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ORIGINAL COMMUNICATION

Defining the clinical-genetic and neuroradiological features in SPG54: description of eight additional cases and nine novel *DDHD2* variants

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Abstract

Recessive mutations in *DDHD2* cause SPG54, a complex hereditary spastic paraplegia (HSP) with less than forty patients reported worldwide. In this retrospective, multicenter study we describe eight additional SPG54 cases harboring homozygous or compound heterozygous *DDHD2* variants. Finally, we reviewed literature data on SPG54, with the aim to better define the phenotype and the brain magnetic resonance imaging (MRI) pattern as well as genotype–phenotype correlations. SPG54 is typically characterized by early-onset (i.e., congenital or, more frequently, infantile) delay in motor and cognitive milestones, coupled or followed by appearance of spasticity. Cognitive impairment is absent in adult-onset cases. Spasticity progresses over time. Abnormal eye movement, found in about 50% of cases, is the feature most frequently associated with spasticity and developmental delay. Cerebellar ataxia is a prominent sign in several patients, including one adult of this study, suggesting to include SPG54 in the differential diagnosis of spastic-ataxia syndromes. Brain MRI shows thin corpus callosum and non-specific periventricular white matter lesions in about 90% and 70% of cases, respectively. Brain MR spectroscopy reveals abnormal lipid peak in 90% of investigated patients. Twenty-one pathogenic changes have been reported so far, many of which are nonsense or small deletion/duplication. Most mutations appear to be private, with only two mutations recurring in three (i.e., R287*) or more families (i.e., D660H). The identification of nine novel variants expands the molecular spectrum of *DDHD2*-related HSP and corroborates the notion of a quite homogeneous clinical and neuroradiological phenotype in spite of different genotypes.

Keywords HSP · SPG54 · Leukodystrophy · Hereditary spastic paraparesis · Thin corpus callosum

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Introduction

Hereditary spastic paraplegias (HSP) are neurodegenerative disorders with more than 80 disease genes identified so far, with both autosomal recessive (AR), autosomal dominant (AD), and X-linked inheritance pattern. Currently, eighty different forms are included in the Online Mendelian Inheritance in Men (OMIM) classification (e.g., SPG1 → SPG80). Clinical heterogeneity of HSP is characterized by variability in age of onset (i.e., from congenital to late-adulthood forms) and features, with pure HSP (pHSP) consisting of isolated pyramidal syndrome and complex HSP (cHSP) characterized by a more composite neurological phenotype [1].

SPG54 is a complex form of HSP caused by recessive mutations in the *DDHD2* gene, which encodes the DDHD-domain-containing two proteins, one of the three intracellular phospholipase A1 (iPLA1) families of proteins (DDHD1, DDHD2 and SEC23IP) that are involved in organelle biogenesis and membrane trafficking between the endoplasmic reticulum and Golgi body [2]. Thirty-three SPG54 patients have been reported worldwide [2–12].

In this paper, we describe eight additional SPG54 cases harboring homozygous or compound heterozygous *DDHD2* variants, including nine novel variants. Finally, we reviewed the literature data on SPG54, with the aim to better define the phenotype and brain magnetic resonance imaging (MRI) pattern as well as genotype–phenotype correlations.

Patients and methods

This multicenter, retrospective study was carried out at the Unit of Neuromuscular and Neurodegenerative diseases, Bambino Gesù Research Hospital of Rome. Inclusion criteria were the presence of bi-allelic mutations in *DDHD2* and accurate recording of at least one neurologic examination, at least one 1.5 T brain MRI, and clinical follow-up. Age at onset and type of symptoms, neurological examinations, brain MRI findings and genetic data were collected and analyzed for each case.

