Taylor & Francis
Taylor & Francis Group

Expert Review of Neurotherapeutics

ISSN: 1473-7175 (Print) 1744-8360 (Online) Journal homepage: https://www.tandfonline.com/loi/iern20

Advances in genetic testing and optimization of clinical management in children and adults with epilepsy.

Marcello Scala, Amedeo Bianchi, Francesca Bisulli, Antonietta Coppola, Maurizio Elia, Marina Trivisano, Dario Pruna, Tommaso Pippucci, Laura Canafoglia, Simona Lattanzi, Silvana Franceschetti, Carlo Nobile, Federico Zara, Pasquale Striano, Roberto Michelucci & Antonio Gambardella on the behalf of Genetic Commission of Italian League Against Epilepsy (LICE)

To cite this article: Marcello Scala, Amedeo Bianchi, Francesca Bisulli, Antonietta Coppola, Maurizio Elia, Marina Trivisano, Dario Pruna, Tommaso Pippucci, Laura Canafoglia, Simona Lattanzi, Silvana Franceschetti, Carlo Nobile, Federico Zara, Pasquale Striano, Roberto Michelucci & Antonio Gambardella on the behalf of Genetic Commission of Italian League Against Epilepsy (LICE) (2020): Advances in genetic testing and optimization of clinical management in children and adults with epilepsy., Expert Review of Neurotherapeutics, DOI: 10.1080/14737175.2020.1713101

To link to this article: https://doi.org/10.1080/14737175.2020.1713101

Accepted author version posted online: 16 Jan 2020.

Publisher: Taylor & Francis & Informa UK Limited, trading as Taylor & Francis Group

Journal: *Expert Review of Neurotherapeutics*

DOI: 10.1080/14737175.2020.1713101

Advances in genetic testing and optimization of clinical management in children and adults with epilepsy.

Marcello Scala[1,2], Amedeo Bianchi[3], Francesca Bisulli[4], Antonietta Coppola[5], Maurizio Elia[6], Marina Trivisano[7,8], Dario Pruna[9], Tommaso Pippucci[10], Laura Canafoglia[11], Simona Lattanzi[12], Silvana Franceschetti[11], Carlo Nobile[13], Federico Zara[14], and Pasquale Striano[1,2], Roberto Michelucci[15], Antonio Gambardella[16], on the behalf of Genetic Commission of Italian League Against Epilepsy (LICE).

1. Pediatric Neurology and Muscular Diseases Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy

2. Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy.

3. Division of Neurology, Hospital San Donato Arezzo, Arezzo, Italy.

4. IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy; Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Bologna Italy.

5. Department of Neuroscience and Reproductive and Odontostomatological Sciences, Federico II University, Naples, Italy.

6. Unit of Neurology and Clinical Neurophysiopathology, IRCCS Oasi Research Institute, Troina, Italy.

7. Neurology Unit, Department of Neuroscience, IRCCS Bambino Gesù Children's Hospital, Rome, Italy.

8. Clinic of Nervous System Diseases, University of Foggia, Foggia, Italy.

9. Epilepsy Unit, A. Cao Hospital, Cagliari, Italy.

10. Medical Genetics Unit, Polyclinic Sant' Orsola-Malpighi University Hospital, Bologna, Italy.

11. Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy.

12. Neurological Clinic, Department of Experimental and Clinical Medicine, Marche Polytechnic University, Ancona, Italy.

13.CNR-Neuroscience Institute and Department of Biomedical Sciences (C.N.), University of Padua, Italy.

14. Laboratory of Neurogenetics and Neuroscience, IRCCS Istituto Giannina Gaslini, Genoa, Italy.

15. IRCCS Istituto delle Scienze Neurologiche di Bologna, Ospedale Bellaria, Bologna, Italy.

16. Dipartimento di Scienze Mediche e Chirurgiche, Università della Magna Graecia, Catanzaro, Istituto di Scienze Neurologiche CNR Mangone, Cosenza, Italy.

***Corresponding author:**

Pasquale Striano

Pediatric Neurology and Muscular Diseases Unit, IRCCS Istituto "G. Gaslini",

Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genova, Via Gaslini 5, 16148 Genova, Italy

Telephone: +39(0)1056362758; fax: +39(0)108612070

strianop@gmail.com

Abstract

Introduction: Epileptic disorders are a heterogeneous group of medical conditions with epilepsy as the common denominator. Genetic causes, electro-clinical features, and management significantly vary according to the specific condition.

