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Epilepsia

RESEARCH ARTICLE

Developmental epileptic encephalopathy in *DLG4*-related synaptopathy

Benedetta Kassabian[1,2,*] | Amanda M. Levy[3,*] | Elena Gardella[1,4] | Angel Aledo-Serrano[5] | Amitha L. Ananth[6] | Alejandro J. Brea-Fernández[7,8] | Roseline Caumes[9] | Nicolas Chatron[10,11] | Alice Dainelli[12] | Matthias De Wachter[13] | Anne-Sophie Denommé-Pichon[14,15] | Thomas J. Dye[16,17] | Elisa Fazzi[18,19] | Roxanne Felt[20] | Alberto Fernández-Jaén[21,22] | Montse Fernández-Prieto[7,8] | Emily Gantz[6] | Piotr Gasperowicz[23] | Antonio Gil-Nagel[24] | David Gómez-Andrés[25] | Hansel M. Greiner[16,17] | Renzo Guerrini[12] | Maria K. Haanpää[26] | Minttu Helin[27] | Juliane Hoyer[28] | Anna C. E. Hurst[29] | Staci Kallish[30] | Shefali N. Karkare[31] | Amjad Khan[32,33] | Lotte Kleinendorst[34,35] | Johannes Koch[36] | Sanjeev V. Kothare[31] | Suzanna M. Koudijs[37,38] | Lieven Lagae[39] | Phillis Lakeman[34] | Kathleen A. Leppig[40] | Gaetan Lesca[10,11] | Diego Lopergolo[41,42] | Laina Lusk[43] | Alex Mackenzie[44,45] | Davide Mei[12] | Rikke S. Møller[1,4] | Elaine M. Pereira[46] | Konrad Platzer[47] | Chloe Quelin[48] | Anya Revah-Politi[49] | Sylvain Rheims[50] | Agustí Rodríguez-Palmero[51,52] | Andrea Rossi[19] | Filippo Santorelli[42] | Syndi Seinfeld[53] | Erick Sell[54] | Donna Stephenson[55] | Krzysztof Szczaluba[23,56] | Eugen Trinka[57,58] | Muhammad Umair[59,60] | Hilde Van Esch[61] | Mieke M. van Haelst[34,35] | Danielle C. M. Veenma[38,62] | Sacha Weber[63,64] | Sarah Weckhuysen[65,66] | Pia Zacher[67] | Zeynep Tümer[3,68,#] | Guido Rubboli[1,68,#]

Correspondence
Zeynep Tümer, Department of Clinical Genetics, Kennedy Center, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark.
Email: zeynep.tumer@regionh.dk

Guido Rubboli, Department of Epilepsy Genetics and Precision Medicine, Danish Epilepsy Center Filadelfia, Dianalund, Denmark.
Email: guru@filadelfia.dk

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Abstract

Objective: The postsynaptic density protein of excitatory neurons PSD-95 is encoded by discs large MAGUK scaffold protein 4 (*DLG4*), de novo pathogenic variants of which lead to *DLG4*-related synaptopathy. The major clinical features are developmental delay, intellectual disability (ID), hypotonia, sleep disturbances, movement disorders, and epilepsy. Even though epilepsy is present in 50% of the individuals, it has not been investigated in detail. We describe here the phenotypic spectrum of epilepsy and associated comorbidities in patients with *DLG4*-related synaptopathy.

Methods: We included 35 individuals with a *DLG4* variant and epilepsy as part of a multicenter study. The *DLG4* variants were detected by the referring

*Benedetta Kassabian and Amanda M. Levy contributed equally.

#Zeynep Tümer, and Guido Rubboli contributed equally to this work.

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laboratories. The degree of ID, hypotonia, developmental delay, and motor disturbances were evaluated by the referring clinician. Data on awake and sleep electroencephalography (EEG) and/or video-polygraphy and brain magnetic resonance imaging were collected. Antiseizure medication response was retrospectively assessed by the referring clinician.

Results: A large variety of seizure types was reported, although focal seizures were the most common. Encephalopathy related to status epilepticus during slow-wave sleep (ESES)/developmental epileptic encephalopathy with spike–wave activation during sleep (DEE-SWAS) was diagnosed in >25% of the individuals. All but one individual presented with neurodevelopmental delay. Regression in verbal and/or motor domains was observed in all individuals who suffered from ESES/DEE-SWAS, as well as some who did not. We could not identify a clear genotype–phenotype relationship even between individuals with the same *DLG4* variants.

Significance: Our study shows that a subgroup of individuals with *DLG4*-related synaptopathy have DEE, and approximately one fourth of them have ESES/DEE-SWAS. Our study confirms DEE as part of the *DLG4*-related phenotypic spectrum. Occurrence of ESES/DEE-SWAS in *DLG4*-related synaptopathy requires proper investigation with sleep EEG.

KEYWORDS

DEE-SWAS, epilepsy, ESES, PSD-95, SHINE syndrome

1 | INTRODUCTION

The postsynaptic submembrane space in excitatory neurons contains a multiprotein complex, designated the postsynaptic density (PSD), which supports integration and plasticity of the glutamatergic synapses. The PSD contains several scaffolding proteins, including PSD-95, encoded by discs large MAGUK scaffold protein 4 (*DLG4*). PSD-95 plays a major role in synaptic maturation and dendritic morphology and interacts with both transmembrane and cytoplasmic proteins. The glutamate N-methyl-D-aspartic acid (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptors and K_v1 potassium channels are directly or indirectly anchored to the postsynaptic membrane by PSD-95, which thereby regulates their function. In the cytoplasm, PSD-95 associates with synaptic Ras GTPase-activating protein 1 (SYNGAP1) and SH3 and multiple ankyrin repeat domain 3 (SHANK3; the latter via SAPAPs [SAP90/PSD-95-associated proteins]), which are both encoded by genes that lead to synaptopathies when disrupted.[1–5] Pathogenic variants in *DLG4* have recently been identified in individuals with the autosomal dominant brain disorder *DLG4*-related synaptopathy,[6–12] with overlapping features observed in *SYNGAP1*- and *SHANK3*-related synaptopathies. The predominant clinical features of *DLG4*-related synaptopathy, together with the underlying genetic defects, have been described by our group,[6] and comprise global developmental delay, intellectual disability (ID), autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), hypotonia, and epilepsy in approximately 50% of the affected individuals.[6] As *DLG4*-associated epilepsy has not yet been described in detail, here we aim to describe the phenotypic spectrum of *DLG4*-related synaptopathy with a special focus on the epilepsy phenotype in correlation with the underlying genetic defect and to explore the developmental epileptic encephalopathy (DEE) component.

Key points

- *DLG4*-related synaptopathy is a rare brain disorder
- Developmental delay, intellectual disability, behavioral problems, sleeping disturbances, hypotonia, and epilepsy are major features
- A subgroup of individuals with *DLG4*-related synaptopathy have DEE
- A substantial number of individuals with DEE have ESES/DEE-SWAS
- ESES/DEE-SWAS should be explored in individuals with *DLG4* variants, and *DLG4* variants should be considered in ESES/DEE-SWAS

2 | MATERIALS AND METHODS

2.1 | Individuals included in the study

Clinical and genetic data of 23 individuals with *DLG4* variants and epilepsy were collected through an international network of epilepsy and genetic centers, the European Reference Networks (ERNs) ERN-EpiCARE (www.epi-care.eu) and ERN-ITHACA (www.ern-ithaca.eu), GeneMatcher (www.genematcher.org),[13] and the patient advocacy organization SHINE Syndrome Foundation (www.shinesyndrome.org). For one of these individuals, only the *DLG4* variant, but not the clinical data, was previously published.[14] Furthermore, clinical data of 12 individuals previously published by our group[6,15] were updated. All the affected individuals were unrelated except for the proband #25 and her mother (#25m). Seizures and epilepsy syndromes were classified according to the International League Against Epilepsy (ILAE) position papers.[16] Data on neurodevelopmental delay (NDD) and ID were collected. The degree of ID at the last follow-up was ranked as none, mild, moderate, or severe ID by the referring physician. Formal neuropsychological testing was not possible particularly in the active phase of encephalopathy related to status epilepticus during slow-wave sleep (ESES)/DEE with spike–wave activation during sleep (DEE-SWAS) because of the deterioration of the cognitive status and/or affected individual's lack of cooperation. The cognitive assessment was thus based on clinical evaluation, parental reports, and information on school performance and achievements. Motor development and language abilities were also evaluated by the referring clinician. Disabling motor disturbances with or without the capacity to perform activities of daily living (e.g., to eat or to walk) were defined as moderate or severe, respectively. Expressive verbal impairment was classified as none, moderate (pronunciation of words and sentence content), or severe (nonverbal or only able to articulate sounds).

