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Epilepsy phenotypes across the different age-ranges in *IQSEC2*-related encephalopathy: An Italian multicentre retrospective cohort study

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ABSTRACT

Background: Epilepsy is a hallmark of *IQSEC2*-related encephalopathy within a phenotypic variability ranging between early onset epileptic and developmental encephalopathy and X-linked intellectual disability with epilepsy.

Patients and Methods: Data including demographic aspects, gene variants, seizure semiology and timing, EEG features, neuroimaging and response to therapy were retrospectively collected in patients with *IQSEC2*-related epilepsy referring to 8 Italian tertiary centres.

Results: The reported cohort included 11 patients (8 males and 3 females). Mean age at the onset of epilepsy was 3.90±2.80 years. No cases were reported in the first year of life. No specific epileptic syndromes were recognized. Predominant seizure-types in the age range 12–36 months included focal onset tonic seizures with impaired awareness, myoclonic seizures, and late onset spasms. Generalized motor seizures were predominant in patients between 3 and 6 years and between 12 and 18 years while focal motor seizures with impaired awareness were the most represented types between 6 and 12 years. No patients experienced status epilepticus. EEG patterns included a delayed maturation of EEG organization, irregular focal or diffuse slow activity, multifocal or diffuse epileptiform abnormalities. No structural epileptogenic lesions were detected at MRI. Valproate, lamotrigine, clobazam, topiramate and levetiracetam were the most used antiseizure medication. Complete seizure freedom was achieved only in 2 patients.

Conclusions: Onset of epilepsy after the first year of age, predominance of focal seizures with impaired awareness and generalized motor seizures, no pathognomonic underlying epileptic syndrome and infrequent occurrence of status epilepticus emerged as the main features of *IQSEC2*-related epilepsy phenotype.

1. Background

The *IQSEC2* gene on chromosome Xp11.22 encodes a guanine nucleotide exchange factor that activates small GTPases belonging to the ARF family and interacts with proteins of the PSD-95 complexes in the postsynaptic spaces of excitatory synapsis [1]. The encoded protein is

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Table 1
Demographic features and epilepsy phenotypes of previously published patients with *IQSEC2*-related encephalopathy.

Refs.	Number of patients	Males	Females	Age at onset of epilepsy (years)	Patients with epilepsy	Patients without epilepsy	Main seizure Types (number of patients when available)	Main EEG features (number of patients when available)	Main neuroimaging features (number of patients when available)	Response to treatment
Morleo et al. [14]	1	0	1	1	1	0	GOMM GOMES	H	Diffuse brain atrophy	DR
Gilissen et al. [15]	1	0	1	18	1	0	GOMTC	NA	NA	NA
Tran Mau-Them et al. [16]	3	3	0	3.5	2	1	GOMM FMS	NA	Diffuse brain atrophy Periventricular white matter changes	NA
Fieremans et al. 2015[17]	1	0	1	NA	0	1	NA	NA	NA	NA
Moey et al. [18]	4	4	0	5	1	3	GOMM	SBA DSAFD.	NA	DR
Kalscheuer et al. [19]	14	7	7	NA	1	13	NA	NA	NA	NA
Zerem et al. [2]	18	12	6	4.1	18	0	GOMA (4 onset+6 FU) GONMA (1 onset+3FU) GOMM (2 onset+3 FU) GOMES (2 onset + 3 FU) GOMTC (1 onset+ 3 FU) FS (0 onset + 1 FU)	SBA H DEA MFEA	White matter changes. Diffuse brain atrophy	DR
Madrigal et al. [20]	6	3	3	NA	0	6	NA	NA	NA	NA
Ewans et al. [21]	5	0	5	2.7	4	1	FMS	NA	NA	NA
Helm et al. [22]	6	5	1	1.4	6	0	NA	NA	Diffuse brain atrophy. Anterior cortical abnormalities. Thinning white matter.	DR (good response to levetiracetam in one case)
Zipper et al. [23]	1	1	0	0.7	1	0	FMS GOMTC	FEA	NA	DR
Radley et al. [24]	14	6	8	NA	11	3	GOMTC GOMM	NA		NA
Mignot et al. [1]	42	22	20	3.4	35	7	GONMT (17) GOMTC (13) GOMT (12) GOMA (11) GOMM (10) GOMES (9) FOMCD (4).	SBA (16) MFEA (8) FEA(6) DEA (8) FSA (5). H(4)	Normal (21) Mild brain atrophy (10) Thin corpus callosum (NA) White matter changes (NA).	DR (15/27 cases)
Wayhelova et al. [25]	2	2	0	4.5	2	0	GOMA FMS	SBA INFEA IDEA	Periventricular White matter changes	DR
Lopergolo et al. [4]	12	4	8	2	7	5	GOMTC GONMA	SBA	Diffuse brain atrophy	DR (good response to leveticetam in one case)
Liu et al. [26]	4	4	0	2	4	0	GOMES GOMTC	INFEA	Diffuse brain atrophy	DR
Leoncini et al. [3]	19	9	10	NA	18	1 F	NA	NA	NA	DR
Nagabushana et al. [27]	1	1	0	0.25	1	0	FMS GOMES	H	Normal	good response to steroids
Nakashima et al. [28]	1	1	0	2.5	1	0	NA	SBA	0	DR