Mutations in *DDHD2* were detected by means of targeted next-generation sequencing (tNGS) panels designed for HSP genes in all but one case (patient 7, Table 1), in which a whole exome sequencing (SureSelectXT Human All Exome V5, Agilent) was performed. Detailed methods for tNGS analysis have been previously described (in [13] for patients 1–3; in [3] for patients 6 and 8) or are available as supplementary file 1 (for patient 4 and 5). Segregation of the identified variants was investigated in the parents. Pathogenicity of the missense variants was investigated by in silico prediction tools, including PolyPhen-2 (https://genetics.bwh.harvard.edu/pph2/), SIFT (https://sift.jcvi.org/), Mutation Taster (https://www.mutationtaster.org/), Alamut (https://www.interactive-biosoftware.com/) and CADD (https://cadd.gs.washington.edu/hom). Variants were annotated with reference to the following transcripts: *DDHD2* (NM_015214.2). Signed written informed consent for genetic analysis was obtained from all the parents of individuals enrolled in the study. The local ethical committee of all the involved centers approved the study.

Results

Data from eight Caucasian patients (six men, two women), aged between 8 and 56 years, were collected. Clinical features, brain MRI, and genetic data of the reported patients are summarized in Table 1, while more detailed clinical descriptions of medical histories are available as supplementary file 2. Most of patients developed first symptoms of the disease in pediatric age, with congenital onset (i.e., < 1 year of age) and early infantile onset in three cases each. Remaining two patients manifested spastic or spastic-ataxia syndrome in the fourth decade. Among pediatric-onset cases, delay in cognitive and motor milestones was the most common presentation, whereas adult-onset cases lack of cognitive impairment but manifested gait difficulties for spasticity of the lower limbs. A relatively small cohort of other neurological signs and symptoms was also recorded, such as prominent cerebellar ataxia in one of the two adults. Clinical follow-up revealed an overall progressive course of the disease in most of cases (7 out of 8, 87.5%). Although many patients who presented with global developmental delay were able to achieve independent ambulation, they also manifested a progressive worsening of spasticity with loss of ability to walk unaided in a period between 6 and 15 years. Brain magnetic resonance imaging (MRI) anomalies were detected in seven out of eight cases (87.5%), with thinning of the corpus callosum (7/8) and periventricular white matter (WM) anomalies in FLAIR and T2-weighted images (6/8) as the most common findings (Fig. 1a–d). Interestingly, in both adult-onset patients (6 and 8) periventricular WM anomalies were absent. Moreover, brain proton magnetic resonance spectroscopy (^{1}H-MRS) performed in patient 8 was normal without evidence of the pathologic lipid peak.

Genetic results of our and previously reported patients are summarized in Tables 1, 2, Fig. 2 and Supplementary file 2. We identified ten different *DDHD2* variants in eight patients. Nine variants (9/10) were novel, whereas one (D660H) had been previously reported [2]. Mutations were homozygous in five cases and compound heterozygous in three. We detected only three missense variants (G197R,

Table 1 Main clinical, genetic and neuroradiological features of the eight newly reported patients with SPG54