Data on awake and sleep electroencephalography (EEG)/video-polygraphy and brain magnetic resonance imaging (MRI) were collected. Whenever possible, original EEG studies were evaluated by three neurologists with EEG expertise (E.Gar., G.R., and B.K.) and classified according to the ILAE recommendations.[17] For ESES/DEE-SWAS diagnosis, a spike–wave index (SWI) of at least 50% of spike–wave activity in non-rapid eye movement (REM) sleep was used. EEG investigations were primarily performed due to epilepsy onset, but in some individuals due to regression that started prior to epilepsy. Antiseizure medication (ASM) response was retrospectively assessed by the referring clinician. Patients with EEGs showing extreme activation of epileptic discharges during slow-wave sleep and associated cognitive/behavioral regression were diagnosed with ESES. This condition has recently been renamed DEE-SWAS.[18–20] Local ethical committees approved this study. All probands or parents/legal guardians provided consent to participate in the study.

2.2 | Identification and classification of the *DLG4* variants

DLG4 variants were identified using next generation sequencing-based technologies (clinical exome or genome sequencing). Parental testing for segregation analysis was carried out for all individuals, except for one individual whose parents were deceased and another whose father was unavailable for testing. All variants are annotated using the NM_001365.4/ENST00000648172 (GRCh38/hg38) *DLG4* transcript, which is now designated as MANE (matched annotation from NCBI and EMBL-EBI) Plus Clinical transcript (an update will be publicly reported after the next MANE version release). Variants are described following the Human Genome Variation Society recommendations (www.varnomen.hgvs.org) and are confirmed by VariantValidator (www.variantvalidator.org). The presence of each variant in control populations was assessed in the Genome Aggregation Database (gnomAD v3.1.2, www.gnomad.broadinstitute.org), and the splice variant prediction tool SpliceAI (www.github.com/Illumina/SpliceAI) was used to predict the effect of the intronic, synonymous, and missense variants on splicing. The 55-bp rule[21] was employed to predict whether the protein-truncating variants (PTVs) would be subjected to nonsense-mediated decay (NMD). The potential pathogenicity of *DLG4* variants was assessed using Combined Annotation Dependent Depletion (www.cadd.gs.washington.edu) and Rare Exome Variant Ensemble Learner (www.sites.google.com/site/revelgenomics) scores. The *DLG4* variants were classified according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) criteria.[22]

3 | RESULTS

The present cohort comprises 35 individuals, including 23 newly reported individuals and updated clinical data on 12 previously described individuals.[6,15] The male:female ratio is 19:16, and the median age at inclusion was 13 years (range = 1.7–61 years). All individuals are alive at the time of writing, except for #16, who died at the age of 61 years because of status epilepticus following acute brain injury. Hereafter, denominators indicate the number of individuals from whom a given clinical feature is available. Data on the whole cohort can be found in Tables 1–4.

TABLE 1 Frequencies of the clinical features.

Category	Clinical feature	Individuals [$N = 35$], n (%)	
Sex	Male	19/35 (54.3)	
	Female	16/35 (45.7)	
Age	Median	13 years	
	Range	1.7–61 years	
Cognitive function	Normal[a]	2/35 (5.7)	
	ID	33/35 (94.3)	
	Mild	10/33 (30.3)	
	Moderate	14/33 (42.2)	
	Severe	8/33 (24.2)	
	Unspecified	1/33 (3.0)	
Motor impairment	None	8/35 (22.9)	
	Moderate	21/35 (60.0)	
	Severe	6/35 (17.1)	
Verbal impairment	None	5/35 (14.3)	
	Moderate	21/35 (60.0)	
	Severe	9/35 (25.7)	
Initial neurodevelopment	Normal	6/31 (19.4)	
	Delayed	25/31 (80.6)	
Psychiatric/behavioral problems	ASD + ASD features	25/35 (71.4)	
	ADHD	20/34 (58.8)	
Neurological features	Ataxia	12/35 (34.3)	
	Apraxia	18/33 (54.5)	
	Tremor	9/34 (26.5)	
	Dystonia	8/32 (25.0)	
	Hypotonia	22/35 (62.9)	
	Insomnia	24/35 (68.6)	
	Initiating sleep	17/24 (70.8)	
	Maintaining sleep	21/24 (87.5)	
	Early awakening	15/24 (62.5)	
Regression		+ESES/DEE-SWAS, n (%)	÷ESES/DEE-SWAS, n (%)
	Verbal	2/9 (22.2)	2/23 (8.7)
	Motor	2/9 (22.2)	1/24 (4.2)
	Verbal and motor	4/9 (44.4)	5/23 (21.7)
	Other	1/9 (11.1)	3/23 (13.0)

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; ESES/DEE-SWAS, encephalopathy related to status epilepticus during slow-wave sleep/developmental encephalopathy with spike–wave activation during sleep; ID, intellectual disability.

[a]The two individuals are from family 25, where the mother has normal cognitive function, but the daughter has low average intelligence quotient and alertness significantly below average.

3.1 | Clinical features

3.1.1 | Epilepsy electroclinical features

The median age at epilepsy onset was 7 years (range = 3 months–16 years). Seizure types were described in 33 individuals with the following distribution: focal in 18 (54.5%) individuals, focal to bilateral tonic–clonic in three (9.1%), generalized tonic–clonic (GTC) in 13 (39.4%), absences in four (12.1%, two individuals with atypical absences), tonic in two (6.1%), myoclonic in three (9.1%), epileptic spasms in two (6.1%), atonic in one (3%), and seizure with unknown onset in one (3%). Ten of 33 (27.3%) individuals demonstrated more than one seizure type. Only one individual presented with febrile seizures in the first year of life and infantile spasms (#27). Individual

TABLE 2 Frequencies of the electroclinical features.

Category	Electroclinical feature	Individuals, *n* (%)
Seizure type [*n* = 33]	Focal	18 (54.5)
	Focal to bilateral	3 (9.1)
	Tonic–clonic	13 (39.4)
	Absences	4 (12.1)
	Myoclonic	3 (9.1)
	Tonic	2 (6.1)
	Spasms	2 (6.1)
	Atonic	1 (3.0)
	Unknown onset	1 (3.0)
	Status epilepticus	1 (3.0)
Seizure frequency at last follow-up [*n* = 32]	Seizure-free	19 (59.4)
	Rare	4 (12.5)
	Monthly	4 (12.5)
	Daily	3 (9.4)
	Weekly	2 (6.3)
Interictal EEG [*n* = 30]	Multifocal	17 (56.7)
	Focal	5 (16.7)
	Generalized	2 (6.7)
	Encephalopathic	2 (6.7)
	Hypsarrhythmia	1 (3.3)
Ictal EEG [*n* = 6]	Electrodecremental	3 (50.0)
	Multifocal	3 (50.0)
	Generalized	2 (33.3)
	Focal	2 (33.3)
Mean SWI in ESES/DEE-SWAS individuals [% of NREM sleep; *n* = 6]		70; range = 40–100
Median age at ESES/DEE-SWAS onset, years		7; range = 3–8

Abbreviations: EEG, electroencephalogram; ESES/DEE-SWAS, encephalopathy related to status epilepticus during slow-wave sleep/developmental epileptic encephalopathy with spike–wave activation during sleep; NREM, non-rapid eye movement; SWI, spike–wave index.

#25 m, who has normal cognition and is currently seizure-free, originally presented with myoclonic seizures and generalized EEG epileptic abnormalities compatible with juvenile myoclonic epilepsy. A single episode of nonconvulsive status epilepticus was reported in one individual (#22). Eighteen of 32 individuals (58.1%) experienced sleep-related seizures, described as GTC in seven, focal in five, tonic in three, focal to bilateral in two, and unknown seizure onset in one.

The seizure frequency at last follow-up for 32 individuals was daily in three (9.4%), weekly in two (6.3%), monthly in four (12.5%), and rare (once per year or rarer) in four (12.5%). Nineteen individuals of 32 (59.4%) were seizure-free at the last follow-up (median time elapsed since previous evaluation in years = 6.5, range = 1–20). Two individuals had a follow-up of <1 year (#3 and #9). Among the seizure-free individuals, three (#4, #21, and #29; 9.4%) had a single seizure, and one (#16; 3.1%) had two seizures throughout their entire life. Both individuals with epileptic spasms, #11 with severe ID and an abnormal EEG without epileptic abnormalities, and #27 featuring mild developmental delay, achieved seizure freedom. No correlation between epilepsy severity and age at onset was observed.

EEG data were collected in 30 of 35 (85.7%) individuals. Epileptiform abnormalities were described as focal in six (20%) individuals, multifocal in 17 (56.7%), and generalized in one (3.3%). One individual (#27) presented with hypsarrhythmia and epileptic spasms at 7 months; at 13 months, the EEG showed multifocal spike/polyspike–wave discharges. Only six of 35 individuals had ictal EEG recordings, which showed a focal (#3 and #20), multifocal (#3, #26 and #33), and/or generalized (#3 and #24) onset, or an electrodecremental pattern (#3, #27, and #33), with two individuals presenting with more than one ictal pattern.