Areas Covered: Relevant diagnostic advances have been achieved thanks to the advent of Next Generation Sequencing (NGS)-based molecular techniques. These revolutionary tools allow to sequence all coding (whole exome sequencing, WES) and non-coding (whole genome sequencing, WGS) regions of human genome, with a potentially huge impact on patient care and scientific research.

Expert Opinion: The application of these tests in children and adults with epilepsy has led to the identification of new causative genes, widening the knowledge on the pathophysiology of epilepsy and resulting in therapeutic implications. This review will explore the most recent advancements in genetic testing and provide up-to-date approaches for the choice of the correct test in patients with epilepsy.

Keywords: Epilepsy; Genetic testing; Next Generation Sequencing; Whole Exome Sequencing; therapy, Antiepileptic drugs; surgery.

Article highlights:

- Epilepsy is the common denominator of a heterogeneous group of medical conditions (epileptic disorders) with different genetic causes, electro-clinical features, and management.
- Several genetic tests are currently available to investigate patients with epileptic conditions with presumed genetic basis.
    - The choice of the most appropriate test in each patient plays a primary role to achieve a good diagnostic yield.
- The advent and improvement of Next Generation Sequencing (NGS)-based techniques have provided a significant advancement in the diagnosis of epileptic patients.
- NGS has made possible to sequence all the coding regions of human genome through whole exome sequencing (WES).
- Recent advances of NGS-based techniques have further allowed to sequence the non-coding regions of human genome through whole genome sequencing (WGS).
    - The correct use of NGS-based tests has led to the identification of new causative and putative genes, allowing us to understand new pathophysiological mechanisms underlying epilepsy.
    - The advancements achieved through the recent genetic techniques have significantly improved the possibilities of precision medicine and patient care.

1. Introduction

Epilepsies are a heterogeneous group of medical conditions differing in several aspects, including aetiology, clinical manifestations, therapeutic approaches, and prognosis. The genetic component of these conditions appears increasingly important. Indeed, genetic causes have been identified not only in those forms that were previously classified as “idiopathic”, but also in focal and lesional epilepsies. Currently, it is estimated that “genetic epilepsies” occur in about 0.4% of population worldwide, representing more than 30% of all epilepsies [1]. From a clinical perspective, this group includes a heterogeneous subset of syndromes with related electroencephalographic (EEG) patterns and focal or generalized onset.

Over the last two decades, several genetic alterations have been associated with epileptic syndromes [2, 3]. Most genetic changes have been identified in genes coding for ion channels and result in neuronal hyperexcitability and/or dysfunction of inhibitory systems, leading to seizures generation [4]. More recently, mutations in non-ion channels coding genes have been reported in association with specific epilepsies and encephalopathies [5]. A Mendelian inheritance pattern can be identified in a limited number of genetic epilepsies, whereas complex inheritance patterns might explain most of these disorders [6]. Nowadays, several genetic tests are available, including targeted assays (low-cost tests, useful when a specific condition is suspected) and genome-wide analysis (expensive but suitable when no specific disorder is suspected) [7]. This paper aims to review the electro-clinical features of genetic epilepsies and provide useful hints to drive the diagnostic path in the clinical practice.

2. Clinical setting

A comprehensive study of the clinical phenotype is the first and pivotal step to select the most appropriate test in the individual patient (Supplementary Table 1). The phenotypic evaluation encompasses 5 cardinal points: family history, personal history, clinical examination, seizures semiology, and instrumental findings [8].

2.1. Epilepsy features

Epilepsy history is fundamental and should be as much detailed as possible. Epilepsy and seizure types should be classified according to the current International League against Epilepsy (ILAE) classification, on the basis of the age of onset, frequency, and response to treatment [9, 10]. Ideally, an ictal video-EEG should be obtained, but home-video recordings might prove very useful as well. Other relevant features to be accurately collected include the duration of the episodes (status epilepticus), possible auras, triggering factors, and relationships with wake-sleep cycle. With regards to the triggering factors, it is important to specifically investigate the setting of the seizures (focusing on the presence of flickering lights and/or sounds, cognitive performances, etc.). Drug resistance criteria should be used to evaluate drug response [11, 12] and possible paradoxical aggravation should be documented. The overall prognosis usually depends on the response to the treatment as well as the presence of cognitive impairment and possible associated organ dysfunctions.