Patient	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Sex, age (years)	F, 14	M, 19	M, 23	M, 14	F, 16	M, 46	M, 8	M, 56
Genetic variant	c.1978G>C/c.1978G>C	c.759delT/c.759delT	c.38delA/c.334C>T	c.589G>A/c.589G>A	c.1978G>C/c.1978G>C	c.339_340insACG/c.2096A>G	c.340-341insA/c.340-341insA	c.942delC/c.806C>T
Protein	p.D660H/p.D660H	p.F253Lfs*13/p.F253Lfs*13	p.Q13Rfs*16/p.R112*	p.G197R/p.G197R	p.D660H/p.D660H	p.C113_T114insT/p.Y699C	p.T114Nfs*11/p.T114Nfs*11	p.T314*/p.P269L
Inheritance	Sporadic	AR[a]	Sporadic	Sporadic	Sporadic	Sporadic	Sporadic	Sporadic
Reference	Schuurs-Hoeijmakers et al. [2]	Novel	Novel	Novel	Schuurs-Hoeijmakers et al. [2]	Novel	Novel	Novel
Age of onset	6 months	11 months	6 months	15 months	2 years	40 years	Neonatal period	45 years
First sign/symptom at onset	Swallowing difficulty, psychomotor delay	Psychomotor delay	Psychomotor delay	Psychomotor delay	Psychomotor delay	Gait problem in the right leg	Spasticity of the LL	Gait unsteadiness
Sitting unsupported	12 months	16 months	12 months	Not acquired	Not acquired	Normal	7 months	Normal
Independent ambulation	4 years	5 years	4 years	Only with support at 3 years	Not acquired	Normal	2 years 4 months	Normal
First words	9 months	12 months	15 months	15 months	2 years	Normal	21 months	Normal
Abnormal eye movements	No	No	Gaze palsy, strabismus	Nystagmus	No	No	Horizontal gaze palsy	Slow saccade
Microcephaly	No	Yes	No	No	No	No	No	No
Pyramidal signs[b]	Yes (LL)	Yes (LL)	Yes (LL)	Yes (UL, LL)	Yes (LL)	Yes (LL)	Yes (LL)	Yes (LL)
Extrapyramidal signs	No	No	Hypomimia	No	Bradykinesia, hypomimia	No	No	No
Cerebellar ataxia	–	–	–	–	–	–	–	Ataxia (limb and trunk), dysmetria
Bulbar signs	Dysphagia	Dysphagia	Dysphagia	–	–	No	No	Dysphagia
Cognitive delay	Yes (severe)	Yes (severe)	Yes (moderate)	Yes (severe)	Yes (severe)	No	Yes (NS)	Impairment
Behavioral problems	Mood disorders	Aggressive behaviour (severe)	Yes (NS)	No	No	No	Yes (ADHD, aggressive behaviour, relational problems)	No
Additional clinical findings	Optic nerve hypoplasia	Frequent infections	Foot contractures	Postural kyphosis with hypotonic trunk and neck hypostenia; dysarthria	Dysarthria, foot contractures	Celiac disease	No	Dysarthria
Course (loss of ambulation)	Progressive (9 years)	Progressive (7 years)	Progressive (16 years)	Progressive (14 years)	Progressive (not applicable)	Progressive (not applicable)	Non-progressive (not applicable)	Progressive (not applicable)

Table 1 (continued)

Patient	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
SPRS score (age at evaluation)	38/52 (14 years)	34/52 (16 years)	37/52 (17 years)	37/52 (8 years)	46/52 (14 years)	NA	NA	15/52 (56 years)
MRI findings	CC hypoplasia, agenesis of genu and splenium; dysmorphic LLVV; hyperintensity of PV WM; cerebral peduncles hypoplasia	CC hypoplasia; WM hyperintensity in frontal horns of LLVV	PV WM hyperintensity	Enlarged cerebellar SAS; CC hypoplasia; minimal dysmorphism of LLVV; PV WM hyperintensity	CC hypoplasia; PV WM hyperintensities	Normal	Low-descending occipital lobe; PV WM hyperintensities	CC hypoplasia (splenium and body)
Additional instrumental findings	EEG slow posterior waves; NCS normal; SSEP normal	–	–	EEG slow background activity; NCS normal	NCS normal; SSEP normal	NCS mixed motor polyneuropathy	–	NCS sensory axonal neuropathy; ^{1}H-MRS normal

NA not available, *NS* not specified in detail, *LL* lower limbs, *UL* upper limbs, *WM* white matter, *PV* periventricular, *SAS* sub arachnoid spaces, *LLVV* lateral ventricles, *TW* toe walking

[a] Autosomal recessive inheritance is supposed in this patients since his sister manifested a similar condition but died before achieving a diagnosis

[b] Pyramidal signs included Babinski sign and spasticity in all cases, increased osteo-tendinous reflexes in patients 4–6, ankle clonus in patients 4, 7 and 8

P269L and Y699C), while the remaining six were nonsense or frameshift. All variants co-segregated with the disease; parents were always heterozygous for one *DDHD2* mutation while all affected members carried two mutations.