TABLE 3 Predicted effects of the 30 *DLG4* variants in 35 individuals.

Individual # (ID2021)	Inh	gDNA Chr17 (GRCh38)	cDNA NM_001365.4	Exon/*intron*	Predicted effect on PSD-95	PSD-95 dom	Predicted coding effect	CADD (REVEL)	ACMG/AMP
1	dn	g.7208176G>A	c.223C>T	4	p.(Gln75*)		Nonsense	39	P
2 (ID-7)[6]	dn	g.7203784dup	c.372dup	7	p.(Gly125Trpfs*3)	PDZ1	Frameshift	32	P
3 (ID-9)[6]	dn	g.7203702del	c.455del	7	p.(Gly152Alafs*12)	PDZ1	Frameshift	32	P
4	dn	g.7203331T>C	c.635-2A>G	*IVS8*	p.?		Intronic[a]	33	LP
5	dn	g.7202899T>C	c.916+4A>G	*IVS10*	p.?		Intronic[a]	24	VUS
6	dn	g.7197044G>A	c.925C>T	11	p.(Gln309*)		Nonsense	39	P
7 (ID-20)[6]	dn	g.7196915G>A	c.1054C>T	11	p.(Arg352*)		Nonsense	37	P
8	dn								
9	mat[b]	g.7196909del	c.1061del	11	p.(Pro354Argfs*5)		Frameshift	33	P
10	dn	g.7196903G>A	c.1066C>T	11	p.(Arg356*)	PDZ3	Nonsense	39	P
11	dn								
12 (ID-22)[6]	dn	g.7196850G>A	c.1119C>T; r.1118_1212del	11	p.(Gly373=)/p.(Gly373Glnfs*11)	PDZ3	Synonymous/frameshift[c]	13.7	P
13[14]	dn	g.7196840C>T	c.1129G>A	11	p.(Glu377Lys)	PDZ3	Missense[a]	33 (.379)	VUS
14	dn	g.7196768G>A	c.1201C>T	11	p.(Gln401*)	PDZ3	Nonsense	40	P
15	dn	g.7196557G>A	c.1231C>T	12	p.(Arg411*)	PDZ3	Nonsense	41	P
16	uk	g.7196212_7196228del	c.1424_1430+10del	13	p.(Tyr475Trpfs*15)	SH3	Frameshift	32	P
17	dn	g.7194415_7194418del	c.1510_1513del	14	p.(His504Serfs*41)	SH3	Frameshift	33	P
18 (ID-36)[6]	dn	g.7194302_7194317del	c.1607+4_1607+19del	*IVS14*	p.?		Intronic	24.9	VUS
19	dn	g.7193993G>A	c.1615C>T	15	p.(Arg539*)	SH3	Nonsense	44	P
20 (ID-42)[6]	dn	g.7193712G>A	c.1675C>T	17	p.(Arg559*)		Nonsense	45	P
21	not mat[d]								
22 (ID-43)[6]	dn	g.7193585C>T	c.1721-1G>A	*IVS17*	p.?		Intronic[a]	35	LP
23 (ID-44)[6]	dn	g.7193504T>A	c.1801A>T	18	p.(Lys601*)	GK	Nonsense	44	P
24	dn	g.7193483G>A	c.1822C>T	18	p.(His608Tyr)	GK	Missense	32 (.266)	VUS
25	mat	g.7193055G>A	c.1885C>T	19	p.(Arg629Trp)	GK	Missense	24.9 (.285)	VUS
25 m	uk								
26	dn	g.7193054C>T	c.1886G>A; r.1823_1887del	19	p.(Arg629Gln)/p.(His608Argfs*14)	GK	Missense/frameshift[e]	32	P
27	dn								
28	dn	g.7192962G>A	c.1978C>T	19	p.(Arg660*)	GK	Nonsense	41	P
29 (ID-51)[6]	dn	g.7192957del	c.1984del	19	p.(Val662Trpfs*41)	GK	Frameshift	33	P
30	dn	g.7192943A>G	c.1995+2T>C	*IVS19*	p.?		Intronic[a]	33	LP

TABLE 3 (Continued)

Individual # (ID2021)	Inh	gDNA Chr17 (GRCh38)	cDNA NM_001365.4	Exon/*intron*	Predicted effect on PSD-95	PSD-95 dom	Predicted coding effect	CADD (REVEL)	ACMG/AMP
31 (ID-52)[6]	dn	g.7191991G>T	c.2007C>A	20	p.(Cys669*)	GK	Nonsense	40	P
32[15]	dn	g.7191658G>A	c.2105+235C>T; r.[2105_2106ins2105+1_2105+233, 2105_2106ins2105+91_2105+233]	*IVS20*	p.[(Glu703*; Glu703Thrfs*10)]	GK	Intronic[f]	4.6	P
33 (ID-53)[6]	dn	g.7190805_7190809delinsA	c.2203_2207delinsT	22	p.(Val735Trpfs*12)	GK	Frameshift[g]	34	P
34	dn	g.7147006_7439965dup	c.?		p.?		293-kb duplication	NA	VUS

Note: Individual 25 m is the mother of individual 25.

Abbreviations: ACMG/AMP, variant classification according to American College of Medical Genetics and Genomics/Association for Molecular Pathology criteria; CADD, Combined Annotation Dependent Depletion; delins, deletion–insertion; dn, de novo; dom, domain; GK, guanylate kinase-like; ID2021, identification number in Rodríguez-Palmero et al.[6]; Inh, inheritance; IVS, intervening sequence; LP, likely pathogenic; mat, maternal; NA, not available; P, pathogenic; PDZ, PSD-95/discs large/zonula occludens 1; REVEL, Rare Exome Variant Ensemble Learner; SH3, SRC homology 3; uk, unknown; VUS, variant of unknown significance.

[a]Variant is predicted to affect splicing.

[b]Parent is mosaic.

[c]RNA analysis showed abnormal splicing of *DLG4*, resulting in a truncated protein product r.1118_1212del:p.(Gly373Glnfs*11).

[d]Variant is not maternal; father is not available for testing.

[e,f]RNA analysis showed abnormal splicing of *DLG4* resulting in (e) a truncated protein product r.1823_1887del:p.(His608Argfs*14) and (f) two out-of-frame transcripts (r.[2105_2106ins2105+1_2105+233,2105_2106ins2105+91_2105+233]).

[g]Variant is predicted to escape nonsense-mediated decay.

TABLE 4 Overview of the genotype and phenotype of 35 individuals.

