Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA IN

Scienze Biomediche e Neuromotorie

Ciclo XXXII

Settore Concorsuale: 06/D6

Settore Scientifico Disciplinare: MED/26

EPILEPTIC AND DEVELOPMENTAL ENCEPHALOPATHIES: CLINICAL AND GENETIC STUDY OF ADULT PATIENTS ATTENDING A TERTIARY EPILEPSY CENTER

Presentata da: Carlotta Stipa

Coordinatore Dottorato

Prof. Pietro Cortelli

Supervisore

Prof. Paolo Tinuper

Co-supervisore

Prof. Pietro Cortelli

Esame finale anno 2019

INDEX

1. INTRODUCTION

1.1. The concept of Epileptic and Developmental Encephalopathy

1.1.1. *Epilepsy and intellectual disability*

Epilepsy is a disorder resulting in abnormal discharges of electrical activity in neurons of certain parts of the brain, which may sometimes spread to the entire brain. With respect to the general population, people with epilepsy have been recognised to have more cognitive and behavioural problems (1). These difficulties are the consequence of overlapping factors, including ictal and interictal abnormal neuronal activity and also underlying brain structural abnormalities and antiepileptic drugs.

Approximately a quarter of all people with epilepsy have intellectual disability (ID) and one fifth of people with intellectual disability have epilepsy. The incidence of epilepsy rises as the severity of ID increases (2).

According to the American Association on Intellectual and Developmental Disabilities (AAIDD), the term ID, which has replaced the term "mental retardation", indicates a neurodevelopmental disorder that occurs in childhood and adolescence, characterized by limitations in both intellectual functioning (IQ<70) and in adaptive behaviour. Intellectual functioning refers to general mental capacity, such as learning, reasoning, problem solving. Adaptive behaviour is the collection of conceptual, social, and practical skills that are learned and performed by people in their everyday lives.

Based on the intellectual functioning, quantified in terms of intelligence quotient (IQ), there are 4 different levels of severity:

1) Mild ID: IQ between 55 and 70
2) Moderate ID: IQ between 40 and 55
3) Severe ID: IQ between 25 and 40
4) Profound ID: IQ less than 25

In the general population, the estimated prevalence of ID is about 1 to 3% (3), the prevalence of epilepsy is between 0.6 and 1% (4).

In people with ID epilepsy is more frequent and affects 22.2% of patients (5). Regarding people with epilepsy, 16% have an ID (5).

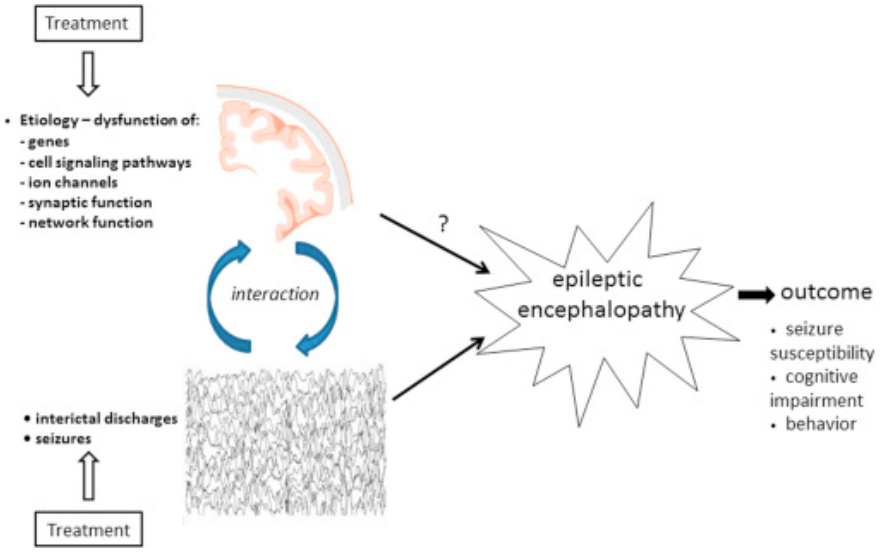
A multicentre Italian study on more than 1.000 patients with drug-resistant epilepsy (SOPHIE study, Study of Outcome of Pharmacoresistance in Epilepsy) showed that 22.8% of adult

patients with epilepsy have intellectual disability and 32.7% have a psychiatric comorbidity (6), especially mood disorders, anxiety and relational troubles.
Psychiatric disorders are more frequent with respect to the general population also in patients with ID (5).

1.1.2. *The definition of epileptic encephalopathy*

The concept of epileptic encephalopathy (EE) has been continuously reviewed from the first description in 1841, when WJ West described the syndrome that took his name, characterized by muscular spasms, psychomotor regression and hypsarrhythmia on the EEG as well.
Boundaries of epileptic encephalopathies were gradually outlined thanks to the discovery of many other syndromes.
Since 1957 Landau and Kleffner have speculated that a cognitive delay could be provoked by epileptic activity (7). Gastaut et al. in 1966 used for the first time the term EE to report that the epileptic activity itself can contribute to the impairment of psychomotor development, a condition that occurs for example in children with early onset severe epilepsy and frequent epileptiform activity (8).
This idea was developed also by Dulac in 2001 (9). In the proposal of the International League Against Epilepsy (ILAE) classification in 2001 the term EE is used for conditions in which epileptiform abnormalities themselves are believed to contribute to the progressive disturbance in cerebral function (10). The ILAE in 2010 added the concept that in EE the epileptic activity itself may contribute to cognitive and behavioural impairments above and beyond what might be expected from the underlying pathology alone, and these can worsen over time (11).
Pathophysiological mechanisms that relate the epileptic activity to cognitive impairment are unknown: interictal epileptiform discharges may have a transient interference with cognitive processes or more lasting effects, both in the involved cerebral areas and in areas that are far from the epileptogenic focus but connected with it (12).
An important consequence of this definition is that this problem is potentially reversible, at least in part. There is direct evidence of this statement in some children with a history of cognitive or language problems in which a partial reversibility of the intellectual disability has been obtained after improvement of the EEG following a surgical (13) (14) or pharmacological (15) treatment.
As illustrated in Figure 1, the definition of EE should include only the situations in which the epileptiform activity causes the cognitive and behavioural deficit (16).

Figure 1 Schematic representation of epileptic encephalopathies (Shao, 2016)



In 2017 the ILAE commission for terminology classification reviewed the concept of EE, stating that "a lot of epileptic syndromes associated with encephalopathy have a genetic aetiology and in these cases cognitive and behavioural problems result not only from the effect of the frequent ictal and interictal epileptic activity, but also from the direct effect of the genetic mutation".

These two variables can contribute to determining the phenotype in different ways.

Developmental delay can be clear since the beginning and it can get worse or plateau when the seizures begin. A well characterized example of this situation is Dravet Syndrome, where a developmental slowing and/or regression appears between 1 and 2 years of age, before seizures become an important aspect of the syndrome. When the seizures start, they cause a worsening of cognitive skills, suggesting that both factors have an important role in the progression of the neuropsychological delay.

In other situations, the developmental delay can occur on a normal background, before the seizures and the epileptic EEG activity start.

Based on these concepts, the terminology of EE has been redefined in the following way (17):

1) Developmental encephalopathy (DE): when the developmental delay depends on the underlying condition and the epileptic activity, ictal or interictal, is not responsible for a further slowing or regression;
2) Epileptic Encephalopathy (EE): when there is no delay before the seizure onset;
3) Epileptic and developmental encephalopathy: when both factors play a role.

It is important to distinguish between these different encephalopathies, even if it is not always easy to, because it is not only a terminology matter, but also a practical one: treatment of EE must be more aggressive because the worsening of the cognitive skills depends on the epileptic activity, and by reducing this activity it is possible to obtain an improvement of cognitive performances.

In developmental encephalopathies, where the cognitive impairment depends on the underlying condition more than on the epileptic activity, overtreatment cannot be necessary. It is well known that antiepileptic drugs, especially polytherapy, can worsen the neuropsychological development (18).

With regard to the aetiology, identifiable causes of the EE and DE are numerous and heterogeneous, acquired or genetic, and it is not always possible to recognize an underlying cause or neurological disease.

Even though some syndromes are more frequently associated with an EE, this particular clinical picture can potentially, be associated with any kind of epilepsy (19). An EE can appear at any age, even if it is more often associated with severe epilepsies with infant onset (20).

Table 1 shows electroclinical syndromes based on ILAE classification.

Table 1 - Electroclinical syndromes considered “epileptic encephalopathies” by the International League Against Epilepsy Classification (Kalser 2018)

Electroclinical syndrome	Age of onset	Prevalent seizure type	EEG	Imaging	Aetiology	Treatment			Seizure and neurodevelopmental outcome
						1st line	2nd line	Avoid	
NEONATAL AND INFANTILE ONSET									
Ohtahara syndrome or early infantile epileptic encephalopathy	<3 months	Tonic spasms 1/3 develop other SZ type: focal motor, generalized tonic [clonic]	Interictal: suppression-burst pattern in wakefulness and sleep Ictal: attenuation/low-voltage fast activity with tonic SZ	Normal Nonspecific brain abnormality: HIE, MCD, agenesis of corpus callosum or of mammillary bodies, porencephaly, others	Structural brain abnormality Genetic: *STXBP1, KCNQ2, SCN2A, ARX*, genes causing structural brain abnormalities Metabolic	CS LEV	KD ZNS VGB PB		Ongoing pharmacoresistant SZ Evolution into West syndrome (~75%) and further into LGS (~12%) Limited development with severe cognitive and motor impairment Reduced life expectancy with early death in infancy
Early myoclonic encephalopathy	<3 months	Erratic fragmentary myoclonі 80% with focal tonic, focal nonmotor autonomic, tonic spasms	Interictal: suppression-burst pattern, sleep>>wakefulness, suppression up to 20 s Ictal: no correlation to erratic myoclonus	Often normal at onset May develop cerebral atrophy	Metabolic: nonketotic hyperglycinemia, pyridoxine/P5P disorders, methylmalonic/propionic acidemia Genetic: *ERBB4, SLC25A22*	[a]	KD	VGB	Ongoing pharmacoresistant SZ EEG: suppression-burst pattern, possible transitory atypical hypsarrhythmia (3–5 months) Neurological impairment from the onset and limited neurodevelopment Reduced life expectancy (~50% die within the 2nd year of life
Epilepsy of infancy with migrating focal seizures or migrating partial seizures of infancy	<6 months (mean 3 months)	Migrating focal motor SZ (involvement of multiple independent cortical regions) May have SZ with autonomic features Frequent SE	Interictal: may be normal at onset. Diffuse slowing of background activity, multifocal spikes enhanced in sleep Ictal: migrating epileptic foci involving independent cortical regions randomly and consecutively	Normal at onset May develop cerebral/cerebellar atrophy, MTS	Genetic: *KCNT1* (~50%), *SCN2A* (~25%), others	LEV CLZ	STP KD CS KBr		Ongoing pharmacoresistant SZ Infrequent evolution into West syndrome or decrease of SZ >1 year Possible normal development at onset, then stagnation/regression, subsequent severe cognitive/motor impairment, acquired microcephaly Reduced life expectancy
West syndrome	2–12 months	Epileptic spasms May have focal	Interictal: may be normal at onset. Hypsarrhythmia, enhanced in sleep Ictal: high amplitude sharp-slow wave discharge followed by fast activity/diffuse voltage attenuation	Normal Nonspecific brain abnormality: pre/perinatal injuries (HIE, PVL, encephalitis, stroke), developmental anomalies (MCD, TSC, Aicardi syndrome)	Structural brain abnormality (see imaging) Chromosomal syndromes: Down, Miller-Dieker Genetic: *CDKL5, ARX, STXBP1, SPTAN1*, other Metabolic	CS[b] VGB	TPM KD ZNS BDZ VPA LEV	CBZ	May develop other epilepsy-type Possible evolution into LGS Normal or delayed development prior onset, then stagnation/regression, variable developmental outcome. ~80% with known aetiology have cognitive, behavioural ± motor impairment
Dravet syndrome	4–15 months	Febrile/afebrile prolonged unilateral/generalized clonic/tonic clonic	Interictal: normal at onset. Diffuse slow background activity, generalized and multifocal spike-wave discharges enhanced in sleep Photosensitivity Ictal: variable according to SZ type	Normal at onset Possible development of cerebral atrophy, MTS	Genetic: *SCN1A* (~90%), *PCDH19, GABRA1, GABRG2, HCN1, STXBP1*	VPA[c] CLB[c] STP[c] TPM	KD CBD KBr FEN	CBZ OXC LTG PHT PGB TGB VGB GBP	Ongoing pharmacoresistant SZ: myoclonus, focal, atonic, atypical absences ± nonconvulsive SE, SE May have less SZ >5 year Normal early development, slowing/regression 1–2 year, then cognitive/behavioural impairment of various degree, seldom normal Normal neurological examination at onset, later ataxia and pyramidal signs Risk of SUDEP and death during SE

Electroclinical syndrome	Age of onset	Prevalent seizure type	EEG	Imaging	Aetiology	Treatment			Seizure and neurodevelopmental outcome
						1st line	2nd line	Avoid	
CHILDHOOD ONSET									
Epilepsy with myoclonic-atonic seizures or Doose syndrome	6months–6years (peak 3–4 years)	Myoclonic-atonic often preceded by febrile SZ/ generalized tonic-clonic Also: atonic, myoclonic, atypical absence ± nonconvulsive SE, generalized tonic-clonic	Interictal: normal or slow background activity (parietal theta), generalized spike/ polyspike-wave, enhance in sleep, ± photosensitivity Ictal: myoclonic-atonic SZ with generalized spike/ polyspike-wave followed by high-voltage slow-wave	Normal	Genetic (~10%): *SLC2A1*, *SLC6A1*, *CHD2*, *GABRA1*, *GABRG2*, *SCN1A*, *SCN1B*, *KCNA2*	VPA LTG KD	CLB ESM RFM FLB	CBZ PHT VGB	Frequently pharmacoresistant SZ, subsequent remission within 3–5 years or persistence of epilepsy with nocturnal tonic SZ Normal development prior onset in most, possible stagnation/ regression with onset, variable cognitive and behavioural outcome
Lennox-Gataut syndrome	1–8 years (peak 3–5 years)	Tonic from sleep ± tonic SE Multiple SZ type: generalized (tonic)-clonic, atypical absence, atonic, spasms, focal, nonconvulsive SE, myoclonic, myoclonic-atonic	Interictal: focal/diffuse slow background activity, slow spike wave and paroxysmal fast activity in slow-wave sleep, focal/ multifocal spike waves Ictal (according to SZ type): electrodecrement with tonic SZ, slow spike-wave with atypical absences	Normal Nonspecific brain abnormality (see West syndrome)	Structural brain abnormality (see imaging) Metabolic Genetic: various genes	VPA LTG	TPM RFM FLB CLB LEV CS KD VNS	BDZ[d]	Ongoing pharmacoresistant SZ Developmental delay of various degree prior onset, then stagnation/regression, subsequent cognitive and behavioural impairment
Landau-Kleffner syndrome	2–8 years (peak 5–7 years)	Not present in all patients Focal (rolandic)	Interictal: waken EEG normal or focal/diffuse slowing, focal uni-/bilateral temporal-parietal sharp/ spike-waves. Strong activation with NREM sleep Ictal: according to SZ type	Normal	Genetic: *GRIN2A* (~10–20%)	CS CLB STM	VPA ETM LEV	CBZ OXC	EEG-changes and SZ are self-limiting in mid-teens in most Normal development prior onset, then progressive speech and language regression ± other cognitive/ behavioural impairment Variable residual language impairment after offset
Epileptic encephalopathy with continuous spike-wave during slow wave sleep (ECSWS)	2–12 years (peak 4–5 years)	Not present in all patients Multiple SZ type: focal, typical/ atypical absences, atonic, negative myoclonus	Interictal: waken EEG may be normal or focal/diffuse slowing, focal sharp/ spike-waves, poor sleep architecture, diffuse synchronous slow spike-waves in >50% NREM sleep Ictal: according to SZ type	Normal Nonspecific brain abnormality: pre/perinatal injuries (stroke, thalamic injury), developmental anomalies (MCD)	Structural brain abnormality (see imaging) Genetic (see LKS)	CS CLB STM	VPA ETM LEV	CBZ OXC	EEG-changes and SZ are self-limiting before adulthood in most Normal development prior to onset (when no structural lesion is present), cognitive/ motor/behavioural regression with onset, common language impairment and/or other neuropsychological sequelae after offset

ASD, autism spectrum disorder; BDZ, benzodiazepines; CBZ, carbamazepine; CLB, clobazam; CLZ, clonazepam; CS, corticosteroids; EEG, electroencephalography; ESM, ethosuximide; FEN, fenfluramine; FLB, felbamate; FU, follow up; GBP, gabapentin; HIE, hypoxic ischemic encephalopathy; KBr, potassium bromide; KD, ketogenic diet; LEV, levetiracetam; LGS, Lennox-Gastaut syndrome; LTG, lamotrigine; MCD, malformation of cortical development; MTS, mesial temporal sclerosis; OXC, oxcarbazepine; PB, phenobarbitone; PGB, pregabalin; PHT, phenytoin; RFM, rufinamide; SE, status epilepticus; STM, sulthiame; STP, stiripentol; SUDEP, sudden unexpected death in epilepsy; SZ, seizure; TGB, tiagabine; TPM, topiramate; TSC, tuberous sclerosis complex; VGB, vigabatrin; VPA, valproic acid; ZNS, zonisamide.