2.2. Personal history

Collecting information on pregnancy (weeks of gestation, abortion threats, fetal movements, infections, gestosis, etc.) and delivery (eutocic or dystocic, Apgar score, respiratory distress, peri- and neonatal course, etc.) can be relevant, especially in the neonatal setting. Psychomotor development should be accurately evaluated, especially with regards to motor and language skills. In case of impaired cognition, it is essential to establish if this impairment started (psychomotor regression) or worsened by the time seizures manifested. Associated neurological conditions such as neurodevelopmental disorders should be accurately investigated, including attention deficit hyperactivity disorder (ADHD), ASD, and movement disorders. Non-neurological comorbidities should also be interrogated, especially metabolic disorders and organ dysfunctions or structural abnormalities.

2.3. Family history

A detailed family history may provide clues on the possible inheritance pattern and relevant information about the genetic risk in the relatives of the proband. For example, first-degree relatives of a patient with idiopathic epilepsy (in whom genetic aetiology is suspected) have higher chance of developing epilepsy as compared to the general population. The patient's siblings will have a 3-5% higher risk of developing epilepsy in comparison with the overall risk of about 1-2% in the general population. If the affected parent is the mother, siblings have a 4-6% higher risk. In symptomatic epilepsies, the familial risk is only slightly higher than in general population (1-2%) [13, 14].

Focused history taking should start from a classic three-generation pedigree, which can be extended to further generations if more information becomes available.

Standardized human pedigree nomenclature according to the recommendations of the National Society of Genetic Counselors (NSGC) should be used [15]. Particular attention should be paid to possible parental consanguinity, twin pregnancies, abortions or miscarriages, and infantile deaths. Whenever possible, the sex of aborted/miscarried foetuses should be specified, since this data might provide clues towards a specific inheritance pattern (e.g., recurrent miscarriages of male foetuses might suggest a male-lethal X-linked condition). Affected individuals should be characterized according to the type of epilepsy and, ideally, seizure semiology. The occurrence of provoked and febrile seizures (sometimes requiring specific investigation) should also be reported. Neurocognitive comorbidities, including intellectual disability (ID) and ASD, should be indicated as well. When interpreting a pedigree, the occurrence of distinct epileptic syndromes in different family members (phenotypic variability) and non-genetic conditions (e.g., post-traumatic epilepsy) mimicking the studied epileptic disorder (phenocopy) should always be considered. Eventually, it is always advisable to update the pedigree at each follow-up evaluation.

2.4. Physical and neurological examination

General physical examination should be performed to evaluate growth parameters (weight, height, and occipito-frontal circumference), facial dysmorphic features, and associated abnormalities of the trunk and limbs. Pictures of the patient (face, hands, and feet) can be collected to improve the genetic evaluation. Neurological examination should be as complete as possible. Focal dysfunctions and movement disorders

(involuntary, paroxysmal, or stereotyped) should be carefully checked. When appropriate, standardized scales should be used to evaluate cognitive function.

2.5. Instrumental investigations

EEG is the gold standard exam for epilepsy diagnosis. In the best-case scenario, pathognomonic EEG abnormalities may be found (e.g., 3 Hz spike-wave complexes during hyperpnoea are suggestive of childhood absence epilepsy, whereas centro-temporal spikes during sleep might suggest rolandic epilepsy). A slowing of the background activity, photoparoxysmal response, and event-related EEG potentials may all represent significant hints towards the correct diagnosis. Symmetrical and synchronous 3-Hz spike-wave activity sometimes mixed with polyspike-wave discharges on a normal background points towards a typical absence seizure [16]. An ictal EEG activity characterized by high-amplitude polyspikes and polyspike-wave discharges of 3-6 Hz lasting up to 6 seconds and triggered by intermittent photic stimulation, eye closure, or hyperventilation, might suggest an eyelid myoclonia with absences (Jeavons syndrome) [17]. The identification of an interictal EEG activity characterized by high voltage, asynchronous slow waves with multi-focal spikes varying in duration and location (hypsarrhythmia) in a child with epileptic spasms and developmental regression suggest a diagnosis of West syndrome [18]. Highly stereotyped spike-wave complexes with a normal interictal background in a patient without significant intellectual impairment or behavioural disturbances suggest a benign childhood focal epilepsy [19, 20]. Of note, EEG can be also performed to ascertain possible abnormalities in unaffected members of certain families. However, it is

important to bear in mind that EEG has technical and temporal limits, such as the presence of age-dependent abnormalities (e.g., 3 c/sec spike-wave complexes disappearing in adulthood) and inter- and intra-individual variability.

