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FULL-LENGTH ORIGINAL RESEARCH

Epilepsia®

Neonatal developmental and epileptic encephalopathy due to autosomal recessive variants in *SLC13A5* gene

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Abstract

Objective: Autosomal recessive pathogenic variants of the *SLC13A5* gene are associated with severe neonatal epilepsy, developmental delay, and tooth hypoplasia/hypodontia. We report on 14 additional patients and compare their phenotypic features to previously published patients to identify the clinical hallmarks of this disorder.

Methods: We collected clinical features of 14 patients carrying biallelic variants in *SLC13A5* and performed a PubMed search to identify previously published patients.

Results: All patients presented clonic or tonic seizures in the first days of life, evolving into status epilepticus in 57%. Analysis of seizure frequency and developmental milestones divided into five epochs showed an evolutionary trajectory of both items. In the first 3 years of life, 72% of patients had weekly/monthly seizures, often triggered by fever; 14% were seizure-free. Between the ages of 3 and 12 years, 60% become seizure-free; in the following years, up to age 18 years, 57% were seizure-free. After the age of 18 years, all three patients reaching this age were seizure-free. Similarly, 86% of patients at onset presented mild to moderate developmental impairment and diffuse hypotonia. In late childhood, all had developmental delay that was severe in most. Benzodiazepines, phenobarbital, phenytoin, and carbamazepine were the most effective drugs. Eight probands carried heterozygous compound variants, and homozygous pathogenic variants occurred in six. Literature review identified 45 patients carrying *SLC13A5* gene pathogenic variants whose clinical features overlapped with our cohort. A peculiar and distinguishing sign is the presence of tooth hypoplasia and/or hypodontia in most patients.

Significance: Autosomal recessive pathogenic variants in *SLC13A5* are associated with a distinct neonatal epileptic encephalopathy evolving into severe cognitive and motor impairment, yet with seizures that settle down in late childhood. Tooth

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hypoplasia or hypodontia remains the peculiar feature. The *SLC13A5* gene should be screened in neonatal epileptic encephalopathies; its recessive inheritance has relevance for genetic counseling.

KEYWORDS
autosomal recessive, development, epileptic encephalopathy, neonatal, *SLC13A5* gene, tooth hypoplasia

1 | INTRODUCTION

Developmental and epileptic encephalopathies (DEEs) are a spectrum of severe neurological disorders sharing developmental delay or regression and early onset intractable seizures, but with highly heterogeneous clinical features and genetic etiology. The genetic architecture of DEEs is currently being shaped thanks to the rising availability of high-throughput DNA sequencing approaches. An increasing number of DEEs have been associated with pathogenic variants in hundreds of different genes. Recent studies combining next generation sequencing (NGS) epilepsy gene panels, whole exome sequencing, and genome sequencing demonstrate that the genetic diagnostic puzzle might be solved in up to 50% of patients with DEEs.[1,2]

Autosomal recessive inherited loss-of-function variants of the *SLC13A5* gene (NM_177550) have been reported to cause epileptic encephalopathy, early infantile 25 (MIM 615905).[3,4] Patients carrying biallelic variants in *SLC13A5* have neonatal onset intractable seizures, subsequent developmental delay, and tooth hypoplasia or hypodontia.[3,4] The *SLC13A5* gene is located at chromosome 17p13.1, consists of 11 exons, and has both eukaryotic and prokaryotic homologs. It encodes a highly conserved homodimeric plasma membrane sodium-dependent citrate transporter (Na^+/CT), which is expressed in the brain, liver, and testis.[5]

The aim of this study is to collect an international cohort of patients carrying the *SLC13A5* gene pathogenic variants, to describe the clinical features of this disorder, and to correlate genotype-phenotype findings. We performed a literature review and compared the phenotypic features of previously published patients with our cohort to define distinctive features and clinical hallmarks of this disorder that might help clinicians in its early recognition and genetic diagnosis. The recessive inheritance of this condition enables focused genetic counseling for the families.

Key Points

- Biallelic pathogenic variants in *SLC13A5* are associated with a distinct neonatal onset epileptic encephalopathy
- Fever sensitivity, hypotonia with moderate to severe developmental impairment, and tooth hypoplasia are additional distinctive hallmarks
- The evolutionary trajectory shows that over time seizures tend to settle down, and in late childhood, seizure freedom may be achieved
- Cognitive and motor impairment evolve to plateauing in late childhood, yet most patients stabilize into a severe impairment
- The *SLC13A5* gene should be screened in neonatal epileptic encephalopathies

2 | MATERIALS AND METHODS

2.1 | Study design and setting

We performed a multicenter retrospective observational cohort study, selecting patients carrying *SLC13A5* gene pathogenic variants. Patients were collected by contact via email, principal national and international genetic laboratories and epilepsy centers, including collaborators of epilepsy and genetic networks. Nine epilepsy centers in Italy, France, Romania, Denmark, Croatia, and Germany responded to our call. Genetic and clinical findings were retrieved from medical records, and data were collected using a specific form and stored in a database.

The Marche region ethics committee approved this study.

2.2 | Study setup and data collection

2.2.1 | Clinical and laboratory findings

For each enrolled patient, we recorded demographic data including age at the time of the study, gender, age at genetic diagnosis, family history, and antenatal and perinatal history. The following clinical variables were assessed at disease onset and during follow-up: age at seizure onset, seizure type and frequency, seizure duration, episodes of status epilepticus, triggering factors, electroencephalographic (EEG) and brain magnetic resonance imaging (MRI) findings, treatment and response to treatment, neurological findings, motor and cognitive development, and additional features. We divided the assessment of clinical variables into different epochs according to the patient's age and last available follow-up: from 0 to 3 years (first epoch), older than 3 to 6 years (second epoch), older than 6 to 12 years (third epoch), older than 12 to 18 years (fourth epoch), and older than 18 years (fifth epoch). Seizure types were classified according to the 2017 Operational Classification of the International League Against Epilepsy,[6] and seizure frequency was reported as daily, weekly, monthly, or sporadic. Treatment was classified as effective when leading to seizure freedom or significant seizure reduction, noneffective when seizures persisted at the same frequency or worsened. The motor and cognitive development was defined as normal or delayed with mild, moderate, or severe impairment.