LEGEND: GOMA= Generalized Onset Motor Atonic; **FMS**=Focal Motor Seizures; **NA**=not available; **GOMM**= Generalized Onset Motor Myoclonic; **GOMES**= Generalized Onset Motor Epileptic Spasms; **GOMTC**= Generalized Onset Motor Tonic-Clonic; **GONMA (ATYPICAL ABSENCES)**=Generalized Onset Non Motor Atypical (Absences); **GONMT (TYPICAL ABSENCES)**=Generalized Onset Non Motor Typical (Absences); **GOMT**=Generalized Onset Motor Tonic; **FS** = Focal Seizures; **FOMCD**= Focal Onset Motor Clonic And Dyscognitive; **SBA**= Slowing Background Activity; **INFEA**= Interictal Focal Epileptiform Abnormalities; **IDEA**= Ictal Diffuse Epileptiform Abnormalities; ***H***= Hypsarrhythmia; **FU**=Follow-Up; **DEA**=Diffuse Epileptiform Abnormalities (generalized spike and waves, polyspikes and

waves, slow spikes and waves); **MFEA**= Multifocal Epileptiform Abnormaslities**; FSA**= Focal Slow Activity**; DSAFD**= Diffuse Slow Abnormalities With Frontal Dominance.

involved in the construction of dendritic spine morphology and in the regulation of glutamate-mediated excitatory synaptic transmission [1, 2].

A PubMed search (last access=April 11 th, 2024) has found evidence of 155 published patients (84 males and 71 females) with *IQSEC2*-related encephalopathy and an estimated prevalence ranging between 7.0 and 7.9 × 10–74 was recently calculated after a recent structured survey on 19 Italian patients (Table 1) [3–5].

The clinical spectrum of *IQSEC2*-related encephalopathy includes variable degree of severity ranging from early onset epileptic and developmental encephalopathy in males to X-linked intellectual disability with epilepsy in females [2].

This study retrospectively evaluated the clinical distribution of epilepsy phenotypes across the different age ranges in a cohort of Italian patients with *IQSEC2*-related encephalopathy.

2. Patients and methods

Patients with a diagnosed *IQSEC2*-related developmental and epileptic encephalopathy referring to 8 Italian tertiary centres were considered eligible for the study.

A digital data sheet was filled by the specialized physicians involved in the management of epilepsy for each patient. The collected data included demographic aspects, gene variants, epilepsy onset and evolution over time, seizure types, presence of specific epileptic syndromes, occurrence of status epilepticus, developmental phenotypes, EEG features across different age-ranges, neuroimaging, response to therapy.

Gene variants were automatically interpreted with Franklin by Geenox (https://franklin.genoox.com/clinical-db/home) and manually refined according to the American College of Medical Genetics and Genomics (ACMG) criteria and ACGS Best Practice Guidelines [6,7].

A detailed description of non-neurological features of almost all the patients reported here were previously reported in another previously published collaborative study [3].

Seizure types and epilepsy syndromes were classified according to the 2017 ILAE Commission for Classification and Terminology taxonomy [8,9].