Individual #	Variant	Age at inclusion, years	Sex	Development in the first years	First concern, months	Motor impairment	Verbal ability impairment	Regression	Cognition (estimated ID)	Psychiatric and behavioral symptoms	Neurological signs	Age at epilepsy onset, years	Types of seizures	Febrile seizures	Nocturnal seizures	Seizure frequency	EEG	ESES/ DEE-SWAS (diagnosis age, years)
1	Gln75*	10	M	N/A	6	Mod	-	Fluctuating cognitive performance	Mild	Anxiety, ASD, ADHD	Hypotonia	7	GTC	-	GTC	SF	Multifocal	+ (8)
2	Gly125Trpfs*3	10	M	Delay	42	Mod	Mod	Motor, verbal, and coordination, self-skills	Mod	Anxiety, ASD, ADHD	Hypotonia, apraxia, insomnia	5	Atypical absence	-	-	Rare	Multifocal	+ (5)
3	Gly152Alafs*12	14	M	Delay	6	Severe	Severe	Motor and verbal	Severe	Anxiety, ASD, ADHD	Hypotonia, ataxia, apraxia, insomnia	9	Focal, focal to bilateral, tonic	-	Tonic	SF	Multifocal	-
4	c.635-2A>G	22	F	Delay	9	Mod	-	-	Mod	-	-	10	Absence, GTC	-	-	SF	Multifocal	-
5	c.916+4A>G	11	M	Delay	36	Mod	Mod	Motor, verbal, and adaptive functions and cognitive profile	Mod	Anxiety	Hypotonia, apraxia, tremor, dystonia	8.5	Focal, focal to bilateral	-	GTC	N/A	Multifocal	-
6	Gln309*	20	F	Delay	6	Severe	Severe	Motor	Mod	Anxiety, ASD, ADHD	Hypotonia, ataxia, apraxia, tremor, dystonia, insomnia	.3	Myoclonic	-	-	SF	Unremarkable	N/A
7	Arg352*	15	F	Delay	.25	Mod	Mod	N/A	Severe	Anxiety, ASD, ADHD	Hypotonia, apraxia, insomnia	9	Focal	-	N/A	SF	N/A	N/A
8	Arg352*	17	M	N/A	18	Mod	Severe	Verbal	Severe	Anxiety, ASD, ADHD	Hypotonia, apraxia, insomnia	10	Focal, GTC	-	Focal to bilateral	SF	Generalized	+ (8)
9	Pro354Argfs*5	13	F	Delay	9	Mod	Mod	-	Mod	-	Hypotonia	11	Focal	-	N/A	SF	Focal	-
10	Arg356*	13	M	Delay	8	Mod	Mod	-	Mild	ASD	Hypotonia, ataxia, tremor, insomnia	2	Focal	-	-	Weekly	Focal	-
11	Arg356*	7	M	Delay	2	Mod	Severe	Motor and verbal	Severe	ASD, ADHD	Hypotonia, ataxia, apraxia	.4	Spasms	-	-	SF	Encephalopathic	N/A
12	Gly373=/ Gly373Glnfs*11	14	M	Normal	36	Mod	Severe	Motor and verbal	Severe	ASD	Insomnia	12	Undetermined	-	Undetermined, GTC	Monthly	Encephalopathic	-
13	Glu377Lys	26	F	Normal	8	-	Severe	Verbal	Severe	Anxiety, ASD	Hypotonia, insomnia	8	Focal	-	Focal	SF	Multifocal	-
14	Gln401*	27	F	Delay	15	-	Mod	-	Mild	Anxiety, ADHD	Hypotonia, dystonia, insomnia	4	Focal	-	Focal	SF	N/A	-
15	Arg411*	15	M	N/A	24	Mod	Mod	-	Mild	Anxiety, ASD, ADHD	Tremor, dystonia, insomnia	8	GTC	-	GTC	SF	Focal	-
16	Tyr475Trpfs*15	61	F	Delay	4	-	-	Following depression in adulthood (e.g., needs help with feeding)	Mod	-	-	9	N/A	-	-	SF	N/A	N/A
17	His504Serfs*41	9	F	Normal	25	-	Mod	-	Mild	ASD, ADHD	Insomnia	7	Focal	-	Focal	Monthly	Multifocal	-
18	c.1607+4_1607+19del	13	F	Delay	11	Mod	Mod	Writing skills	Mod	Anxiety, ASD	Hypotonia, ataxia, insomnia	9.9	Focal	-	Focal	SF	N/A	-
19	Arg539*	11	M	Delay	17	Mod	Severe	Verbal and social interactions	Mod	Anxiety, ASD, ADHD	Apraxia, tremor, insomnia	7	N/A	-	-	N/A	Focal	+ (7)
20	Arg559*	16	F	Delay	6	Severe	Mod	-	Mod	Anxiety, ASD, ADHD	Insomnia	2	Myoclonic, absences	-	-	Weekly	Unremarkable	-

(Continues)

TABLE 4 (Continued)

Individual #	Variant	Age at inclusion, years	Sex	Development in the first years	First concern, months	Motor impairment	Verbal ability impairment	Regression	Cognition (estimated ID)	Psychiatric and behavioral symptoms	Neurological signs	Age at epilepsy onset, years	Types of seizures	Febrile seizures	Nocturnal seizures	Seizure frequency	EEG	ESES/ DEE-SWAS (diagnosis age, years)
21	Arg559*	8	M	Delay	15	Mod	Mod	-	Mild	Anxiety, ASD, ADHD	Hypotonia, apraxia, insomnia	7	GTC	-	GTC	SF	Multifocal	N/A
22	c.1721-1G>A	17	F	Delay	24	Severe	Mod	-	Mild	ADHD	Ataxia, dystonia, spastic tetraparesis	8	Focal, GTC	-	GTC, focal to bilateral	Monthly	Multifocal	-
23	Lys601*	25	F	Delay	10	Severe	Mod	Motor and verbal	Mod	Anxiety, ASD	Hypotonia, ataxia, apraxia, tremor, dystonia, insomnia	.3	Atonic, focal, GTC	-	Focal	Rare	Multifocal	N/A
24	His608Tyr	8	M	Delay	N/A	Mod	Mod	-	Mild	ASD, ADHD	Hypotonia, apraxia, insomnia	3	Atypical absence	-	N/A	Daily	Generalized	N/A
25[a]	Arg629Trp	12	F	Normal	N/A	-	Mod	Fluctuating cognitive performance, motor and verbal	Low average[b]	Anxiety	Apraxia, insomnia	8	Focal	-	-	Daily	Multifocal	+ (8)
25 m[a]	Arg629Trp	36	F	Normal	-	-	-	-	Normal	-	-	13	Myoclonic, GTC	-	-	SF	Unremarkable	-
26	Arg629Gln/ His608Argfs*14	10	M	Delay	N/A	Severe	Mod	Motor	Mod	ASD, ADHD	Ataxia, apraxia, tremor	6	Focal, GTC	-	GTC	N/A	Multifocal	+ (8)
27	Arg629Gln/ His608Argfs*14	2	M	Delay	5	Mod	Mod	-	Mild	-	Hypotonia, insomnia	.5	Spasms	+	Tonic	SF	Hypsarrhythmia to multifocal	-
28	Arg660*	30	M	Normal	36	Mod	Mod	Motor	Severe	Anxiety, ASD	Hypotonia, apraxia, tremor, insomnia	3	GTC	-	-	Rare	N/A	+ (3)
29	Val662Trpfs*41	13	M	Delay	12	-	Mod	-	Mild	Anxiety, ADHD	Hypotonia, apraxia	12	Focal	-	Focal	SF	Multifocal	-
30	c.1995+2T>C	27	F	Delay	4	Mod	Mod	-	Mod	Anxiety, ASD	Ataxia, insomnia	16	GTC	-	GTC	Rare	Unremarkable	-
31	Val662Trpfs*41	11	F	N/A	12	Mod	-	-	ID[c]	ASD, ADHD	-	7	Focal	-	-	SF	Focal	-
32	c.2105+235C>T	10	M	Delay	Since birth	Mod	Severe	Motor and verbal	Mod	Anxiety, ASD, ADHD	Hypotonia, ataxia, apraxia, tremor, dystonia, insomnia	4	Focal	-	-	Monthly	Multifocal	+ (4)
33	Val735Trpfs*12	9	M	Delay	6	Mod	Severe	Motor and verbal	Severe	Anxiety, ASD	Hypotonia, ataxia, apraxia, dystonia, insomnia	5	Focal, tonic, focal to bilateral	-	Tonic	Daily	Multifocal	+ (4)
34	293-kb duplication	14	M	Delay	10	-	Mod	Verbal	Mod	Anxiety, ASD, ADHD	Hypotonia, ataxia, apraxia, insomnia	.6	GTC	-	N/A	SF	Multifocal	-

Note: The "p." prefix is omitted from coding variants for simplicity.

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; EEG, electroencephalogram; ESES/DEE-SWAS, encephalopathy related to status epilepticus during slow-wave sleep/developmental epileptic encephalopathy with spike–wave activation during sleep; F, female; GTC, generalized tonic–clonic; ID, intellectual disability; M, male; Mod, moderate; N/A, not available; SF, seizure-free.

[a]Individuals #25 m and #25 are mother and daughter.

[b]Individual #25 has low-average intelligence quotient (83) and alertness significantly below average.

[c]ID not specified.

3.1.2 | ESES/DEE-SWAS

Because we observed ESES/DEE-SWAS in our previous study,[6] we investigated the possible occurrence of ESES/DEE-SWAS in 24 individuals from whom sleep EEGs were available. Nine of 24 (37.5%) presented with a striking enhancement of epileptiform activities during non-REM sleep associated with the appearance of cognitive and behavioral regression, thus consistent with the diagnosis of ESES/DEE-SWAS (Figure 1).[18,20,23] The median age at diagnosis of ESES/DEE-SWAS was 7 years (range = 3–8 years). Raw EEG data were available for reanalysis in seven individuals; the topography of the sleep-related EEG abnormalities was focal in four, multifocal in two, and focal-to-diffuse in one. The SWI, reported in six of nine, ranged from 40% to 100% (a 40% SWI was observed in an individual who was already in clinical remission). Individual #19 presented with an exaggeration of sleep-related epileptic discharges associated with cognitive regression, thus fulfilling the diagnosis of ESES/DEE-SWAS, without any evidence of clinical seizures, whereas two other individuals with epileptic seizures exhibited a striking activation of EEG during slow-wave sleep, resembling an ESES/DEE-SWAS EEG pattern, without being associated with any overt cognitive/behavioral regression, thus not fulfilling the diagnostic criteria of ESES/DEE-SWAS.