All missense variants were predicted to be pathogenic using standard in silico prediction tools. Among missense variants, only one was absent in available databases, while two were found at very low frequency (i.e., MAF < 0.01) in the ExAC database.

Discussion

Spastic paraplegia type 54 has been reported in 33 patients from 15 unrelated families, and some clinical and neuroradiological clues have been highlighted for this condition [2–11], such as psycho-motor delay with early-onset spasticity, thin corpus callosum and white matter anomalies on brain MRI. By reviewing the current literature (Table 2 and supplementary file 3 [Table S1]), it emerges that almost the totality of patients develops symptoms between the first and the tenth year of life, with only two cases presenting adult-onset spastic-ataxia syndrome. In the congenital- or infantile-onset form, the cognitive and motor developments are almost inexorably delayed. Spasticity and pyramidal signs are more often confined to lower limbs. Various additional features may contribute to complete the clinical picture; among these, abnormal ocular movements, mainly strabismus, have been reported in about 50% of cases. Several cases may also show signs of cerebellar ataxia. Although optic nerve hypoplasia and short stature were hypothesized as possible frequent clues for SPG54 in the first descriptions [2, 4], they have been reported occasionally in the following cases. Similarly, other features may be variably encountered such as bulbar signs, dysarthria, microcephaly, or signs of neuropathy on nerve conduction studies. The clinical features observed in our eight cases are quite similar to those reported in the literature, thus indicating that, although the overall low number (i.e., 41 cases) of patients, the SPG54 phenotype is quite homogeneous in pediatric-onset cases, and clinical variability seems to be linked to the "minor" features above reported or to onset in adulthood. Interestingly, none of the four SPG54 adult-onset cases (i.e., patients 6 and 8 in this series and the two reported by Doi et al. [7]) manifested cognitive impairment, and three of them presented with an ataxic-spastic syndrome. One case (patient 6 in Table 1) manifested an adult-onset spastic paraplegia without abnormalities in the brain MRI; after a follow-up of 6 years no other symptoms and signs appeared, except for evidence of a mild and asymptomatic polyneuropathy on nerve conduction studies, thus mimicking a pure phenotype.

Analysis of brain MRI data indicates that SPG54 is associated with a thin corpus callosum (TCC) in the vast