The analysed age-ranges for the evaluation of EEG features were a) premature born at 24–27 weeks of gestation; b) premature born at 28–31 weeks of gestation; 3) premature born at 32–36 weeks of gestation, 4) term newborns (0–28 days of life); 5) 29 days-12 months; 6) 12–36 months; 7) 3–6 years; 8) 6–12 years; 9) 12–18 years; 10) > 18 years. EEG evaluation included maturation/organization of the traces (i.e., consistency with the physiological age-related EEG structure), the presence of specific EEG pathological patterns at onset and during the follow-up (i.e., suppression burst, hypsarrhythmia, etc.), the distribution of the EEG abnormalities (e.g. focal/multifocal or focal secondarily generalized/diffuse abnormalities) [10–12].

The Italian *IQSEC2* parents' association supported the data collection providing the details about the physicians and the site of the follow-up for epilepsy of each patient [13].

The study received an ad-hoc IRB approval (PNRR-MR1-2022-12376284) and a parental written informed consent was obtained for each one of the included patients.

3. Results

3.1. Cohort composition

The analysed cohort included 11 patients (8 males and 3 females) with a mean age of 12.45±9.89 years (Table 2). All patients but one were born at term, five male patients presented with a congenital microcephaly while another male was small for gestational age (Table 2). Five patients experienced various perinatal problems including jaundice, feeding difficulties, necrotizing enterocolitis, and hypoglycaemia.

Mean age at molecular genetic diagnosis was 8.09±3.75 years with no significant differences between male and females. The detected *IQSEC2* gene variants were summarized in Table 3. Eight variants were de novo while an X-linked inheritance was assessed in two cases (Table 3). Franklin Automatic ACMG Classification predicted 4 variants as likely pathogenic, 4 variants as VUS and 3 variants as pathogenic (Table 3). Four VUS shifted to likely pathogenic with the manual application of ACMG rules (Table 3).

A delay in the achievement of developmental milestones was assessed before the onset of seizures in all patients (Table 4). Independent walking was achieved in 5 patients. Motor behaviour Assessment Scale scores ranged between 16 and 61 (mean 39.22±2.12). A severe intellectual disability was diagnosed in 9 patients while two patients (Patient 7 and 8 in Table 2) had a borderline cognitive functioning (Table 2). Autistic traits were noticed in two patients (Patient 2, 3 and 5 in Table 2).

3.2. Epilepsy phenotypes

Mean age at the onset of epilepsy was 3.90±2.80 years while mean duration of epilepsy (i.e. age at last seizure-age at the onset of seizures) was 6.06±3.66 years (Table 3). No specific epileptic syndromes were recognized. None of them had seizures during the first year of life. (Table 4). Predominant seizure-types in the age range 12–36 months included focal onset tonic seizures with impaired awareness, myoclonic seizures, and late onset spasms (Table 4). Generalized motor seizures were predominant in patients between 3 and 6 years and in the age range 12–18 years while focal motor seizures with impaired awareness were the most represented types in children between 6 and 12 years (Table 4). All patients experienced recurrent clusters of seizures in the different age-ranges even if in any case in-hospital acute emergency treatments were required. Three patients (patients 2, 6 and 11) presented with recurrent paroxysmal non-epileptic events (mainly including prolonged motor stereotypes, dystonic postures and staring) requiring LTM-video EEG monitoring recordings to be differentiated from epileptic seizures.

The EEG was characterized by signs of delayed maturation, involving both posterior background activity in wake as well as the sleep architecture, and excess of irregular slow activity in almost all patients across all the analysed age-ranges (Table 4). A significant interhemispheric asynchrony was recorded in the only patient who underwent EEG during the neonatal period (Patient 1 in Table 4). Interictal and/or ictal epileptiform abnormalities were mainly focal, especially after the age of 6, with the predominant involvement of frontal and, less frequently, occipital regions (Table 4). Multifocal and diffuse abnormalities were, respectively, predominant in the age range 3–6 years and 6–12 years and less frequent in patients older than the age of 12 (Table 4, Fig. 1, Fig. 2).

No patients had evident epileptogenic lesions on brain MRI. Two patients presented with a mild diffuse cortical and subcortical atrophy (Patients 3 and 4), two exhibited cortical atrophy in the frontal areas (Patients 1 and 7).

Valproate was the most used antiseizure medication (9 out of 11 patients with periods of seizure control of variable duration in 4 cases) followed by lamotrigine, clobazam, topiramate and levetiracetam (Table 4). Ketogenic diet was administered in one patient but it was suspended because of inefficacy (Patient 10 in Table 4). Complete seizure freedom was achieved only in 2 patients (Patient 4 and 10 in Table 4).