Standard laboratory tests should always be performed in patients with epilepsy with drug-resistant seizures, especially if developmental delay or progressive neurological deterioration is associated [21]. Indeed, a comprehensive metabolic workup might reveal an underlying metabolic condition with a possible aetiology-specific treatment. An early diagnosis and a timely start of the most appropriate treatment are essential in the management of treatable metabolic epilepsy syndromes, allowing to stabilize or reverse neurological and systemic symptoms [21, 22]. In particular, standard laboratory tests (blood glucose, electrolytes, and ammonia) should be supported by a first-line metabolic screening including plasma and urine amino acid levels, urine organic acids, blood spot acylcarnitine profile, and urine creatine/creatinine ratio [22]. If a specific disorder is suspected according to the patient's electroclinical features, second-line tests can be performed. As an example, the dosage of plasma and urine biotin as well as the analysis of serum biotinidase enzyme activity may be fundamental to diagnose the underlying metabolic disorder in a child developing seizures, neuro-ophthalmological, and cutaneous manifestations in the first months of life [22]. In this case, an early diagnosis of biotinidase deficiency allows to start a prompt biotin supplementation (5-20 mg/day), which leads to seizure control, stabilization of neurological complications, and reversal of neuroradiological abnormalities [22, 23].

Neuroimaging may play a relevant role in the diagnosis of the brain abnormalities associated with epileptic disorders. Brain magnetic resonance imaging (MRI) is

preferred to computed tomography (CT) due to a better overall diagnostic accuracy. However, CT plays a primary role in specific conditions, including suspected acute haemorrhage and brain calcifications [24]. Brain MRI is essential to rule out cortical migration defects, cortical development abnormalities, vascular anomalies, and defects of the corpus callosum and cerebellum. Contrast enhancement study is generally not necessary for a first-level evaluation. In certain conditions, brain MRI findings alone may help in the choice of the best genetic test [25]. For example, the presence of bilateral periventricular nodular heterotopias (PNH) in a female might suggest to search for *FLNA* mutations through gene panel testing for neuronal migration defects [26].

Other instrumental studies are not advisable as first-line approach in patients with epilepsy, but may be performed according to the specific clinical presentation and indications in the single case. For example, somatosensory evoked potential may disclose a giant potential and suggest a possible new-onset progressive myoclonic epilepsy (PME) in a patient with drug-resistant generalized epilepsy. In some circumstances, furthermore, instrumental findings may be very specific and point towards the clinical diagnosis. As an example, the evaluation of the *fundus oculi* may disclose a red-cherry spot (highly suggestive of sialidosis) in patients with PME.

3. Genetic testing

Over the last years, the relevant advances in the field of molecular biology, genomics, and related technologies have significantly increased our understanding of the molecular mechanisms behind the pathogenesis of epileptic disorders. A variety of genetic tests are currently available to investigate the cause of epileptic disorders [27].

In general, each test should be chosen according to the expected advantages in the diagnosis of a particular class of epileptic disorders and its specific limitations (Figure 1). Either cytogenetic analysis or DNA sequencing may be performed on the basis of the clinical presentation (Table 1). Genetic tests are available for most developmental epileptic encephalopathies (DEE) and several idiopathic epilepsies, playing a pivotal role in the diagnosis of these complex conditions. Furthermore, the results of genetic tests may positively influence treatment strategies and, hence, improve patient care. A thorough electro-clinical characterization of the patient is essential to guide the choice of the best genetic test and provide a relevant support in genetic data interpretation. In addition, regular clinical evaluations significantly improve this complex diagnostic process.

3.1. Cytogenetic analysis

Cytogenetic studies may be particularly helpful in patients with epilepsy and ID, especially when dysmorphic features coexist or a definite syndromic epilepsy is not recognizable.