2.2.2 | Genetic analysis

Genetic variants were identified using direct Sanger sequencing for the candidate gene, NGS gene target panels, or whole exome sequencing (WES), performed in different research or diagnostic laboratories. Variants with minor allele frequency > 1% reported in the dbSNP (https://www.ncbi.nlm.nih.gov/projects/SNP/), 1000 Genomes Project (https://www.internationalgenome.org/), Exome Variant Server database (evs.gs.washington.edu), ExAC database (https://gnomad.broadinstitute.org), and Varsome (https://varsome.com/) were considered benign variants and excluded from the report.

The genetic variants were classified as "pathogenic" (class 5), "likely pathogenic" (class 4), "variants of unknown significance" (class 3), and "unlikely pathogenic variants" (class 2), according to criteria derived from a modified version of the classification proposed by Antoniadi et al.[7]

The traditional approach of Sanger sequencing was used to confirm all variants and to perform segregation analysis.

2.3 | Literature review

We searched PubMed using the term "*SLC13A5*" and included all studies reporting patient-relevant information, which were collected in a separate database.

3 | RESULTS

We included 11 unreported and three previously described patients (Family B and D in Hardies et al and Family 5 in Weeke et al).[4,8]

Six were male and eight were female, with a current median age of 12 years (range = 3-24, interquartile range [IQR] = 6-16). The median age at genetic diagnosis was 7 years (range = 3-22, IQR = 6-16). The family history for comorbid neurological conditions was negative in all subjects. All but one were born full-term (one late preterm), after an uneventful pregnancy. Twelve had an uncomplicated delivery, whereas two presented mild suffering with fetal tachycardia. All had normal birth weights and good Apgar scores. Tables 1 and 2 summarize relevant clinical information at onset and during follow-up.

3.1 | Clinical features at onset

All patients presented with neonatal onset epilepsy, 10 patients manifesting seizures in the first day of life, the remaining four during the second day of life. Seizures were focal motor and focal to bilateral, featuring clonic jerks in the majority (11/14, 79%) or, less frequently, tonic semiology (3/14, 21%). All patients had daily seizures, each lasting a few minutes but rapidly evolving into status epilepticus in 57% during the neonatal period. EEG recordings at onset showed slow background activity in three patients (21%). Epileptiform abnormalities were detected in 10 patients (72%), including

TABLE 1 Clinical findings at disease onset

Patient ID	Gender	Age at onset	Seizure type at onset	Frequency at onset	SE during neonatal age	Treatment at onset	Neurological examination at onset
1	M	D2	Focal motor, focal to bilateral clonic	Daily	Yes	DZP, PB, pyridoxine, PHT	Hypotonia, poor spontaneous movements
2	M	D2	Focal motor, tonic	Daily	Yes	PB	Hypotonia
3	F	D1	Focal motor, focal to bilateral clonic	Daily	Yes	PB	Hypotonia
4	M	D1	Focal motor, focal to bilateral clonic	Daily	Yes	PB, PHT, pyridoxine, LEV, lidocaine	Hypotonia
5	F	D1	Focal motor, focal to bilateral clonic	Daily	No	PB	Normal
6	F	D1	Focal motor, focal to bilateral clonic	Daily	No	PB	Normal
7	F	D1	Focal to bilateral tonic	Daily	Yes	PB, pyridoxine, PHT	Hypotonia
8	F	D1	Focal to bilateral tonic	Daily	Yes	PB, pyridoxine, PHT	Hypotonia
9	M	D1	Focal motor, focal to bilateral clonic	Daily	No	PB, MDZ, PHT, LEV	Hypotonia, poor spontaneous movements
10	F	D2	Focal motor, focal to bilateral clonic	Daily	No	PB	Hypotonia
11	F	D2	Focal to bilateral clonic	Daily	No	PB	Hypotonia
12	F	D1	Focal motor, clonic (alternating side)	Daily	Yes	PB, PHT	Hypotonia
13	M	D1	Focal motor, clonic (alternating side)	Daily	Yes	PB, PHT	Hypotonia
14	M	D1	Focal motor, focal to bilateral clonic	Daily	No	PB, PHT, pyridoxal phosphate, folinic acid	Hypotonia

Abbreviations: D1 = day 1; D2 = day 2; DZP = diazepam; F = female; LEV = levetiracetam; M = male; MDZ = midazolam; PB = phenobarbital; PHT = phenytoin.

focal (4/10, 40%), multifocal (5/10, 50%), and diffuse (2/10, 20%). Brain MRI was not informative at epilepsy onset in any patient but one, presenting with punctate white matter lesions, which disappeared by the age of 6 months. Neonates received treatment with several antiseizure medications, including phenobarbital (PB) that controlled seizure in six, and phenytoin (PHT) that controlled seizures in four. The neonatal period was marked by hypotonia in all patients but two (86%), associated with poor spontaneous motor movements in two (14%; Table 1).

3.2 | Clinical features at different epochs

Overall, patients had a median time of follow-up of 12 years (range = 3-24, IQR = 6-16). All of them were followed up to the first epoch (0 to 3 years), 13 up to the second >3 to 6 years), ten up to the third (>6 to 12 years), seven up to the fourth (>12 to 18 years), and three up to the fifth (>18 years of age; Table 2, Figure 1).