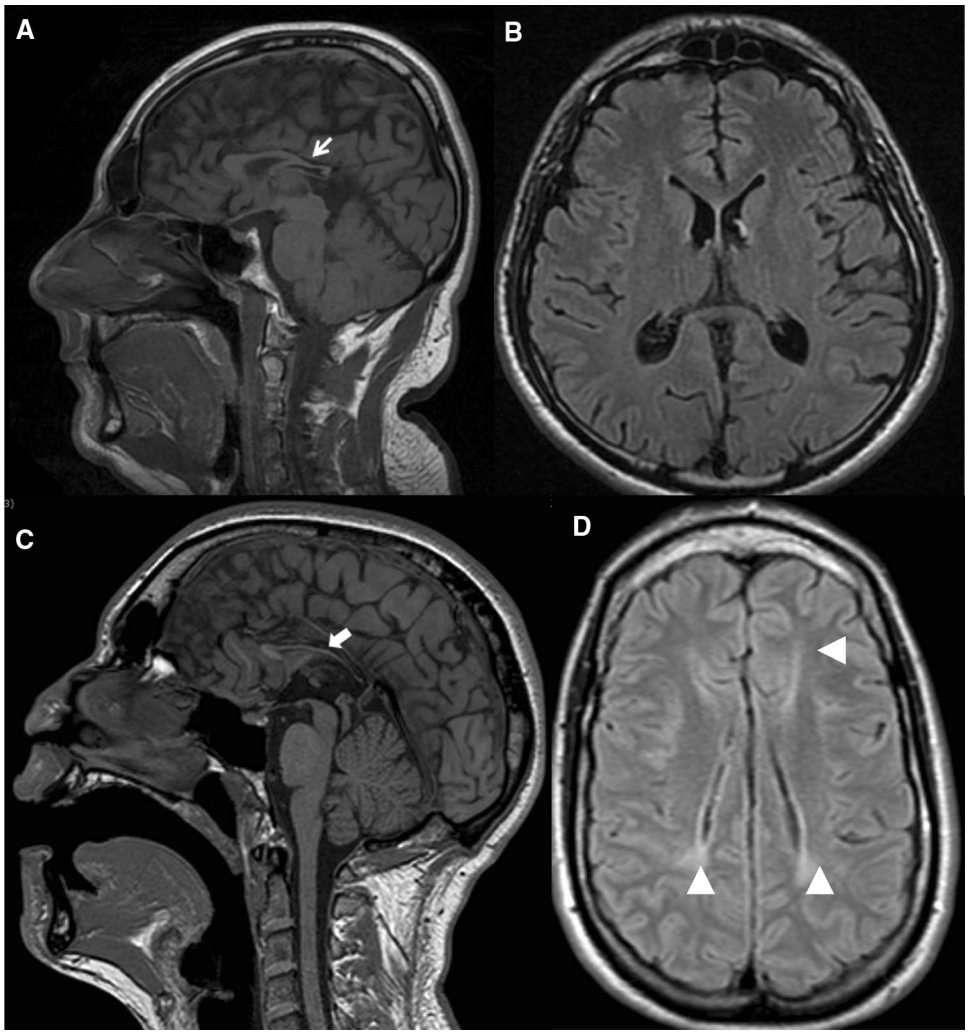

Fig. 1 **a**–**d** Brain MRI, sagittal T1-weighted (**a**, **c**) and axial FLAIR sections (**b**, **d**); patient 8 (performed at age of 51) showed thin corpus callosum (arrows) without WM abnormalities. Thin corpus callosum with agenesis of splenium (arrows) and periventricular WM hyperintensity (arrowhead) are observed in a 24-year-old boy with SPG54 (patient HSP-4 reported in [3])

majority of the patients (90%) and presents periventricular white matter changes (WMCs) in about over 70% of cases. TCC is a well-known biomarker of a group of HSP which includes, besides SPG11/Spatacsin and SPG15/Spastizin [1, 14], also other forms including SPG7/Paraplegin, SPG21/ACP33, SPG35/FA2H, SPG44/GJC2, SPG46/GBA2, SPG47/AP4B1, SPG48/AP5Z1, SPG49/TECPR2, SPG56/CYP2U1, SPG63/AMPD2, and few others [1]. Thinner corpus callosum is frequent but not mandatory to support the molecular diagnosis in most of these forms and, with few exceptions as in SPG11 and SPG15, clinical and other neuroradiological features often differ among forms [1]. Additionally, the involvement of WM has been well defined only in few HSP (e.g., "ears of lynx" sign in SPG11 and SPG15) [15]. This is not the case of SPG54, in which the WMCs appear as mild hyperintensities in the T2-weighted and FLAIR images mainly involving the periventricular posterior areas or they may also be missing as in our adult-onset patients. In regard of a neuroradiological marker, use of ^{1}H-MRS is considered useful in SPG54 since it has identified abnormal lipid peak at 1.3 ppm (in the basal ganglia and the thalamus) in all but one (i.e., patient 8 in our series) of the explored patients [2, 7, 8, 11]. This finding is considered to be related to the underlying pathophysiological mechanism of SPG54, as observed in other diseases caused by abnormal lipid metabolism (e.g., Sjogren–Larsson syndrome) [7]. It is not clear if the spectroscopic data are exclusive of SPG54 or may be shared by others HSP linked to genes involved in lipid metabolisms (e.g., SPG5/CYP7B1, SPG28/DDHD1, SPG35/FA2H, SPG39/PNPLA6, SPG46/GBA2, and SPG56/CYP2U1) [1]. None of these have been studied by mean of ^{1}H-MRS, with the exception of a single case report for SPG28 in which no abnormal lipid peak was detected [16]. On the other hand, in our cohort the only patient that was investigated with ^{1}H-MRS was negative to this biomarker, suggesting that the specificity of this finding needs confirmation in a larger number of patients.