Table 2
Demographic and neurodevelopmental features of the reported cohort.

PATIENTS	SEX	Age (Years)	Age at molecular genetic diagnosis (Years)	Gestational age at birth	Weight at birth	Length at birth	OFC At birth	Perinatal problems	Developmental milestones before the onset of seizures	Higher Motor Function Achieved (Age at achievement)	Motor Behaviour Assessment Scale	Higher Language development Achieved (Age at achievement)	Cognitive outcome	Autism Spectrum Disorder
1	M	3	2	38+1	2170	45	32	SGA Cardiotocographic abnormalities Failure to progress during delivery Jaundice Hypoglycaemia	Delayed	Rolling (2.8y)	54	Vocalization (7 m)	Severe Intellectual disability	Yes
2	M	16	10	39	3560	53	36	None	Delayed	Autonomous Walking (2.5 y)	34	Vocalization, Babbling (2.8 y)	Severe Intellectual disability	No
3	F	16	9	39	3100	50	34	None	Delayed	Walking with support (age 2.8)	61	Vocalization (3 y)	Severe Intellectual disability	Yes
4	M	17	11	39	2140	45	31	Suction difficulties	Delayed	Autonomous walking (16 m)		No language	Severe Intellectual disability	Yes
5	F	14	10	38	2600	47	31	None	Delayed	Autonomous walking (age?)	44	No language	Severe Intellectual disability	Yes
6	M	3	2	41 + 6	3580	50	34,5	Failure to progress during delivery	Delayed	Sitting Position (3y) Rolling (3.1y)		Bubbling (3.2y)	Severe Intellectual disability	No
7	M	16	11	30	749	32	24,5	Prematurity Jaundice Necrotizing enterocolitis CMV infecttion	Delayed	Autonoumous walking (3.5 y)	21	No language	Borderline functioning	No
8	M	9	7	40	3600	50	35	None	Delayed	Autonoumous walking (3.2)	16	No language	Borderline functioning	No
9	F	13	6	38	3140	49	34,5	Jaundice	Delayed	18 m	31	Less than 30 words (4y)	Severe Intellectual disability	No
10	M	13	7	39	3400	50	33	None	Delayed	Quadrupedal walking (6y)	41	Bubbling (3y)	Severe Intellectual disability	No
11	M	17	14	39	3090	49	32	Feeding difficulties	Delayed	Partially autonomous Walking (2.6)	51	Vocalization (age 2.8)	Severe Intellectual disability	No

Table 3
Gene variants detected in the reported cohort.