The array comparative genomic hybridization (array CGH) is a molecular cytogenetic method suitable for DNA copy number variants (CNVs) analysis, such as deletions or duplications. This technique is based on the quantitative comparison between test DNA extracted from peripheral blood lymphocytes of the proband and reference DNA from healthy donors, using competitive fluorescence in situ hybridization (FISH). The resolution power is variable, ranging from 1 Mb to 100 kb, but it is at least 100-fold higher than traditional cytogenetics. Rare CNVs, some of which involve known morbid

genes, contribute to approximately 10% of infantile epilepsies and 5% of epileptic encephalopathies, overall [28]. In large cohorts, array CGH studies further showed recurrent deletions in 15q13.3, 16p13.11, and 15q11.2 in patients with focal epilepsies or generalized epilepsies with ID, suggesting that these rearrangements might represent susceptibility factors for these conditions [29-31]. When epilepsy is not associated with ID or dysmorphic features/malformations, the diagnostic impact of array CGH appears to be lower [32, 33].

As a second step, karyotype may be helpful to identify possible chromosome rearrangements that are not detectable by array CGH, such as translocations. FISH is only indicated in selected cases to search for known deletions or duplications in specific syndromes (e.g., 22q11.2 deletion in suspected Di George syndrome) or to better define chromosome abnormalities identified with other techniques (e.g., the complex rearrangements in the duplication/inversion 15q11 or isodicentric 15 chromosome syndrome). Eventually, multiplex ligation-dependent probe amplification (MLPA) allows to detect deletions and duplications of several exonic sequences, and can be used for the screening of entire genes in the same experimental session. Furthermore, MLPA plays a pivotal role in the identification of intragenic deletions in cases where Sanger sequencing and array CGH result negative (e.g., epilepsy due to *SCN1A* or *CDKL5* intragenic deletions).

3.2. Next generation sequencing

Through traditional Sanger sequencing, it is possible to directly determine the nucleotide sequence of the exons of a single gene. Although this technique may reveal

useful in some cases, in recent years it has been replaced by next generation sequencing (NGS), an innovative sequencing technology which allows to simultaneously sequence many genes at relatively low costs. The number of genes that may be sequenced by NGS ranges from dozens of clinically or functionally related genes organized in panels to nearly all human genes [34, 35].

Gene panel testing usually costs less than WES since fewer genes are sequenced, even though the improvement of the sequencing technique and the increasing diffusion of WES is leading to a significant cost reduction [36]. Furthermore, gene panels can be used to validate WES findings and potentially identify additional variants in candidate genes [36]. Depending on the type of panel and the characteristics of the investigated population, the diagnostic yield of gene panels is around 30% (ranging from 10% to 50%). Thanks to targeted NGS panels, the diagnostic yield in patients with childhood epilepsy has improved up to 28% [37, 38]. NGS-based gene panels testing has proven to be particularly useful in the diagnosis of infantile epilepsies, with a diagnostic yield up to 50% [39-41]. Accordingly, several studies support a prioritization of this group of disorders for epilepsy gene panel testing [39-41]. However, the limited number of genes that can be sequenced for each panel and the need for a continuous updating of the included genes represent major limitations that have been brilliantly overcome by Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS).

The advent of NGS have made feasible the sequencing of the exons (coding sequences, approximately 1% of the whole genome) of all human genes through WES, as well as the sequencing of the entire genome (including non-coding regions) through

WGS. Although WGS is very powerful, its application in clinical practice is still limited due to the higher costs than WES, the technical difficulties resulting from the handling of huge amounts of sequencing data, and the complexity of variant interpretation in non-coding regions. Both gene panel sequencing and WES are being increasingly used in the clinical diagnosis of epileptic disorders with a presumed genetic basis [42-44].

The possibility to sequence the entire genome through Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS) have further increased the diagnostic yield in recent years in comparison to NGS-based panel testing. The use of WES has led to an approximately 2-fold increase in the diagnostic yield in patients with epilepsy in comparison to NGS-based panel testing, allowing to obtain a diagnostic rate of around 40% [37, 38]. Furthermore, WES allows to sequence many genes at the same time, with relevant advantages in both clinical settings (e.g., no need to periodically update gene panels) and scientific research (e.g., possible discovery of new candidate genes for a specific disorder) [38, 45, 46].