[a]Treatment of metabolic disorder where possible.

[b]ACTH or Prednisolone.

[c]Combination treatment.

[d]Small risk of precipitating tonic SZ.

1.1.3. *The aetiology of epileptic encephalopathy*

EE can have multiple causes: acquired structural causes (hypoxic-ischemic encephalopathy, trauma, infection and stroke), infectious (meningitis, encephalitis), metabolic (folate deficit, pyridoxine-dependent epilepsy), autoimmune (coeliac disease) and genetic (17).

This group is the most numerous. Even in the presence of a structural abnormality or metabolic, an underlying genetic cause should be searched for (17).

A genetic aetiology for EE was discovered for the first time in 2001, when a de novo *SCN1A* mutation was found in seven children with Dravet syndrome (21).

With the advent of molecular techniques, such as chromosomal microarray and next generation parallel sequencing of multiple genes, a rapid growth in gene discovery for epileptic encephalopathies has occurred (22).

The percentage of patients with genetically determined EE where it is possible to identify a causative genetic abnormality is between 17% and 40% in different studies (23). De novo mutations in affected patients are the most common. Rare cases are due to chromosomal abnormalities, copy number variations (CNVs), inborn errors of metabolism and cortical malformations (22).

Phenotypic pleiotropy

Phenotypic heterogeneity or pleiotropy, in which mutations in a single gene cause different phenotypes, is increasingly recognised in epilepsy.

The epilepsy syndromes associated with a gene might range from a benign seizure disorder to an epileptic encephalopathy, as exemplified by several of the ion channel genes (eg, *KCNQ2*, *SCN1A*, *SCN2A*). Mutations in KCNQ2, for example, are associated with self-limited syndrome benign familial neonatal epilepsy and with a severe neonatal onset epileptic encephalopathy, characterised by tonic seizures and profound developmental impairment. Mutations in SCN1A are reported in genetic epilepsy with febrile seizures plus, a mild self-limited epilepsy that often does not need treatment, and in Dravet syndrome (22).

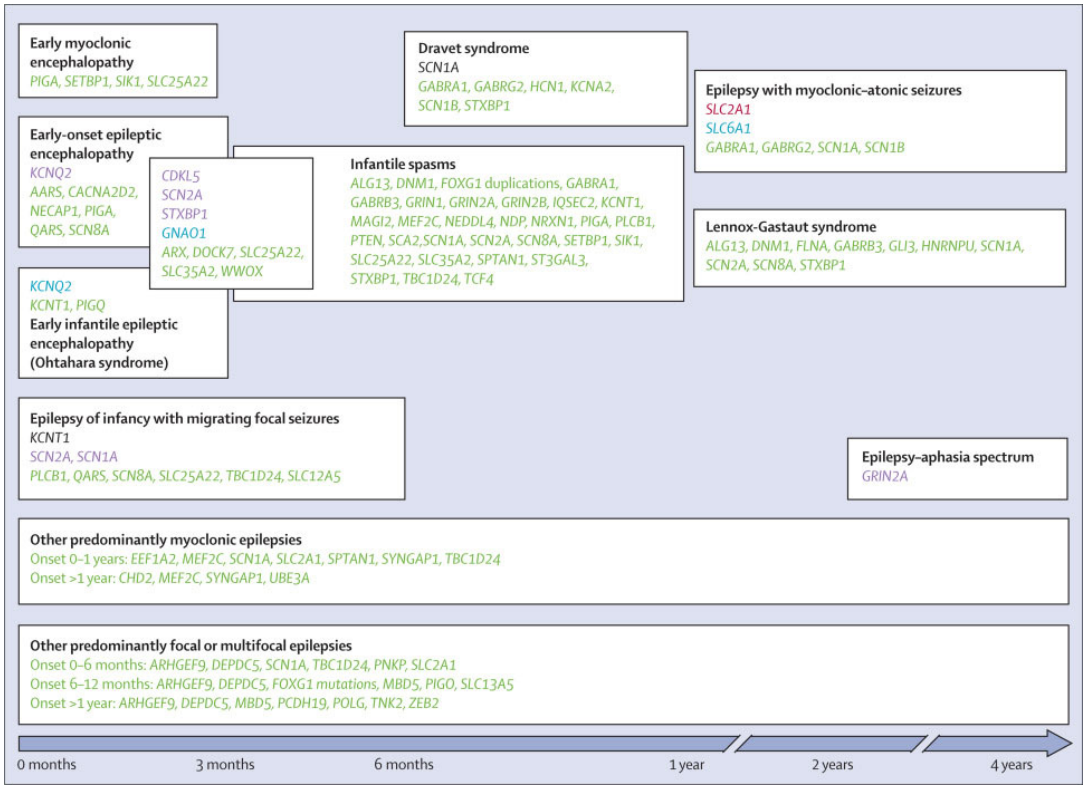
Many factors contribute to phenotypic heterogeneity, including type and timing of mutations during development, timing and location of physiological gene expression, epigenetic factors and modifier genes.

Genetic heterogeneity

Genetic heterogeneity occurs in every epilepsy syndrome. Even in the prototypical genetic epileptic encephalopathy, Dravet syndrome, in which more than 80% of patients have an *SCN1A* mutation, other genes (eg, *STXBP1* and *GABRA1*) are found in a small proportion of cases.

Analysis of larger numbers of genetically homogeneous cases could demonstrate clinical features that distinguish the phenotype. For example, epilepsy with myoclonic-atonic seizures, described by Doose (24), is associated with mutations in *CHD2* or *SLC2A1* in a small proportion (4%) of cases. *CHD2* is associated with clinical photosensitivity and *SLC2A1* is associated with paroxysmal exercise-induced dyskinesia (25) (26).

Figure 2 - Phenotypic pleiotropy genetic heterogeneity in genetic of EE (Mc Tague, 2016)



1.2. The challenge of adult patients

One third up to 50% of children with epilepsy will continue to have drug-resistant seizures in adulthood and this percentage is higher in patients with intellectual disability (27). Therefore, they need to be referred to an adult neurologist and it is

not infrequent that these patients come to their first visit in an adult epilepsy centre without an established aetiological diagnosis.

In adult patients, identifying aetiological causes is particularly challenging as they usually have a long history of epileptic seizures and complex antiepileptic therapies. These can disguise possible syndromic features and make it more difficult to retrace the clinical history (28).

Furthermore, some syndromes with onset in childhood continue into adulthood with different features. For example, some features of Lennox-Gastaut syndrome (LGS) change overtime, therefore in adult patients LGS is less easily identifiable than in children (29).

For patients referring for the first time to an adult epilepsy centre, detailed medical records are often not available, thus the collection of the medical history relies on the family or the caregiver. Unfortunately, parents may not be alive or able to recall the patient's medical history and the caregiver may have limited knowledge of the patient, hence it is frequent that important diagnostic details are missing (30): for example, a history of seizures in association with fever that would suggest Dravet syndrome.

These patients represent the "lost generation": if they had been seen as children today, they would have probably undergone a targeted genetic investigation (31). Since the first years of life, clinical and genetic features of their disease could have been defined, probably avoiding numerous useless investigations and non-targeted therapy. Unfortunately, at the time of their disease onset, knowledge was not so advanced as to allow the understanding of their disease's aetiology and as time passed it has become more difficult to recognize distinctive features of their syndrome.

Even if it occurs after many years since the disease onset, establishing the diagnosis has important benefits for the patients, especially if genetic. It represents the end of the 'investigative odyssey' and allows the counselling of patients and their families in terms of prognosis, targeted drug options and risks for future generations (32).

For this reason, at the time of transition to adult care, a full revaluation of these patients should be performed (28).

1.3. Therapy

The new concept introduced by Berg according to which the epileptic activity itself can contribute to developing ID and that situation can worsen over time has crucial consequences regarding the therapeutic approach (11).

Antiepileptic drugs can stop or reduce intellectual disability and a therapy targeted to the underlying condition, such as a metabolic disorder or a surgical treatable lesion can represent a solution not only for the epilepsy but also for the secondary ID.

EE is a condition with onset typically during infancy, with drug-resistant seizures and psychomotor delay.

The main target of their treatment is the reduction of neurological or intellectual deficit, decreasing seizure frequency. Therapy efficacy can be evaluated based on the following targets: seizure control, reduction of EEG abnormalities and ID improvement.

One of the major problems is to quantify treatment efficacy in ID. On the one hand, it is possible to quantify therapy efficacy in seizures and EEG abnormalities, but on the other it is difficult to understand how much the epileptic activity is responsible for the ID. Despite the scientific community interest on this subject, evidences regarding treatment efficacy is limited and therapeutic options are mostly based on case reports, expert consensus and clinicians' personal experiences (33).

1.3.1. EE therapy

EE treatment is based on the syndrome, if the diagnosis is available (34).

McTague and Cross summarized in one table the recommendations of the Expert Consensus of National Institute for Clinical Excellence (NICE) on therapy selection for EE (Table 2).

Table 2 –Treatment of EE By Syndrome **(34)**

Syndrome	First-line treatment	Other treatments to consider	Treatments to be avoided (may worsen seizures)
Ohtahara[a]	Corticosteroids	Ketogenic diet	
	Levetiracetam	Zonisamide	
		Vigabatrin	
		Phenobarbitone	
EMEE[a]	Dextromorphan/ketamine/sodium benzoate (if due to non-ketotic hyperglycinaemia)	Ketogenic diet	Vigabatrin
MPSI[a]	Levetiracetam	Stiripentol	
	Clonazepam	Ketogenic diet	
		Corticosteroids	
		Bromides	
West	Corticosteroids (prednisolone/ ACTH)	Benzodiazepines (nitrazepam)	Carbamazepine
	Vigabatrin	Topiramate	
		Zonisamide	
		Ketogenic diet	
Dravet	Sodium valproate	Clobazam	Carbamazepine
	Topiramate	Stiripentol	Gabapentin
		Ketogenic diet	Lamotrigine
		Bromides	Oxcarbazepine
			Phenytoin
			Pregabalin
			Tiagabine
			Vigabatrin
LGS	Sodium valproate	Topiramate	Benzodiazepines: small risk of precipitating tonic status epilepticus
	Lamotrigine	Rufinamide	
		Felbamate	Gabapentin/oxcarbazepine/ carbamazepine/lamotrigine: may worsen myoclonic seizures if prominent
		Clobazam	
		Levetiracetam	
		Corticosteroids	
		Ketogenic diet	
		Vagal nerve stimulation	
LKS	Corticosteroids	Valproate	
		Clobazam	
		Sulthiame	
		Multiple sub-pial transection	
ESES/CSWS	Corticosteroids	Valproate	
	Clobazam	Ethosuximide	
		Sulthiame	
		Ketogenic diet	
Doose/MAE	Sodium valproate	Clobazam	Carbamazepine
	Lamotrigine	Rufinamide	Phenytoin
	Ketogenic diet	Felbamate	Vigabatrin
		Ethosuximide	

[a] Limited evidence from case reports/series and generally poor response in the early infantile epileptic encephalopathies

ACTH adrenocorticotropic hormone, *EMEE* early myoclonic epileptic encephalopathy, *ESES/CSWS* electrical status epilepticus in slow wave sleep/ continuous spike wave in slow wave sleep, *LGS* Lennox Gastaut syndrome, *LKS* Landau Kleffner syndrome, *MAE* myoclonic astatic epilepsy, *MPSI* migrating partial seizures of infancy

1.3.2. Precision therapy

Thanks to the ever-increasing knowledge of epilepsy genetic causes and to an improvement in understanding underlying mechanisms, in the last years precision medicine has been developed for the treatment of genetic epilepsies.
So far, precision therapy has been available for a small number of patients.

SCN1A

SCN1A gene encodes a sodium channel alpha1 subunit and its mutations are found in 80% of patients with Dravet syndrome.
Many patients with *SCN1A* positive Dravet syndrome experience a paradoxical effect of seizure increase after sodium channel blocking drug administration, like carbamazepine, lamotrigine or phenytoin, which usually are effective antiepileptics (35).
Stiripentol is particularly effective in patients with Dravet syndrome, when added to valproic acid and clobazam (36): although the reason for its effectiveness in Dravet syndrome has not been clearly elucidated yet, it could be hypothesized that the GABAergic effect of stiripentol compensates for the decreased activity of inhibitory interneurons. However, other effects of stiripentol may play a role as well (37).
Recent data suggest that fenfluramine may have outstanding efficacy in patients with Dravet syndrome, but the anticonvulsant mechanism has not been clarified (37).

SLC2A1 (GLUT1)

SLC2A1 gene encodes for GLUT1, which is the glucose transporter that is required to transport glucose across the blood–brain barrier. Affected patients have reduced cerebral glucose availability.
The ketogenic diet represents the standard therapy for the classical GLUT1 deficiency. It consists of a high-fat diet with reduced carbohydrates which induces the production of ketone bodies. These cross the blood–brain barrier independent of GLUT1 and can be used as an energy source by the brain. The ketogenic diet, therefore, bypasses the defective glucose transport and provides an alternative energy supply to the brain (37).

KCNQ2

The gene *KCNQ2* encodes a subunit of the voltage-gated potassium channel.

Mutations in *KCNQ2* epileptic encephalopathy usually result in a reduction of channel current. The antiepileptic drug retigabine is an opener of *KCNQ2*- and *KCNQ3*-encoded potassium channels and has been shown to partially reverse the effect of KCNQ2 mutations in cell models (37).
Pisano et al. recently reported a study conducted on 15 patients that shows efficacy of sodium-blocker antiepileptic drugs on *KCNQ2*-related EE (38).

TSC1, TSC2

Heterozygous mutations in the tumour suppressor genes TSC1 and TSC2 cause tuberous sclerosis. TSC1 and TSC2 are negative regulators of the mTOR pathway, that regulates cell growth and cell proliferation.
Mutations in TSC1 and TSC2 lead to mTOR overactivity resulting in the occurrence of tumours in varying locations. The mTOR inhibitor everolimus is an established precision medicine approach in tuberous sclerosis. It has effects on systemic tumour size, reduces subependymal giant-cell astrocytoma volume and seizure frequency in patients with tuberous sclerosis by inhibiting the overactive mTOR pathway (37).
The recently conducted EXIST-3 trial provided evidence that everolimus treatment leads to a significant seizure reduction in patients with tuberous sclerosis and drug-resistant epilepsy as compared to placebo (39).

KCNT1

The gene *KCNT1* encodes the sodium-dependent potassium channel and is responsible for the slow hyperpolarization of the transmembrane potential during action potentials. Mutations in *KCNT1* typically are gain of function.
Proposed therapies for *KCNT1* related epilepsies include potassium-blocker drugs such as quinidine, which is an antimalarial and antiarrhythmic drug with a specific inhibitory effect on *KCNT1* (37).

SCN8A

SCN8A encodes the voltage-dependent sodium channel Nav1.6. It is located in inhibitory and excitatory neurons and is essential for the initiation and generation of action potentials. Mutations in SCN8A were found in 0.6–2.4% of cases with early infantile epileptic encephalopathy (37).

Sodium channel blockers have been suggested as an effective precision medicine treatment in patients with SCN8A mutations (37).

GRIN2A/GRIN2B

GRIN2A and *GRIN2B* encode the GluN2A and GluN2B subunits of the N-methyl-D-aspartate (NMDA) receptor, which play a major role in excitatory pathways and have important effects on synaptogenesis and synaptic plasticity.

NMDA receptor antagonists have been suggested as a therapeutic option in *GRIN2A/2B* mutations. The NMDA receptor antagonist memantine, which is an approved drug for the treatment of Alzheimer's dementia, seem to reduce seizures frequency in these patients (40), even though these are still preliminary results and other studies are needed to validate this therapeutic option.

1.3.3. Future directions

iPSCs

Advances in cellular reprogramming have made it possible to generate virtually any cell type from induced Pluripotent Stem Cells (iPSCs), which represent an attractive model for neurologic disease, where access to live human tissue suitable for culture is extremely limited. Some recent studies have used iPSCs to model epilepsy mechanisms in Dravet syndrome. These data suggest that epilepsy syndrome specific iPSC-derived neurons are useful for modelling epileptic-like hyperactivity, which offers a platform for screening new antiepileptic therapies (41).

Gene therapy

Gene therapy holds much promise for treatment-resistant epilepsies. However, many obstacles remain: delivery of large molecules and transcripts across the blood–brain barrier and into cells is challenging (22).

SINEUPs are an example of genic therapy, they are a synthetic antisense lncRNAs that stimulate the translation of sense mRNAs. These molecules are the combination of 2 RNA elements: the Binding Domain (BD) with a sequence similar to the target mRNA and the Effector Domain (ED), which contains the element *inverted SINEB2* that activates the protein synthesis (42).

The combined activities of the 2 domains predict that by swapping the BD with an appropriate sequence it is possible to increase the amount of proteins encoded by the mRNAs of choice acting at post-transcriptional level.

SINEUPs have 2 major advantages: 1) they modulate translation of target mRNAs without introducing stable genomic changes into target cells; 2) their induction of selected proteins is typically in a more physiological range (2-fold) than most conventional gene replacement strategies.