Patient (NGS methods)	Gene Variant NM_001111125.3 (NP_001104595.1) Ref. 10	Mutation type	Inheritance	Prediction softwares	Ref	ClinVar/ dbSNPrs	Genomic Position (GRch37/hg19)	Exon	Franklin Automatic ACMG Classification	ACMG rules applied manually
1 **Whole Exome Sequencing ClinEx pro kit 4 bases NovaSeq6000 platform (Illumina)**	c.2459+1G>*A*	Splicing	Maternal	dbscSNV: Deleterious Splice AI: Splice-altering / Strong		/	chrX-53,277,902 C > T	6	Likely Pathogenic PVS1 PM2	
2 **Whole Exome Sequencing ClinEx pro kit 4 bases NovaSeq6000 platform (Illumina)**	c.2750–2A>*G*	Splicing	De Novo	dbscSNV Deleterious Splice AI: Splice-altering / Strong		/	chrX-53,272,655 T > C	9	Likely Pathogenic PVS1 PM2	*PS2 (de novo confirmed) Pathogenic
3 **Whole exome sequencing Platform Ion Torrent Ion AmpliSeq™Exome Kit**	c.3011T>C (p. Leu1004Pro)	Missense	De Novo	Revel Deleterious (Moderate) MetaLR Deleterious		/	chrX-53,270,970 *A* > *G*	10	VUS PM2PP3PM1	*PS2 (de novo confirmed) Likely Pathogenic
4 **Epilepsy gene panel Mi-Seq (Illumina), Paired-End 150 bp Protocol**	c.1133G>*T* p. (Arg378Leu)	Missense	De Novo	Revel Uncertain MetaLR Benign (Low)		/	chrX-53,283,980 C > A	4	VUS PM2	*PS2 (de novo confirmed) -> *Likely Pathogenic*
5 **Epilepsy gene panel Mi-Seq (Illumina), Paired-End 150 bp Protocol.**	c.2272C>T (p. Arg758*)	Nonsense	De Novo	/	1, 2, 3	280,180 Pathogenic criteria provided, multiple submitters, no conflicts /dbSNPrs886041433	chrX-53,279,486 *G* > *A*	5	Pathogenic PVS1PM2PS4PP5	*PS2 (de novo confirmed) Pathogenic
6 **Clinical exome "Gexome Clinical"- Eurofins Genoma Group**	c.2507C>T (p. Ala836Val)	Missense	De Novo	Revel Deleterious (Moderate) MetaLR Deleterious	4, 5	377,978 Pathogenic/Likely pathogenic criteria provided, multiple submitters, no conflicts /dbSNPrs782099475	chrX-53,277,371 *G* > *A*	7	Likely Pathogenic PS2PP3PM2PP5	*PS2 (de novo confirmed) PM1 (Sec7 domain) Pathogenic
7 **Whole Exome Sequencing ClinEx pro kit 4 bases NovaSeq6000 platform (Illumina)**	c.526G>C (p. Gly176Arg)	Missense	De Novo	Revel Benign (Moderate) MetaLR Benign		/	chrX-53,349,796 C > G	1	VUS PM2	*PS2 (de novo confirmed) Likely Pathogenic
8 **Whole Exome Sequencing ClinEx pro kit 4 bases NovaSeq6000 platform (Illumina)**	c.2488_2490delTCC (p. Ser830del)	In frame deletion	Maternal	/		/	chrX-53,277,387 TGGA>*T*	7	VUS PM2PM4	*PM1 (Sec7 domain) Likely Pathogenic
9 **Epilepsy gene panel Mi-Seq (Illumina), Paired-End 150 bp Protocol**	c.4110_4111del p. (Tyr1371Glnfs*15)	Frameshift	De Novo				chrX-53,263,756 TAC>T	15	Likely Pathogenic PVS1PM2	*PS2 (de novo confirmed) Pathogenic
10 **Epilepsy gene panel Mi-Seq (Illumina), Paired-End 150 bp Protocol**	c.854del (p. Pro285Leufs*21)	Frameshift	De Novo		6,7,8	619,985 Pathogenic criteria provided, multiple submitters, no conflicts / dbSNPrs782460038	chrX-53,285,126 AG>*A*	3	Pathogenic PVS1PS2PM2PP5	*PS2 (de novo confirmed) Pathogenic
11 **Clinical Exome ClinEx pro kit 4 bases NovaSeq6000 platform (Illumina)**	c.2984G>*A* (p.Arg995Gln)	Missense	Maternal	Revel Deleterious (Supporting) MetaLR Deleterious (Low)	9	280,590 Pathogenic/Likely pathogenic criteria provided, multiple submitters, no conflicts / rs886041767	chrX-53,270,997 C > T	10	Pathogenic PS4PM2PM5PM1PP3PP5	

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1) 10.1038/s41436–018–0268–1; 2) 10.1186/s13229–019–0284–2; 3) 10.26508/lsa.201900386; 4) https://doi.org/10.1016/j.ejmg.2019.103735; 5) https://doi.org/10.1111/cge.13507; 6) 10.1038/s41598–021–86131–3; 7) 10.1002/ajmg.a.62138; 8) 10.1038/s41436–018–0268–1; 9) 10.1002/mgg3.1880; 10) PMC6752297.
NOTE: According to ACGS PM2 is applied at moderate strength.

Table 4
Epilepsy phenotypes in the reported cohort.