However, the interpretation of WES data per se may be difficult because variants output can be very large, requiring complex filtering procedures to identify disease-causative variants. The variant data set from each proband is first compared with polymorphic variants in the general population through the genome aggregation database gnomAD (http://gnomad.broadinstitute.org/), which is the largest database available worldwide (including about 125,000 individuals). Then, all polymorphic variants with minor allele frequency (MAF)>1-5% are filtered out. Only those variants that are absent or very rarely observed in the general population (according to gnomAD database) are considered candidate causative disease variants, especially for

autosomal dominant disorders [47]. Depending on the type of epilepsy and its inheritance, putative variants are prioritized based on segregation: de novo variants, which are carried by the proband but absent in the parents; variants co-segregating with inherited epilepsy in affected families. The resulting variants can be classified based on their nature (missense, nonsense, and frameshift) and effects on protein structure and function.

Genetic variants should be classified according to the American College of Medical Genetics and Genomics (ACMG) guidelines [48]. Apart from benign/likely benign and pathogenic/likely pathogenic variants, NGS-biased techniques may also lead to the identification of variant of unknown significance (VUS). Indeed, the predicted functional effect of the mutation and the available scientific literature may not be sufficient to establish if the variant is clearly pathogenic or benign. In these circumstances, accurate genetic counseling for the family is fundamental to help parents understand the meaning and the actual limits of this finding. Furthermore, a periodic review of the VUS in light of the current scientific literature should be performed in order to try to improve the variant interpretation and provide the best care to the family.

Eventually, when proband-WES is performed alone (e.g., trio-WES is not feasible), the segregation analysis of the candidate variants in the parents through Sanger sequencing is essential to establish the cis/trans status and a possible *de novo* status [38]. Given their complexity, the results of NGS tests should be evaluated by a medical geneticist with expertise in genome analysis in cooperation with the neurologist.

Before any test is carried out, patients should always be informed on the expected results and test limitations.

Although the role of array-based techniques is still relevant in patients with epilepsy with neurodevelopmental comorbidities or dysmorphic features, the perspectives on the efficiency of WES and WGS in detecting CNVs are very promising thanks to the combination of read depth and allele frequency approaches [45]. Indeed, these techniques have proven to be successful in the detection of both single nucleotide variants (SNVs) and CNVs, especially WGS [49, 50]. Although WGS has the potential to overcome array-based techniques in the comprehensive profiling of the whole range of CNVs, the existing analytic methods and the current computational approaches still lack sufficient accuracy and reliability. The development of a single informatics method for all the structural DNA variations, standard protocols, and extensive laboratory validations will need to be addressed for the accurate detection of CNVs in a computationally feasible manner through WGS [51, 52].

Despite the current health economic evidence base to support the use of WES and WGS in clinical practice is limited, WES has proven to result in lower long-term charges and more timely diagnosis in comparison to the current second tier testing (e.g., single gene testing or NGS panels) [53, 54]. However, the decreasing technical costs and the relevant clinical implication deriving from WES/WGS (e.g., genetic counseling, family planning, personalized medicine, and systemic investigation for comorbidities) are fundamental drivers toward the widespread diffusion of these techniques and contribute to justify the allocation of healthcare resources in their employment in clinical settings [53].

4. Candidate epileptic disorders for genetic testing

4.1. Idiopathic epilepsies

Idiopathic (or genetic) epilepsies are a group of age-related disorders characterized by distinctive electro-clinical features, with known or presumable genetic aetiology [55]. Most of these conditions have an early onset (neonatal-infantile), but some may have onset in adolescence-adulthood. The use of NGS-based techniques has allowed the identification of the genetic causes of several idiopathic epilepsies. In most cases, mutations in genes encoding membrane proteins with peculiar functional roles have been identified, especially ion channels (e.g., *SCN2A*, *SCN8A*, *KCNQ2*, and *KCNQ3*) and synaptic receptors (*CHRNA4*, *CHRNB2*, and *CHRNA2*). Mutations in proteins secreted within the nervous system and exerting relevant, but yet unclear functions (e.g., *LGI1* and *RELN*) have also been reported (Table 2).