During the first epoch (all patients), seizures persisted from weekly to monthly frequency in most patients (10/14, 72%) and were often triggered by fever (10/14, 72%). Nine patients (64%) also experienced episodes of status epilepticus. Seizure semiology was focal motor (Figure 2A) and focal to bilateral, with a prevalent clonic component. Only two patients (14%) were seizure-free, and they discontinued treatment at 2 and 5 months. The other patients received polytherapy; valproate (VPA; 5/10, 50%) and carbamazepine (CBZ; 3/8, 37.5%) were the most effective.

Longitudinal EEG recordings revealed a normal (6/13, 46%) or slow (7/13, 54%) background activity, with focal (3/13, 23%), multifocal (4/13, 31%), or diffuse (2/13, 15%) epileptiform abnormalities.

TABLE 2 Clinical information during disease course and at last available follow-up

Patient ID	Current age, y	Seizure type during FU	Frequency at last FU	Fever sensitivity	Treatment during FU	Development delay at FU	Additional features
1	16	Focal motor and nonmotor	Sporadic	Yes	PB, LEV, VPA, CBZ, ZNS, HC, CLB, OXC	Severe	Tooth hypoplasia, scoliosis, dystonia, dyskinesias, tetraparesis
2	6	Focal to bilateral	Free	No	VPA	Severe	Tooth hypoplasia, hypotonia, ataxia, brisk reflex, microcephaly
3	10	Focal nonmotor	Free	Yes	PB, LEV, VPA	Severe	Tooth hypoplasia and hypodontia, ataxia, mild pyramidal signs
4	8	Focal to bilateral	Monthly	Yes	PHT, KD, VPA, CNZ, CLB, VGB, TPM	Severe	Tooth hypoplasia and hypodontia, hypotonia
5	5	Free	Free	No	None	Normal	None
6	3	Free	Free	No	None	Normal	None
7	24	Focal to bilateral	Free	Yes	PB, CLB, CBZ	Severe	Tooth hypoplasia and hypodontia, dyskinesias, tetraparesis
8	24	Focal to bilateral	Free	Yes	PB, CBZ	Severe	Tooth hypoplasia and hypodontia, dyskinesias, tetraparesis
9	5	Focal to bilateral	Sporadic	Yes	LEV, VPA, ZNS	Moderate	Tooth hypoplasia and hypodontia, ataxia, pyramidal signs
10	18	Focal to bilateral	Free	Yes	CBZ, VPA	Moderate	Tooth hypoplasia and hypodontia, axial hypotonia, ataxia, peripheral hypertonia, scoliosis
11	16	Focal to bilateral	Free	No	CBZ, TPM, CLB, NTZ, LEV, VPA	Severe	Tooth hypoplasia and hypodontia, ataxia, hypotonia
12	13	Focal motor and nonmotor	Free	Yes	VPA, CNZ, OXC, TPM	Moderate	Tooth hypoplasia and hypodontia, pyramidal and extrapyramidal signs
13	15	Focal motor and nonmotor	Free	Yes	VPA, OXC, TPM	Severe	Tooth hypoplasia and hypodontia, pyramidal and extrapyramidal signs
14	11	Focal motor and nonmotor	Free	Yes	PB, PHT, bromide, TPM, LEV, CLB, RUF, LTG, VPA, acetazolamide	Severe	Tooth hypoplasia and hypodontia, ataxia, dystonia, dyskinesias, delayed skeletal age

Abbreviations: CBZ, carbamazepine; CLB, clobazam; CNZ, clonazepam; FU, follow-up; HC, hydrocortisone; KD, ketogenic diet; LEV, levetiracetam; LTG, lamotrigine; NTZ, nitrazepam; OXC, oxcarbazepine; PB, phenobarbital; PHT, phenytoin; RUF, rufinamide; TPM, topiramate; VGB, vigabatrin; VPA, valproate; ZNS, zonisamide.

Brain MRI was performed in 11 patients and was normal in seven of them (64%). In three patients (27%), nonspecific hypersignal in T2 and fluid-attenuated inversion recovery (FLAIR) sequences in the periventricular white matter and mild corpus callosum hypoplasia were observed.

Examination revealed hypotonia in 12 patients (86%), associated with dystonia (3/12, 25%), dyskinesias (4/12,

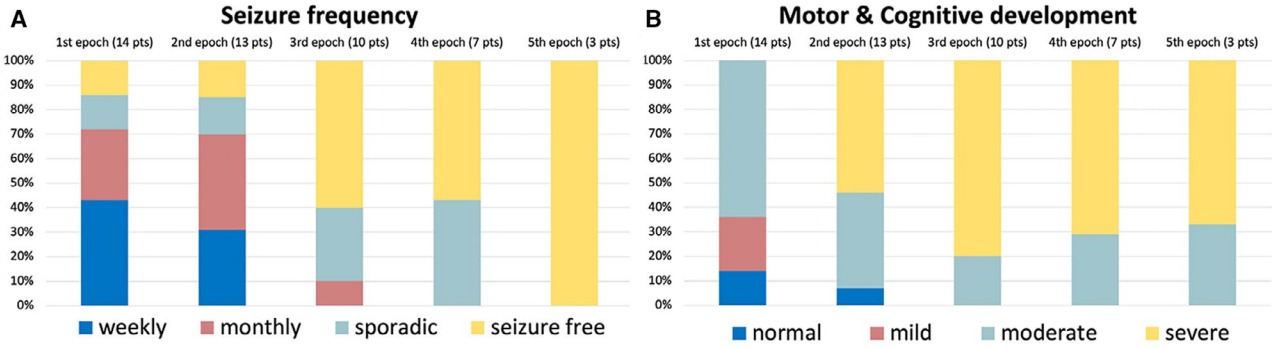

FIGURE 1 A, Seizure frequency outcome at different epochs. B, Motor and cognitive development outcome at different epochs. Patients (pts) were divided into the first (0 to 3 years), the second (>3 to 6 years), the third (>6 to 12 years), the fourth (>12 to 18 years), and the fifth epochs (>18 years of age)