	EPILEPSY TIMING		SEIZURE TYPES						EEG							ASM
PATIENTS	Age at the onset of seizures	Age at the achievement of seizure freedom	0–28 days	29 days-12 months	12 months-3 years	3–6 years	6–12 years	12–18 years	0–28 days	29 days-12 months	12 months-3 years	3–6 years	6–12 years	12–18 years	>18 years	Effective treatments
1	1	NA	None	None	FOIAMOT GOMM GOMES	None	None	None	IA MVDW EAFR	ISAMO	HVDW MFEA DEA	ISAMO MFEA	NP	NP	NP	HC CLZ
2	11	NA	None	None	None	None	None	FOIAMOM GOMTC GOMT GONMEM UOMTC	NP	NP	NP	NP	DSA MBA DLW DEA	DSA	NP	VPA LEV
3	1,5	NA	None	None	FOIAMOT FOIANMOBA GOMT	FOIANMOBA GOMT	FOIANMOBA GOMT	FOIANMOBA	NP	NP	IA ISAMO EAFR	ISAMO MFA EAFR	IA ISAMO MFA EAFR MFEA	IA MVDW MFA ISAMO EAFR MFEA	NP	None
4	4	7	None	None	None	UOMTC	UOMTC	None	NP	NP	NP	MFEA		EAFR DEA	NP	LEV
5	4	NA	None	None	None	FOANMOA	FOIANMOBA	None	NP	NP	NP	DSA MFEA	DSA MFEA	DSA MFEA	NP	VPA
6	2,16	NA	None	None	FOAMOT GOMT GOMM GOMMA GOMA GOMES	None	None	FOIANMOBA	NP	ISAMO	ISAMO	NP	NP	NP	NP	VPA LTG
7	2,58	NA	None	None	FOIAMOTA, FOIAMOT	FOIAMOA	FOIAMOA	FOIAMOA GOMT	NP	NP	OEA	OEA	OEA	OEA	NP	None
8	6,16	NA	None	None	None	FOIAMOC	None	None	NP	NP	NP	EAFR	EAFR	NP	NP	None
9	3,25	NA	None	None	None	FOIAMOT	FOIAMOT	FOIAMOT	NP	NP	NP		MFA EAFR	DSA MFA EAFR MFEA	NP	VPA
10	2,33	NA	None	None	FOIAMOA, FOIAMOT	FOIAMOA GOMC	FOIAMOA GOMC	FOIAMOA GOMC GOMT	NP	NP	NP	MFEA	DSA MFA MFEA ISAMO OEA DEA	ISAMO MFA EAFR	NP	TPM CLB
11	5	11	None	None	None	GOMA GOMMA	None	None	NP	NP	NP	DSA DEA	DSA DEA	DSA DEA	DSA	VPA LTG

LEGEND= NA=Not achieved; Focal Onset Impaired Awareness Motor Onset Tonic= **FOIAMOT;** Generalized Onset Motor Myoclonic=**GOMM;** Generalized Onset Motor Epileptic Spasms= **GOMES;** Focal Onset Impaired Awareness Non Motor Onset behaviour Arrest=**FOIANMOBA;** Generalized Onset Motor Tonic=Gomt; Focal Onset Aware Motor Onset Tonic=**FOAMOT;** Generalized Onset Motor Myoclonic Atonic=**GOMMA;** Generalized Onset Motor Atonic=**GOMA;** Generalized Onset Motor Epileptic Spasms**: GOMES;** Focal Onset Impaired Awareness Motor Onset With Automatisms=**FOIAMOTA;** Focal Onset Impairment Awareness Motor Onset Atonic=**FOIAMOA;** Unknown Onset Motor Tonic-Clonic=**UOMTC;** Focal Onset Aware Non Motor Onset Autonomic=**FOANMOA;** Focal Onset Impaired Awareness Motor Onset Clonic=**FOIAMOC;** Generalized Onset Motor Clonic=**GOMC;** Focal Onset Aware Non Motor Onset Autonomic=**FOANMOA;** Focal Onset Impaired Awareness Motor Onset Myoclonic=**FOIAMOM;** Generalized Onset Motor Tonic-Clonic=**GOMTC;** Generalized Onset Non Motor (Absence) Eyelid Myoclonia= **GONMEM; IA**= Interhemispheric asynchrony**; MVDW**=Moderate voltage delta waves**; EAFR**= Epileptiform abnormalities in frontal regions**; ISAMO**-=Irregular Slow Activity Mainly Occipital**; HVDW**= High Voltage Delta Waves**; MFEA**= Multifocal epileptiform abnormalities**; DEA**= Diffuse epileptiform abnormalities; **OEA**= Occipital epileptiform abnormalities**; MFA**= Moderate fast activity**; DSA**= Diffuse slow activity**; MBA**= Moderate beta activity**; DLW**= Diffuse low voltage**; NP**= Not performed**; HC**= Hydrocortisone**; CLZ**=Clonazepam**; VPA**=Valproic Acid**; LEV**= Levetiracetam**; LTG**= Lamotrigine**; TPM**= Topiramate**; CLB**= Clobazam.

