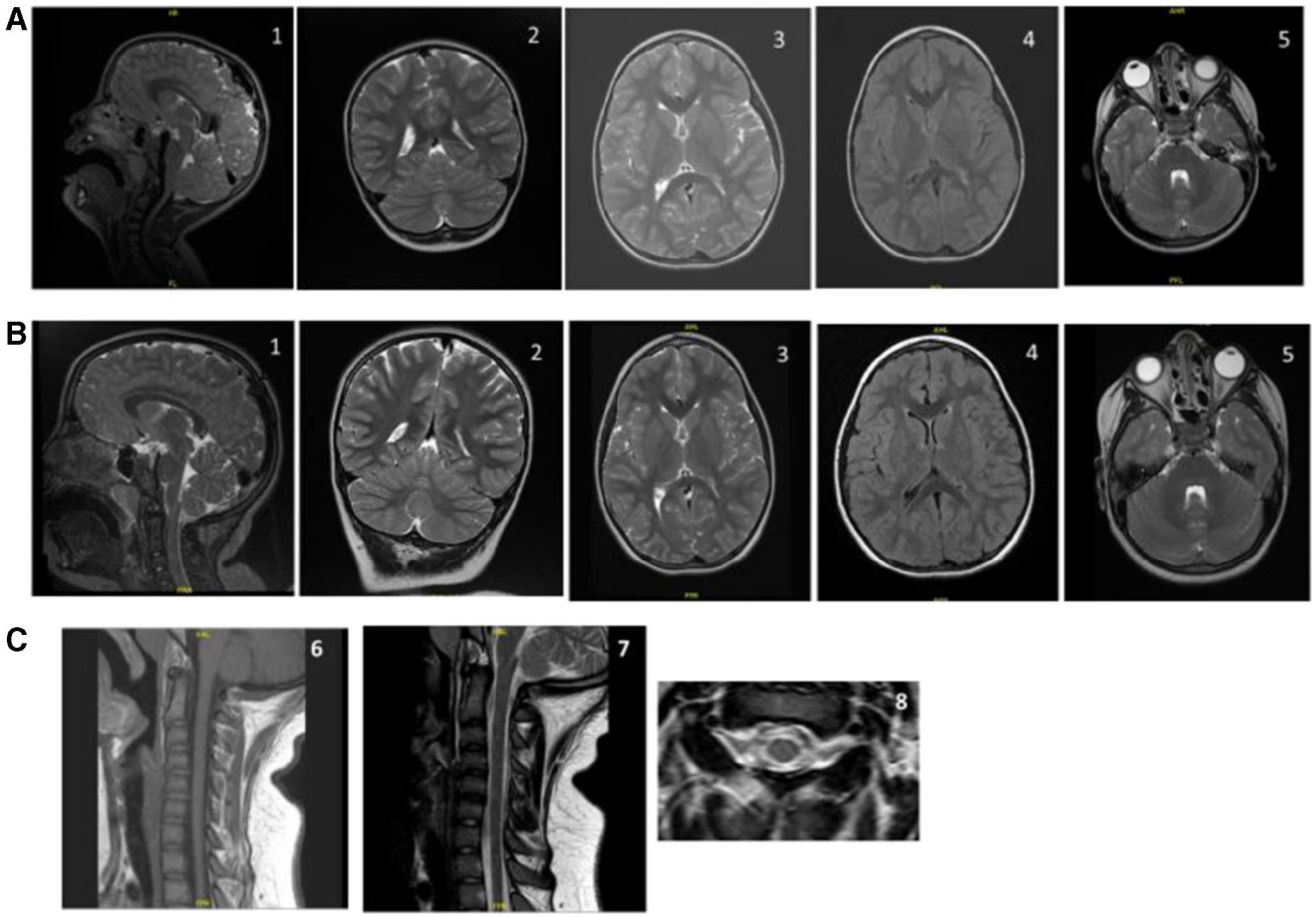

Fig. 2 Longitudinal MRI images of patient 1 performed at 5 years (**A**), 8 years (**B**), and 14 years (**C**). The images show the sagittal T2-weighted image (labeled as 1), coronal T2-weighted image (labeled as 2), axial T2-weighted image at the basal ganglia level (labeled as 3), axial fluid attenuation inversion recovery (FLAIR) (labeled as 4), axial T2-weighted image at the level of the pons (labeled as 5), sagittal T1-weighted (labeled as 6), sagittal T2-weighted (labeled as 7), and axial T2-weighted images of the cervical spinal cord (labeled as 8) at age 1. The following distinctive features were observed on the MRI scans: (i) absence of cerebral, cerebellar, or pontocerebellar atrophy with a normal-sized brain stem (A1-A3, A5, B1-B3, B5); (ii) normal white matter signal on FLAIR images (a4, b4); (iii) normal T2 signal in the basal ganglia (a3, b3); and (iv) normal size of the spinal cord with no further atrophy (c6, 7, 8). Note the normal-sized lower part of the cerebellum (c6, c7)

limbs. Muscle tone was increased in the lower limbs (Modified Ashworth Scale 1+ in knee flexion and extension, ankle dorsiflexion) with normal range of motion in all joints and no muscle weakness. Pyramidal signs manifested as hyperreflexia and sustained clonus were noted with flexor plantar response. There was no evidence of ataxia or other cerebellar dysfunction. She was able to run and alternately climb stairs with no support and was able to hop on one foot. Anal tone was normal. Sensory examination did not reveal any sensory deficit, but the examination was limited due to her cognitive abilities.

Since then, her motor function has gradually declined, being able to walk only for a short distance. This was accompanied by urinary incontinence. Cognitive deterioration was noted by increased comprehension difficulties and decline in school performance. Repeat neuropsychological evaluation diagnosed moderate ID. Epilepsy was well controlled, with no interictal epileptic activity on subsequent EEG at age 12 that showed only mild intermittent frontotemporal left background slowing. On the last examination at the age of 12 years, her weight was 52 kg, with normal height of 149 cm (39th percentile) and BMI of 22.5 (88th percentile, overweight). HC was 51.7 cm (20th percentile). Neurological examination revealed mild dysarthria with hypernasal speech, normal gag reflex, and palate movement. Mild difficulty with tongue movement was noted with no tongue fasciculation or atrophy. There was no evidence of dysphagia. She had mild rigidity in the upper limbs without evidence of parkinsonism, with brisk reflexes in all four limbs, more evident in the lower limbs, with sustained clonus and flexor plantar response in