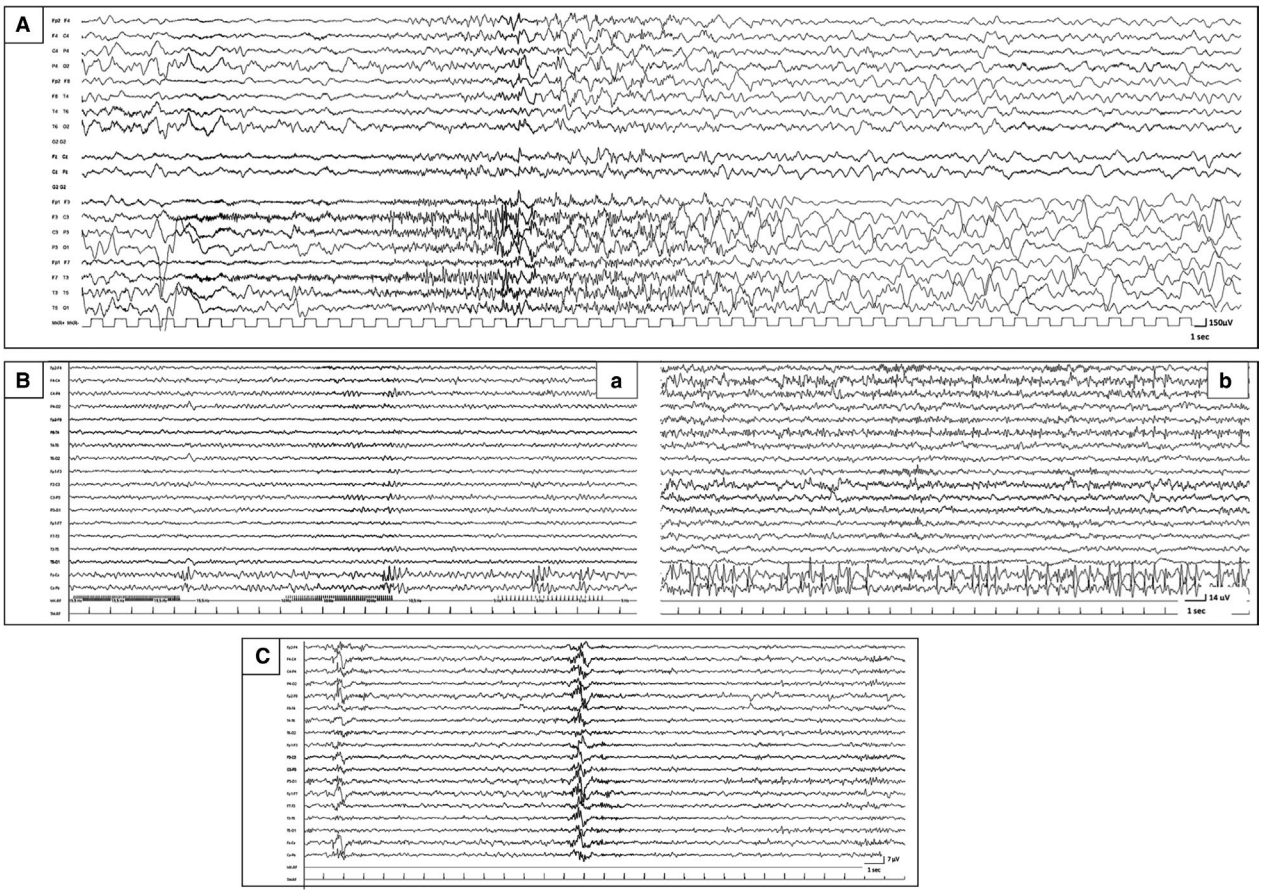

FIGURE 2 A, Electroencephalographic (EEG) recording of Patient 1 at age 1 year showing a focal seizure characterized by fast low-voltage epileptiform activity over the left centrotemporal region, evolving into a rhythmic discharge of polyspikes and sharp waves, followed by high-voltage slow waves over the same region. B, EEG recording of Patient 9 at age 4 years showing (a) during wakefulness, normal background activity and intermittent spike and sharp wave complexes over the vertex region; and (b) during sleep, such epileptiform activity increased in amplitude and frequency to become almost continuous over the vertex and centrotemporal regions with a right predominance. C, EEG recording of Patient 10 at age 18 years during sleep showing spikes, and sharp waves with anterior predominance, intermingled with spindles and positive occipital sharp transients of sleep

33%), ataxia (3/12, 25%), brisk reflexes (5/12, 42%), and microcephaly (1/12, 8%), variably combined. Motor and cognitive development was impaired in 12 patients (12/14, 86%), ranging from mild (3/12, 22%) to moderate (9/12, 64%) delay. Two patients (2/14, 14%) had a normal neurological examination and development. All patients but two (12/14, 86%) had tooth hypoplasia or hypodontia (Figure S1).