SINEUPs could be useful in autosomal dominant diseases, when the mutation causes protein *loss of function*, among which the Dravet syndrome. By using *SINEUPs* it is possible to increase protein expression and, theoretically, to cure the disease (42).

1.4. Genetic Tests

Genetic tests available so far are summarized in Table 3.

Table 3 – Modified by LICE genetic commission 2016

Exam	Description	When to use
Karyotype analysis	Identifies possible numeric (trisomy or monosomy) or structural (translocations, deletions or inversions) abnormalities	In patients with dysmorphism or multiple congenital abnormalities; suspicion of monosomy, trisomy or chromosomal rearrangements
Array-CGH	Identifies sub-microscopic chromosomal rearrangements like CNVs, in different loci simultaneously	When epilepsy is associated with intellectual disability, autism and/or dysmorphism
Fluorescent in situ hybridization (FISH)	Using fluorescent probes identifies sub microscopic rearrangements not detectable with karyotype	Confirms a deletion/duplication in specific regions, e.g. 22q11
Methylation study	Evaluates methylation abnormalities in specific chromosomal regions	Suspicion of methylation abnormalities, like Prader-Willi or Angelman syndrome
Single gene sequencing	Identifies variants in a specific gene sequence	Suspicion of one abnormality in a specific gen (e.g. *SLC2A1* for glucose transport deficit)
Multiplex Ligation-dependent Probe Amplification (MLPA)	Identifies little CNVs in a specific gene	Suspicion of one abnormality in a specific gene, but negative sequencing

Next Generation Sequencing (targeted- resequencing)	Identifies variants with simultaneous sequencing of numerous genes	Disorders associated with numerous genes, like epileptic encephalopathy
Whole exome or genome sequencing	Identifies variants in all genes codifying regions (exome) or entire genome	Strong suspect of genetic disease but all the previous genetic tests are negative

1.4.1. Karyotype analysis

This analysis identifies numeric (like trisomy or monosomy) or structural (translocation, deletion, inversion) chromosomal abnormalities.

For epilepsy molecular diagnosis, karyotype allows the identification of chromosomal rearrangements, like ring chromosome (ring 14, ring 20), not detectable with Array-CGH (43).

1.4.2. Array-CGH and FISH

Array-Comparative Genomic Hybridization (Array-CGH) is a technique for detection of small numeric chromosomal abnormalities (copy number variations, CNVs), like duplications/amplifications or deletions. It is based on the quantitative comparison of the DNA under examination (DNA test) with a reference DNA of an unaffected subject (reference DNA) (44).

Array-CGH allows the analysis of the entire human genome, the exact location of the altered genomic region with all the contained genes.

The resolution of this analysis is variable: for diagnostic purposes, we use array between 1 Mb and 100 kb, with a resolution 100 times higher than for traditional cytogenetic.

This is now a routine test, thanks to its low cost and reliability, but that has limits such as the difficulties in interpretation of variants of unknown significance (VUS) and the impossibility of showing genetic testing not altering the total quantity of material (example: balanced translocation) (43).

It represents the first test for patients with ID (45), with a diagnostic rate of 12% (46).

Regarding epilepsy, CNVs role has been well studied in children with seizures, less in adults: in a cross-sectional study conducted by Borlot et al., they examined 143 patients' Array-CGH finding pathogenic or likely pathogenic CNVs in 16.1% (47).

Fluorescence in situ hybridization (FISH) uses fluorescent probes to detect and localize the presence or absence of little trisomy or partial deletions. It is useful in select cases, in order to verify specific diagnostic suspicion of microdeletion syndrome (like Angelman syndrome) and to better characterize chromosomal abnormalities detected with other techniques (for example chromosome 15 inversion/duplication) (43).

1.4.3. Methylation studies

This study allows the evaluation of mutilation abnormalities in specific chromosomal regions and diagnosis of clinical syndromes like Angelman, that in 2-5% of cases can be due to an imprinting defect on 15q11 region.

1.4.4. Single gene sequencing and MLPA

The technique of First Generation DNA Sequencing, developed by Sanger in 1977, is indicated in case of suspicion of epilepsy caused by mutation in one known gene (43).

MLPA technique (Multiplex Ligation- Dependent Probe Amplification) allows the identification of deletions/duplications in the exon sequence of specific genes. It can be useful to detect cases of epilepsy with intragenic deletion, that turned out negative on Sanger sequencing or array-CGH (43).

1.4.5. Gene Panel

The first study on a gene panel was published by Lemke at al. in 2012, using a 265 genes panel on 33 patients with variable phenotypes of epilepsy (48).

Since then, gene panels have revolutionized the diagnostic approach to people with epilepsy and so far they have been the most useful choice for genetic diagnosis in epilepsy due to the lower cost and higher coverage of the technology (49).

In a study by Chambers et al. in 2016 (50), authors compared different gene panel available for epilepsy, characterized by an extreme variability in the number of gene selection (from 70 to 465).

Using a panel with a higher number of genes is controversial: Mercimek-Mahmutoglu's study on gene panel diagnostic rate (51) showed a rate between 10 and 48.5%, with the higher rate associated with the panel with a larger number of genes (265). Another study reported a 47% diagnostic rate using a 67 gene panel

(47), showing that a large number of genes is not always necessary to obtain a diagnosis (49).

Diagnostic rate seems to be higher in cohorts including drug-resistant patients with early onset epilepsy, and in particular with EE (52).

Gene panel has possible diagnostic pitfalls: it allows only the detection of coding region variants that impair protein function, excluding regulatory regions (example: promoter, microRNA). Some genomic regions are difficult to target because of their high guanine-cytosine (GC) content or their proximity to repeated sequences, resulting in missed or reduced sequencing

Lastly, a gene panel can miss the identification of the structural variations, including insertions, deletions and duplications. This is why this technique should be associated with other tests like MLPA and array-CGH (52).

1.4.6. *Whole Exome Sequencing*

Introduced in 2011, WES technique refers to entire exome sequencing, that is the functional part of the genome, that codes for proteins.

Human exome include about 1% of genome and the 85% of mutations associated with diseases.

For every analysed subject, this technique allows the comparison of the identified genetic variants with the polymorphic variants (non-pathogenic) of the general population.

Exome variants absent or extremely rare in the general population will be considered as putative mutations (43). Currently, WES has a 25% diagnostic rate (53).

WES use in clinical practice can reduce the number of different molecular analyse, which most patients with rare diseases underwent, resulting in time and cost savings.

The main problem is the interpretation of numerous variants that can be identified and the incidental findings.

Some helpful tools for variant interpretation are represented by publicly available resources such as the ExAC (http://exac.broadinstitute.org), the gnomAD (http://gnomad.broadinstitute.org) or the 1000 Genomes Project (http://www.internationalgenome.org) databases, which list variants, and their allele frequencies, observed in large populations (52).

The freely available ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php)

or several locus-specific databases aggregate useful information about genomic variation and its relationship to human health (44).

Furthermore, segregation analysis performed in parents or other relatives to determine the carrier status or de novo occurrence of a variant is often helpful to provide further evidence for or against pathogenicity (52).

2. MATERIAL AND METHODS

We prospectively and retrospectively recruited all adult patients with intellectual disability and epilepsy at the moment of their first evaluation or control visit at the Epilepsy Centre "G.M. Corsino" (IRCCS Institute of Neurological Science of Bologna –DIBINEM) from October 2016 until May 2019 based on the following criteria:

- Patients aged more than 18 years;
- Personal history of epilepsy: clinical history of at least the unprovoked epileptic seizures occurring more than 24 hours apart (54);
- Epilepsy onset in paediatric age (0-18 years);
- Intellectual disability.

The study was approved by the local ethics committee (cod. CE:16057).

2.1. Clinical work-up

Recruited patients underwent full clinical evaluation including

- familial anamnesis;
- physiological anamnesis, with particular attention to pregnancy, delivery, peri- and post-natal period and psychomotor development;
- Epileptic history, in particular age at the onset, seizure semiology, frequency and evolution of the seizures.

Clinical assessment was performed, including the search for dysmorphism. A genetic evaluation was requested in case of dysmorphic findings.

Developmental and intellectual levels were evaluated on the basis of schooling and developmental milestones. As for the neuropsychological assessment, severe and complex patients were tested during clinical examination by the neurologist personally, whereas most of patients showing compliance underwent a neuropsychological testing with intelligence quotient (IQ) evaluation.

Electroencephalogram was performed and categorized as "normal" or "pathologic"; further "pathologic" EEGs were divided into two different groups: with nonspecific EEG abnormalities (e.g. background activity slowing) and with epileptic abnormalities (e.g. sharp/sharp-waves).

Computed tomography scan (CT) and magnetic resonance imaging (MRI) were analysed. We divided patients' imaging results into three groups: "lesional", "aspecific" and "normal". The term "lesional" was used when the lesion found in the brain scan was potentially responsible for the clinical picture, whereas the term "aspecific" was used when the abnormality did not clearly correlate with the clinical picture.

Based on clinical pictures, other exams were performed, such as coeliac disease screening, electromyography, evoked potentials, lumbar puncture.

All data regarding therapeutic history were reviewed, with attention to drugs that worsened seizures.

2.2. Genetic study

A genetic screening was suggested for patients without a specific diagnosis. Based on the clinical suspicion and when available on the genetic evaluation, we performed different genetic exams.

2.2.1. Constitutional karyotype, fluorescent in situ hybridization (FISH)

They were proposed in patients without family history, with ID and dysmorphism. Karyotype was also performed in all patients with EEG suggestive of ring 20 chromosome (55).

2.2.2. Sanger sequencing

Performed in patients that had some clinical elements suggesting a specific gene mutation.

2.2.3. Array-CGH

Array-CGH was proposed to non-lesional cases without familial history, with ID and, when present, dysmorphism.

Alterations were categorized into three different groups: "pathogenic", "unknown meaning" and "benign". This categorization has been made on the basis of public CNV databases, familial segregation, alteration size and the genes contained.

Public databases used are: Database of Genomic Variants (http://projects.tcag.ca/variation/), DECIPHER (http://decipher.sanger.ac.uk/) and TROINA (http://dbcnv.oasi.en.it/gvarianti/index.php).

2.2.4. Targeted Next Generation Sequencing (tNGS)

Targeted-resequencing was carried out using a custom epilepsy multi-gene panel for the screening of all the coding sequence and exon flanking region of all included genes (see tables 4 and 5). Libraries were obtained using Nextera Rapid Capture enrichment kit (Illumina Inc., Santa Clara, CA) following manufacturer's instructions and were sequenced on an Illumina MiSeq sequencing platform (Illumina Inc., Santa Clara, CA). Reads alignment and variant calls were obtained using default parameters with BWA and GATK on the Base Space analysis platform and variant annotation and filtering were applied using the software Variant Studio 3.0 (Illumina Inc., Santa Clara, CA).

We annotated all the identified variants, considering the longest transcripts, using the online version of Variant Effect Predictor (VEP) for human GRCh37 (http://grch37.ensembl.org/Homo_sapiens/ Tools/VEP). Population allele frequencies for each variant were extracted from the Genome Aggregation Database browser (gnomAD, http://gnomad.broadinstitute.org/; accessed in July 2019). We used in silico prediction tools to assess the pathogenicity of variants: M-CAP (Mendelian Clinically Applicable Pathogenicity) for missense variants; HSF (Human Splice Finder v3.0) for splice-region variants. All variants were classified, following the American College of Medical Genetics (ACMG) guidelines (56), as pathogenic, likely pathogenic, variants of uncertain significance (VoUS), likely benign or benign. Sanger sequencing was used to carry out validation and segregational study for all the pathogenic, likely pathogenic variants and VoUSs.

Table 4 – Gene panel, 43 genes

Gene	**Phenotype - principal category**
ARX	Epileptic encephalopathy, early-infantile (EIEE); Lissencephaly, X-linked
CDKL5	EIEE
SLC25A22	EIEE
STXBP1	EIEE
SPTAN1	EIEE
KCNQ2	EIEE

PLCB1	EIEE
PNKP	EIEE
MECP2	EIEE
PNPO	Pyridoxamine 5-primephosphate oxidase deficiency
ALDH7A1	Epilepsy, pyridoxine-dependent
SCN1A	EEIE, severe myoclonic epilepsy in infancy (SMEI)/GEFS+
SCN1B	SMEI/GEFS+
SCN2A	SMEI/GEFS+
SCN8A	EIEE
GABRG2	SMEI/GEFS+
CHD2	Epileptic encephalopathy, childhood onset
UBE3A	Angelman syndrome
PCDH19	EIEE
SLC2A1	GLUT1 deficiency syndrome
PRRT2	Paroxysmal kinesigenic dyskinesias (PKD); benign familial infantile seizures (BFIS); infantile convulsions with paroxysmal choreoathetosis (ICCA)
TSC1	Tuberous sclerosis
TSC2	Tuberous sclerosis
LIS1	Malformation of cortical development (MCD)
DCX	MCD
FLNA	MCD
ARFGEF2	MCD
RELN	MCD
TUBA1A	MCD
TUBB3	MCD
TUBB2B	MCD
SRPX2	MCD
GPR56	MCD
DEPDC5	AD Focal Epilepsy (ADFE)
LGI1	AD Focal Epilepsy (ADFE)
CHRNA4	AD Focal Epilepsy (ADFE)
CHRNB2	AD Focal Epilepsy (ADFE)
CHRNA2	AD Focal Epilepsy (ADFE)
KCNT1	AD Focal Epilepsy (ADFE)
GRIN2A	Epilepsy, focal with speec disorder ± ID
CACNA1A	Familial hemiplegic Migraine (FHM)
ATP1A2	FHM; Alternating Hemiplegia of Childhood (AHC)
ATP1A3	AHC

Table 5 – Gene panel, 79 genes

Gene	**Phenotype - principal category**
ARX	Epileptic encephalopathy, early-infantile (EIEE); Lissencephaly, X-linked
CDKL5	EIEE
SLC25A22	EEIE

STXBP1	EEIE
SPTAN1	EEIE
SCN1A	EEIE, severe myoclonic epilepsy in infancy (SMEI)/GEFS+
KCNQ2	EEIE
PCDH19	EIEE
PKNP	EEIE
SCN2A	EIEE
PLCB1	EEIE
SCN8A	EIEE
KCNA2	EIEE
FOXG1	EIEE
GABRA1	EIEE
TBC1D24	EIEE
SYNGAP1	EIEE
HCN1	EIEE
MECP2	Rett syndrome, atypical, with preserved speech variant
PNPO	Pyridoxamine 5-primephosphate oxidase deficiency
ALDH7A1	Epilepsy, pyridoxine dependent
CDH2	Epileptic encephalopathy, childhood onset (myoclonic seizures, photosensitivity)
UBE3A	Angelman syndrome
TSC1	Tuberous sclerosis
TSC2	Tuberous sclerosis
LIS1	Malformation of cortical develepment
RELN	MCD
TUBA1A	MCD
DCX	MCD
FLNA	MCD
ARFGEF2	MCD
TUBB3	MCD
RTUBB2B	MCD
SRPX2	MCD
GPR56	MCD
CNTNAP2	MCD/EE/FE
DEPDC5	AD Focal Epilepsy
LGI1	AD Focal Epilepsy
CHRNA4	AD Focal Epilepsy
CHRNB2	AD Focal Epilepsy
CHRNA2	AD Focal Epilepsy
KCNT1	AD Focal Epilepsy
NPRL2	FE
NPRL3	FE
GRIN2A	Epilepsy, focal with speec disorder ± ID
SLC2A1	GLUT1 deficiency syndrome
PRRT2	Paroxysmal kinesigenic dyskinesias (PKD); benign familial infantile seizures (BFIS); infantile convulsions with parosysmal choreoathetosis (ICCA)

SCN1B	GEFS +
GABRG2	GEFS+
CACNA1A	Familial hemiplegic Migraine (FHM)
ATP1A2	FHM; Alternating Hemiplegia of Childhood (AHC)
ATP1A3	AHC
CSTB	Myoclonic epilepsy of Unverricht and Lundborg
EPM2A	Lafora progressive myoclonus epilepsy
NHLRC1	Progressive myoclonic epilepsy (PME)
PRICKLE1	Myoclonus epilepsy with ataxia
SCARB2	Action myoclonus–renal failure (AMRF) syndrome
CLN1/PPT1	Neuronal Ceroid Lipofuscinosis (NCL) with onset in infancy, or any age up to adulthood
CLN2/TPP1	NCL with onset in late infancy, or at later ages
CLN3	NCL with juvenile onset
CLN4/DNAJC5	AD PME, Parry disease
CLN5	NCL with onset in late infancy, or at later ages up to adulthood
CLN6	NCL with onset in late infancy, or at later ages up to adulthood including Kufs type A
CLN7/MFSD8	NCL with onset in late infancy, or at later ages
CLN8	NCL with onset in late infancy, or a very different disease, Northern epilepsy
CLN9/CTSD	NCL with early onset
CLN11/GRN	NCL with adult onset
CLN12/ATP13A2	NCL with juvenile onset
CLN13/CTSF	NCL with adult onset, including some Kufs type B
CLN14/KCTD7	NCL with infantile onset
NEU1	PME - sialidosis
GBA	PME - Gaucher
GM2A	PME - Gangliosidosis
KCNC1	PME
GOSR2	PME
NPC1	Niemann-Pick disease type C1
NPC2	Niemann-Pick disease type C2
MEF2C	Mental retardation, autosomal dominant 20 (MRD20)
GNAO1	Neurodevelopmental disorder with involuntary movements (NEDIM)

2.2.5. Whole Exome Sequencing (WES)

We performed WES studies as part of the project "Epi25 Collaborative for Large-Scale Whole Exome Sequencing in Epilepsy", approved in 06/16//2016 by Area Vasta Emilia Centro Ethic Committee (CE 16057). All patients or their legal guardian signed a specific informed consent.