Fig. 1. EEG of patient 10 at the age of 7 showing multifocal spike and waves with a predominance in the anterior regions.

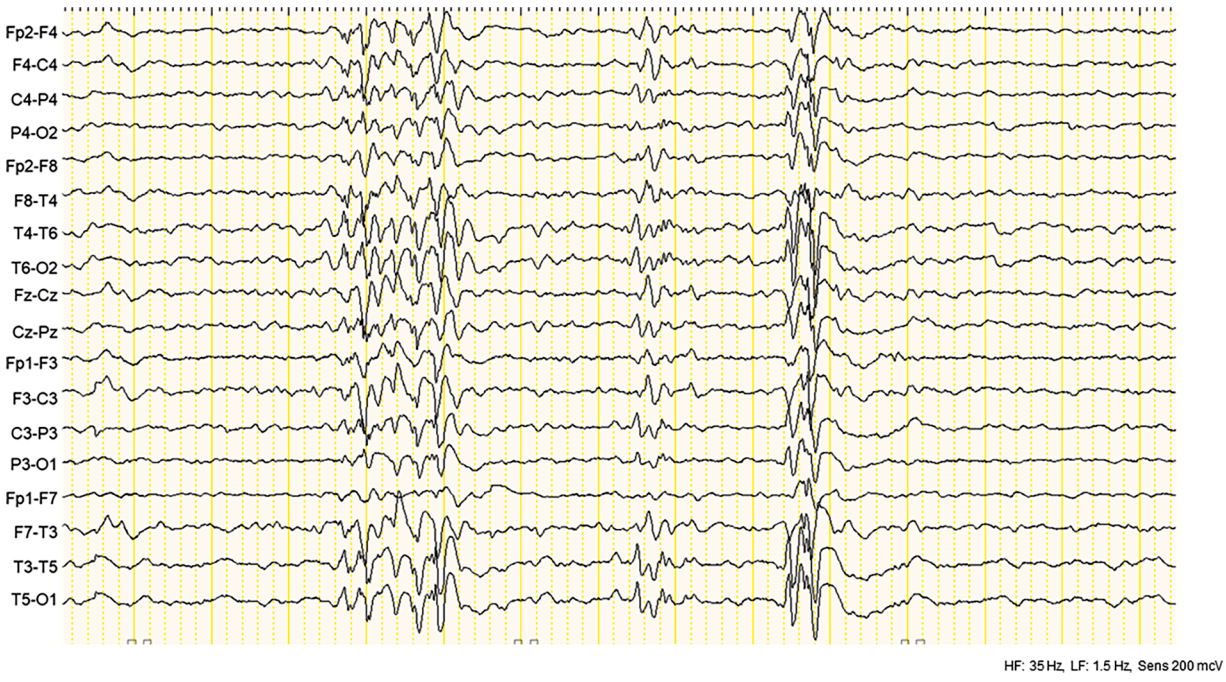

Fig. 2. EEG of patients 11 at the age of 14 showing diffuse spike and waves discharges.

4. Discussion

Epilepsy is a hallmark of *IQSEC2*-related encephalopathy affecting 114/145 published patients, namely up to 70 % of females and 95 % of males (Table 1) [1–4]. Drug-resistance has been assessed in most patients (Table 1) [1–4]. Recent *in vitro* studies, based on patient-derived induced pluripotent stem cells, have suggested that the main epileptogenic mechanisms might be represented by a neuronal hyperexcitability due to increased sodium and potassium currents in the early stages of neurodevelopment and to a concomitant reduction in the number of GABA expressing neurons [5]. This early hyperexcitability progressively attenuates with a subsequent deficient synaptic transmission and increased neuronal mortality in the later stages yielding the neurodevelopmental impairment beyond the effects of epilepsy itself [5].

The analysis of the herein reported cohort highlighted variable presenting patterns of epilepsy phenotypes with some peculiar aspects including onset of epilepsy after the first year of age, predominance of focal motor/non motor seizures with impaired awareness and generalized motor seizures, no pathognomonic underlying epileptic syndrome and infrequent occurrence of status epilepticus (Table 4). The onset of seizures mainly after the first year of age and the predominance of motor seizures overlapped with the ones of previously published patients while the frequency of focal seizures was higher and represented the first manifestation and the main seizure types in all the age-ranges (Table 1, Table 4) [1–4,14–28]. Unlike in other published series, no relevant

differences were noticed between males and females in terms of age at epilepsy onset, seizure frequency and response to treatment and it was not demonstrated an evident correlation between the severity of intellectual disability and the course of epilepsy[1–4,14–28].