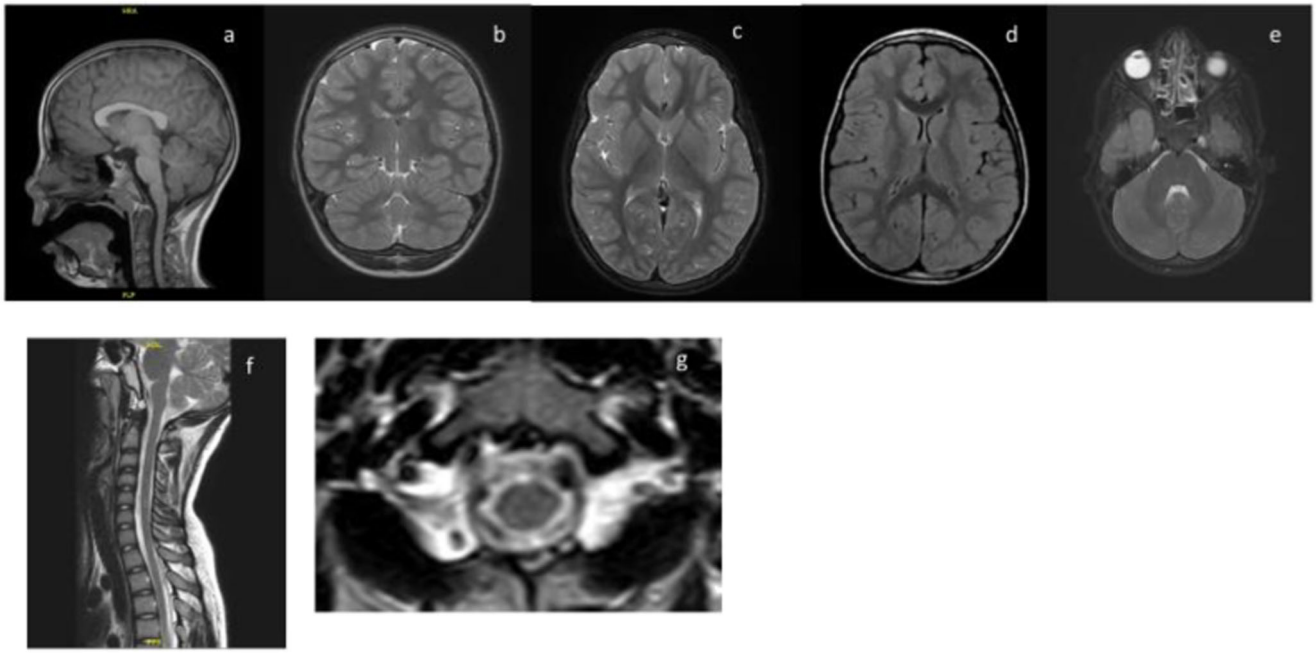

Fig. 3 MR images of patient 2 at age 6.7 years (**a**–**e**) and at age 12 (**f**,**g**). Sagittal T1-weighted (**a**) and coronal T2-weighted images (**b**) at 6.7 years of age showing normal appearance of the cerebellum with no evidence of atrophy of the cerebellum or the brainstem and normal appearance of the pons. T2-weighted axial image at the level of the basal ganglia shows no cerebral atrophy with normal signal within the basal ganglia (**c**). Axial fluid attenuation inversion recovery (FLAIR) shows normal white matter signal with no white matter hyperintensity lesions in the cerebral white matter (**d**). Transverse T2-weighted image at the level of the pons shows normal size of the pons (**e**), sagittal (**f**), and axial (**g**) T2-weighted spine MR images at 12 years of age showing no cervical spinal cord atrophy with normal anteroposterior diameter of the cervical spinal cord. Note the normal appearance of the cerebellum and the anterior curvature of the pons (**f**)

lower extremities. She had minimal lower limbs weakness (hip flexion and extension, hip abductors, knee flexion and extension, ankle dorsiflexion), with normal range of motion in all joints and no apparent change in the degree of spasticity. There was no evidence of ataxia or sensory disturbances. Anal tone and wink were normal. She was able to run and jump cumbersomely and needed assistance to climb stairs reciprocally.

Whole exome sequencing and data analysis

Both patients underwent WES to seek for molecular diagnosis, considering their clinical phenotype. Saliva from parents, the affected siblings, and from a healthy sibling was used for DNA extraction. Sample enrichment was done using Agilent SureSelect v5 kit (Agilent Technologies, Santa Clara, CA, USA). Sequencing was performed using Illumina HiSeq4000 with 108-bp paired-end reads. Reads were aligned to GRCh37 with Novoalign (Novocraft Technologies). Single-nucleotide variants and small indels were identified using GATK Unified Genotyper and filtered according to the Broad Institute's best-practice guidelines v3 [18]. Variants registered in dbSNP137 (http://www.ncbi.nlm.nih.gov/projects/SNP/) were filtered. The filtered variants were annotated using ANNOVAR according to the dbSNP database (build 135), based on Human Genome GRCh38 assembly version. The mean allele frequency was determined by the following data bases: dbSNP, 1000 genomes [19], The Exome Aggregation Consortium (ExAC) [20], NHLBI GO Exome Sequencing Project (ESP) (Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/)), and the Variantyx allele frequency database. Only variants located in the candidate regions (which are highlighted by linkage mapping/homozosity mapping) were focused. Reported variants were detected, analyzed, and reported using the Variantyx Genomic Intelligence platform version 1. 15.0.0_1. To maintain the most up-to-date annotations, the Variantyx database is updated quarterly. Variants were deemed to be potentially pathogenic as determined by a combination of curated databases of disease association and predicted severity of the variants. The TraP score was used for prediction of pathogenicity, (score between 0 and 1). The higher the score, the higher the damage the variant is predicted to have [21].

Sanger sequencing

Sanger sequencing was performed to validate the variants in the patients and their parents.