During the second epoch (13 patients), seizures diminished in frequency, and 54% (7/13) of patients had monthly and sporadic events. All patients but one, who was drug-free, received polytherapy, and VPA (5/12, 42%), CBZ (2/12, 17%), clobazam (CLB; 1/12, 8%), acetazolamide (1/12, 8%), and zonisamide (1/12, 8%) were the most effective drugs. Only one patient (8%) presented an episode of status epilepticus triggered by fever. EEG recordings were marked by slow background activity (5/13, 39%), with focal (4/13, 31%), multifocal (5/13, 39%; Figure 2B), or diffuse (2/13, 15%) epileptiform abnormalities. Repeated MRI in one patient showed nonspecific high signal in T2 and FLAIR sequences in the periventricular white matter, and three patients had mild corpus callosum hypoplasia. Motor and cognitive development ranged from normal (1/13, 7%) to moderate (5/13, 39%) and severe (6/13, 54%) impairment. Language skills were seriously impaired, particularly in patients with severe intellectual disability, yet despite such a severe degree of compromise, these patients maintained some social skills.

During the third epoch (10 patients), more than one-half of the patients (6/10, 60%) became seizure-free. In the remaining, sporadic seizures (3/10, 30%) were triggered by fever or poor adherence to treatment; only one patient had monthly seizures (1/10, 10%). No status epilepticus was recorded during this epoch. All patients but one received polytherapy with medications variably combined; the remaining patient received monotherapy. Therapy included PB (4/10, 40%), CBZ (3/10, 30%), VPA (6/10, 60%), levetiracetam (2/10, 20%), oxcarbazepine (OXC; 2/10, 20%), topiramate (2/10, 20%), PHT (1/10, 10%), CLB (1/10, 10%), clonazepam (CNZ; 1/10, 10%), and ketogenic diet (1/10, 10%). EEG recordings were marked by monomorphic or slow background activity (5/10, 50%), with focal (7/10, 70%) and multifocal (5/10, 50%) epileptiform abnormalities. Four patients underwent repeated MRI revealing a nonspecific hypersignal in T2 and FLAIR sequences in the periventricular white matter in one, and mild corpus callosum hypoplasia in three. Examination was characterized by hypotonia (2/10, 20%), ataxia (4/10, 40%), and by mixed pyramidal and extrapyramidal signs (9/10, 90%) variably combined in each patient. Motor and cognitive impairment ranged from moderate (2/10, 20%) to severe (8/10, 80%). Some social skills such as congruent smiling were present, but language was limited to a few words.

During the fourth epoch (seven patients), four (57%) patients were seizure-free, and three (43%) presented only sporadic seizures. The therapeutic drug load was progressively reduced, leaving patients on bitherapy (6/7, 86%) or monotherapy (1/7, 14%). The drugs included PB (3/7, 43%), CBZ (3/7, 43%), VPA (4/7, 57%), OXC (3/7, 43%), and CLB (1/7, 14%). EEG recordings were marked by monomorphic or slow background activity (4/7, 57%), with rare focal (4/7, 57%) and multifocal (3/7, 43%) epileptiform abnormalities. Repeated MRI in two revealed cortical atrophy.

Examination was characterized by hypotonia (1/7, 14%), by ataxia (2/7, 29%), and by mixed pyramidal and extrapyramidal signs (5/7, 72%). Motor and cognitive impairment ranged from moderate (2/7, 29%) to severe (5/7, 71%)

Three patients had a follow-up to the fifth epoch, and all were seizure-free on medication that included PB (2/3, 67%), CBZ (2/3, 67%), and VPA (1/3, 33%). EEG recordings were marked by monomorphic or slow background activity, with rare focal (1/3, 33%) and multifocal (2/3, 67%) epileptiform abnormalities (Figure 2C). Repeated MRI confirmed cortical atrophy in two patients.

Examination was characterized by pyramidal signs (1/3, 33%), mixed with extrapyramidal signs (2/3, 67%). Motor and cognitive impairment ranged from moderate (1/3, 33%) to severe (2/3, 67%). The latter were nonverbal but with some social skills.

3.3 | Genetic findings

NGS gene panels identified genetic variants in nine patients, WES detected variants in four (Patients 1, 12, 13, and 14), and direct gene sequencing, by Sanger approach, uncovered pathogenic variants in the last one (Patient 3). All variants identified by NGS gene panel were subsequently confirmed by direct Sanger sequencing. Six probands (6/14, 43%) were sporadic, whereas the remaining eight (8/14, 57%) belonged to four families with two affected siblings each. Two were monozygotic twins (Patients 7 and 8), and their parents were first cousins. All other families but one (Patient 14), had unrelated parents (Table 3). Genetic studies uncovered heterozygous compound pathogenic variants for two different missense variants in six subjects, including a family with two affected siblings (Table 3, Patients 5 and 6) and three sporadic probands. The sixth proband was a heterozygous compound for a missense variant and a multiple exon deletion (Patient 2). Two further siblings were heterozygous compound for a nonsense variant and a frameshift variant (Patients 12 and 13). Five subjects, including four siblings of two unrelated families and a single proband, were homozygous for two missense mutations and one nonsense stop variant. A sixth proband was homozygous for a novel splicing variant.