All samples were sequenced at the Broad Institute of Harvard and MIT on the Illumina HiSeq X platform, with the use of 151 bp paired-end reads. Exome capture was performed with Illumina Nextera® Rapid Capture Exomes or TruSeq Rapid

Exome enrichment kit (target size 38 Mb), except for three control cohorts (MIGen ATVB, MIGen Ottawa, and Swedish SCZ controls) for which the Agilent SureSelect Human All Exon Kit was used (target size 28.6 Mb – 33 Mb). Sequence data in the form of BAM files were generated using the Picard data-processing pipeline and contained well-calibrated reads aligned to the GRCh37 human genome reference. Samples across projects were then jointly called via the Genome Analysis Toolkit (GATK) best practice pipeline24 for data harmonization and variant discovery. This pipeline detected single nucleotide (SNV) and small insertion/deletion (indel) variants from exome sequence data.

All the interesting variants identified during WES analysis were validated with Sanger sequencing in probands, to exclude false positive results, and then we conducted segregation study in parents' DNA, when available.

All identified variants were classified on the basis of the guidelines proposed by the ACMG in 2015.

They define 28 criteria based on population data, functional data, computational prediction, allelic data, segregation study and de novo observations. Combination of these criteria allows variant classification in the following 5 categories: pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign and benign (57).

3. RESULTS

3.1. General population data

We recruited 232 patients with epilepsy and ID.

Among them:

- 10 were excluded because seizure onset occurred after 18 years old:
- 21 were excluded because of lack of information regarding clinical history;
- 1 was excluded because <18 years of age.

200 patients were included, 109 males and 91 females.

Mean age at the first visit was 28 years old (range 6-62 years).

The majority of patients had already underwent a neurologic or child neurologic evaluation: 83 patients (41.5) were referred by child neurologist whereas 155 (77.5%) had already performed a neurological visit in a different centre.

Mean age at the last visit was 36 years old (range 18-65 years). Mean follow-up is 8.5 years (range between 1 and 43 years).

3.2. Clinical work-up

Family history was positive in 50 patients (25%): in 37 for epilepsy, in 13 for febrile seizures. Among these 50 patients, 6 also had positive family history for ID.

Sixty-five patients (32.5%) were born by dystocic delivery.

Regarding psychomotor development, 139 patients (69.5%) had a delay, 49 (24.5%) had a normal development, whereas in 12 patients (6%) these data were not available.

In 30 out of the 49 patients who had a normal psychomotor development, a regression or a plateau occurred, in 18 of them concurrently with seizure onset.

Forty-five patients (22.5%) had febrile seizures (FS). Mean age of FS onset was 14 months (range first days of life - 36 months).

Mean age at seizure onset is 4 years old (range first days - 17 years old). Sixteen patients (8%) presented with seizures in the neonatal period (within the first 30 days of life); 58 (29%) in the infant period (1-12 months): 67 (33.5%) had the first seizure between 2 and 5 years of age; 45 (22.5%) between 6 and 12 years and 14 (7%) between 13 and 18 years.

Eighty-five patients (42.5%) had generalized seizures at onset, whereas 57 (28.5%) had focal seizures, of which 28 with evolution into bilateral tonic-clonic seizures. Fifty-eight patients (29%) had unknown onset seizures at onset (47 with motor symptoms).

In 107 patients (53.5%) seizures at onset had a daily or weekly frequency, in 32 (16%) frequency was monthly. In 40 cases seizures were rarer with a yearly or sporadic rate. In 21 patients (10.5%), frequency was not available.

At the first visit in our epilepsy centre, 67 patients (33.5%) had generalized seizures, 73 (36.5%) had focal seizures (31 with evolution into bilateral tonic-clonic seizure) and 28 patients (14%) unknown onset seizures. Thirty-two patients (16%) were seizure free.

Patients with monthly or more frequent seizures were 142 (71%), 69 of them (34.5%) with daily seizures.

At the last visit, 54 patients (27%) had generalized seizures, 62 (31%) focal seizures of which 24 with evolution into bilateral tonic-clonic seizure and 26 (13%) unknown onset seizures. Seizure-free patients were 59 (29.5%).

Seizures were monthly or more frequent in 109 patients (54.5%), of which 39 (19.5%) had daily seizures.

Neurological examination revealed craniofacial dysmorphia in 51 patients (25.5%).

Based on clinical information, patients were divided into 3 categories:

- 54 patients (27%) with EE
- 94 patients (47%) with DE
- 52 patients (26%) with developmental and epileptic encephalopathy.

3.3. Psychiatric comorbidity

A total of 95 patients (47.5%) had a psychiatric comorbidity: behavioural disorder in 73 patients, depression in 11 patients and autistic spectrum disorder in 8 patients.

The behavioural disorders most often reported are verbal or physical aggressiveness, psychomotor agitation or obsessive behaviour.

Thirty patients were on psychiatric co-therapy: 26 patients were taking neuroleptics, 4 patients anti-depressants.

3.4. Neuropsychological study

All patients had an intellectual disability (ID).

Nine patients (4.5%) had a borderline ID, 49 (24.5%) had a mild ID. In 77 (38.5%) the ID was moderate and in the remaining 65 (32.5%) it was severe.

In 43 patients (21.5%) intelligence quotient (IQ) was evaluated with neuropsychological testing. Mean scoring was 53.3 (range 35-78). Nine patients had a borderline IQ, 13 mild, 19 moderate and 2 severe.

Regarding the remaining 150 patients, 37 patients had an ID unquantifiable with standardized tests because of their severity. The remaining 113 patients have been assessed by the clinician.

3.5. Neurophysiological study

All recruited patients performed at least one EEG recording:

- 159 patients (79.5%) had epileptiform abnormalities;
- 38 patients (19%) had non-specific abnormalities;
- 3 patients (1.5%) had a normal EEG.

3.6. Neuroradiologic study

A proportion of 196 patients (98%) performed Magnetic Resonance Imaging (MRI) or computed tomography scan (CT) at least once during their life. In 56 (28%) patients, there was a lesion, more frequently dysplasia, tuberous or perinatal ischemic lesions. Sixty-nine patients (34.5%) had non-specific abnormalities whereas 71 patients (35.5%) had a normal exam (Table 4). Cerebral lesions are resumed in Table 6.

Table 6 – Cerebral lesions on neuroimaging

Lesion	Patients	%
Dysplasia	12	6%
Ischaemic lesion	11	5.5%
Tuberous	8	4%
Gyration abnormalities	5	2.5%
Cortical malformation	4	2%
Heterotopia	4	2%
Post- haemorrhagic	3	1.5

Complex cerebral malformation	3	1.5%
Hemiatrophy	3	1.5%
Multicystic encephalomalacia	1	0.5%
Microcephaly	1	0.5%
Meningioma removal	1	0.5%

3.7. Genetic study

Patients that underwent at least a genetic exam are 128 (64%).

In Table 7 all genetic exams performed, the number of analysed patients and positive patients for each exam are reported.

Table 7 – Genetic exams

Exam	Associated syndromes	Analysed	Positive
Karyotype		**19**	4
chr 20	Ring 20	19	4
FISH		**6**	1
Uniparental dysomy chr 15	Angelman syndrome	3	0
del22q11.2	Velocardiofacial syndrome	3	1
CGH-array		**42**	1
MLPA	Rett syndrome	**2**	1
Single gene test		**40**	3
FRAXA	X-fragile syndrome	14	0
SCN1A	Dravet syndrome	10	2
SLC2A1	GLUT1 deficiency syndrome	7	1
PCDH19	Dravet-*like* syndrome	4	0
CDKL5	West, Rett syndrome	2	0
POLG	Alpers disease	1	0
ARX	West syndrome	4	0
CHD7	CHARGE syndrome	1	0
Cystatin B	Unverricht-Lundborg syndrome	1	0
Mitochondrial diseases genes	MELAS, MERRF, Leber	2	0
NGS Panel		**25**	8
43 genes		21	7
79 genes		3	0
Galliera Hospital		1	1
Whole Exome Sequencing		**68**	17

Karyotype analysis was performed in 19 patients and in 4 of them identified a ring 20 syndrome.

FISH was performed in 3 patients and in one patient detected a microdeletion on 22q (velocardiofacial syndrome).

For the suspicion of X-fragile syndrome, in 14 patients the specific exam was performed, and it was negative in all patients.

MLPA analysis performed in 2 patients showed a duplication in Xq28 involving *MECP2* gene, including also other genes (*GD1*, *FLNA*, *IRAK1*, *L1CAM* and *IDH3G*).

In 10 patients SCN1A gene analysis has been performed and turned out positive in 2 patients.

DNA of 42 patients (21%) was analysed with CGH-array and in 21 cases CNVs were found. Clinical meaning of these alterations has been classified as follows:

- *Benign:* 1;
- *Potentially benign:* 6;
- *Uncertain:* 11;
- *Potentially pathogenic: 2*;
- *Pathogenic: 1*;

The alterations detected are reported in Table 8.

Table 8 – CGH-array alterations

Patient	Variant	Type of alteration	Dimensions	Meaning	Clinic
1	dup6q27	Duplication	210 kb	Benign	DE
2	dup15q25.2	Duplication	180 kb	Potentially benign	DEE
3	dup7p22.2	Duplication	247 kb	Potentially benign	DEE
4	dup8q21.2	Duplication	107 kb	Potentially benign	EE
5	trp9q22.2	Triplication	214 kb	Potentially benign	DEE
6	del2p11.2	Deletion	792 kb	Potentially benign	EE
7	delXq23	Deletion	130 kb	Potentially benign	DE
8	del6q16.1	Deletion	562 kb	Potentially benign	DEE
9	dup17q24.2	Duplication	121 kb	Uncertain	DE
10	dup16p13.1	Duplication	1.900 kb	Uncertain	DEE
11	delXp22.33	Deletion	69 kb	Uncertain	DE
12	dup17p13.2	Duplication	16 kb	Uncertain	EE
13	del9p21.1	Deletion	130 kb	Potentially benign	DE
	dup16p12.3	Duplication	90 kb	Uncertain	
	dupXp22.33	Duplication	250 kb	Potentially benign	
14	dup20q13. 2	Duplication	718 kb	Potentially benign	DE
15	dup16q23.2	Duplication	110 kb	Uncertain	EE
	dup19p12	Duplication	75 kb	Potentially benign	
16	del2q37.3	Deletion	18 kb	Potentially benign	DE
	dup3q27.2	Duplication	192 kb	Uncertain	
	dup15q24.3q24.2	Duplication	707 kb	Uncertain	
17	trp1p22.1	Triplication	94 kb	Uncertain	DE
18	dup5p14.1p13.3	Duplication	759 kb	Uncertain	DE
19	del3q29	Deletion	1570 kb	Potentially pathogenic	EE
20	del15q11.2q13.1	Deletion	4830 kb	Potentially pathogenic	DEE
21	del17q21.31	Deletion	442 kb	Pathogenic	DE

EE: epileptic encephalopathy; DE: developmental encephalopathy; DEE: developmental and epileptic encephalopathy

Next Generation Sequencing (NGS) panel was performed in 25 patients and it results positive in 8 patients (*SCN1A* in 2 patients, *SLC2A1* in 2 patients, *TSC2* in one patient, *SCN8A* in one patient, *PCDH19* in one patient, *DCX* in one patient).

Whole Exome Sequencing (WES) was performed in 68 patients. In 26 patients we found one or more than one interesting genetic variants, in particular 21 variants in

genes with autosomal dominant inheritance, 8 in genes with recessive inheritance and 2 in genes with *X-linked* inheritance.

We divided the variants on the basis of gene function (Table 9).

Table 9 – Variants detected divided on the basis of gene function

Ionic transport	**Synaptic transmission**	**Neuronal migration**	**DNA repair Chromatin remodelling DNA methylation**	**Transcriptional regulator**	**Intracellular signal**	**Others**
CACNA1E *KCNB1* *SCN8A* *SCN9A* *CACNA1A* *KIAA2022* *SCN2A* *SCN1A* *KCNH5* *KCNQ2*	*STXBP1* *GRIN2A* *GABRG2* *GABRG1* *GRIN1* *GABRB2* *KIF1A*	*LAMB1* *APC2*	*PNKP* *SMC1A*	*ZBTB20* *ANKRD11*	*DYRK1A*	*PRODH* *RARS2* *TRIP12* *DYNC1H1* *AARS* *EEF1A2*

3.7.1.*Pathogenic or likely pathogenic variants*

On the basis of ACMG guidelines, two of the missense variants found in this study in two patients were classified as likely pathogenic, in genes *STXBP1* and *GABRB2*. They are 2 variants localized in the same position of other 2 variants already reported in literature, but with different amino acid substitution, in which it was not possible to perform segregation study.

Always based on the same guidelines, we classified as pathogenic 21 variants in 15 patients: *RARS2* (2), *CACNA1E*, *PNKP* (2), *DYRK1A*, *LAMB1* (2), *SMC1A*, *KIAA2022*, *ZBTB20*, *APC2* (2), *PRODH* (2), *KCNB1*, *DYNC1H1*, *KCNH5*, *SCN1A*, *ANKRD11*, *GABRG2*.

All these variants were confirmed with Sanger method and, where possible, segregation study in parents was performed.

Even if it was not possible to perform a segregation study on all these variants, we decided anyway to classify them as pathogenic because they were already described as causative in literature and they were in genes characterized by incomplete penetrance and responsible for phenotypes extremely similar to our probands'.

Regarding the variants in which segregation studies were performed, it was found that: 6 autosomal dominant transmission variants and X-linked transmission were de novo, autosomal recessive transmission variants were in 4 cases one maternal and one paternal, and in one case one de novo and one paternal (Table 10).

Table 10 - Pathogenic or likely pathogenic variants

TRANS	PHENOTYPE	GENE	IDENTIFIED MUTATIONS				INHERITANCE	gnomAD	M-CAP	ACMG	
			Chromosomal position (GrCH37)	c.DNA nucleotidic change	Protein aminoacidic change	Function		Frequency/ Allele count(AC)	PRED	SCORES	
AR	EE	*RARS2* (NM_020320)	g .88228541C>T	c.1305+1G>A	p.(?)	Splice donor variant	paternal	1	nd	PVS1, PM2, PM3, PP3	P
			g .88231191C>T	c.1026G>A	p.Met342Ile	missense	maternal	69	D	PS1, PM3, PM5, PP3, PP5	P
AR	DE	*PRODH* (NM_016335)	g.18905859G>A	c.1397C>T	p.Thr466Met	missense	maternal	1454	D	PS1, PS3, PM3, PP3	P
			g.18905934A>G	c.1322T>C	p.Leu441Pro	missense	paternal	1279	D	PS1, PS3, PM3, PP3	P
AR/AD	DE	*APC2* (NM_005883)	g.1457098G>A	c.1063G>A	p.Val355Ile	missense	maternal	11	T	PM2, PM3, PP4	VUS / LP
			g.1469920C>T	c.6620C>T	p.Pro2207Leu	missense	paternal	nf	D	PM2, PM3, PP3, PP4	LP
	DE	*GABRG2* (NM_198903)	g.161578735G>T	c.1249-1G>T	p.(?)	Splice Acceptor	paternal	2		PVS1, PS1, PS3, PM2, PP3	P
AD	EE	*DYNC1H1* (NM_001376)	g.102446852G>A	c.926G>A	p.Arg309His	missense	Not completed (mother wt)	nf	D	PS1, PM2, PP3, PP4, PP5	LP
XL	DE	*SMC1A* (NM_006306)	g.53439920C>A	c.784G>T	p.Glu262*	Stop gained	De novo	nf	nd	PVS1, PM2, PM6, PP3, PP4	P
AD	DEE	*KCNB1* (NM_004975)	g.47991181G>A	c.916C>T	p.Arg306Cys	missense	unknown	nf	D	PS1, PM1, PM2, PP3, PP5	P
AD	DE	*KCNH5* (NM_139318)	g.63417240C>T	c.980G>A	p.Arg327His	missense	unknown	nf	D	PS1, PS3, PM2, PP3	P
AD	DEE	*STXBP1* (NM_003165)	g.130425623G>A	c.569G>A	p.Arg190Gln	missense	unknown	nf	D	PM2, PM5, PP3, PP4, PP5	LP
AD	DEE	*SCN1A* (NM_006920.5)	g.166894441G>A	c.2758C>T	p.Arg920Cys	Missense	unknown	nf		PS1, PM5, PM1, PM2, PP2, PP3, PP5	P

AD	DEE	*ZBTB20* (NM_001348805)	g.114058135G>A	c.1724C>T	p.Ser575Phe	missense	De novo	nf	D	PM2, PM6, PP2, PP3, PP4	LP
AR	DEE	*LAMB1* (NM_002291)	g.107564647_107564698del	c.5201_5224+28del	p.(?)	Splice donor variant	De novo	nf	nd	PVS1+PM2+PM3+PP4	P
			g.107600231T>C	c.2363A>G	p.Asn788Ser	Missense	Paternal	0.0000119/(3)	T	PM2+PM3+PP4+BP4	VUS/LP
XL	EE	*KIAA2022* (NM_00100853)	g.73962676_73962679del	c.1713_1716del	p.Ser571Argfs*13	Frameshift	De novo	nf	nd	PVS1+PM2+PM6+PP4	P
AD	EE	*CACNA1E* (NM_001205293)	g.181726221G>A	c.4288G>A	p.Gly1430Arg	Missense	De novo	nf	D	PS1+PM1+PM2+PM6+PP3+PP4 + PP5	P
AD	DE	*GABRB2* (NM_021911)	g.160886715C>G	c.373G>C	p.Asp125His	Missense	Unknown	nf	D	PM2+PM5+PP3+PP4	LP
AD	DE	*ANKRD11* *(NM_001256183)*	g.89351632G>A	c.1318C>T	p.Arg440*	Nonsense	De novo	nf	nd	PVS1+PM2+PP3+PP5	P
AD	DE	*DYRK1A* (NM_001396)	g.38878524delC	c.1669delC	p.Gln557Argfs*35	Frameshift	De novo	nf	nd	PVS1+PM2+PM6+PP3	P
AR	DE	*PNKP* (NM_007254)	g.50367645G>C	c.514C>G	p.Leu172Val	Missense	Maternal	Not found	D	PVS1+PP4	P
			g.50365057delinsGTTGTCGATGGCGACCC	c.1270delAinsGGGTCGCCATCGACAAC	p.Thr424Glyfs Ter75	Frameshift	Paternal	41	nd	PM2+PM3+PP3+PP4	LP

Abbreviations: M-CAP: Mendelian Clinically Applicable Pathogenicity; D: possibly pathogenic variant.