In this study, we identified ten *DDHD2* variants of which nine were novel, raising to 21 the number of distinct pathogenic changes so far reported [2, 4, 9]. Most variants appear

Table 2 Summary of main clinical, neuroradiological and genetic features of SPG54 patients

Features	This paper	Reported patients	Total (%)
Sex (M/F)	6 M, 2 F	14 M, 19 F	41
Sporadic/familial	7 sporadic, 1 familial	3 sporadic, 30 familial (15 families)	10 sporadic 31 familial
Age of onset			
< 1 year	3/8	7/33	10/41 (24.4)
1–5 years	3/8	23/33	26/41 (63.4)
6–10 years	–	1/33	1/41 (2.4)
11–20 years	–	–	–
> 20 years	2/8	2/33	4/41 (9.8)
Cognitive delay	6/8	31/33	37/41 (90)
Motor delay	6/8	18/22 + (NR 11)	24/30 (80)
Abnormal eye movements	4/8	18/33	22/41 (53.6)
Microcephaly	1/8	3/33[a]	4/41 (9.8)[a]
Pyramidal signs	8/8	33/33	41/41 (100)
Extrapyramidal signs	2/8	1/33[a]	3/41 (7.3)[a]
Bulbar signs	4/8	7/33[a]	11/41 (27)[a]
Behavioral problems	4/8	3/33[a]	7/41 (17)[a]
Phenotype			
Complex HSP	7/8	33/33	40/41 (97.5)
Pure HSP	1/8	–	1/41 (2.5)
Course			
Progressive	7/8	28/28 (NR 5)	35/36 (97)
Non-progressive	1/8	–	1/36 (3)
MRI findings			
Normal	1/8	0/28	1/36 (2.7)
CC hypoplasia	5/8	25/28	30/36 (83.3)
CC dyplasia	1/8	2/28	3/36 (8.3)
WM hyperintensity	6/8	19/28	25/36 (69.4)
WM reduction	–	4/28	4/36 (11.1)
Cerebellar atrophy	–	4/28	4/36 (11.1)
Brainstem atrophy	–	3/28	3/36 (8.3)
Syrinx	–	2/6	2/6 (33.3)
MRS lipid peak	0/1	10/10	10/11 (91)
Type mutation			
Missense	3/9	3/12	6/21 (28.6)
Nonsense	2/9	3/12	5/21 (23.8)
Frameshift	3/9	4/12	7/21 (33.3)
Duplication	1/9	–	1/21 (4.7)
Splice site	–	2/12	2/21 (9.5)

NR not reported

[a]These data may be underestimated since in many cases clinical descriptions are scarce and many features are not clearly specified or not reported

to be private, with only two mutations recurring in three (R287*) or more families (D660H) [2, 5, 6]. A broad range of mutations have been reported, most of which were nonsense or small deletion/duplications. These variants lead to the formation of a premature stop codon in the open reading frame of *DDHD2* mRNA, suggesting a loss of function mechanism due to the production of an inactive protein or to the degradation of mRNA by nonsense-mediated mRNA decay [2, 9]. A total of six missense changes were described; among these, the D660H was the most frequent recurring in 5 of 22 families [2, 5, 6]. This mutation falls within a highly conserved residue in the DDHD domain of the DDHD2 protein. This multifunctional domain, together with the SAM domain, is essential for the phospholipase activity (PLA_1)