3.3.1 | Novel or recurrent single variants

Two missense variants were not described in association with the disease: c.1421C>T (p.Pro474Leu) and c.943G>A (p.Ala315Thr, rs201857984). The in silico analysis (using

TABLE 3 Summary of the heterozygous compound and homozygous pathogenic variants

Patient ID	cDNA change	Protein change	Mode of inheritance
1	c.655G>A rs144332569 CM146657	p.Gly219Arg	Heterozygous compound
	c.1421C>T Novel	p.Pro474Leu	
2	c.655G>A rs144332569 CM146657	p.Gly219Arg	Heterozygous compound
	del exons 1-5 Novel	del exons 1-5	
3	c.680C>T rs587777577 CM146656	p.Thr227Met	Heterozygous compound
	c.1570G>C rs863225448 CM1511264	p.Asp524His	
4	c.655G>A rs144332569 CM146657	p.Gly219Arg	Heterozygous compound
	c.1280C>T rs548065551 CM1511261	p.Ser427Leu	
5[a]	c.655G>A rs144332569 CM146657	p.Gly219Arg	Heterozygous compound
	c.943G>A rs201857984	p.Ala315Thr	
6[a]	c.655G>A rs144332569 CM146657	p.Gly219Arg	Heterozygous compound
	c.943G>A rs201857984 (n.d.)	p.Ala315Thr	
7[b]	c.997C>T rs773770609	p.Arg333*	Homozygous
8[b]	c.997C>T rs773770609	p.Arg333*	Homozygous
9[c]	c.425C>T rs761917087 CM1511263	p.Thr142Met	Homozygous
10[c]	c.425C>T rs761917087 CM1511263	p.Thr142Met	Homozygous
11	c.1475T>C rs1057519449	p.Leu492Pro	Homozygous
12[d]	c.1022G>A rs150203483	p.Trp341*	Heterozygous compound
	c.1207_1217dup11 rs863225447	p.Pro407Argfs*12	
13[d]	c.1022G>A rs150203483	p.Trp341*	Heterozygous compound
	c.1207_1217dup11 rs863225447	p.Pro407Argfs*12	
14	c.369-2A>G	—	Homozygous

Abbreviations: del, deletion; n.d., not described.

[a]Siblings.

[b]Siblings.

[c]Siblings.

[d]Siblings.

PolyPhen-2 analysis, MutationTaster analysis, and SIFT analysis) for these novel variants revealed a possibly damaging effect for the mutated protein (score = 1.000, 0.999, and 0.0, respectively). One novel splicing variant (c.369A>G) has not been described so far; in silico analysis (using splice site prediction [Berkeley Drosophila Genome Project] and Alternative Splice Site Predictor) showed a possible damaging effect for the abolition of the physiological acceptor splicing site.

The remaining nine single nucleotide substitutions and one deletion for 1-5 exons of the gene were previously described. Particularly, the c.655G>A (p.Gly219Arg, rs144332569) variant was identified in four subjects of our cohort and reported in 17 previously described probands either in homozygous form or in heterozygous form.[3,4,8–12] A recurrent single missense variant including the c.680C>T (p.Thr227Met, rs587777577) was reported in one of our probands (already described both in Hardies et al and in Weeke et al)[4,8] and in eight other published patients (one in homozygous form).[3,8,9,11] The c.1475T>C (p.Leu492Pro, rs1057519449) was detected in homozygous form in one of our patients and, as heterozygous compound in two previously described patients.[9,11] The c.425C>T (p.Thr142Met, rs761917087) was identified in two patients of our series and in three previously described.[4,10] The c.1280C>T (p.Ser427Leu, rs548065551) was detected in one patient of our series and in three previously described.[7] The nonsense stop variant c.997C>T (p.Arg333*, rs773770609), identified in homozygous form in two monozygotic twins, was also on record in three previously described patients.[10,11] One of our patients was heterozygous compound for a missense variant c.655G>A (p.Gly219Arg rs144332569) and an intragenic deletion of exons 1-5. Lastly, two already reported siblings were heterozygous compound for a nonsense stop variant c.1022G>A (p.Trp341*, rs150203483) and a frameshift variant c.1207_1217dup11 (p.Pro407Argfs*12, rs863225447).[4]

In Table 4 are reported all *SLC13A5* pathogenic variants identified in our cohort and comparison with literature.

TABLE 4 Summary of *SLC13A5* variants identified in our cohort and comparison with literature

Nucleotide substitution	AA variant	Mutation type	Present study: pts, n	Previous studies; pts, n	Reference
c.425C>T rs761917087 CM1511263	p.Thr142Met	Missense	2 pts (siblings)	3 pts	Hardies et al 2015,[4] Schossig et al 2017,[10]
c.655G>A rs144332569 CM146657	p.Gly219Arg	Missense	4 pts (2 siblings)	17 pts	Thevenon et al 2014,[3] Hardies et al 2015,[4] Klotz et al 2016,[9] Weeke et al 2017,[8] Schossig et al 2017,[10] Bainbridge et al 2017,[1] Anselm et al 2017[12]
c.680C>T rs587777577 CM146656	p.Thr227Met	Missense	1 pt[a]	8 pts	Thevenon et al 2014,[3] Hardies et al 2015,[4] Weeke et al 2017,[8] Schossig et al 2017,[10] Klotz et al 2016,[9] Bainbridge et al 2017[1]
c.943G>A rs201857984	p.Ala315Thr	Missense	2 pts (siblings)	—	Not described PolyPhen-2: probably damaging (score = 1.000) MutationTaster: disease-causing (score = 0.999) SIFT: damaging (score = 0.0)
c.997C>T rs773770609	p.Arg333*	Nonsense Stop codon	2 pts (siblings)	3 pts	Schossig et al 2017,[10] Bainbridge et al 2017[1]
c.1022G>A rs150203483	p.Trp341*	Nonsense Stop codon	2 pts (siblings)[a]	2 pts	Hardies et al 2015[4]
c.1207_1217dup11 rs863225447	p.Pro407Argfs*12	Frameshift	2 pts (siblings)[a]	2 pts	Hardies et al 2015[4]
c.1280C>T rs548065551 CM1511261	p.Ser427Leu	Missense	1 pt	3 pts	Weeke et al 2017[8]
c.1421C>T Novel	p.Pro474Leu	Missense	1 pt	—	Novel missense PolyPhen-2: probably damaging (score = 0.999) MutationTaster: disease-causing (score = 0.999) SIFT: damaging (score = 0.02)
c.1475T>C rs1057519449	p.Leu492Pro	Missense	1 pt	2 pts	Klotz et al 2016,[9] Bainbridge et al 2017[1]
c.1570G>C rs863225448 CM1511264	p.Asp524His	Missense	1 pt[a]	—	Hardies et al 2015,[4] Weeke et al 2017[8]
Exons 1-5 del		Exons del	1 pt	—	Novel del
c.369-2A>G		Splicing	1 pt	—	Novel spicing variant Splice site prediction (BDGP), ASSP Acceptor site prediction: abolished

Splice site prediction (BDGP): http://www.fruitfly.org/seq_tools/splice.html; ASSP: http://wangcomputing.com/assp/index.html.