4.1.1. Generalized idiopathic epilepsies

A hereditary predisposition has been hypothesized in childhood absence epilepsy (CAE), but specific causative genes have not been identified yet. Mutations in *SCL2A1*, encoding the cerebral glucose transporter Glut1, have been reported in 10% of patients with early-onset absence epilepsy (EOAE) [56, 57]. Juvenile myoclonus epilepsy (JME) is a genetically heterogeneous disorder. Mutations in *GABRA1*, *GABRG2*, *GABRB3*, and *GABRD* (encoding the subunits A1, G2, B3, and D of GABA-A receptor, respectively), as well as in *EFHC1* (encoding myoclonin) have been reported in some families with autosomal dominant transmission and incomplete penetrance [58].

Nevertheless, the clinical impact of genetic testing in patients with generalized idiopathic epilepsies is still insufficient.

4.1.2. Self-limiting epilepsies in the first year of life

These epileptic disorders occur in otherwise healthy infants, usually within 8 months of age, and are characterized by focal seizures, usually occurring in clusters. The clinical course is usually benign, with spontaneous resolution within the age of 2 years and normal psychomotor development in most cases. Based on the age of onset, focal epilepsies in the first year of life can be classified as benign familial neonatal seizures (BFNS), benign familial neonatal-infantile seizures (BFNIS), or benign familial infantile seizures (BFIS).

Several genes associated with these conditions have been identified in the past few years, explaining around 90% of familial cases [59] and 20-30% of sporadic cases [60]. The inheritance pattern is autosomal dominant. More than 80 distinct pathogenic variants in *KCNQ2* (voltage-gated potassium channel, subfamily Q, member 2, OMIM *602235) have been reported in BFNS patients, but mutations in *KCNQ3* (voltage-gated potassium channel, subfamily Q, member 3, OMIM *602232) have also been described in some cases. Occasional episodes of febrile and afebrile seizures during the follow-up have been reported in 15% of BFNS patients, especially in individuals carrying *KCNQ2* mutations [61]. In these subjects, persistent seizures may occur in up to 30% of cases [61]. Furthermore, *KCNQ2* mutations have been recently associated with a severe clinical course (epileptic encephalopathy, persistent epilepsy, ID, and behavioural disturbances) in some patients. Unfortunately, clear genotype-phenotype correlations

are not yet available to identify patients with a greater risk of developing epileptic encephalopathy. Of note, sodium channel blockers (e.g., phenytoin and carbamazepine) appear particularly effective in these encephalopathic patients [62], potentially suggesting candidate genes for further investigations.

Most BFIS patients carry mutations in *PRRT2* (proline-rich transmembrane protein 2, OMIM *614386), localized at 16p11.2 and encoding a membrane protein which interacts with synaptosomal associated protein 25 kDa (SNAP25). Interestingly, some patients also show paroxysmal kinesigenic dyskinesia (PKD, OMIM #128200), which is typically caused by *PRRT2* mutations [60]. Globally, pathogenic variants in *PRRT2* are responsible for 80-90% of familial BFIS, PKD, and infantile convulsions with paroxysmal choreoathetosis (ICCA) cases, as well as for 20-30% of sporadic cases [60]. In some families, individuals carrying *PRRT2* variants may suffer from migraine, occurring as isolated symptom or in association with epilepsy, and presenting with or without aura and/or hemiplegia [63]. Mutations in *SCN2A* (already associated with BFNIS) and, although rarely, in *KCNQ2* and *KCNQ3* have also been reported in association with BFIS [59]. Recently, *CHRNA2* (cholinergic receptor nicotinic alpha polypeptide 2, OMIM *118502) pathogenic variants have been described in a BFIS family with autosomal dominant transmission [64]. Eventually, the missense change c.4447G>A, p.(Glu1483Lys) in *SCN8A* (NM_001330260.2) has been described in some *PRRT2*-negative families [60]. This variant is classified as pathogenic according to the ACMG guidelines. It is absent in gnomAD database, is reported as pathogenic in ClinVar (VCV000253195.1), falls in a hot-spot for pathogenic variant of length 61 base-pairs, and is predicted pathogenic by several bioinformatic tools [48, 60].