ACGM scores for asses the variant pathogenicity according the Americans College of Medical Genetics guideline:

PVS1: Null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease;

PM1: Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation;

PM2: Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium;

PM6: Assumed de novo, but without confirmation of paternity and maternity;

PP1: Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease. Note: May be used as stronger evidence with increasing segregation data.

PP2: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease;
PP3: Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.);
PP4: Patient's phenotype or family history is highly specific for a disease with a single genetic etiology;
PP5: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.

3.7.2. *Variants of Unknown Significance (VUS)*

We identified 13 variants of unknown significance in: *SCN2A*, *TRIP12*, *KIF1A*, *AARS*, *SCN1A*, *GABRG1*, *GRIN2A*, *CACNA1A*, *SCN9A*, *EEF1A2*, *KCNQ2*, *GRIN1*, *SCN8A*.

Some of these variants are compatible with patient phenotype, but they proved inherited and/or with an allelic frequency different from zero in databases reporting frequency data in control populations; some of them have been identified in genes characterized by incomplete inheritance, therefore it would be useful to extend the segregation study to a larger number of relatives to better define if they are pathogenic or not.

We also classified other variants as VUS if we were unable to extend the study for lack of parent's biological material (Table 11).

Table 11 - VUS

TRANS	**PHENOTYPE**	**GENE**	**REFSEQ**	**IDENTIFIED MUTATIONS**				**INHERITANCE**	**gnomAD**	**ACMG SCORES**
				Chromosomal position (GrCH37)	**c.DNA nucleotidic change**	**Protein aminoacidic change**	**Function**		**Frequency/ Allele count(AC)**	
AD	DE	*SCN2A*	NM_001040143.1	g.166210846C>T	c.3064C>T	p.Arg1022Cys	missense	inc (brother wt)	not found	PM2, PP2, PP3
AR/AR	EE	*TRIP12*	NM_004238.2	g.230724057G>A	c.332C>T	p.Ser111Leu	Missense	inc (mother wt)	3	PP3, BS2
		KIF1A	NM_001244008.1	g.241710430G>C	c.1299C>G	p.Ile433Met	Missense	maternal	2	PM2, PP2, BS2
AR/AD	EE	*AARS*	NM_001605.2	g.70296392G>A	c.1528C>T	p.Arg510Cys	Missense	unknown	16	PM2, PP3

				g.70316554G>A	c.113C>T	p.Thr38Ile	Missense	unknown	1	PM2, PP3
		SCN1A	NM_006920.5	g.166915084G>C	c.379C>G	p.His127Asp	Missense	unknown	20	PP2, PP3, PP5, BS2
AD	EE	*GABRG1*	NM_173536.4	g.46060358delA	c.792delT	p.Phe264LeufsTer3	frameshift	unknown	30	PM2, PP3
AD/AD	DE	*GRIN2A*	NM_001134407.3	g.9858605C>G	c.2796G>C	p.Met932Ile	Missense	inc (mother wt)	2	PM2, PP3
		CACNA1A	NM_001127221.1	g.13320155G>A	c.6500C>T	p.Ser2167Phe	Missense	inc (mother wt)	not found	PM2, PP3
AD	DE	*SCN9A*	NM_002977.3	g.167055561A>C	c.5555T>G	p.Met1852Arg	Missense	unknown	3	PM2, PP3
AD	EE	*EEF1A2*	NM_001958.4	g.62126283G>A	c.496C>T	p.Arg166Cys	Missense	inc (mother and brother wt)	not found	PM2, PP2, PP3
AD/AD	DE	*KCNQ2*	NM_172107.3	g.62062720G>C	c.1121C>G	p.Ser374Trp	Missense	maternal	not found	PM2, PP3, PP2
		GRIN1	NM_001185090.1	g.140033940T>A	c.2T>A	p.Met1Lys	start_lost	maternal	not found	PVS1, PM2, BS1, BS2
AD	EE	*SCN8A*	NM_014191.3	g.52200785G>A	c.5515G>A	p.Gly1839Arg	Missense	unknown	not found	PM2, PP2, PP3

3.8. Therapy

At our last visit, 192 patients are taking at least one antiepileptic drug.

In particular, patients taking only one antiepileptic drug (AED) are 40 (20%), patients taking 2 AEDs are 68 (34%). Patients on 3 AEDs are 51 (25.5%) and patients on more than 3 AEDs are 33 (16.5%).

Four patients are on a ketogenic diet, 17 have a vagus nerve stimulator, currently switched off because of inefficacy in 3 of them.

Table 12 shows the number of AEDs taken by patients with EE, patients with DE and patients with developmental and epileptic encephalopathy (DEE). Mean number of AEDs was 3 in patients with EE and 2 in patients with DE.

Table 10 – Number of AEDs at last visit

AEDs	EE		DE		DEE	
	Patients	%	Patients	%	Patients	%
1	3	1.5%	32	16%	5	2.5%
2	19	9.5%	33	16.5%	16	8%
3	21	10.5%	14	7%	16	8%
>3	11	5.5%	8	4%	14	7%
None	0	-	7	3.5%	1	0.5%

At our last visit, we evaluated the response to therapy that was less than 50% in 54 patients (27%) and more than 50% in 58 patients (29%). Seizure frequency was unaltered in 29 patients (14.5%), considered not responders. A total of 59 patients (29.5%) had been seizure-free for one year at least (mean 9 years; range 1-52) (Table 13). We also divided response to therapy based on the diagnosis into 3 groups of patients (Table 14).

Table 11 – Response to therapy at last visit

Response to therapy	Patients	%
Non-responder	29	14.5%
<50%	54	27%
>50%	58	29%
Seizure-free	59	29.5%

Table 12 – Response to therapy in 3 different group of patients

Response to therapy	EE		DE		DEE	
	Patients	%	Patients	%	Patients	%
Non-responder	10	5%	11	5.5%	8	4%
<50%	25	12.5%	15	7.5%	14	7%
>50%	16	8%	20	10%	22	11%
Seizure-free	3	1.5%	48	24%	8	4%

In 174 patients we were able to trace the therapy at the moment of the first visit: in 49 patients therapy was not modified, whereas in the remaining 125 therapeutic changes were made. In 27 patients we simplified therapy by reducing antiepileptic drugs, in particular in 11 patients phenobarbital was discontinued.

In 9 patients we implanted a vagus nerve stimulator (VNS).

Ketogenic diet was prescribed to 4 patients.

Seizure-free patients at our first visit were 32 (16%), whereas at the last visit they were 59 (29.5%).

A total of 24 patients or their relatives reported a global improvement after therapy change, in terms of cognitive performances, daily life activities and relationship skills.

We statistically compared seizure improvement across three groups of patients: patients with a novel etiological diagnosis, patients with an already known etiological diagnosis and patients without etiological diagnosis. We considered patients with a seizure reduction of more than 50%. The result of this comparison did not show any statistically significant difference between these three groups in terms (p: 0.985).

3.9. Diagnosis

Sixty-nine patients (34.5%) came to our attention with an aetiological diagnosis:

- 42 patients with a lesional diagnosis, of which 8 anoxic-ischemic lesions, 2 perinatal haemorrhage and 6 patients with tuberous sclerosis
- 20 patients with a genetic diagnosis
- 6 patients with post-infectious encephalopathy
- one patient with suspected mitochondrial encephalopathy

All patients were re-evalued and in 28 patients we performed genetic tests. In 7 patients we found a genetic aetiology:

Pt 1 (patient with lesion on the MRI, suspected for tuberous), we found a mutation in TSC2 gene.

Pt 2 (diagnosis of mitochondrial encephalopathy): we found an intronic de novo mutation in *SCN1A* gene, pathogenic.

Pt 3 (patient with lesion on the MRI, compatible with polymicrogyria): we found a pathogenic mutation in *DYNC1H1* gene.

Pt 4 (lesional MRI): we found a microdeletion 17q21.3, responsible for di Koolen de Vries syndrome.

Pt 5 (post-infective encephalitis): we found a pathogenic mutation in *SCN1A* gene.

Pt 6 (lesional MRI): we found a pathogenic mutation in *ZBTB20* gene.

Pt 7 (lesional MRI, double cortex): we found a pathogenic mutation in *DCX* gene.

Regarding the 131 patients which came to our attention without an aetiological diagnosis, 65 patients (32.5%) underwent neuroimaging exams and in 101 (50.5%) patients we performed genetic testing.

Genetic tests allowed the identification of the aetiological cause of the clinical picture in 28 patients. In particular: 4 patients had a ring 20 chromosome, one patient had *MECP2* duplication syndrome, one patient had a microdeletion in 22q11.2 responsible for velocardiofacial syndrome and the remaining 20 patients had pathogenic mutations in the following genes: *SLC2A1* (3), *SCN1A* (3), *SCN8A* (1), *PCDH19* (1), *CACNA1E* (1), *RARS2* (1), *PNKP* (1), *ACP2* (1), *KIAA2022* (1), *PRODH* (1), *SMC1A* (1), *GABRB2* (1), *KCNB1* (1), *KCNH5* (1), *STXBP1* (1), *LAMB1* (1), *DYRK1A* (1), *ANKRD11* (1).

Neuroimaging exams allowed us to identify in 8 patients (4%) a lesion potentially responsible for the clinical picture. In Table 15 we reported the results of the MRI and the diagnostic delay for each patient.

Table 13 – Neuroimaging results and diagnostic delay

Patient	MRI	Diagnostic delay
1	Polymicrogyria	36
2	Cortical dysplasia (left temporal)	33
3	Cortical dysplasia and left mesial temporal sclerosis	29
4	Cortical dysplasia (right temporal)	49

5	Cortical dysplasia (bilateral)	29
6	Tuberous sclerosis	18
7	Heterotopia	30
8	Cortical dysplasia (bilateral) and heterotopia	37

In 2 patients video-EEG recording with recording of typical "seizures" proved diagnostic for non-epileptic episodes. In these patients the antiepileptic therapy was discontinued.

Overall, we were able to reach an aetiological diagnosis in 45 patients (22.5%) out of our 200 patients' cohort:

- 35 patients with a genetic aetiology;
- 8 patients with a cerebral lesion;
- 2 patients with psychogenic non epileptic seizures.

In particular, in 7 patients with a genetic mutation, we found variants with a potential consequence for antiepileptic drug treatment and clinical approach, that is SCN1A (5), SLC2A1 (3) and SCN8A (1).

3.10. Practical implications

For 11 of the 45 patients in which we clarified the aetiological diagnosis, the new diagnosis might have a consequence for antiepileptic treatment, management of comorbidities and risk of transmission of the disease to children.

We identified 2 patients with Dravet syndrome (ID 14 and 36) in which the diagnosis allowed a therapeutic change. In the patient with ID 14 we discouraged use of phenytoin for the treatment of his status epilepticus, which usually determined worsening of seizures, and we suggested use of benzodiazepines, with which he is successfully treated at home, avoiding hospitalization. In the patient with ID 36 we introduced topiramate, second line drug for Dravet syndrome, and the patient became seizure free.

In patients with Glut1 deficiency syndrome (ID 15, 25 and 35) we suggested ketogenic diet, with seizure improvement and in one patient (ID 25) seizure freedom.

One patient (ID 12) has an SCN8A mutation for which in literature good response to sodium blocker drugs has been reported.

In the patient with ID 11 we started a trial with high doses of B6 vitamin after we clarified the mitochondrial nature of his encephalopathy.

Also in the patient with ID 1 the variant found can affect therapeutic management, because good therapeutic response is reported in patients treated with topiramate.
In the patient with ID 20 we requested preconceptional counselling in order to clarify transmission risks to her kids.
Patient with ID 43 underwent further diagnostic investigation in order to exclude comorbidities described in association with DYRK1A gene mutation.
Furthermore, in the patient in which video-EEG allowed us to clarify the non-epileptic nature of her seizures (ID 30), we withdrew antiepileptic drugs, with cognitive skills improvement.

4. CLINICAL CASES

Table 16 summarizes all patients in which we have found an aetiological diagnosis.

ID	Sex, age	Age at onset (months)	Psychomotor development	ID	Phenotype	Psychiatric comorbidity	Seizures at onset	Seizures at last visit	Epileptiform abnormalities	MRI	Diagnosis	Therapy	Response to therapy	Diagnostic exam	Aetiology
1	M, 25	6	Delay	Severe	EE		F motor	F motor	Yes	Normal	*CACNA1E*	VPA, LTG	<50%	Exome	Genetic
2	M, 38	24	Delay	Moderate	DE		Unknown onset motor	Unknown onset motor	Yes	Lesional	Multifocal polymicrogyria	LEV, LTG, AZT, VPA, CLB	NR	MRI	Lesional
3	F, 56	48	Regression	Severe	EE		G motor	G nonmotor	Yes	Non-specific	Ring20	VPA, LTG, PRP	<50%	Karyotype	Genetic
4	M, 38	6	Delay	Moderate	DE	Aggressive behaviour	G motor	G motor	Yes	Lesional	Dysplasia	VPA, PHT	>50%	MRI	Lesional
5	M, 51	36	Delay	Mild	DE	Aggressive behaviour	G motor	Absent	Yes	Non-specific	*SCN1A*	LTG, LEV, AZM	SF	Gene panel	Genetic
6	M, 50	12	Delay	Moderate	DEE		G nonmtor	G nonmotor	Yes	Lesional	Dysplasia + temporal sclerosis	LTG, OXC	>50%	MRI	Lesional
7	F, 32	132	NA	Moderate	EE		G nonmtor	G nonmotor	Yes	Normal	Ring20	LTG	<50%	Karyotype	Genetic
8	M, 43	3	Delay	Severe	EE		Unknown onset motor	F motor + tc	Yes	Lesional	*TSC2*	LTG, VPA, CLB	>50%	Gene panel	Genetic
9	M, 65	48	Normal	Mild	DE	Depression	Unknown onset motor	No	Yes	Lesional	Dysplasia	LTG, PRP, CLB	SF	MRI	Lesional
10	M, 27	1	Delay	Borderline	DE	Depression	G motor	No	No	Normal	Not seizures	OXC, LTG, CLB	SF	Video-EEG	Not seizures
11	M, 26	8	Delay	Mild	EE		Unknown onset motor	Unknown onset motor	Yes	Non-specific	*RARS2*	VPA, LTG, CNZ	<50%	Exome	Genetic
12	M, 22	36	Delay	Severe	DEE		G motor	G motor	Yes	Non-specific	*SCN8A*	VPA, ZNS, ETS, BRV	>50%	Gene panel	Genetic
13	F, 31	12	Delay	Mild	DE		F motor	No	Yes	Normal	*PNKP*	LEV, LTG	SF	Exome	Genetic
14	M, 38	6	Regression	Severe	DEE		F motor + tc	F motor + tc	Yes	Non-specific	*SCN1A*	LEV, LTG, VPA, PRP	>50%	Sanger sequencing	Genetic
15	M, 26	6	Delay	Severe	EE		G motor	G motor	Yes	Normal	*SLC2A1*	VPA, LTG, KD	<50%	Gene panel	Genetic