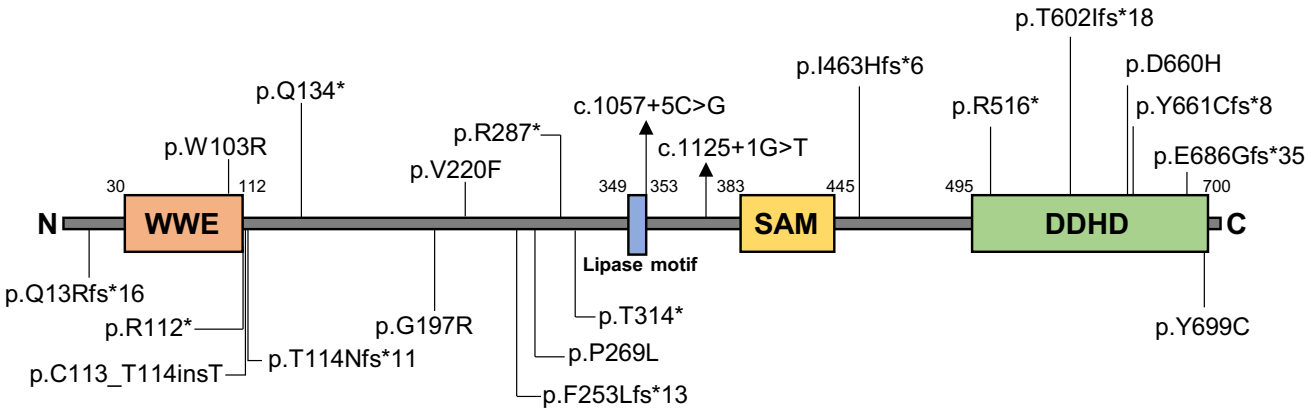

Fig. 2 Schematic representation of DDHD2 protein. Novel variants identified in this paper are reported below in the figure, while previously described mutations are shown above. Arrows indicate splicing mutations

of the DDHD2 protein [7]. Functional studies have shown a marked reduction in PLA_1 activity for D660H and two other missense variants falling with the SAM–DDHD domain, indicating that the loss of PLA_1 activity significantly contributes to SPG54 pathogenicity [7]. In this study, we report three novel missense variants, of which only one (Y699C) resides in the DDHD domain and for which it is possible to hypothesize the same pathogenic mechanism. The other two mutations not falling into the SAM–DDHD domain (G197R and P269L) are predicted to have deleterious effect on protein function by different in silico tools. In the future, functional studies will help us to understand the pathogenic mechanism of these missense variants. As shown by our series and by review of published cases, the *DDHD2*-associated phenotype is quite homogeneous regardless of the type of mutation. A total of five variants have been described in the four adult-onset cases, and all but the p.Y699C fall in a region of the protein located between the domains WWE and lipase motif (Fig. 2). However, five other variants occurring in pediatric-onset cases have been found in the same region and at present no further conclusions can be drawn about the genotype–phenotype correlations or potential pathomechanisms for adult-onset SPG54 patients.

Conclusion

SPG54 represents a subtype of complex HSP with TCC. Phenotype is rather homogeneous and characterized by early-onset (i.e., congenital or, more frequently, infantile) delay in motor and cognitive milestones, coupled or followed by appearance of spasticity. Patients show progression of spasticity over time, and they may eventually require a wheelchair. Abnormal eye movements, found in about 50% of cases, are the feature most frequently associated with the spastic paraplegia and developmental delay. Other neurological symptoms may include cerebellar ataxia, and *DDHD2* analysis should be also considered in the differential diagnosis of spastic-ataxia syndromes, especially for adult cases. Brain MRI shows thin corpus callosum and non-specific periventricular WMCs in most cases; brain ^{1}H-MRS can be a useful biomarker of this condition, but the specificity of this technique needs confirmation in a larger number of patients. The identification of novel variants expands the array of *DDHD2* mutations and suggest that all lead to a common loss-of-function mechanism giving rise to the specific features seen in SPG54.