Results

A nonsynonymous homozygous variant in exon 19 of the *VPS53* gene (c.2084A>G: 2084A>G)) p.Gln695Arg) was found in both patients. The mean coverage for this locus was 52.9, with a median coverage of 49. Ninety-seven percent of samples were over 20X coverage. The homozygous variant was identified in two affected sisters, while their asymptomatic parents and tested sibling were carriers of the variant. This missense variant is commonly found in the Moroccan-Jewish population (carrier rate of ~ 1:71). It replaces the medium size and polar amino acid glutamine to large size and basic arginine at position 695/833 in a highly conserved region of VPS53's protein C-terminal. This variant had a zero-allele frequency in the population database (GnoMad-http://gnomad.broadinstitute.org/), and in ClinVar, it is denoted as pathogenic. In silico prediction tools predicted this variant to be deleterious; SIFT (sorts intolerant from tolerant) score was 0.003 (range 0 to 1 with values less than 0.05 usually considered functionally intolerant), and Transcript-inferred Pathogenicity (TraP) score was 0.95, (range 0–1), which qualifies this variant as probably damaging. Hence, the variant is defined as pathogenic based on the ACMG guidelines [22]. In addition, the two sisters shared another relatively common homozygous variant in the *DNAH11* gene (c.4919A>G. p. Asp1640Gly), which was also detected in a heterozygous form in both parents and their healthy sister. This second variant is relatively frequent in the general population with an estimated maximal allele frequency of ~ 5%. This variant has not been previously reported as pathogenic in the literature. Other variants in this gene were reportedly associated with primary ciliary dyskinesia in patients with respiratory symptoms and situs inversus.

Discussion

We report herein a homozygous variant in *VPS53* gene in two sisters with spastic paraparesis featuring mildly progressive spasticity and mild to moderate ID, in the presence of normal HC and brain MRI. The combination of slowly progressive lower limb spasticity, incontinence, and mild ID with no evidence of perinatal insult leads to the working diagnosis of complicated HSP. The fact that both asymptomatic parents and a healthy sibling are carriers of this variant is compatible with segregation of the variants with the disease, in an AR inheritance manner. This is the first report of VPS53 gene in association with HSP. The only previously known disease associated with pathogenic variants in the VPS53 gene is progressive cerebello-cerebral atrophy (PCCA) type 2 [23], which is a severe neurodegenerative disorder characterized by progressive microcephaly due to cerebral and cerebellar atrophy, spasticity, severe to profound ID, and early-onset epilepsy. PCCA syndrome was originally described in 2003 by Ben-Zeev et al. [24] in seven patients of Moroccan and Iraqi Jewish ancestry, who presented with AR-inherited phenotype of nondysmorphic profound ID, progressive microcephaly, severe spasticity, generalized seizures, and brain MRI demonstrating ongoing atrophy of the cerebellum and cerebrum [24]. The causative gene was later found to be SEPSEC, in which mutations disrupt the generation of selenoproteins in humans [25]. In 2014, Feinstein et al. [23] described the allelic PPCA type 2 syndrome, with a phenotype similar to PCCA type 1, of which the known causative gene is VPS53. In both syndromes, the patients usually reach minimal developmental milestones and are not ambulatory. Cerebral and cerebellar gray and white matter atrophy is already evident during the second year of life and is progressive. These findings were not apparent in our patients. Indeed, both patients had a relative decrease in the HC percentiles compared with the reported values at birth. However, the HC was still within normal limits for age and gender and was stable since early infancy. In addition, the maternal HC was in the 30th percentile, and the healthy brother had a HC in the 25th percentile. Taken together with the normal repeated imaging which clearly shows no signs of cerebral atrophy, we tend to believe that this downward trend reflects their genetic potential rather than a phenotypic trait resulting from the VPS53 pathogenic variant.