16	F, 26	108	Delay	Mild	DE		G nonmtor	No	Yes	Normal	*APC2*	LEV, ETS	SF	Exome	Genetic
17	M, 36	18	Delay	Moderate	EE		Unknown onset nonmotor	Unknown onset motor	Yes	Lesional	*DYNC1H1*	LTG, VPA	NR	Exome	Genetic
18	F, 48	24	Delay	Severe	EE		G motor	G motor	Yes	Normal	*KIAA2022*	VPA, LTG, RFN	<50%	Exome	Genetic
19	M, 28	6	Delay	Moderate	EE	Autism	Unknown onset motor	No	No	Normal	*PCDH19*	VPA, LEV	SF	Gene panel	Genetic
20	F, 34	24	Regression	Borderline	DE	Oppositive behaviour	G motor	No	Yes	Non-specific	*PRODH*	-	SF	Exome	Genetic
21	F, 37	84	Delay	Severe	DE		G motor	G motor	Yes	Normal	*SMC1A*	LEV, CLB, LTG, TPM	<50%	Exome	Genetic
22	F, 43	60	Regression	Mild	DE		Unknown onset nonmotor	No	Yes	Normal	*GABRB2*	VPA	SF	Exome	Genetic
23	F, 49	12	Regression	Moderate	DEE	Behavioural disorder	Unknown onset motor	No	Yes	Normal	*KCNB1*	-	SF	Exome	Genetic
24	F, 53	96	Delay	Moderate	DEE		G nonmtor	G nonmotor	No	Lesional	Dysplasia	VPA, LTG, PRP, AZT, DZP	NR	MRI	Lesional
25	F, 41	12	Delay	Moderate	EE		G nonmtor	No	Yes	Non-specific	*SLC2A1*	VPA, RFN, KD, VNS	SF	Gene panel	Genetic
26	M, 26	12	Delay	Borderline	DE	Behavioural disorder	G motor	No	No	Normal	*SCN1A*	LEV, VPA	SF	Sanger sequencing	Genetic
27	M, 60	1	Normal	Mild	DE		G motor	No	Yes	Non-specific	*KCNH5*	LTG	SF	Exome	Genetic
28	M, 37	96	Normal	Borderline	DEE	Behavioural disorder	F nonmotor	F nonmotor	Yes	Normal	Ring20	RFN, OXC, CLB	<50%	Karyotype	Genetic
29	M, 24	84	Delay	Moderate	DE		Unknown onset motor	No	Yes	Lesional	Microdeletion 17q21.3	LTG, CLB	SF	Array-CGH	Genetic
30	F, 26	60	Normal	Moderate	DE	Behavioural disorder	G nonmtor	No	No	Non-specific	Not seizures	-	SF	Video-EEG	Not seizures
31	M, 27	12	Normal	Mild	DEE	Depression	F motor + tc	F motor + tc	Yes	Non-specific	*STXBP1*	CBZ, TPM, LCS, CLB	<50%	Exome	Genetic

32	M, 40	8	Normal	Borderline	DEE	Depression	F nonmotor + tc	F motor	Yes	Lesional	Tuberous sclerosis	LTG, VPA, CLB	<50%	MRI	Lesional
33	M, 52	48	Delay	Moderate	DE	Depression	G motor	No	No	Non-specific	Microdeletion 22q11.2	PB	SF	FISH	Genetic
34	M, 56	6	Delay	Severe	DEE		F motor + tc	F motor + tc	Yes	Normal	*SCN1A*	PB, VPA, AZT, CLB	>50%	Exome	Genetic
35	M, 19	12	Regression	Borderline	DEE		G motor	G motor	Yes	Normal	*SLC2A1*	LTG, VPA, PRP, KD	>50%	Sanger sequencing	Genetic
36	M, 50	4	Delay	Severe	DEE		Unknown onset motor	No	No	Non-specific	*SCN1A*	VPA, TPM, CLB	SF	Gene panel	Genetic
37	F, 30	30	Delay	Severe	DE		G motor	No	No	Non-specific	*ANKRD11*	LTG, ETS	SF	Exome	Genetic
38	M, 31	72	Delay	Severe	DEE	Behavioural disorder	G motor	G motor	Yes	Non-specific	*ZBTB20*	LTG, VPA, CBZ	<50%	Exome	Genetic
39	M, 52	132	Delay	Borderline	DEE		F motor + tc	No	Yes	Normal	Ring20	PHT, LTG, VPA	SF	Karyotype	Genetic
40	M, 54	4	Delay	Mild	DEE	Behavioural disorder	F motor + tc	F motor + tc	Yes	Lesional	*LAMB1*	LTG, PB, RFN, PRP	<50%	Exome	Genetic
41	F, 35	36	Normal	Moderate	DEE		F	F	Yes	Lesional	Heterotopia	OXC, VPA	<50%	MRI	Lesional
42	F, 43	12	Normal	Moderate	DE	Autism	G motor	F	Yes	Lesional	Dysplasia + heterotopia	LTG	<50%	MRI	Lesional
43	F, 28	24	Delay	Moderate	DE		G motor	G motor	Yes	Non-specific	*DYRK1A*	LTG, VPA	NR	Exome	Genetic
44	F, 31	36	Regression	Severe	DEE		Unknown onset motor	Unknown onset motor	Yes	Lesional	*DCX*	RFN, VPA, GBP, CLB	>50%	Gene panel	Genetic
45	M, 22	60	Delay	Severe	EE		Unknown onset nonmotor	Unknown onset motor	Yes	Lesional	*MECP2* duplication	RFN, VPA, LTG, PRP, NTP	NR	MLPA	Genetic

F: focal, G: generalized, TC: tonic-clonic, MLPA: Multiplex Ligation-dependent Probe Amplification, VPA: valproic acid, LTG: lamotrigine, LEV: levetiracetam, AZT: acetazolamide,

CLB: clobazam, PRT: perampanel, PHT: phenytoin, OXC: oxcarbazepine, CNZ: clonazepam, ETS: ethosuximide, ZNS: zonisamide, BRV: brivaracetam, KD: ketogenic diet, RFN: rufinamide, TPM: topiramate, DZP: diazepam, VNS: vagus nerve stimulator, LCS: lacosamide, PB: phenobarbital, GBP: gabapentin, NTP: nitrazepam

Below, we describe in detail some clinical cases in which the clarification of aetiological diagnosis allowed us to change the patient's management, with improvement of seizures or comorbidities.

4.1. ID 1, male, 25 years old

Negative family history for epilepsy.

Born at 41 weeks, presented perinatal suffering (umbilical cord around his neck). He presented with congenital strabismus and cryptorchidism.
Psychomotor development was normal since seizure onset at 5 and a half months, then he presented with severe delay (seated position at 1 and a half year, walking at 4 years, language almost absent, no sphincterial control).
At 5 and a half months he started having prolonged spasms. He was diagnosed as West syndrome and treated with ACTH.
At the age of 3 he started having motor arrest and eye deviation episodes. At the age of 6 during these episodes he could have repetitive arms movements. He also had a focal seizure with evolution in bilateral tonic-clonic seizure and staring seizures.
He continues presenting with seizures characterized by frightened expression, screaming, impaired awareness, sweating and pallor. Frequency is every 3-4 months.
Neurological examination showed congenital strabismus, walking with enlarged base, on his toe.
Brain MRI was normal. Karyotype, fragile X and array-CGH were negative.
Exome study showed a mutation on *CACNA1E* gene, localized on chromosome 1q 25.3, coding for subunit $\alpha_{1\varepsilon}$ of channel $Ca_v2.3$.
In 2018 Helbig et al. described 30 patients with *CACNA1E* gene de novo mutations (EIEE69 on OMIM) (58). Clinical picture of patients is characterized by refractory infantile-onset seizures, severe hypotonia, and profound developmental impairment, often with congenital contractures, macrocephaly and hyperkinetic movement disorders.

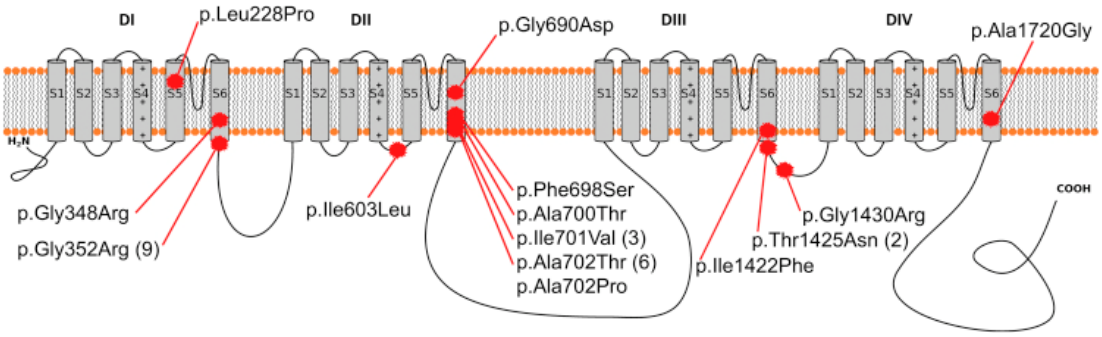


Figure 3 - Secondary Structure of the Cav2.3 Channel with the Distribution of Disease-Causing Missense Variants (Helbig 2018)

Our patient presented a de novo missense mutation in c.4231G>A position (p.Gly1411Arg), predicted to be pathogenic by CADD and PP2.0 and not present in ExAC and gnomAD databases. This mutation is on S6 segment of the third domain. Phenotype of our patient is similar to the ones described by Helbig and colleagues. Our patient is currently on VGB and LTG. In Helbig's study, 5 patients became seizure free after taking topiramate, which acts on $Ca_v2.3$ channels reducing type R calcium current (58).

In the future, we will try topiramate in our patient as a therapeutic option.

4.2. ID 11, male, 28 years old

Familial history negative for neurologic diseases.

Born with caesarean section because of previous abortion at VIII month for gestosis.

He presented psychomotor delay since the first months of life.

At the age of 8 months he started having spasms, treated successfully with B6.

At the age of 8 years old he presented with tonic-clonic seizures when he was falling asleep and atonic seizures.

He started LTG, with worsening. Despite different antiepileptic drugs (ACZ, PCT, CLB, CLZ, LEV, VPA, RFM) he continued having seizures every 2-3 months.

He is now on VPA, CLB and LTG and he is only having blinking monthly episodes.

Neurological exams revealed arms myoclonus, dysarthria.

EEG showed temporo-parieto-occipital epileptiform abnormalities and photo paroxysmal response to intermittent photic stimulation (IPS).

MRI was normal.

Array-CGH, single gene sequencing (ARX, SLC2A1, ALDH7A1) and 43 gene panel were negative.

WES identified mutation in RARS2 nuclear gene.

Mutations in this gene were associated with pontocerebellar hypoplasia type 6 (PCH6), associated with hypoplasia and multiple mitochondrial defects in respiratory chain.

Our patient has a normal MRI, that is already described in literature. In a review of 23 patients pontocerebellar hypoplasia was described in 80% of cases (59). Our patient's clinical phenotype of these 23 patients reported, with early onset epileptic seizures (within the first year of life), hypotonus and ID.

The diagnosis can improve patient's management, because of:

- better phenotypic characterization, with evaluation of high lactate and hypoacusia
- muscular biopsy in order to evaluate respiratory chain function
- therapy modification: VPA withdrawal, because it can worsen mitochondrial diseases and antioxidant diseases: coenzyme Q, thiamine, riboflavin), that could help in mitochondrial diseases.

4.3. ID 13, female, 31 years old

Familial history negative for neurologic diseases.

She presents microcephaly, mild intellectual disability. Walking at 14 months, language delay, she cannot write.

At the age of 11 months she presented her first generalized seizure, with eyelid myoclonus. She had the second seizure at 14 months, and then again at 2 years and 5 months. Then she continued having focal seizures with impaired awareness, with monthly frequency.

She is now on LEV and LTG, seizure free since 2012.

EEG showed epileptiform abnormalities on right posterior regions. Brain MRI was reported as normal (no report available). Array-CGH showed 7q36.1 (151,832,037-152,008,417) x 4 maternal, unknown significance.

WES disclosed *PNKP* gene mutation. *PNKP* gene codes for a protein with a kinase domain and a phosphatase domain, involved in DNA repair of damage caused by oxidative stress (60).

Homozygous or heterozygous mutations have been associated by Shen in 2010 with congenital microcephaly, epileptic seizures and psychomotor delay (MCSZ/EIEE10,

OMIM 613402) (61) and by Bras in 2015 to oculomotor ataxia-apraxia (AOA4, OMIM 616267) (62).

In our proband composed heterozygosis consists of pathogenic variant p.Thr424fs (paternal) and missense variant p.L172V (maternal) not found in GNOMAD.

The frameshift starting with Thr424 codon replaces this amino acid with a glycine residue and create a premature stop codon in position 49 of the new lecture frame (p.Thr424GlyfsX49). This variant is predicted to cause loss of protein function with truncation, because the last 98 amino acids are substituted by aberrant residues (62).

Clinical picture of our patient, with microcephaly, developmental and language delay and early onset seizure (11 months) is consistent with the MSCZ phenotype.

For a full patient's assessment, it will be useful in the future:

- new brain MRI, in order to exclude AOA4 presence and a degenerative picture as the one described by Poulton (63);
- alpha-fetoprotein measurement, as it was elevated in some patients (62);
- cutaneous fibroblast culture to show reduced activity of PNKP, increased apoptosis susceptibility, that could be the mechanism at the basis of the microcephaly in the MCSZ.

4.4. ID 14, male, 38 years old

This is a 32-year-old male born at term by normal pregnancy after prolonged labor. A significant global developmental delay had been recognized since 6 years of age.

At age 5 months he experienced the first epileptic seizure during hyperpyrexia characterized by a left hemiclonic seizure during sleep followed by a bilateral convulsive seizure. Since then he had monthly generalized motor seizures, with or without fever, mainly arising from sleep. Seizures became weekly despite trials of several antiepileptic drugs. Recurrent episodes of intractable status epilepticus requiring accesses to hospital and worsened by diphenilidanthoyn were also reported.

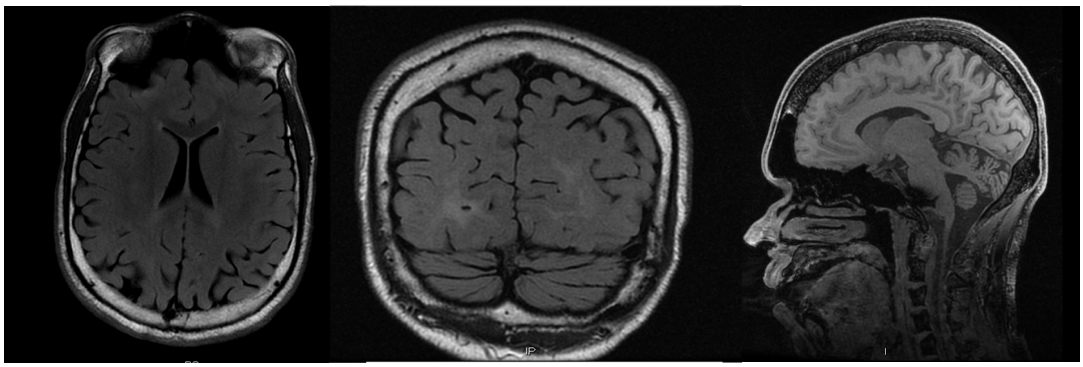
At the age of 17 the patient underwent muscle biopsy showing a similar ragged red aspect and a defect in mitochondrial electron transport chain complex I activity. A diagnosis of mitochondrial encephalopathy was made. At our first assessment (32 years of age) the patient was fully re-evaluated.

Neurological examination showed craniofacial dysmorphism and global severe delay. During prolonged video-EEG monitoring we recorded one of his typical status

epilepticus episodes clinically characterized by left arm clonic seizures and bilateral convulsive seizures.

Because of the previous diagnosis of mitochondrial disease, the patient repeated muscle biopsy and the result was negative. Furthermore, acid lactic dosage on serum and liquor was normal and main mutations of mitochondrial DNA were excluded. Electromyography and somatosensory evoked potentials were normal. Brain MRI disclosed a mild cerebellar atrophy (Figure 3).

Figure 4 – Brain MRI



Molecular analysis of SCN1A gene identified a de novo intronic mutation, causing nucleotidic substitution c.383+3A>C and exon skipping on exon 2, thus a diagnosis of Dravet syndrome has been made.

Since the diagnosis was made, use of sodium blocker drugs has been discouraged and the patient presented with a reduction in the number of seizures and status epilepticus episodes, well treated at home with benzodiazepines.

4.5. ID 20, female, 35 years old

Positive family history for epilepsy in her sister, which was on antiepileptic therapy for some years, then discontinued.

Since infancy, her relatives noticed learning difficulties and behavioural disorder.

She had a positive personal history of migraine.

At the age of one year and 8 months she presented an episode with impaired awareness, facilitated by high environmental temperature.

One year later she presented 3 tonic seizures during fever. After other seizures also without fever and after epileptiform abnormalities on EEG, she started antiepileptic

therapy at the age of 3 and a half years (TPM, VPA, CLB, LTG), which she discontinued at the age of 28. In the last years she did not present clinical relevant episodes, but she reports nocturnal episodes of auditory hallucinations, which wake her up (these episodes do not seem to have an epilpeptic origin).

On EEG she has epileptiform diffuse abnormalities with erratic photoparoxysmal response to intermittent photic stimulation.

Brain MRI showed might asymmetry of the temporal corn of the lateral ventricle and slight hippocampal asymmetry, with no signal alteration.

She underwent karyotype and *SLC2A1* gene mutation research, both negative.