3.1.3 | Epilepsy treatment

Data on epilepsy treatment were available in 34 of 35 individuals (97.1%). Complete seizure abatement was achieved in 12 individuals with monotherapy and in four with polytherapy; the drugs most commonly effective in controlling seizures (alone or in combination) were valproate (VPA; $n = 6$), levetiracetam (LEV; $n = 3$), and lamotrigine (LTG; $n = 3$). Individual #27 with epileptic spasms was seizure-free with vigabatrin (VGB) and VPA. Therapy was tapered in five individuals (#4, #11, #14, #25 m, and #28) and they are currently untreated and seizure-free at 22, 6.5, 27, 36, and 30 years of age, respectively. Among them, only individual #28 relapsed, presenting with rare GTC seizures. In individual #11, epileptic spasms at 7 months of age were controlled by VGB (which was tapered at 3 years of age) and a short course of steroids at 6 months of age.

Of the nine individuals with ESES/DEE-SWAS, steroids were employed in four, high-dose benzodiazepines in one, clobazam in six, and sulthiame in two. Sleep-related EEG abnormalities disappeared in two individuals (one treated with steroids, the other with lamotrigine); however, the behavioral clinical picture remained unchanged in both. In four individuals, medication neither improved the EEG nor lessened the degree of regression. Response to the treatment was not

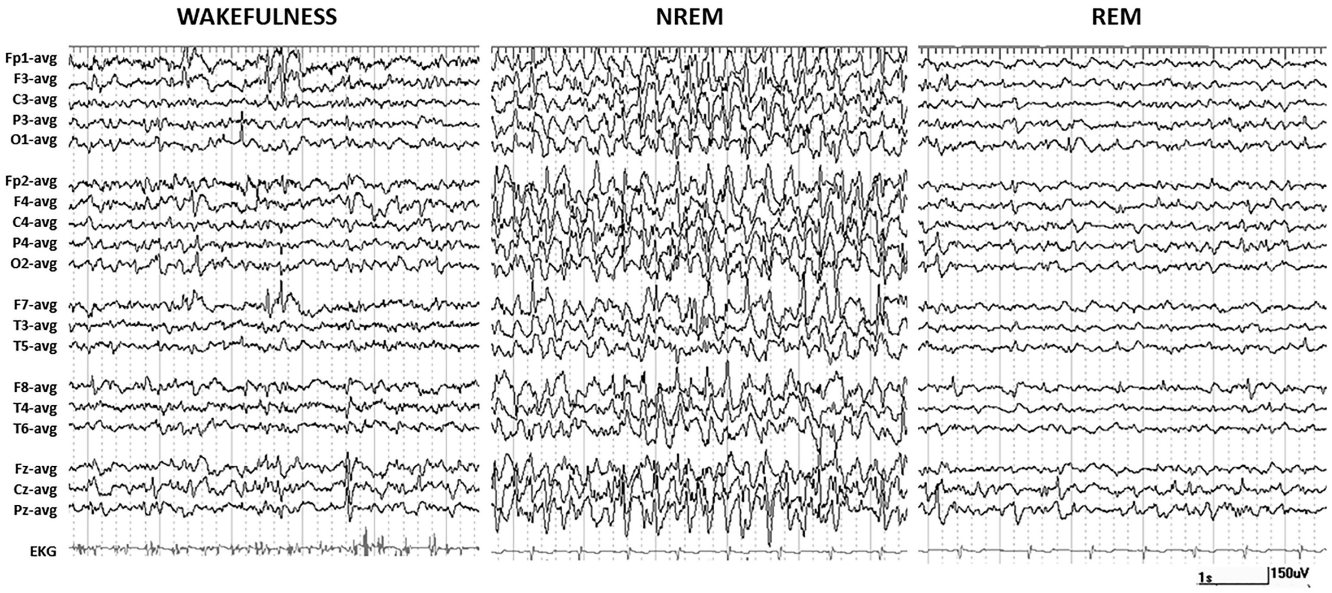

FIGURE 1 Awake and sleep electroencephalogram (EEG) of individual 33 with encephalopathy related to status epilepticus during slow-wave sleep (ESES)/developmental epileptic encephalopathy with spike–wave activation during sleep (DEE-SWAS) at the age of 8 years. EEG during wakefulness showed multifocal asynchronous spikes in both hemispheres (left panel). Sleep EEG during ESES/DEE-SWAS showed a striking activation of epileptic abnormalities during non-rapid eye movement (NREM) sleep (spike–wave index = 80%) with right-side predominance (middle panel). During rapid eye movement (REM) sleep, remarkable attenuation of multifocal epileptic abnormalities was observed (right panel).

available in three patients. Cognitive improvement after resolution of ESES/DEE-SWAS was not observed in any individual.

Among four individuals treated with a ketogenic diet, only one (#6) became seizure-free. In individual #33, employment of vagal nerve stimulation temporarily reduced SWI in ESES/DEE-SWAS and improved daytime irritability, which, although difficult to quantify, may have been part of the ESES/DEE-SWAS. In this individual, deep brain stimulation resulted in reduction of tonic seizures and improvement of gait.

3.1.4 | Neuroimaging

Neuroimaging data (MRI) were collected from all individuals; white matter hyperintensity was observed in four (11.8%), cerebral atrophy in three (8.8%), and thinning of corpus callosum, cerebellar atrophy, and incomplete inversion of the hippocampus in single individuals (2.9% each). The eldest (#16), at 61 years, demonstrated parieto-occipital corticosubcortical edema during an ultimately fatal episode of status epilepticus. One individual (#32) had a nonprogressive, static spinal lesion.

3.1.5 | NDD, ID, and regression

Initial neurodevelopment was reported as normal in six of 31 (19.4%) individuals (#12, #13, #17, #25, #25 m, and #28; first developmental concern at a median age of 15 months, range = 8–36 months), where two subsequently developed ESES/DEE-SWAS. All the remaining individuals (25/31, 80.6%) showed delay in the acquisition of psychomotor milestones and cognitive abilities early in life with a broad spectrum of severity (ranging from individuals without grasping reflex at birth to individuals with delayed milestones). When last examined, all individuals but one (#25 m) manifested NDD. Over the course of the disease, cognitive and behavioral regression appeared in 20 of 32 (60.6%), including the nine individuals with ESES/DEE-SWAS. At the last follow-up, ID was defined as mild in 10 of 33 (30.3%), moderate in 14 of 33 (42.2%), and severe in eight of 33 (24.2%) individuals, and one individual was reported as having unspecified ID. Individual #25 m had normal neurodevelopment and cognition; her daughter (#25) had an intelligence quotient of 83 at last evaluation but presented a fluctuating cognitive performance and below average linguistic skills.

ESES/DEE-SWAS individuals exhibited verbal regression (#8 and #19), motor regression (#26 and #28), or both (#2, #25, #32, and #33); fluctuations of cognitive performance not further specified were experienced by individuals #1 and #25. Individual #2 also showed regression in coordination and basic activities of daily living, and individual #19 also showed a reduction of social interactions. Regression of cognitive abilities was also observed in individuals without ESES/DEE-SWAS; verbal regression was reported in two individuals (#13 and #34), motor regression in one (#6), and both verbal and motor regression in five (#3, #5, #11, #12, and #23). In addition, regression in writing abilities, behavior, and general performance in adulthood following depression were reported in one individual each (#18, #29, and #16, respectively).

3.1.6 | Neurological and psychiatric features

Hypotonia was detected in 22 of 35 individuals (62.9%). Apraxia and gait ataxia were found in 18 of 33 (54.5%) and 12 of 35 (34.3%), respectively, whereas tremor and dystonia were observed in nine of 34 (26.5%) and eight of 32 (25%), respectively. Insomnia was reported in 24 of 35 individuals (68.6%); difficulties in initiating or maintaining sleep in 17 of 24 (70.8%) and 21 of 24 (87.5%) individuals, respectively, and early awakening in 15 of 24 (62.5%).

ASD or ASD features and ADHD were diagnosed in 25 of 35 (71.4%) and 20 of 34 (58.8%) individuals, respectively. Among the nine individuals with ESES/DEE-SWAS, eight (88.9%) had ASD or ASD features and six had ADHD (66.7%). Stereotypies were reported in 15 individuals, 14 of whom had ASD or ASD features. Anxiety was reported in 22 of 34 individuals (64.7%).