Interestingly, all of the described patients with PCCA2 [23] were combined heterozygous for the same VPS53 c.2084A>G [p.(Gln695Arg)] missense variant which was found in our patients and for an intronic variant (c.1556+5G>A). The c.2084A>G [p.(Gln695Arg)] variant reported in our patients in the homozygous form replaces a highly conserved amino acid in VPS53 C-terminal, whereas the latter is in the 5th nucleotide of intron 14 and is expected to disrupt one of *VPS53* splice-donor sites. Carrier rate in Jewish Moroccan controls was found to be 2:143 for the c.2084A>G variant and 2:156 for the c.1556+5G>A variant (~ 1:37 carrier rate for *VPS53* pathogenic variants). Our study identified for the first time a true homozygous pathogenic variant in VPS53 gene. This VPS53 gene variant is located at position 69 of 70 on exon 19 of 22 exons. Despite its designation as a missense variant, it is located only 2 base pairs prior to the exon-intron boundary, which implies that it serves as a truncating canonical splice-site variant, rather than a missense variant, and has a larger effect size. Importantly, although recessively inherited truncating variants tend to have a deleterious effect on the phenotype, with usually severe features, splice-site variants tend to be leaky, which might explain the relatively milder phenotype compared with PCCA type 2. In addition, the c.1556+5G>A splice-site variant described in the patients with PCCA2 [23] was found to generate an unstable transcript that is predicted to encode a truncated protein, resulting in a dysfunctional Golgi-

associated retrograde protein)GARP(, which could also contribute to the more severe phenotype in the cohort reported by Feinstein et al. [23].

The microdeletion in chromosome 2, which was found in both siblings, was inherited from the mother, who is healthy. This microdeletion or the genes included in this microdeletion were not previously associated with a disease according to OMIM. Considering the absence of neurological symptoms in the healthy adult carrier (mother), it is highly implausible that this deletion is associated with the abovementioned phenotype. The homozygous variant in the *DNAH11* gene detected in both our patients in addition to the *VPS53* pathogenic variant has an unknown contribution to their phenotype. This variant is relatively frequent in the general population, with estimated maximal allele frequency of ~ 5%, and has not been previously reported in peer-reviewed clinical literature. Other variants in this gene were reported to be associated with primary ciliary dyskinesia and situs inversus which clinically present completely differently than the patients discussed here [26]. Importantly, none of the two sisters presented with respiratory symptoms or dextrocardia as reported in patients with other variants in this particular gene, so that the role of *DNAH11* is seemingly low key.

We reviewed the 17 gene panels for HSP currently in the NCBI: Genetic Testing Registry (GTR) and none of the genetic panels includes testing for variants in the *VPS53* gene, which confirms that the HSP phenotype is currently not reported. With a carrier rate of *VPS53* mutations of ~ 1:37 among Jews of Moroccan ancestry, routine carrier screening for pathogenic variants of *VPS53* gene should be considered in this ethnic group to identify patients with PCCA, as well as HSP.

The *VPS53* gene is a vacuolar gene that encodes for one of the four subunits of the GARP [27, 28]. The GARP complex has a role in intracellular cholesterol transport and sphingolipid homeostasis by mediating retrograde trafficking from endosomes to the Golgi [29]. This is mediated by soluble Niemann-Pick C2 (NPC2), which facilitates the passage of low-density lipoprotein-derived cholesterol from lysosomes [30, 31]. GARP complex deficiency compromises sphingolipid and sterol homeostasis and results in the accumulation of sphingolipid synthesis intermediates in the lysosomes, which leads to lysosomal dysfunction [30]. The pathogenicity of the c.2084A>G VPS53 variant reported hereby is supported by a substitution of a neutral amino acid by a basic one, within the second helix of the surface of VPS53's conserved C-terminal, in one of the most conserved positions of the molecule [32]. This variant is predicted to affect the C-terminal domain of VPS53, known to be critical to its role as part of the GARP complex.

The more severe phenotype seen in PCCA2 is probably related to the intronic variant located in one of VPS53's splice-donor sites, resulting with no functional VPS53 C-terminus and partially dysfunctional GARP. In fibroblasts of patients with PCCA2 [23] as well as in yeast cells carrying the same compound heterozygous pathogenic variant [30], loss of the GARP complex resulted in reduced levels of complex sphingolipids and the accumulation of toxic sphingolipid synthesis intermediates, which correlated with abnormal vacuolar morphology and function and causes cellular dysfunction of many proteins, as shown by Fröhlich et al. [30]. In keeping with the role of VPS53 in maintaining the GARP complex function as an evacuator of cellular waste in the lysosome, and even though none of the patients had typical features of a lysosomal storage disorder, it is reasonable to assume that the *VPS53* pathogenic variants can cause a form of lysosomal disease. The proposed common pathology is retrograde axonal degeneration of the posterior columns and cortical-spinal tracts in the spinal cord [33] which was recently related to lysosomal dysfunction in various studies [34–38]. Both SPG11and SPG15 result from defects in proteins involved in lysosome shaping; spatacsin (SPG11) and spastizin (SPG15) account for proteins involved in the formation of lysosomes and their recycling [35, 36] and interact with components of late endosomes [38].