Exome analysis showed mutation c.1397C>T (p.Thr466Met) and c.1322T>C (p.Leu441Pro) in *PRODH* gene in compound heterozygosity, inherited by her mother and her father.

Both these variants are already reported in literature (64) (65) as associated with hyperprolinemia type 1, a congenital abnormality of proline metabolism characterized by high levels of this aminoacid in blood and urine. Transmission is autosomal recessive.

This disease is usually benign, but can be associated with epileptic seizures, renal abnormalities, neuropsychological and behavioural disorders.

This patient underwent plasmatic and urinary aminoacid dosing, showing high levels of proline (501; range 152-226), cysteine (64; range 32-55) and tyrosine (102; range 50-76).

Our patient was planning to become pregnant, therefore she underwent genetic counselling, which identified a low transmission risk, because of the rarity of the disease in the general population.

Furthermore, we extended genetic analysis to patient's sister.

4.6. ID 24, female, 41 years old

Negative family history for epilepsy.

Febrile seizure (39.5°) at 8 months, characterized by loss of awareness for 10 minutes. The day after she presented with right arm paralysis, never solved.

Since this episode, a psychomotor delay became evident (poor language, walking at 2 years). She had moderate intellectual disability.

At 10 months she started having head falling episodes, with eyes rolling, eyelid myoclonus, lasting 5 minutes. They occurred when awake and were rare. She started sultiame and became seizure-free.

Since the age of 6 years she started having migraine, sometimes followed by left arm and leg hyposthenia.

Since the age of 9 she presented brief seizures when she woke up, characterized by rapid head and shoulder movement, staring, some eyelid myoclonus, sometimes lasting hours. Despite the high number of antiepileptic drugs attempted, she continued presenting seizure.

The EEG showed diffuse epileptiform abnormalities.

In this patient, we performed a 43 gene panel and we found an SLC2A1 gene mutation, responsible for GLUT1 deficiency syndrome.

After the diagnosis, patients started ketogenic diet and she became seizure-free after a 40-years-long disease.

4.7. ID 30, female, 26 years old

This is a 25-year-old female who had since 6 years of age a developmental delay. Since then her mother started to notice episodes characterized by staring and eyes deviation towards left. She underwent an EEG showing epileptiform abnormalities. Despite numerous antiepileptic drugs, she continued presenting daily episodes.

She came to our attention at the age of 21 years old, with a diagnosis of epileptic encephalopathy of unknown cause. Neurological exam showed a moderate intellectual disability. She was taking two antiepileptic drugs and having daily seizures.

Brain MRI, genetic analysis (karyotype and SLC2A1 analysis) and metabolic testing were normal.

Video-EEG allowed the recording of several episodes. During these episodes no epileptic discharges were recorded, therefore an epileptic origin was excluded.

Antiepileptic drugs have been withdrawn without any effect on episodes, which we interpreted as voluntary eye movements in a globally delayed patient. After the antiepileptic drugs discontinuation, the patient's mother noticed some improvement in patient's alertness and participation in daily life activities.

5. DISCUSSION

The primary aim of this study is the clinical and genetic characterization of adult patients with paediatric onset epilepsy and ID.

Management of these patients, who in the past was a prerogative of child neurologists, is becoming an emerging problem, because transition to the adult services due to reached age limits is an increasing practice.

A lot of these patients continue having seizures not controlled by therapy also in adulthood, thus they came to adult epileptologist's attention.

Since the first visit, recruited patients have been studied with a homogeneous diagnostic protocol, that aimed to provide an accurate electro-clinical characterization that in some cases was helpful in establishing aetiological diagnosis, unknown in the majority of our patients.

Even in patients with an aetiological diagnosis, often this was made several years before, and sometimes was based on summary anamnestic data and without accurate diagnostic exams (neuroimaging or genetic exams), that could not be available at the moment of the disease onset. Many patients came to our attention with anoxic-ischemic encephalopathy diagnosis, based only on dystocic delivery history.

Syndromic definition and aetiological diagnosis in adult patients with ID and epilepsy is particularly challenging, because the long disease history and the effects of a long-lasting antiepileptic therapy can mask the original clinical situation, as recently reported by Catarino et al. (66).

Some infantile onset syndromes have typical characteristics that modify over the years. For example, some important aspects of Lennox Gastaut syndrome, useful for diagnosis, change over time; typical EEG abnormalities and seizure semiology change in adult patients, therefore in adult patients LGS is less easily identifiable than in children (29).

For patients referring for the first time to an adult epilepsy centre, detailed medical records are often not available, thus the collection of the medical history relies on the family or the caregiver. Unfortunately, parents may not be alive or able to recall the patient's medical history and the caregiver may have limited knowledge of the patient, hence it is frequent that important diagnostic details are missing.

Institutionalized patients are escorted by social workers and often they have a limited knowledge of their clinical history.

Difficulties in recording medical history depend on the particular kind of patient, which in the majority of cases is not able to provide their clinical information, and timing of our first visit, performed after many years from the disease onset. In our cases, years between seizure onset and our first observation were 23.5 (range 0-58).

For these reasons, some important aspects regarding family history and patients' clinical history were often unavailable (in particular regarding the perinatal period, epilepsy onset and pharmacological history). Some important information for aetiological suspicion, such a history of seizures in association with fever that would suggest Dravet syndrome, can be completely missing (30).

Almost all our patients before accessing care in our centre had already been evaluated by other neurologists or child neurologists, but in the majority of cases, despite a complex diagnostic and therapeutic process, neither an aetiological diagnosis nor a good therapeutic response were obtained.

In the majority of cases research of the aetiological cause was abandoned and the focus became therapeutic management, often limited to adding a new drug and/or benzodiazepines, with the result of a complex polytherapy, sometimes with low rated drugs and with a major risk of side effects.

This approach in patients with epilepsy and ID is common in clinical practice, because often the specialist renounces the research of the cause, believing that any effort in this direction is disappointing and has no useful implication for the patient.

Actually, the importance of the identification of an aetiological cause, especially if genetic, in previously undiagnosed patients has been recently emphasized because of the chance of setting up a focused drug therapy, a better knowledge of comorbidities and their prevention and the possibility of a family genetic counselling (28).

For these reasons, a full clinical re-evaluation must be guaranteed to all patients with epilepsy and ID, even if already followed up by an epilepsy clinic and with an aetiological diagnosis, which should be always re-evaluated in view of new diagnostic techniques. In fact, in our series we re-evaluated 69 (34.5%) patients which came to our attention with an aetiological diagnosis and in 7 (10%) the diagnosis, formulated years ago, was wrong and we were able to reach a genetic diagnosis.

Patients with epilepsy and ID represent what Galizia defined the “lost generation”, i.e. people who, if seen as a children today, would have probably undergone specific genetic exams in order to reach a diagnosis (31). Since their first years of life, clinical and genetic characteristics of their disease should be framed from a diagnostic point of view, probably avoiding a large number of useless genetic analysis and non-targeted therapies. The “lost generation” fits into a particular historical period, when scientific knowledge, especially in the genetic field, was not so advanced at the moment of the disease onset to define the aetiology and later the clinical picture evolution masked peculiar characteristics, making the diagnostic assessment harder.
In some cases, even EEG, which is an easy to perform, fast and cheap exam, can help in formulating a diagnosis: for example, it can show a peculiar pattern suggestive of ring chromosome 20 syndrome.
Thanks to neuroimaging progress, it is now possible to identify structural abnormalities, not visible before. Many of our patients performed a neuroradiologic study only at the disease onset, often limited to a CT scan or a low-field MRI, later not repeated because of the patients’ behavioural problems or poor collaboration, requiring sedation or general anaesthesia.
With regard to the disease onset and the first visit, new syndromes have been identified. There are new diagnostic methods available, such as genetic tests.
Genetic exams should be targeted based on the clinical suspicion, but in adult patients this can be challenging, because in adult some peculiar syndromic characteristics typical of the paediatric age can be missing.
Cytogenetic studies are really helpful in patients with epilepsy and ID, especially in case of major malformation or somatic dysmorphisms.
Gene panel is a useful approach, with a diagnostic rate variable between 10 and 48.5% (51). Also an advanced technique such as WES has become helpful in clinical practice, with a 25% diagnostic rate (53).
Full exome analysis has the advantage of the possible identification of novel or very recently discovered new genes for phenotypes with both ID and epilepsy that have not yet been included in current panel releases. In addition, full exome analysis allows a genome-wide analysis for chromosomal copy number variations and can thereby replace separate chromosomal analysis by array analysis (67).
Despite the high yield of WES, it is important to realize that some genetic causes of ID and epilepsy cannot be detected by this approach. WES will not detect

trinucleotide repeats, as for example a CGG repeat expansion in the *FMR1* gene in fragile X syndrome. Also balanced chromosomal rearrangements, such a ring chromosome 20, will not be detected using WES (68).

In both these exams, a substantial limit is represented by the findings of unknown significance, especially in a cohort of patients like ours, where parents are often unavailable for testing (31).

In our series, we performed WES in 69 patients and in 17 of them we were able to identify a genetic aetiology. WES diagnostic field in our study is 24.6%, not different from literature data of 25%.

Performing genetic studies as gene panel and WES in adult patients has some challenging implications: finding of unknown significance (VUS) are frequent, and relatives, necessary for extending genetic exams, sometimes can be missing.

In future studies, such as functional studies and studies in larger patient groups, it will be possible to re-evaluate these variants and further delineate the related phenotype.

Clarifying the genetic aetiology, even if many years after the disease onset, has positive implications for patients and their family (32).

Benefits include targeted antiepileptic drug in some syndromes (for example Dravet syndrome and GLUT1 deficiency syndrome) and a better knowledge of possible comorbidities (for example dysphagia and walking difficulties in Dravet syndrome). Furthermore, aetiological diagnosis brings an end to the "diagnostic Odyssey" and other unnecessary and maybe invasive investigations (31).

In our series, we did not find a statistically significant difference (p:0.985) in good response to therapy (seizure improvement of more than 50%) between three groups of patients: patients with a novel etiological diagnosis, patients with an already known etiological diagnosis and patients without etiological diagnosis.

A difficulty we found in our population, is the lack of clear diagnostic criteria for EE. The majority of our patients came to our attention classified as EE.

We tried, basing on the new classification (17), to divide our patients into 3 categories: epileptic encephalopathy, developmental encephalopathy and epileptic and developmental encephalopathy.

This is not only a simple definition, because based on the diagnosis, the treatment can change. In EE it is defined that the epileptic activity itself contributes to the intellectual disability, therefore it is important to treat and reduce seizures, in order

to improve the cognitive skills. Instead, in people with DE using an aggressive approach to reduce the number of seizures can be counter-productive, considering the possible negative effects (especially on cognitive skills) of antiepileptic drugs (18).

Besides neurological problems, many of these patients also have comorbidities, that need to be handled. Psychiatric comorbidity is really important and can associated with a large number of early onset epilepsies, but it is more frequent in patients with severe epilepsy (27). A proportion of 47.5% of our patients presented this comorbidity, the majority of them had behavioural problems. In literature it is reported that in this particular kind of patients, often the psychiatric comorbidity is not treated, because of the fear of using drugs that potentially can worsen epileptic seizures (69).

Another important aspect is that in patients with epilepsy and ID sometimes it is difficult to understand whether character modification can be linked to a seizure (ictal or post-ictal) or, on the contrary, some behavioural issues can be interpreted erroneously as epileptic seizures (69).

In our cases, video-EEG performing allowed the identification of 2 patients with paroxysmal non-epileptic episodes. In one of these patients, classified as drug-resistant and on polytherapy, we withdrew antiepileptic drugs with benefits on the cognitive outcome.

Management of this kind of patients requires a lot of resources and it is extremely complex from a clinical perspective (70).

Mean age of our patients at the moment of their first visit in our centre was 28 years old, while mean age at seizure onset was 6 years old. During time between seizure onset and their first visit (25.6 years in our cases), the majority of patients underwent a large number of visits and diagnostic tests, inconclusive in many of them.

An original aspect of our study is the long follow-up. Patients recruited have been followed up in our centre for 8.5 years (range 1 to 43 years) and it was possible to evaluate the trend of the epileptic seizures and the response to therapy.

Therapy management of these patients is really challenging. They often come to the neurologists' attention on polytherapy, with side effects and without a complete seizure control. Re-evaluation of therapy is essential, especially the simplification and reduction of sedative drugs are tricks that improve patients' quality of life (69).

We noted an improving response to therapy, with increase in the number of seizure-free patients, i.e. from 16% at the first visit to 29% at the last visit.

This positive result is probably due to regular clinical visits and optimization of drug therapy, which also reduce side effects on cognitive skills. A proportion of 12% of our patients (or their relatives) reported a global improvement after therapy change, in terms of cognitive performances, daily life activities and relationship skills.

For 11 of the 45 patients in which we clarified the aetiological diagnosis, the new diagnosis might have a consequence for antiepileptic treatment, management of comorbidities and risk of transmission of the disease to children.

In some cases, aetiological diagnosis allowed a targeted therapy approach. The case of the patient with *SLC2A1* mutation is emblematic. We were able to formulate the diagnosis only after 40 years from the disease onset. The patient, who was drug-resistant, became seizure-free after the introduction of the ketogenic diet. If the metabolic deficit had been identified before, targeted therapy would have been able to reduce the cognitive problems.

Reaching a diagnosis also allows giving a definitive answer to the family, which often have some false beliefs regarding the causes of the disease, such as vaccine administration, a minor trauma during delivery or other irrelevant events that happened before symptom onset and believed to be the cause (71).

When the diagnosis is genetic it is important to refer other family member to a genetic counselling.

Our study has some limitations. The population is extremely heterogeneous and, even if we try to standardize the study protocol, different clinicians performed diagnostic exams. Patients, particularly cognitively impaired ones, were not able to perform certain exams, because of their poor collaboration. Concerning the therapy, the follow- up is not the same for all patient and the range is quite wide. Changes in therapy were made not following a specific protocol but basing on good clinical practice.

6. CONCLUSIONS

The transition from paediatric to adult care is an important moment that provides specialists with the opportunity to carefully re-assess all aspects of patient care.

It is particularly essential in patients with unknown aetiology: they should be fully re-evaluated, using MRI and other investigation tools, in order to exclude or detect specific aetiologies that might affect treatment decisions (29).

A detailed collection of clinical history is essential in order to obtain all clinical information useful to formulate a clinical suspicion: for example, a history of seizures facilitated by fever or occurring after a vaccination is suggestive of Dravet syndrome.

In some patients, a simple EEG, an ordinary and non-expensive investigation, can allow physicians to suspect an aetiological diagnosis: an example is the finding of non-convulsive status epilepticus, suggesting ring chromosome 20 syndrome (56).

Because of the rapid advances in genetics, the group of genetic epilepsies is expanding, with new genes identified in rapid succession. Many genes may have been discovered since the patient's first evaluation; therefore genetic investigations focused on the patient's clinical features should be considered (28).

Array-CGH allows the identification of genetic syndromes with copy number variations (duplications and deletions), but variants of uncertain significance are common. When these variants are found, parents testing is essential to determine whether the mutation is inherited or de novo. In these particular adult cases it is not always possible to collect parent's biological material, as parents might not be alive, so it is not infrequent that uncertain findings remain not clarified.

Targeted gene panels can identify causes of disorders related to single gene mutations, with the advantage that they allow a rapid screening of a large number of selected known genes, representing a fast and cost-effective diagnostic method to detect mutations. In our series, gene panel has a 29% diagnostic rate.

Whole exome sequencing is a useful technique, but a clinical geneticist must be involved in data interpretation because variants of unknown significance are common. Also in this kind of test, the major problem in adult cases is the high possibility that parents' biological material might not be available for segregation study, thus sometimes it is not possible to validate the mutations found.

Genetic diagnosis has significant positive implications for patients, even if it has been made after years from the beginning of the disease. A clear example is clinical case with ID 25: this drug-resistant patient received the diagnosis of GLUT1 deficiency syndrome 40 years after the disease onset. She was drug resistant but after introduction of ketogenic diet she became seizure-free.

Another practical example is patient with ID 14: he received the diagnosis more than 30 years after the disease onset, but it has had a positive implication on the correct clinical management. Sodium blocker drugs have been withdrawn and a better therapy for status epilepticus has been suggested.

Benefits for this type of patients include better-targeted antiepileptic drug (AED) choice, sparing of further unnecessary investigations and improved access to therapies or services (32).

Even if now our knowledge of pharmacotherapy in genetic syndromes is not advanced enough to guide AED choice, for some syndromes there is evidence that some AEDs have to be preferred to others.

For example, in Dravet syndrome valproic acid, stiripentol and clobazam should be tried first, whereas the use of lamotrigine and carbamazepine should be discouraged in order to avoid seizure exacerbation. Another example is the use of ketogenic diet (high-fat, carbohydrate-restricted diet) (72) in glucose transporter type 1 deficiency syndrome (GLUT1-DS), which is associated with *SLC2A1* gene mutations.

A re-evaluation of the pharmacological treatment should also be performed to determine if all appropriate AEDs have been tried, if they are being used at the correct dose and if treatment can be simplified in patients on polytherapy, reducing drug interactions and side effects.

Patients should also be reassessed to determine whether surgery or other types of therapy (vagus nerve stimulation, ketogenic diet) should be considered.