3.2 | Spectrum of the 30 *DLG4* variants

Thirty heterozygous *DLG4* variants (14 novel and 16 previously published) were identified in 33 unrelated individuals and in a family (#25 and her mother #25 m; Table 3, Figure 2). All the variants, except for the three missense, are predicted to be PTVs: 11 nonsense, 10 frameshift, and five intronic variants. In addition, one individual has a 293-kb duplication encompassing *DLG4* and two other Online Mendelian Inheritance in Man morbid genes: *EIF5A* and *ACADVL*. One variant was transmitted from a mildly affected mother to her daughter (#25 m and #25, respectively), whereas the variants occurred de novo in 30 individuals. The mother of #9 is mosaic (27%) for the variant, and the parents of two individuals were unavailable for testing. None of the variants is present in control populations in the gnomAD database, except p.(Arg629Trp), which is present at extremely low frequency (.0007%,

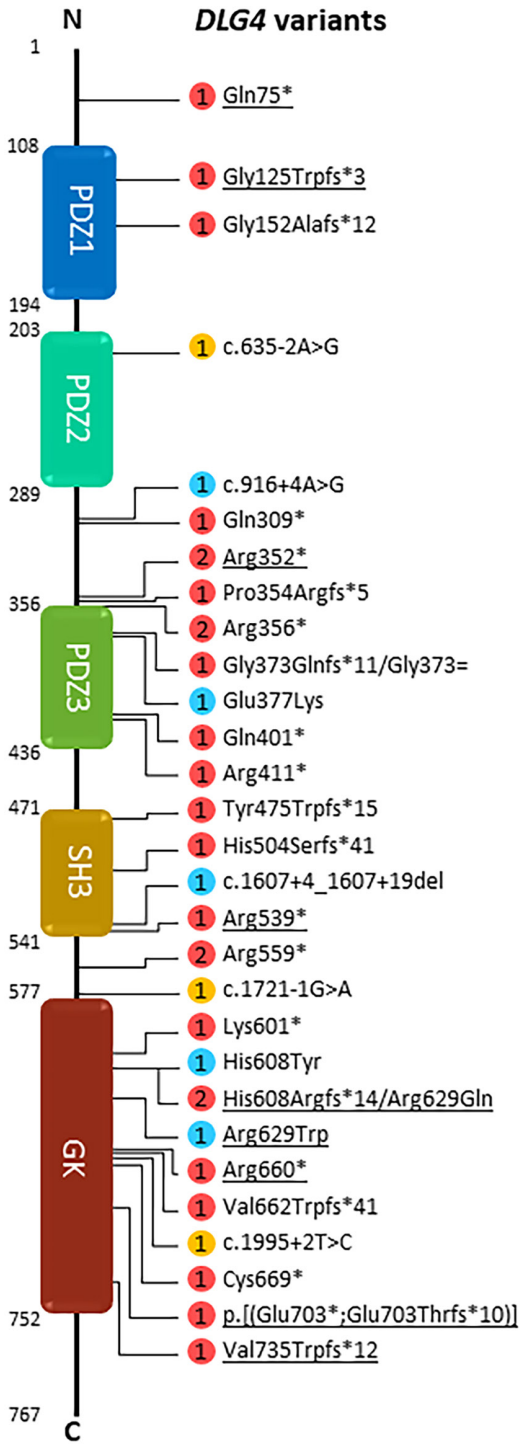

FIGURE 2 PSD-95 domains and *DLG4* variants. All variants are annotated using the NM_001365.4 *DLG4* transcript. The "p." prefix is omitted from coding variants for simplicity, whereas the "c." prefix is included for intronic variants. The 293-kb duplication in individual #34 is not shown. Pathogenicity of the variant is illustrated by a red (pathogenic), yellow (likely pathogenic), or blue (variant of unknown significance) circle. The number in the circle is the number of individuals with the variant. Underlined variants are associated with ESES/DEE-SWAS (encephalopathy related to status epilepticus during slow-wave sleep/developmental epileptic encephalopathy with spike–wave activation during sleep). GK, guanylate kinase-like; PDZ, PSD-95/discs large/zonula occludens 1; SH3, SRC homology 3.

1/152016 alleles). Six variants were observed more than once: p.(Arg352*) in four individuals (#7, #8, and two published [ID-19 and ID-21][6]); p.(Arg356*) in two individuals (#10 and #11); p.(Arg411*) in two individuals (#15 and one published [ID-27][6]); p.(Arg559*) in two individuals (#20 and #21); p.(Arg660*) in three individuals (#28 and two published [ID-49 and ID-50][6]); and p.(Arg629Gln)/p.(His608Argfs*14) in three individuals (#26, #27, and one published [ID-47][6]). All PTVs are predicted to be subject to NMD according to the 55-bp rule,[21] except the frameshift variant p.(Val735Trpfs*12). Three of the five intronic variants are canonical ±1–2 splice-site variants, whereas the two other variants, c.916+4A>G and c.1607+4_1607+19del, are located downstream of the canonical splice sites. They are all predicted to affect splicing. In addition, three single-nucleotide substitutions, originally annotated as synonymous (p.(Gly373=)), missense (p.(Arg629Gln)), or deep intronic (c.2105+235C>T), were also predicted to affect splicing. Previous RNA analyses confirmed that the variants cause alternative splicing, leading to out-of-frame transcripts, and should likely be classified as PTVs.[6,15] According to ACMG/AMP criteria, 21 variants are classified as pathogenic and three as likely pathogenic. Three missense variants, two noncanonical splice-site intronic variants, and the 293-kb duplication, are classified as variants of uncertain significance but are included in this study as the phenotypes of these individuals are in concordance with the overall clinical spectrum (Table 3).

3.3 | Phenotype–genotype comparison

To explore any possible phenotype–genotype correlation, we first compared the phenotypes of the individuals with identical *DLG4* variants. Two individuals (#7 and #8) with epilepsy from the present cohort and two individuals without epilepsy previously published by our group (ID-19 and ID-21)[6] have the same nonsense variant, p.(Arg352*). All four individuals have global developmental delay and moderate to severe ID. ESES/DEE-SWAS was only present in individual #8, whereas it was not assessed in #7. It is notable that among these four individuals with the same variant, the ones with epilepsy present with a more severe phenotype. Individuals #10 and #11 with the p.(Arg356*) nonsense variant have rather different clinical features, as the 13-year-old individual #10 has mild ID without regression, whereas 7-year-old #11 has severe ID and has experienced both motor and verbal regression. Two other individuals (#20 and #21) with the p.(Arg559*) nonsense variant also differ clinically; they both present psychiatric features such as ASD and ADHD and no neurological symptoms (apart from hypotonia in #21). The older girl

(#20) presents a moderate ID and weekly seizures, whereas the younger boy (#21) has mild ID and has had a single bilateral tonic–clonic seizure throughout his life. Two individuals (#26 and #27) with the p.(His608Argfs*14) frameshift variant also have symptoms of different severity; #26 is more severely affected with ESES/DEE-SWAS, neurological symptoms, and moderate ID, whereas the younger individual (#27) is less severely affected, with age-appropriate unsteady walking and a good relationship to the environment, and presented only with epileptic spasms early in life. However, he was 20 months old at the time of last examination, and development of ESES/DEE-SWAS later in life cannot be ruled out.

We also attempted to correlate the clinical severity of the individuals with missense variants ($n = 4$) to those with PTVs, which are found in the majority of individuals ($n = 31$). Individuals #25 m and #25, with the missense variant p.(Arg629Trp), are mother and daughter, respectively, and they are the least affected individuals of the present cohort. Individual #25 m has normal cognition and juvenile myoclonic epilepsy starting at age 13 years, from which she has now been seizure-free for 21 years. Her daughter has moderate NDD, fluctuating performance at the lower border of normal cognition, some sleeping disturbances, epilepsy, and ESES/DEE-SWAS. The two other individuals with missense variants (#13 and #24) are more severely affected compared to #25 and #25 m, and their symptoms in general are comparable to those with PTVs.

4 | DISCUSSION

As it is a recently identified brain disorder, there exist comparatively few studies describing the *DLG4*-related synaptopathy phenotype. In one previous study carried out by our group, epilepsy was diagnosed in approximately 50% of individuals with *DLG4* variants, together with ID and neurological and behavioral disturbances also observed frequently.[6]

In this study, we explore the phenotypic and genotypic spectrum of individuals focusing on the epilepsy phenotype and investigating the presence of DEE. The median age of epilepsy onset was 7 years; however, the age range at onset varied from early infancy to adolescence. A large variety of seizure types was reported, including both focal and generalized onset seizures, occurring either in isolation or in various combinations. Focal onset seizures, observed in >50% of individuals, were the most common seizure, 9.1% having focal to bilateral tonic–clonic seizures. GTC seizures were experienced by one third of individuals, whereas other types of generalized onset seizures (i.e., myoclonic, absences, tonic, atonic) were observed in a limited number of individuals. Infantile spasms were seen in only two individuals. These latter findings align with previously published data.[6] Seizures occurring during sleep were common, affecting approximately 60% of individuals. Fever sensitivity and nonconvulsive status epilepticus occurred in only one individual each. Seizure frequency varied from single or very rare seizures (in approximately 16%) to daily/weekly seizures (16%). Overall, the epilepsy prognosis was favorable, with seizure freedom achieved during the course of the disease in >60% of individuals, most of them treated with a single ASM. The most frequently effective drugs were VPA or LEV, although this finding might be biased, as these two drugs were the most commonly used. In a small proportion of affected individuals, LTG and other sodium channel blockers were effective in obtaining seizure freedom after multiple trials with other ASMs. Finally, in four individuals (11.4%), ASMs were tapered without relapses.