It is of worth to note that the disruption of the GARP subunit VPS54 in mice leads to the "wobbler" phenotype of a neurodegenerative disease that shares characteristics with amyotrophic lateral sclerosis (ALS) [39, 40]. A neuropathological overlap between HSP caused by SPG11 and ALS was recently reported [41]; the hallmark of which was intracytoplasmic granular lysosome-like structures in neurons mainly in supratentorial areas. This continuum of motor neuron degeneration and its underlying mechanism should be further explored, particularly as restoration of sphingolipid balance may be a strategy to treat the diseases due to these defects.

To summarize, this is the first report of a pathogenic variant in the *VPS53* gene as the etiology of AR complicated HSP. This expands the phenotype of *VPS53*-related disease and warrants *VPS53* testing among patients with HSP. The role of GARP complex in neurodegenerative processes should be further elucidated.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

All study protocols were reviewed and approved by the Institutional Review Board at the Tel Aviv Sourasky Medical Center.

References

1. McMonagle P, Webb S, Hutchinson M (2002) The prevalence of "pure" autosomal dominant hereditary spastic paraparesis in the island of Ireland. J Neurol Neurosurg Psychiatry 72(1):43–46
2. Sedel F, Fontaine B, Saudubray JM, Lyon-Caen O (2007) Hereditary spastic paraparesis in adults associated with inborn errors of metabolism: a diagnostic approach. J Inherit Metab Dis 30(6):855–864
3. Harding AE (1981) Hereditary "pure" spastic paraplegia: a clinical and genetic study of 22 families. J Neurol Neurosurg Psychiatry 44: 871–883
4. McDermott CJ, White K, Bushby K, Shaw PJ (2000) Hereditary spastic paraparesis: a review of new developments. J Neurol Neurosurg Psychiatry 69:150–160
5. Depienne C, Stevanin G, Brice A, Durr A (2007) Hereditary spastic paraplegias: an update. Curr Opin Neurol 20:674–680
6. Appleton RE, Farrell K, Dunn HG (1991) 'Pure' and 'complicated' forms of hereditary spastic paraplegia presenting in childhood. Dev Med Child Neurol 33(4):304–312
7. Harding AE (1983) Classification of the hereditary ataxias and paraplegias. Lancet 1(8334):1151–1155
8. da Graça FF, de Rezende TJR, Vasconcellos LFR, Pedroso JL, Barsottini OGP, França MC Jr (2019) Neuroimaging in hereditary spastic paraplegias: current use and future perspectives. Front Neurol 9:1117
9. Lo Giudice T, Lombardi F, Santorelli FM, Kawarai T, Orlacchio A (2014) Hereditary spastic paraplegia: clinical- genetic characteristics and evolving molecular mechanisms. Exp Neurol 61C:518–539
10. Tesson C, Koht J, Stevanin G (2015) Delving into the complexity of hereditary spastic paraplegias: how unexpected phenotypes and inheritance modes are revolutionizing their nosology. Hum Genet 134(6):511–538
11. Souza PVS, Bortholin T, Dias RB, Chieia MAT, Burlin S, Naylor FGM et al (2017) New genetic causes for complex hereditary spastic paraplegia. J Neurol Sci 379:283–292
12. D'Amore A, Tessa A, Casali C, Dotti MT, Filla A, Silvestri G et al (2018) Next generation molecular diagnosis of hereditary spastic paraplegias: an Italian cross-sectional study. Front Neurol 9:981
13. Lynch DS, Koutsis G, Tucci A, Panas M, Baklou M, Breza M et al (2016) Hereditary spastic paraplegia in Greece: characterization of a previously unexplored population using next-generation sequencing. Eur J Hum Genet 24:857–863 10.1038
14. Lu C, Li LX, Dong HL, Wei Q, Liu ZJ, Ni W et al (2018 Jul) Targeted next-generation sequencing improves diagnosis of hereditary spastic paraplegia in Chinese patients. J Mol Med (Berl) 96(7): 701–712
15. Kara E, Tucci A, Manzoni C, Lynch DS, Elpidorou M, Bettencourt C, Chelban V, Manole A, Hamed SA, Haridy NA, Federoff M, Preza E, Hughes D, Pittman A, Jaunmuktane Z, Brandner S, Xiromerisiou G, Wiethoff S, Schottlaender L, Proukakis C, Morris H, Warner T, Bhatia KP, Korlipara LVP, Singleton AB, Hardy J, Wood NW, Lewis PA, Houlden H (2016) Genetic and phenotypic characterization of complex hereditary spastic paraplegia. Brain 139(Pt 7):1904–1918