Abbreviations: AA, amino acid; ASSP, Alternative Splice Site Predictor; BDGP, Berkeley Drosophila Genome Project; del, deletion; pt, patient.

[a]Previously published.

3.4 | Literature review

We identified 45 subjects from 22 different families, and further two sporadic patients from eight previous studies (Table S1).[3,4,8–13] Only three families harbored consanguinity loops.[3,4,10]

All patients presented seizures in the first days of life (91%) or within the first month. Seizure semiology was

focal motor with a prevalent clonic component in most (18 patients), but subclinical focal seizures were also described (13 patients). Early in the disease, a rapid progression into difficult to treat recurrent seizures and status epilepticus were present (17 patients). Multiple seizure types persisted over time from weekly to sporadic, sometimes triggered by fever, and later episodes of status epilepticus were reported.[4,8,9,12,13] In the follow-up, seizure semiology was mostly focal or focal to bilateral, and only one patient presented infantile spasms.[9] Epilepsy settled down in eight patients, and in three of them, it occurred between age 3 and 7 years.[3,4,8,10] Seven patients died, including three during the neonatal period,[8] two during status epilepticus in childhood,[4,8] one patient of sepsis,[8] and the seventh of unknown cause.[3]

EEG at disease onset in 27 patients[3,4,8,13] revealed normal background activity in most, but one had a suppression-burst pattern.[13] Epileptiform abnormalities were multifocal (10 patients) or focal (11 patients).[3,4]

Longitudinal EEG recordings, at variable ages, showed normal (four patients) or slow (eight patients) background activity and multifocal (13 patients) or focal (eight patients) epileptiform abnormalities.[4,9,10,12,13] Brain MRI was normal in the majority of the patients (30 patients). White matter hyperintensities were described in 10. Weeke et al[8] described punctate white matter lesions detected in seven patients during the neonatal age, later evolving into MRI changes suggesting gliotic scarring.

Patients were treated with benzodiazepines (BZP; diazepam, CLB, CNZ, lorazepam, midazolam; 9/39, 23%) and PB (10/27, 37%), as well as the sodium channel blockers PHT (6/8, 75%), CBZ (1/9, 11%), OXC (3/12, 25%), lamotrigine (LTG; 5/8, 62.5%), and lidocaine (3/6, 50%). Myoclonic seizures were exacerbated in some patients on PHT and LTG. [9] Other treatments included acetazolamide (5/5, 100%), VPA (5/14, 35.7%), stiripentol (STR; 3/3, 100%), and vagus nerve stimulation in a single patient. The efficacy of the ketogenic diet is still debated, as improved seizure frequency was reported in some patients (3/9, 33%),[4] whereas others showed increased seizure frequency (4/9, 44%).[9,11,12] Two patients were unsuccessfully treated with triheptanoin.[9]

All patients had motor and cognitive developmental impairment ranging from mild (2%) to moderate (4.5%) to severe (75.5%). So far, autistic traits have not been described in the variety of clinical features of patients, including the nonverbal ones. Developmental impairment in all patients was variably combined with other associated neurological signs and symptoms of different degrees, including axial hypotonia (14 patients), peripheral hypertonia (eight patients), spasticity (four patients), ataxia (five patients), choreoathetosis (five patients), microcephaly (four patients), dystonia (three patients), and dyskinesia (one patient). Moreover, most patients (29/45, 64%) manifested tooth hypoplasia and/or hypodontia (see Figure S1).

4 | DISCUSSION

Our study describes the clinical and genetic features of 14 probands, 11 novel and three included in two previous studies,[4,8] carrying autosomal recessive pathogenic variants in the *SLC13A5* gene. We compared these with the clinical data of 45 probands from previous studies.[3,4,8–13]

The clinical hallmark of all 56 patients was the onset of intractable focal, predominantly clonic, seizures in the first days of life, rapidly evolving into status epilepticus in early life. About 72% of probands presented an increase of seizure frequency with fever.

Following the stormy onset in our cohort, predominant focal and focal to bilateral seizures persisted during early childhood but with decreasing frequency. Subdividing patients into age epochs to match their ages while analyzing seizure frequency, we observed a progressive improvement of their epilepsy, leading either to sporadic or to no seizures in late childhood and early adulthood. Comparison with the literature cohort was not possible because of the limited data of earlier reports, where only eight patients had decreased seizure activity in late childhood.[3,4,8,10]

Similarly, motor and cognitive developmental milestones followed an evolutionary trajectory, as 86% of patients showed mild to moderate impairment with diffuse hypotonia at onset. By late childhood, all patients had a developmental delay that was severe in most, and the developmental curve seemed to reach a plateau in early adolescence. Expressive language was limited to a few words or completely absent in those with severe intellectual disability, but some social skills were preserved. Two patients had normal development in the early stage, eventually regressing or failing to progress before the age of 3 years. These two siblings with a mild clinical spectrum also achieved early seizure freedom; however, their short-term follow-up does not allow a firm conclusion; an evolution from a mild to a severe phenotype that could occur later in life cannot be excluded. About 64% of patients from the literature cohort presented severe motor and cognitive impairment at their last available follow-up. Furthermore, a range of neurological signs and symptoms variably combined have been reported and confirmed in our series, including axial hypotonia, peripheral hypertonia, spasticity, ataxia, choreoathetosis, microcephaly, dystonia, and dyskinesia. Tooth hypoplasia or hypodontia of later onset is a peculiar diagnostic characteristic, and has been described in 65% of the reported patients and confirmed in 86% of ours (Figure S1).