Finally, establishing the genetic aetiology allows the provision of genetic counselling to families and better understanding of comorbidities and prognosis. Determining the aetiology is also important for families, as many of them carry a burden of blame related to mistaken beliefs about disease causation (73).

In our study, we have fully re-evaluated 200 adult patients with epilepsy and ID, the majority of them with unknown aetiology.

All patients underwent a full diagnostic workup and in the majority of them neuroradiologic and genetic investigations were performed.

The patients of our study, defined the lost generation (31), are difficult to manage because of the high number of hospital admission and evaluations they underwent.

Forty-five patients (22.5%) of the recruited patients received an aetiological diagnosis after many years from the disease onset, with a mean diagnostic delay of 30 years since the beginning of epileptic seizures. In 11 patients of the 45 the diagnosis had a consequence for antiepileptic treatment or clinical management.

We statistically compared seizure improvement across three groups of patients: patients with a novel etiological diagnosis, patients with an already known etiological diagnosis and patients without etiological diagnosis. We considered patients with a seizure reduction of more than 50% and we did not find a statistically significant difference (p:0.985) in good response to therapy between the three groups.

We have speculated that one possible reason could be that the majority of patients with a novel etiological diagnosis have received a recent diagnosis and thus a recent change of therapy, therefore it is too early to evaluate their response to it. It is important to follow up these patients in order to evaluate the response to therapy in the future.

Based on the results of our study, it is important to evaluate and characterize in a systematic way these patients, in order to identify an aetiological diagnosis, set up a targeted therapy and evaluate long-term prognosis and response to treatment.

Our results show that this approach results in a clinical improvement with increase of seizure-free patients, which in our series increased from 16% at the first visit to 29.5% at the last follow-up visit. It also results in a global cognitive improvement in 12% of patients.

7. BIBLIOGRAPHY

1. *Neuropsychological effects of epilepsy and antiepileptic drugs.* **Kwan P, Brodie MJ.** Lancet. 2001, Vol. 357(9251), p. 216-22.

2. *Under representation of people with epilepsy and intellectual disability in research.* **Shankar R, Rowe C, Van Hoorn A, et al.** PLoS One 2018, Vol. 13(6).

3. *The genetic basis of non-syndromic intellectual disability: a review.* **Kaufman L, Ayub M, Vincent JB.** J Neurodev Disord. 2010, Vol. 2(4), p. 182-209.

4. *Prevalence of epilepsy among people with intellectual disabilities: A systematic review.* **Robertson J, Hatton C, Emerson E, et al.** Seizure 2015, Vol. 29, p. 46-62.

5. *A systematic review of neuropsychiatric comorbidities in patients with both epilepsy and intellectual disability.* **van Ool JS, Snoeijen-Schouwenaars FM, Schelhaas HJ, et al.** Epilepsy Behav. 2016, Vol. 60, p. 130-137.

6. *Characteristics of a large population of patients with refractory epilepsy attending tertiary referral centers in Italy.* **Alexandre V Jr, Capovilla G, Fattore C, SOPHIE Study Group.** Epilepsia 2010, Vol. 51(5), p. 921-925.

7. *Syndrome of acquired aphasia with convulsive disorder in children.* **Landau WM, Kleffner FR.** Neurology 1957, Vol. 7, p. 523-530.

8. *Epileptic encephalopathy of children with diffuse slow spikes and waves (alias "petit mal variant") or Lennox syndrome.* **Gastaut H, Roger J, Soulavrol R, et al.** Ann Pediatr 1966, Vol. 13(8), p. 489-99.

9. *Epileptic encephalopathy.* **Dulac O.** Epilepsia 2001, Vol. 42(S3), p. 23-6.

10. *A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task Force on Classification and Terminology.* **Engel J.** Epilepsia 2001, Vol. 42(6), p. 796-803.

11. *Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009.* **Berg AT, Berkovic SF, Brodie MJ, et al.** Epilepsia 2010, Vol. 51(4), p. 676-85.

12. *The epileptic encephalopathy jungle - from Dr West to the concepts of aetiology-related and developmental encephalopathies.* **Kalser J, Cross JH.** Curr Opin Neurol. 2018 , Vol. 31(2), p. 216-222.

13. *Reversible acquired epileptic frontal syndrome and CSWS suppression in a child with congenital hemiparesis treated by hemispherotomy.* **Kallay C, Mayor-Dubois C, Maeder-Ingvar M, et al.** Eur J Paediatr Neurol 2009, Vol. 13(5), p. 430-8.

14. *Epilepsy surgery in children with electrical status epilepticus in sleep.* **Loddenkemper T, Cosmo G, Kotagal P, et al.** Neurosurgery 2009, Vol. 64, p. 328-37.

15. *The spectrum of acquired cognitive disturbances in children with partial epilepsy and continuous spike-waves during sleep. A 4 year follow-up case study with prolonged reversible learning arrest and dysfluency.* **Deonna T, Davidoff V, Maeder-Ingvar M, et al.** Eur J Paediatr Neurol 1997, Vol. 1, p. 19-29.

16. *Pediatric Epileptic Encephalopathies: Pathophysiology and Animal Models.* **Shao LR, Stafstrom CE.** Semin Pediatr Neurol. 2016, Vol. 23(2), p. 98-107.

17. *ILAE classification of the epilepsies: Position paper of the ILAE Commission for Classification and Terminology.* **Scheffer IE, Berkovic S, Capovilla G, et al.** Epilepsia 2017, Vol. 58(4), p. 512-521.

18. *Cognitive and memory effects of the new antiepileptic drugs.* **Meador KJ.** Epilepsy Res 2006, Vol. 68(1), p. 63-7.

19. *The epileptic encephalopathies.* **Cross JH, Guerrini R.** Handbook of Clinical Neurology 2013, Vol. 111, p. 619-26.

20. *Age at onset of epilepsy, pharmacoresistance, and cognitive outcomes: a prospective cohort study.* **Berg AT, Zelko FA, Levy SR, et al.** Neurology 2012, Vol. 79(13), p. 1384-91.

21. *De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy.* **Claes L, Del-Favero J, Ceulemans B, et al.** Am J Hum Genet 2001, Vol. 68(6), p. 1327-32.

22. *The genetic landscape of the epileptic encephalopathies of infancy and childhood.* **McTague A, Howell KB, Cross JH, et al.** Lancet Neurol 2016, Vol. 15, p. 304-16.

23. **Shorvon S, Guerrini R, Trinka E, et al.** *The Causes of Epilepsy: Diagnosis and Investigation.* s.l. : Cambridge University Press, 2019.

24. *Myoclonic-astatic epilepsy.* **Doose, H.** Epilepsy Res Suppl 1992, Vol. 6, p. 163-8.

25. *CHD2 myoclonic encephalopathy is frequently associated with self-induced seizures.* **Thomas RH, Zhang LM, Carvill GL, et al, and the EuroEPINOMICS RES Consortium.** Neurology 2015, Vol. 84, p. 951–58.

26. *Glucose transporter 1 deficiency as a treatable cause of myoclonic astatic epilepsy.* **Mullen SA, Marini C, Suls A, et al.** Arch Neurol 2011, Vol. 68, p. 1152–55.

27. *Epidemiologic aspects: Lost in transition.* **Beghi E, Camfield PR, Camfield CS.** Epilepsia 2014, Vol. 55(S3), p. 3-7.

28. *Transition to adult life in the monogenic epilepsies.* **Scheffer IE, Dravet C.** Epilepsia 2014, Vol. 55(3), p. 12-5.

29. *Evolution and management of Lennox-Gastaut syndrome through adolescence and into adulthood: are seizures always the primary issue? .* **Kerr M, Kluger G, Philip S.** Epileptic Disord 2011, Vol. 13(S1), p. S15-S26.

30. *Delivery of epilepsy care to adults with intellectual and developmental disabilities.* **Devinsky O, Asato M, Camfield P, et al.** Neurology 2015, Vol. 85, p. 1-10.

31. *Array comparative genomic hybridization: Results from an adult population with drug-resistant epilepsy and co-morbidities.* **Galizia EC, Srikantha M, Palmer R, et al.** Eur J of Med Gen 2012, Vol. 55, p. 342-8.

32. *Dravet syndrome-From epileptic encephalopathy to channelopathy.* **Brunklaus A, Zuberi SM.** Epilepsia 2014, Vol. 55(7), p. 979-84.

33. *Therapeutic approach to epileptic encephalopathies.* **Vigevano F, Arzimanoglou A, Plouin P, et al.** Epilepsia 2013 , Vol. 54(S8), p. 45-50.

34. *Treatment of Epileptic Encephalopathies.* **McTague A, Cross JH.** CNS Drugs 2013, Vol. 27, p. 175-84.

35. *Prognostic, clinical and demographic features in SCN1A mutation-positive Dravet syndrome.* **Brunklaus A, Ellis R, Reavey E, et al.** Brain J Neurol 2012 , Vol. 135, p. 2329–2336.

36. *Stiripentol in severe myoclonic epilepsy in infancy: a randomised placebo-controlled syndrome-dedicated trial. STICLO study group.* **Chiron C, Marchand MC, Tran A, et al.** Lancet 2000, Vol. 356(9242), p. 1638-42.

37. *Precision medicine in genetic epilepsies: break of dawn?* **Reif PS, Tsai MH, Helbig I, et al.** Expert Rev Neurother 2017, Vol. 17, p. 381–392.

38. *Early and effective treatment of KCNQ2 encephalopathy.* **Pisano T, Numis AL, Heavin SB, et al.** Epilepsia 2015, Vol. 56(5), p. 685-91.

39. *Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study.* **French JA, Lawson JA, Yapici Z, et al.** Lancet 2016, Vol. 388(10056), p. 2153-2163.

40. *GRIN2A mutation and early-onset epileptic encephalopathy: personalized therapy with memantine.* **Pierson TM, Yuan H, Marsh ED, et al.** Ann Clin Transl Neurol 2014 , Vol. 1(3), p. 190-8.

41. *Epileptic encephalopathies: new genes and new pathways.* **Nieh SE, Sherr EH.** Neurother. J. Am. Soc. Exp. Neurother 2014, Vol. 11, p. 796–806.

42. *SINEUPs: A new class of natural and synthetic antisense long non-coding RNAs that activate translation.* **Zucchelli S, Cotella D, Takahashi H, Carrieri C, Cimatti L, Fasolo F, Jones MH, Sblattero D, Sanges R, Santoro C, Persichetti F, Carninci P, Gustincich S.** RNA Biol 2015, Vol. 12, p. 771-779.

43. ***LICE, Commissione Genetica.*** *2016.*

44. The Causes of Epilepsy: Diagnosis and Investigation. ***Shorvon S, Guerrini R, Trinka E, et al.*** *Cambridge University Press, 2019.*

45. Confirmation of chromosomal microarray as a first-tier clinical diagnostic test for individuals with developmental delay, intellectual disability, autism spectrum disorders and dysmorphic features. ***Battaglia A, Doccini V, Bernardini L, et al.*** *Eur J Paediatr Neurol 2013, Vol. 17(6), p. 589-99.*

46. A clinical approach to developmental delay and intellectual disability. ***Vasudevan P, Suri M.*** *Clin Med (Lond) 2017, Vol. 17(6), p. 558-61.*

47. Prevalence of Pathogenic Copy Number Variation in Adults With Pediatric-Onset Epilepsy and Intellectual Disability. ***Borlot F, Regan BM, Bassett AS, et al.*** *JAMA Neurol 2017, Vol. 74, p. 1301-11.*

48. Targeted next generation sequencing as a diagnostic tool in epileptic disorders. ***Lemke JR, Riesch E, Scheurenbrand T, et al.*** *Epilepsia 2012 , Vol. 53(8), p. 1387-98.*

49. Next Generation Sequencing Methods for Diagnosis of Epilepsy Syndromes. ***Dunn P, Albury CL, Maksemous N, et al.*** *Front Genet 2018 , Vol. 7, p. 9-20.*

50. Review of Commercially Available Epilepsy Genetic Panels. ***Chambers C, Jansen LA, Dhamija R.*** *J Genet Couns 2016, Vol. 25, p. 213-7.*

51. Diagnostic yield of genetic testing in epileptic encephalopathy in childhood. ***Mercimek-Mahmutoglu S, Patel J, Cordeiro D, et al.*** *Epilepsia 2015, Vol. 26, p. 707-16.*

52. The Impact of Next-Generation Sequencing on the Diagnosis and Treatment of Epilepsy in Paediatric Patients. ***Mei D, Parrini E, Marini C, et al.*** *Mol Diagn Ther 2017, Vol. 21, p. 357–373.*

53. Molecular findings among patients referred for clinical whole-exome sequencing. ***Yang Y, Muzny DM, Xia F, et al.*** *JAMA 2014 , Vol. 312(18), p. 1870-9.*

54. ILAE official report: a practical clinical definition of epilepsy. ***Fisher RS, Acevedo C, Arzimanoglou A, et al.*** *Epilepsia 2014, Vol. 55(4), p. 475-82.*

55. Sleep in ring chromosome 20 syndrome: a peculiar electroencephalographic pattern. ***Zambrelli E, Vignoli A, Nobili L, et al.*** *Functional Neurology 2013, Vol. 28(1), p. 47-53.*

56. Ring chromosome 20 syndrome: Electroclinical description of six patients and review of the literature. ***Elens I, Vanrykel K, De Waele L, et al.*** *Epilepsy Behav 2012, Vol. 23(4), p. 409-14.*

57. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. ***Richards S, Aziz N, Bale S, et al.*** *Genet Med 2015, Vol. 17(5), p. 405-24.*

58. De Novo Pathogenic Variants in CACNA1E Cause Developmental and Epileptic Encephalopathy with Contractures, Macrocephaly, and Dyskinesias. ***Helbig KL, Lauerer RJ, Bahr JC et al.*** *Am J Hum Genet 2018, Vol. 103(5), p. 666-678.*

59. Distinct magnetic resonance imaging features in a patient with novel RARS2 mutations: A case report and review of the literature. ***Zhang J, Zhang Z, Zhang Y, et al.*** *Exp Ther Med 2018, Vol. 15, p. 1099-1104.*

60. Molecular cloning of the human gene, PNKP, encoding a polynucleotide kinase 3'-phosphatase and evidence for its role in repair of DNA strand breaks caused by oxidative damage. ***Jilani A, Ramotar D, Slack C, et al.*** *J Biol Chem 1999, Vol. 274(34), p. 24176-86.*

61. Mutations in PNKP cause microcephaly, seizures and defects in DNA repair. ***Shen J, Gilmore EC, Marshall CA, et al.*** *Nat Genet 2010, Vol. 42(3), p. 245-9.*

62. Mutations in PNKP cause recessive ataxia with oculomotor apraxia type 4. ***Bras J, Alonso I, Barbot C, et al.*** *Am J Hum Genet 2015, Vol. 96(3), p. 474-9.*

63. Progressive cerebellar atrophy and polyneuropathy: expanding the spectrum of PNKP mutations. ***Poulton C, Oegema R, Heijsman D, et al.*** *Neurogenetics 2013, Vol. 14(1), p. 43-51.*

64. Functional consequences of PRODH missense mutations. ***Bender HU, Almashanu S, Steel G, et al.*** *Am J Hum Genet 2005, Vol. 76(3), p. 409-20.*

65. Type I hyperprolinemia and proline dehydrogenase (PRODH) mutations in four Italian children with epilepsy and mental retardation. ***Di Rosa G, Pustorino G, Spano M, et al.*** *Psychiatr Genet 2008, Vol. 18(1), p. 40-2.*

66. Dravet syndrome as epileptic encephalopathy: evidence from long-term course and neuropathology. ***Catarino CB, Liu JY, Liagkouras I, et al.*** *Brain 2011, Vol. 134(Pt 10), p. 2982-3010.*

67. Detection of clinically relevant copy-number variants by exome sequencing in a large cohort of genetic disorders. ***Pfundt R, Del Rosario M, Vissers LELM, et al.*** *Genet Med 2017, Vol. 19(6), p. 667-675.*

68. Diagnostic exome sequencing in 100 consecutive patients with both epilepsy and intellectual disability. ***Snoeijen-Schouwenaars FM, van Ool JS, Verhoeven JS, et al.*** *Epilepsia 2019, Vol. 60(1), p. 155-164.*

69. What Do You Do When They Grow Up? Approaches to Seizures in Developmentally Delayed Adults. ***Devinsky O.*** *Epilepsia 2002, Vol. 43(3), p. 71-79.*

70. Epilepsy transition: Challenges of caring for adults with childhood-onset seizures. ***Borlot F, Tellez-Zenteno JF, Allen A, et al.*** *Epilepsia 2014, Vol. 55(10), p. 1659-66.*

71. The hidden genetics of epilepsy - a clinically important new paradigm. ***Thomas RH, Berkovic SF.*** *Nature reviews. Neurology 2014, Vol. 10, p. 283-292.*

72. Phenotypic spectrum of glucose transporter type 1 deficiency syndrome (Glut1 DS). ***Pearson TS, Akman C, Hinton VJ, et al.*** *Curr Neurol Neurosci Rep 2013, Vol. 13(4), p. 342.*

73. Severe myoclonic epilepsy of infancy (Dravet syndrome): Recognition and diagnosis in adults. ***Jansen FE, Sadleir LG, Harkin LA, et al.*** *Neurology 2006, Vol. 67, p. 2224-6.*