16. Burguez D, Polese-Bonatto M, Scudeiro LAJ, Björkhem I, Schöls L, Jardim LB, Matte U, Saraiva-Pereira ML, Siebert M, Saute JAM (2017) Clinical and molecular characterization of hereditary spastic paraplegias: a next-generation sequencing panel approach. J Neurol Sci 383:18–25
17. Schüle R, Wiethoff S, Martus P, Karle KN, Otto S, Klebe S, Klimpe S, Gallenmüller C, Kurzwelly D, Henkel D, Rimmele F, Stolze H, Kohl Z, Kassubek J, Klockgether T, Vielhaber S, Kamm C, Klopstock T, Bauer P, Züchner S, Liepelt-Scarfone I, Schöls L (2016) Hereditary spastic paraplegia: clinicogenetic lessons from 608 patients. Ann Neurol 79:646–658
18. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, Jordan T, Shakir K, Roazen D, Thibault J, Banks E, Garimella KV, Altshuler D, Gabriel S, DePristo MA (2013) From FastQ data to high confidence variant calls: the genome analysis toolkit best practices pipeline. Curr Protoc Bioinformatics 43:11.10.1–11.1033
19. 1000 Genomes Project Consortium, Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Abecasis GR et al (2015) A global reference for human genetic variation. Nature. 526(7571):68–74
20. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E et al (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature. 536(7616):285–291. https://doi.org/10.1038/nature19057
21. Ren Z, La Carpia F, Halvorsen M, Wang Q, McSweeney KM et al (2017) Annotating pathogenic non-coding variants in genic regions. Nat Commun 8(1):236
22. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17(5):405–424
23. Feinstein M, Flusser H, Lerman-Sagie T, Ben-Zeev B, Lev D, Agamy O, Cohen I, Kadir R, Sivan S, Leshinsky-Silver E, Markus B, Birk OS (2014) VPS53 mutations cause progressive cerebello-cerebral atrophy type 2 (PCCA2). J Med Genet 51:303–308
24. Ben-Zeev B, Hoffman C, Lev D, Watemberg N, Malinger G, Brand N, Lerman-Sagie T (2003) Progressive cerebellocerebral atrophy: a new syndrome with microcephaly, mental retardation, and spastic quadriplegia. J Med Genet 40(8):e96
25. Agamy O, Ben Zeev B, Lev D, Marcus B, Fine D, Su D, Narkis G, Ofir R, Hoffmann C, Leshinsky-Silver E, Flusser H, Sivan S, Söll D, Lerman-Sagie T, Birk OS (2010) Mutations disrupting selenocysteine formation cause progressive cerebello-cerebral atrophy. Am J Hum Genet 87(4):538–544
26. Knowles MR, Leigh MW, Carson JL, Davis SD, Dell SD, Ferkol TW et al (2012) Mutations of DNAH11 in patients with primary ciliary dyskinesia with normal ciliary ultrastructure. Thorax 67(5): 433–441
27. Bonifacino JS, Hierro A (2011 Mar) Transport according to GARP: receiving retrograde cargo at the trans-Golgi network. Trends Cell Biol 21(3):159–167
28. Schindler C, Chen Y, Pu J, Guo X, Bonifacino JS (2015) EARP, a multisubunit tethering complex involved in endocytic recycling. Nat Cell Biol 17(5):639–650
29. Pérez-Victoria FJ, Bonifacino JS (2009) Dual roles of the mammalian GARP complex in tethering and SNARE complex assembly at the trans-Golgi network. Mol Cell Biol 29:5251–5263
30. Fröhlich F, Petit C, Kory N, Christiano R, Hannibal-Bach HK, Graham M, Liu X, Ejsing CS, Farese RV Jr, Walther TC (2015) The GARP complex is required for cellular sphingolipid homeostasis. eLife 4:e08712
31. Wei J, Zhang Y-Y, Luo J, Wang J-Q, Zhou Y-X, Miao H-H et al (2017) The GARP complex is involved in intracellular cholesterol transport via targeting NPC2 to lysosomes. Cell Rep 19:2823–2835
32. Vasan N, Hutagalung A, Novick P, Reinisch K (2010) Structure of a C-terminal fragment of its Vps53 subunit suggests similarity of Golgi-associated retrograde protein (GARP) complex to a family of tethering complexes. Proc Natl Acad Sci U S A 107:14176–14181
33. Bruyn RP (1992) The neuropathology of hereditary spastic paraparesis. Clin Neurol Neurosurg 94s:s16–s18
34. Novarino G, Fenstermaker AG, Zaki MS, Hofree M, Silhavy JL, Heiberg AD, Abdellateef M, Rosti B, Scott E, Mansour L, Masri A, Kayserili H, al-Aama JY, Abdel-Salam GMH, Karminejad A, Kara