Our previous study revealed the emergence of ESES/DEE-SWAS with disease evolution in some individuals.[6] This finding is confirmed by the present study, in which targeted ESES/DEE-SWAS investigation revealed its presence in >30% of individuals. Occurrence of sleep-related seizures in 60% of individuals and ESES/DEE-SWAS in 30% of individuals suggests a link between epileptic activity and sleep mechanisms and underlines the importance of performing sleep EEG (when possible, 24-h) in all individuals with *DLG4*-related synaptopathy.[17] It has been demonstrated that the generation of sleep slow-wave oscillation and of spike–wave discharges might partially share the same underlying cellular mechanisms,[24,25] and that sleep-related enhancement of sleep-related epileptic activity is an age-dependent phenomenon, presenting in childhood/adolescence. In all nine individuals with ESES/DEE-SWAS, the extreme sleep-related activation of epileptic abnormalities was associated with a regression of cognitive abilities and appearance of behavioral disorders, fulfilling the diagnostic criteria of ESES/DEE-SWAS.[18,20] Such cognitive/behavioral derangement was recently proposed to depend on impairment of the physiological synaptic homeostatic processes, crucial for consolidation of learning and memory processes, caused by exaggerated sleep-related epileptic activity.[26]

With regard to molecular pathophysiology, variants affecting the PDZ (PSD-95/discs large/zonula occludens 1) domains of PSD-95 have been hypothesized to disrupt the function of glutamate receptors AMPA and NMDA or Kv1 channels and could thereby lead to altered excitatory synaptic transmission.[3] Notably, pathogenic variants in *KCNA1*, *KCNA2* (encoding K_v1 channel subunits $K_v1.1$ and $K_v1.2$, respectively), and *GRIN2A* (encoding NMDA receptor subunit GluN2A) have been associated with ESES/DEE-SWAS.[27–30] Furthermore,

dysfunction of the cytoplasmic PSD-95 interaction partners SYNGAP1 and SHANK3 also leads to *SYNGAP1*-DEE and *SHANK3*-DEE (Phelan–McDermid syndrome), respectively. These two latter synaptopathies have been linked to sleep abnormalities,[31–33] similar to the DEE subgroup of individuals with *DLG4*-related synaptopathy. Here, a disruption of physiological sleep homeostasis might be caused by exaggerated epileptic activity during non-REM sleep possibly contributing to the cognitive impairment observed in ESES/DEE-SWAS.[26] Furthermore, *DLG4*-, *SYNGAP1*-, and *SHANK3*-related disorders all have a high prevalence of ID, speech and language impairment, ASD, hypotonia, and regression, suggesting perturbance of postsynaptic stability as a common disease mechanism.

NDD was recognized at approximately 1 year of age in most individuals, in line with our previous data on 53 individuals with *DLG4*-related synaptopathy.[6] All but one individual currently have ID, also in concordance with our previous report, where ID is the most common finding (98%) and is moderate–severe in approximately 60%. These data, together with the frequency of ASD (71.4%) and ADHD (58.8%) in the current cohort, are also consistent with a previous report[6] indicating that ID, ASD, and ADHD are related to PSD-95 dysfunction and are independent of the presence of epilepsy.

Regression was observed in all individuals with ESES/DEE-SWAS but also in some of those without diagnosis of ESES/DEE-SWAS. Regression in motor and/or verbal abilities has also been described in individuals with *DLG4*-related synaptopathy without epilepsy, suggesting that a cognitive compromise might be related to the pivotal role of PSD-95 as an anchor for several transmembrane proteins involved in various functions and pathways subserving cognitive processes.[3]

Finally, hypotonia, tremor, dystonia, and apraxia are the most common neurological features of *DLG4*-related synaptopathy individuals with DEE. Tremor, dystonia, and apraxia are more frequent in individuals with DEE compared to all the previously described individuals with or without epilepsy,[6] suggesting a link between movement disorders and the DEE phenotype.

In conclusion, our study shows that DEE can be part of the clinical spectrum of *DLG4*-related synaptopathy and further delineates the DEE phenotype. In individuals with recurrent variants, the phenotype and disease severity vary considerably, suggesting a complex genotype–phenotype relationship and involvement of other genetic and/or environmental factors. Finally, our findings suggest that a high prevalence of ESES/DEE-SWAS in *DLG4*-related synaptopathy warrants proper investigations for early diagnosis.

AUTHOR CONTRIBUTIONS

Conceptualization: Zeynep Tümer, Guido Rubboli, Rikke S. Møller. *Data curation–genetic and clinical investigations:* Angel Aledo-Serrano, Amitha L. Ananth, Alejandro J. Brea-Fernández, Roseline Caumes, Nicolas Chatron, Alice Dainelli, Matthias De Wachter, Anne-Sophie Denommé-Pichon, Thomas J. Dye, Elisa Fazzi, Roxanne Felt, Alberto Fernández-Jaén, Montse Fernández-Prieto, Emily Gantz, Piotr Gasperowicz, Antonio Gil-Nagel, David Gómez-Andrés, Hansel M. Greiner, Renzo Guerrini, Maria K. Haanpää, Minttu Helin, Juliane Hoyer, Anna C. E. Hurst, Staci Kallish, Shefali N. Karkare, Amjad Khan, Lotte Kleinendorst, Johannes Koch, Sanjeev V. Kothare, Suzanna M. Koudijs, Lieven Lagae, Phillis Lakeman, Kathleen A. Leppig, Gaetan Lesca, Diego Lopergolo, Laina Lusk, Alex Mackenzie, Davide Mei, Elaine M. Pereira, Konrad Platzer, Chloe Quelin, Anya Revah-Politi, Sylvain Rheims, Agustí Rodríguez-Palmero, Andrea Rossi, Filippo Santorelli, Syndi Seinfeld, Erick Sell, Donna Stephenson, Krzysztof Szczaluba, Eugen Trinka, Muhammad Umair, Hilde Van Esch, Mieke M. van Haelst, Danielle C. M. Veenma, Sacha Weber, Sarah Weckhuysen, Pia Zacher. *Data Analysis:* Benedetta Kassabian, Amanda M. Levy, Elena Gardella, Zeynep Tümer, Guido Rubboli. *Project administration:* Benedetta Kassabian, Amanda M. Levy, Zeynep Tümer, Guido Rubboli. *Visualization:* Guido Rubboli, Amanda M. Levy. *Writing–original draft preparation:* Benedetta Kassabian, Amanda M. Levy, Zeynep Tümer, Guido Rubboli. *Writing–review & editing:* All authors. *Fine tuning and final editing of the manuscript:* Benedetta Kassabian, Amanda M. Levy, Alex Mackenzie, Zeynep Tümer, Guido Rubboli.

AFFILIATIONS

[1]Department of Epilepsy Genetics and Precision Medicine, Danish Epilepsy Center Filadelfia, member of the European Reference Network EpiCARE, Dianalund, Denmark
[2]Neurology Unit, Department of Neurosciences, University of Padua, Padua, Italy
[3]Department of Clinical Genetics, Kennedy Center, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
[4]Department of Regional Health Research, University of Southern Denmark, Odense, Denmark
[5]Epilepsy and Neurogenetics Unit, Vithas la Milagrosa University Hospital, Vithas Hospital Group, Madrid, Spain
[6]Division of Pediatric Neurology, University of Alabama at Birmingham, Birmingham, Alabama, USA
[7]Grupo de Genómica y Bioinformática, Centro Singular de Investigación en Medicina Molecular y Enfermedades Crónicas (CiMUS), Centro de Investigación Biomédica en Red de Enfermedades Raras del Instituto de Salud Carlos III (CIBERER-ISCIII), Universidade de Santiago de Compostela, Santiago de Compostela, Spain
[8]Grupo de Genética, Fundación Pública Galega de Medicina Xenómica, Instituto de Investigación Biomédica de Santiago (IDIS), Santiago de Compostela, Spain
[9]CHU de Lille, Clinique de Génétique, Lille, France