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Compliance with ethical standards

Conflicts of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical standards All procedures performed in this paper were in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments and have been approved by the appropriate local ethics committees.

References

1. Lo Giudice T, Lombardi F, Santorelli FM, Kawarai T, Orlacchio A (2014) Hereditary spastic paraplegia: clinical-genetic characteristics and evolving molecular mechanisms. Exp Neurol 261:518–539
2. Schuurs-Hoeijmakers JH, Geraghty MT, Kamsteeg EJ et al (2012) Mutations in DDHD2, encoding an intracellular phospholipase A(1), cause a recessive form of complex hereditary spastic paraplegia. Am J Hum Genet 91(6):1073–1081

3. Travaglini L, Aiello C, Stregapede F et al (2018) The impact of next-generation sequencing on the diagnosis of pediatric-onset hereditary spastic paraplegias: new genotype–phenotype correlations for rare HSP-related genes. Neurogenetics 19(2):111–121
4. Gonzalez M, Nampoothiri S, Kornblum C et al (2013) Mutations in phospholipase DDHD2 cause autosomal recessive hereditary spastic paraplegia (SPG54). Eur J Hum Genet 21(11):1214–1218
5. Citterio A, Arnoldi A, Panzeri E et al (2014) Mutations in CYP2U1, DDHD2 and GBA2 genes are rare causes of complicated forms of hereditary spastic paraparesis. J Neurol 261(2):373–381
6. Magariello A, Citrigno L, Zuchner S et al (2014) Further evidence that DDHD2 gene mutations cause autosomal recessive hereditary spastic paraplegia with thin corpus callosum. Eur J Neurol 21(3):e25–26
7. Doi H, Ushiyama M, Baba T et al (2014) Late-onset spastic ataxia phenotype in a patient with a homozygous DDHD2 mutation. Sci Rep 4:7132
8. Novarino G, Fenstermaker AG, Zaki MS et al (2014) Exome sequencing links corticospinal motor neuron disease to common neurodegenerative disorders. Science 343(6170):506–511
9. Alrayes N, Mohamoud HS, Jelani M et al (2015) Truncating mutation in intracellular phospholipase A_1 gene (DDHD2) in hereditary spastic paraplegia with intellectual disability (SPG54). BMC Res Notes 8:271
10. Kumar KR, Wali GM, Kamate M et al (2016) Defining the genetic basis of early onset hereditary spastic paraplegia using whole genome sequencing. Neurogenetics 17(4):265–270
11. Thabet F, Tlili-Graiess K, Tabarki B (2019) Distinct neuroimaging features of *DDHD2* gene-related spastic paraplegia, a mimicker of cerebral palsy. Arch Dis Child. https://doi.org/10.1136/archdischild-2018-316484
12. Schuurs-Hoeijmakers JH, Vulto-van Silfhout AT, Vissers LE et al (2013) Identification of pathogenic gene variants in small families with intellectually disabled siblings by exome sequencing. J Med Genet 50(12):802–811
13. D'Amore A, Tessa A, Casali C et al (2018) Next generation molecular diagnosis of hereditary spastic paraplegias: an Italian cross-sectional study. Front Neurol 9:981
14. Hensiek A, Kirker S, Reid E (2015) Diagnosis, investigation and management of hereditary spastic paraplegias in the era of next-generation sequencing. J Neurol 262(7):1601–1612
15. Pascual B, de Bot ST, Daniels MR et al (2019) "Ears of the Lynx" MRI sign is associated with SPG11 and SPG15 hereditary spastic paraplegia. AJNR Am J Neuroradiol 40(1):199–203
16. Liguori R, Giannoccaro MP, Arnoldi A et al (2014) Impairment of brain and muscle energy metabolism detected by magnetic resonance spectroscopy in hereditary spastic paraparesis type 28 patients with DDHD1 mutations. J Neurol 261(9):1789–1793