M, Kara B, Bozorgmehri B, Ben-Omran T, Mojahedi F, Mahmoud IGED, Bouslam N, Bouhouche A, Benomar A, Hanein S, Raymond L, Forlani S, Mascaro M, Selim L, Shehata N, al-Allawi N, Bindu PS, Azam M, Gunel M, Caglayan A, Bilguvar K, Tolun A, Issa MY, Schroth J, Spencer EG, Rosti RO, Akizu N, Vaux KK, Johansen A, Koh AA, Megahed H, Durr A, Brice A, Stevanin G, Gabriel SB, Ideker T, Gleeson JG (2014) Exome sequencing links corticospinal motor neuron disease to common neurodegenerative disorders. Science. 343(6170):506–511

35. Chang J, Lee S, Blackstone C (2014) Spastic paraplegia proteins spastizin and spatacsin mediate autophagic lysosome reformation. J Clin Invest 124(12):5249–5262
36. Boukhris A, Schule R, Loureiro JL, Lourenço CM, Mundwiller E, Gonzalez MA, Charles P, Gauthier J, Rekik I, Acosta Lebrigio RF, Gaussen M, Speziani F, Ferbert A, Feki I, Caballero-Oteyza A, Dionne-Laporte A, Amri M, Noreau A, Forlani S, Cruz VT, Mochel F, Coutinho P, Dion P, Mhiri C, Schols L, Pouget J, Darios F, Rouleau GA, Marques W Jr, Brice A, Durr A, Zuchner S, Stevanin G (2013 Jul 11) Alteration of ganglioside biosynthesis responsible for complex hereditary spastic paraplegia. Am J Hum Genet 93(1):118–123
37. Dodge JC (2017) Lipid involvement in neurodegenerative diseases of the motor system: insights from lysosomal storage diseases. Front Mol Neurosci 10:356
38. Hirst J, Irving C, Borner GH (2013) Adaptor protein complexes AP-4 and AP-5: new players in endosomal trafficking and progressive spastic paraplegia. Traffic. 14(2):153–164
39. Perez-Victoria FJ, Abascal-Palacios G, Tascón I, Kajava A, Magadán JG, Pioro EP et al (2010) Structural basis for the wobbler mouse neurodegenerative disorder caused by mutation in the Vps54 subunit of the GARP complex. Proc Natl Acad Sci U S A 107:12860–12865
40. Moser JM, Bigini P, Schmitt-John T (2013) The wobbler mouse, an ALS animal model. Mol Gen Genomics 288:207–229
41. Denora P, Smets K, Zolfanelli K, Ceuterick-de Groote C, Casali C, Deconinck T et al (2016) Motor neuron degeneration in spastic paraplegia 11 mimics amyotrophic lateral sclerosis lesions. Brain. 139:1723–1734