Approximately 30% of early infantile DEEs are caused by pathogenic variants in several genes, including *KCNQ2, SCN2A, KCNT1, KCNA2, STXBP1,* and *ALDH7A1*.[14–19] Pathogenic variants in these genes, either at the onset or during disease evolution, cause migrating focal seizures or a suppression-burst EEG pattern, and such distinctive clinical features might guide the genetic diagnosis. *SLC13A5* variants

instead cause a distinct form of neonatal DEE presenting in the first day of life with focal clonic seizures and frequent status epilepticus but neither associated with suppression-burst pattern nor with migrating focal seizures. A high fever sensitivity was observed both in about 60% of patients in the literature and in our cohort. Dravet syndrome and *PCDH19*-related disorder are the prototype of DEE with high fever sensitivity, in which age at onset and seizure types distinguish these conditions.

Several inborn errors of metabolism might also present with neonatal onset refractory seizures accompanied by suppression-burst pattern on EEG and subsequent neurodevelopmental disorders. These diseases, including PIGA (phosphatidylinositol glycan anchor biosynthesis class A) deficiency[20] and congenital disorders of glycosylation,[21,22] also comprise multiorgan involvement and autosomal recessive or X-linked inheritance.

Despite the stormy neonatal onset of intractable seizures, the DEE related to pathogenic variants in *SLC13A5* is not associated with peculiar interictal EEG patterns; in most patients, EEG showed normal background activity, with sparse slow waves and focal/multifocal epileptiform abnormalities. Such shortage of peculiar interictal EEG features is also observed in patients with other DEEs, including early stages of *CDKL5* deficiency presenting normal background activity and intermittent epileptiform abnormalities[23].

The *SLC13A5* gene encodes Na^+/CT expressed in the plasma membrane of neurons, and loss-of-function mutations result in Na^+/CT dysfunction with complete loss of citrate uptake.[4] However, the molecular mechanisms by which *SLC13A5* deficiency causes epileptic seizures and neuronal impairment in development and functions remain to be elucidated. Experimental evidence suggests that the absence of citrate in neurons might account for neuronal energy failure and for the dysfunction of γ-aminobutyric acid (GABA) signaling and hyperfunction of N-methyl-D-aspartate receptor signaling.[5,8] These findings may open therapeutic approaches aimed at increasing intracellular citrate concentration. Triheptanoin was proposed as an etiology-based treatment that could counteract the dysfunction caused by *SLC13A5* deficiency by potentially increasing the cytoplasmic pool of citrate. However, so far, the prescription of triheptanoin in two anecdotal cases was not effective and even worsened seizures.[9] The ketogenic diet, providing alternative energy sources to the brain, has been postulated to be beneficial,[4] but conflicting results have been reported on its efficacy for *SLC13A5*-associated epilepsy.[5,9,11,12] On the clinical level, improvement in seizures control has been reported in response to GABAergic drugs (PB and BZP), as well as to sodium channel blockers such as PHT, CBZ, and LTG, although in some reports, they may exacerbate myoclonic seizures.[9] In our series and anecdotal cases so far reported,[9] VPA may also be beneficial. STR combined with topiramate and CBZ has been reported to lead to a dramatic improvement in three patients,[13] and acetazolamide improved seizure control in five previously reported patients and one in our cohort.[9] It acts as a carbonic anhydrase inhibitor and may decrease urinary citrate excretion by inducing metabolic acidosis, which probably increases citrate reabsorption by renal NaDc1 transporter (SLC13A2).

Our cohort comprised a population of familial and sporadic patients, more than one-half of them carrying compound heterozygous variants. Autosomal recessive inheritance has to be considered in families with multiple affected siblings, and lack of consanguinity should not rule out genetic testing. Several single missense variants, including Gly219Arg, Thr227Met, Leu492Pro, and Thr142Met, were recurrent in several of our patients and in the literature cohorts. Current clinical and genetic data do not suggest genotype-phenotype correlations.

In conclusion, we have identified 56 patients who carry homozygous or compound heterozygous *SLC13A5* pathogenic variants. Analysis of their clinical data allows the recognition of a distinct neonatal onset DEE characterized by nonmigrating clonic seizures in the first days of life and rapidly evolving into status epilepticus in the absence of a suppression-burst EEG pattern. Subsequent fever sensitivity, hypotonia with moderate to severe developmental impairment, and tooth hypoplasia are additional distinctive hallmarks.

The common evolutionary trajectory is that the seizures tend to settle down, and by late childhood, the majority of patients become seizure-free. Likewise, cognitive and motor impairments plateau in late childhood, leaving patients severely impaired. About 50% of our patients have compound heterozygous variants and no evidence of consanguinity. Therefore, *SLC13A5* variants should be considered and tested in neonatal onset DEEs in families with one or more affected siblings, even in the absence of evident consanguinity. Considering the increasing number of epilepsy genes associated with early onset epileptic encephalopathies, NGS gene panels increase the likelihood of identification of pathogenetic variants. Genetic diagnosis of such infants is, at present, not amenable to tailored treatment, yet it allows focused genetic counseling and avoidance of unnecessary, invasive, and costly additional investigations.

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CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose.

ETHICAL PUBLICATION STATEMENT

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.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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