Interictal EEG patterns in the reported cohort confirmed some previously reported non-specific features including a delayed maturation of EEG organization, irregular focal or diffuse slow activity, multifocal or diffuse epileptiform abnormalities (Tables 1, 4, Figs. 1 and 2). The presence of slow abnormalities was recorded in all the age-ranges with the frequent persistence of diffuse delta waves up to late adolescence (Table 4). The persistence of interictal excessive slow abnormalities across all the lifespan was confirmed in a mouse model of *IQSEC2*- encephalopathy in which no epileptic seizures were recorded in the latter stages as a probable epiphenomenon of cognitive/developmental dysfunctions that are not related to epilepsy [29]. The proportion of interictal epileptiform abnormalities progressively increased after the age of 12 months with a peak between 3 and 12 years and a stabilization in the following age-range (Table 4). A higher frequency of focal epileptiform abnormalities than the ones reported in other case series was observed with the predominant involvement of frontal and occipital regions (Tables 1, 4) [1,2]. These focal EEG abnormalities might result from an altered structural connectivity in pre-frontal regions and visual networks including occipital cortex [30]. A dysfunctional connectivity in these areas were documented in mutant mice carrying the *IQSEC2* variant A350V and correlated with autistic features (mainly including impairment of socio-communicative domains) even if the eventual presence of EEG epileptiform abnormalities was not investigated [30]. Hypsarrhytmia was not detected in none of the patients reported here despite late onset epileptic spasms were observed in two children after the age of 12 months (Table 4). Other previously published cohorts included 13 patients presenting with a classic infantile spasm syndrome associated with hypsarrhytmia (Table 1) [1,2,14,31,32]. The different frequency of this pattern in our cohort is not unusual, as EEGs of up to 40 % of children with infantile spasms does not exhibit hypsarrhythmia at the time of diagnosis and does not correlate with clinical severity [32].

The limited number of patients included in the herein reported cohort did not allow to assess specific genotype-phenotype correlations because the detected variants were distributed throughout the whole gene (Table 3). Conversely, Zerem et al. had reported in a previously published cohort of patients with epilepsy that 71 % of pathogenic variants was concentrated in the functional domains including the IQ like motif, Sec 7, and pH domain[2].

The present study expanded the number of transient effective antiseizure medications with a large variability depending on seizure-type and confirmed an underlying diffuse drug-resistance (Table 4). The recurrent paroxysmal non-epileptic events in 3 patients raise the question whether the incidence epilepsy and the frequency of drug-resistance might be overestimated in *IQSEC2*-related encephalopathy. Precision therapeutic strategies under development might improve the whole patient's outcome in the near future [29,33,34]. Most promising therapeutic targets include Thyrotropin Releasing Hormone (TRH) cascade, AMPA-mediated synaptic transmission and its temperature-related regulation or administration of *IQSEC2* mini-gene via adeno-associated virus (AAV) vectors [29,33,34].

The strength of the study is mainly represented by the collection of data about the epilepsy phenotype of *IQSEC2*-related encephalopathy encompassing different age-ranges with the subsequent possibility to depict a longitudinal portrait. The most important limitations are strictly correlated with its retrospective nature and with the small number of patients included due to the rarity of *IQSEC2*-related encephalopathy: (a) influence of different clinical approaches and diagnostic pathways, follow-up planning and therapeutic schedules by different physicians; (b) heterogeneity in the timing of data collection; (c) high rate of missing data reducing the potential for a longitudinal analysis of neurodevelopmental outcome.

5. Conclusions

The variability of epilepsy phenotypes observed in the reported cohort of patients with *IQSEC2*-related encephalopathy and the evolution of different electroclinical patterns over the years follow the same trajectories of other epileptic and developmental encephalopathies. The previously reported differences in clinical severity between males and females was not confirmed in our experience. The implementation of international patients registries might represent an useful strategy for a better phenotypic characterization.

Declaration of generative AI and AI-assisted technologies in the writing PROCESS

The authors declare that no parts of the manuscript were written by AI devices.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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