[10]Service de Genetique, Hospices Civils de Lyon, Bron, France
[11]Institute NeuroMyoGène, Laboratoire Physiopathologie et Génétique du Neurone et du Muscle, Centre National de la recherche scientifique (CNRS) Unité mixte de recherche (UMR) 5261– L'Institut national de la santé et de la recherche médicale (INSERM) U1315, Université de Lyon–Université Claude Bernard Lyon 1, Lyon, France
[12]Neuroscience Department, Meyer Children's Hospital IRCCS (Istituto di Ricovero e Cura a Carattere Scientifico), member of the European Reference Network EpiCARE, Florence, Italy
[13]Department of Pediatric Neurology, Antwerp University Hospital, University of Antwerp, Edegem, Belgium
[14]Functional Unit for Diagnostic Innovation in Rare Diseases, Fédération Hospitalo-Universitaire Médecine TRANSLationnelle et Anomalies du Développement (FHU-TRANSLAD), Dijon Bourgogne University Hospital, Dijon, France
[15]L'Institut national de la santé et de la recherche médicale (INSERM) Unité mixte de recherche (UMR) 1231, Génétique des Anomalies du Développement (GAD), Fédération Hospitalo-Universitaire Médecine TRANSLationnelle et Anomalies du Développement (FHU-TRANSLAD), University of Burgundy, Dijon, France
[16]Division of Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA
[17]Department of Pediatrics, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA
[18]Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy
[19]Unit of Child Neurology and Psychiatry, Azienda Socio Sanitaria Territoriale (ASST) Spedali Civili of Brescia, Brescia, Italy
[20]Department of Neurology, Kaiser Permanente Bellevue Medical Center, Bellevue, Washington, USA
[21]Department of Pediatric Neurology, Neurogenetics Section, Hospital Universitario Quirónsalud, Madrid, Spain
[22]Facultad de Medicina, Universidad Europea, Madrid, Spain
[23]Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland
[24]Neurology Department, Epilepsy Program, Ruber Internacional Hospital, Madrid, Spain
[25]Child Neurology Unit, Hospital Universitari Vall d'Hebron, Vall d'Hebron Research Institute, Barcelona, Spain
[26]Department of Genomics, Turku University Hospital, Turku, Finland
[27]Department of Pediatric Neurology, Turku University Hospital, Turku, Finland
[28]Friedrich-Alexander-Universität Erlangen Nürnberg, Institute of Human Genetics, Erlangen, Germany
[29]Department of Genetics, University of Alabama at Birmingham, Birmingham, Alabama, USA
[30]Division of Translational Medicine and Human Genetics, Department of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA
[31]Division of Pediatric Neurology, Department of Pediatrics, Cohen Children's Medical Center, New Hyde Park, New York, USA
[32]Department of Zoology, Faculty of Biological Sciences, University of Lakki Marwat, Lakki Marwat, Pakistan
[33]Institute for Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany
[34]Department of Human Genetics, Amsterdam University Medical Center (UMC), University of Amsterdam, Amsterdam, the Netherlands
[35]Emma Center for Personalized Medicine, Amsterdam University Medical Center (UMC), University of Amsterdam, Amsterdam, the Netherlands
[36]University Children's Hospital, Paracelsus Medical University, Salzburg, Austria
[37]Department of Neurology, Erasmus Medical Center (MC) Sophia Children's Hospital, Rotterdam, the Netherlands
[38]Erfelijke Neuro-Cognitieve Ontwikkelingsstoornissen, Rotterdam, Erasmus Medical Center (ENCORE)-GRIN Expertise Center, Rotterdam, the Netherlands
[39]Department of Development and Regeneration, Section Paediatric Neurology, member of the European Reference Network EpiCARE, University Hospitals Leuven, Leuven, Belgium
[40]Genetic Services, Kaiser Permanente of Washington, Seattle, Washington, USA
[41]Department of Medicine, Surgery, and Neurosciences, University of Siena, Siena, Italy
[42]Molecular Medicine for Neurodegenerative and Neuromuscular Diseases Unit, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Stella Maris Foundation, Pisa, Italy
[43]Division of Neurology, Epilepsy Neurogenetics Initiative, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA
[44]Research Institute, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada
[45]Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada
[46]Division of Clinical Genetics, Department of Pediatrics, Columbia University Vagelos College of Physicians and Surgeons and New York-Presbyterian Morgan Stanley Children's Hospital, New York, New York, USA
[47]Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany
[48]Department of Medical Genetics, CHU de Rennes, Rennes, France
[49]Institute for Genomic Medicine, Columbia University Irving Medical Center, New York, New York, USA
[50]Department of Functional Neurology and Epileptology, member of the European Reference Network EpiCARE, Hospices Civils de Lyon and Lyon 1 University, Lyon, France
[51]Paediatric Neurology Unit, Department of Pediatrics, Hospital Universitari Germans Trias i Pujol, Universitat Autònoma de Barcelona, Barcelona, Spain
[52]Grupo de Investigación Biomédica en Red de Enfermedades Raras, Instituto de Salud Carlos III, Madrid, Spain
[53]Department of Pediatric Neurology, Neuroscience Center, Joe DiMaggio Children's Hospital, Hollywood, Florida, USA
[54]Division of Neurology, Children's Hospital of Eastern Ontario, Ottawa, Ontario, Canada
[55]Division of Neurology, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA
[56]Center of Excellence for Rare and Undiagnosed Diseases, Medical University of Warsaw, Warsaw, Poland
[57]Department of Neurology, Neurointensive Care and Neurorehabilitation, Christian Doppler University Hospital, member of the European Reference Network EpiCARE, Paracelsus Medical University, Center for Cognitive Neuroscience, Salzburg, Austria
[58]Neuroscience Institute, Christian Doppler University Hospital, member of the European Reference Network EpiCARE, Paracelsus Medical University, Center for Cognitive Neuroscience, Salzburg, Austria
[59]Medical Genomics Research Department, King Abdullah International Medical Research Center, King Saud Bin Abdulaziz University for Health Sciences, Ministry of National Guard Health Affairs, Riyadh, Saudi Arabia
[60]Department of Life Sciences, School of Science, University of Management and Technology, Lahore, Pakistan
[61]Center for Human Genetics, University Hospitals Leuven, Leuven, Belgium
[62]Department of Pediatrics, Erasmus Medical Center (MC)–Sophia Hospital, Rotterdam, the Netherlands
[63]Service de Génétique, Centre Hospitalier Universitaire (CHU) de Caen–Normandie, Caen, France

[64]Service de Neurologie, Centre Hospitalier Universitaire (CHU) de Caen–Normandie, Caen, France
[65]Applied and Translational Neurogenomics Group, Vlaams Instituut voor Biotechnologie (VIB) Center for Molecular Neurology, Antwerp, Belgium
[66]Department of Neurology, Antwerp University Hospital, Antwerp, Belgium
[67]Center for Adults with Disability (MZEB), Epilepsy Center Kleinwachau, Radeberg, Germany
[68]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

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CONFLICT OF INTEREST STATEMENT

E.T. has received consultancy fees from Arvelle Therapeutics, Argenx, Clexio, Celegene, UCB Pharma, Eisai, Epilog, Bial, Medtronic, Everpharma, Biogen, Takeda, LivaNova, Newbridge, Sunovion, GW Pharmaceuticals, and Marinus; speaker fees from Arvelle Therapeutics, Bial, Biogen, Böhringer Ingelheim, Eisai, Everpharma, GSK, GW Pharmaceuticals, Hikma, LivaNova, Newbridge, Novartis, Sanofi, Sandoz, and UCB Pharma; and research funding (directly or to his institution) from GSK, Biogen, Eisai, Novartis, Red Bull, Bayer, and UCB Pharma outside the submitted work. E.T. receives grants from the Austrian Science Fund, Österreichische Nationalbank, and the European Union. E.T. is the CEO of Neuroconsult. None is related to the present study. P.Z. has received speaking fees from Jazz Pharmaceuticals and Angelini Pharma. S.Wec. has received consultancy fees from UCB, Xenon Pharmaceuticals, Lundbeck, Knopp Biosciences, Encoded Therapeutics, Angelini Pharma, and Roche. G.R. has received speaker honoraria from UCB, Eisai, Angelini Pharma, and UNEEG. The remaining authors have no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available upon request from the corresponding authors. Furthermore, all *DLG4* variants were submitted to the ClinVar database (Tümer Group, Copenhagen University Hospital, www.ncbi.nlm.nih.gov/clinvar/submitters/509010/).

ORCID

Amanda M. Levy https://orcid.org/0000-0001-7863-4300
Elena Gardella https://orcid.org/0000-0002-7138-6022
Angel Aledo-Serrano https://orcid.org/0000-0003-4889-3365
Alice Dainelli https://orcid.org/0000-0002-5876-3508
Anne-Sophie Denommé-Pichon https://orcid.org/0000-0002-8986-8222
Antonio Gil-Nagel https://orcid.org/0000-0003-4515-0793
Hansel M. Greiner https://orcid.org/0000-0001-7479-1384
Lieven Lagae https://orcid.org/0000-0002-7118-0139
Gaetan Lesca https://orcid.org/0000-0001-7691-9492
Davide Mei https://orcid.org/0000-0001-6790-6251
Konrad Platzer https://orcid.org/0000-0001-6127-6308
Sylvain Rheims https://orcid.org/0000-0002-4663-8515
Eugen Trinka https://orcid.org/0000-0002-5950-2692
Zeynep Tümer https://orcid.org/0000-0002-4777-5802
Guido Rubboli https://orcid.org/0000-0002-5309-2514

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