4.1.2.1 Genetic testing

A possible approach would be to investigate *KCNQ2* and *KCNQ3* mutations in BFNS patients, and *SCN2A* and *KCNQ2* mutations in subjects with BFNIS. The most effective approach in terms of cost and time is to perform an NGS-based panel, which is more advisable than a step-wise approach (direct sequencing of target genes followed by NGS panel testing in negative cases). In BFIS, both NGS panel testing and direct sequencing can be performed to detect the most common mutation c.649dupC (p.Arg217ProfsTer8) (NM_001256442.2) in *PRRT2* [39, 65]. This variant is classified as pathogenic according to ACMG guidelines. It is a null variant reported in ClinVar database (VCV000065758.5) and previously identified in several affected individuals [48, 66-68]. If this first step yields negative results, other possible causative genes (primarily *SCN2A* and *KCNQ2*, and consequently *SCN8A*, *KCNQ3*, and *CHRNA2*) should be investigated through NGS panel testing or direct sequencing. In patients with BFIS and PKD, direct sequencing of *PRRT2* should be the first step, followed by *SCN8A* analysis in case of negative results. Importantly, since BFIS represents a phenotypic spectrum, all the above-mentioned genes may be responsible for distinct clinical forms of this condition. Even though NGS panels can facilitate genetic analysis, the neurologist still plays a pivotal role in assisting the geneticist in the choice of the best test to perform according to the specific epileptic phenotype. If NGS tests yield negative results, MLPA should be performed to rule out possible gene deletions. Despite all these efforts, however, no genetic diagnosis is achieved in around 10% of patients with BFNS, BFIS, and BFNIS.

4.1.3. Rolandic epilepsy

Rolandic epilepsy (RE) affects children with normal psychomotor development, with a mean onset of 3-12 years (peak 5-8 years). Seizures predominantly occur during sleep and are characterized by facial clonic seizures and sialorrhea, sometimes followed by secondary generalization. The clinical course is usually benign with spontaneous resolution and normal psychomotor development. However, few children may still experience selective neuropsychologic deficits, especially involving language, attention, and behavior. Although mutations in some genes with autosomal dominant transmission (*PRRT2*, *KCNQ2*, *KCNQ3*, *GRIN2A*, *RBFOX1*, and *DEPDC5*) have been associated with this condition, studies based on large case series have not confirmed these findings. Of note, *GRIN2A* mutations have been identified in 20% of patients with atypical RE associated with cognitive and/or behavioral disturbances [69].

4.1.3.1 Genetic testing

The role of genetic tests in the diagnosis of RE is still insufficient. To date, only few reports on a definite genetic diagnosis are available, but these findings have not been subsequently confirmed in larger case series. Despite that, a possible hint would be to search for *GRIN2A* mutations in patients with atypical RE, since it is mutated in 20% of cases [69].

4.1.4. Sleep hypermotor epilepsy

Sleep hypermotor epilepsy, formerly known as Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), consists of hypermotor or, less frequently, tonic/dystonic seizures predominantly occurring during nocturnal sleep in individuals with normal psychomotor development. The onset is within the first two decades, mostly around the age of 10 years. A minority of patients may also develop cognitive or psychiatric disturbances. There is a good response to carbamazepine [70-72]. In 20% of familial and 5% of sporadic cases, mutations in *CHRNA4* (cholinergic receptor, neuronal nicotinic, alphapolypeptide 4, OMIM *118504) and *CHRNB2* (cholinergic receptor, neuronal nicotinic, beta polypeptide 2, OMIM *118507) with autosomal dominant inheritance have been reported [70-72]. These genes encode the alpha4 and beta2 subunits of neuronal cholinergic receptors (AChR), respectively. Mutations in *CHRNA2* (cholinergic receptor, neuronal nicotinic, alpha polypeptide 2, OMIM *118502), encoding the alpha2 subunit of the AChR, have been further described in a family with atypical ADNFLE characterized by fear sensation and wandering [73]. More recently, WES has led to the identification of pathogenic variants in *KCNT1* (potassium channel, subfamily t, member 1, OMIM *608167) in sporadic and familial cases of ADNFLE with earlier age of onset and increased risk of developing cognitive and psychiatric disturbances [74]. Eventually, mutations in *DEPDC5* and *NPRL3* (encoding subunits of GATOR1 protein complex, involved in mTOR regulation) have been reported in patients with frontal epilepsy and malformations of cortical development (MCDs) [75].

4.1.4.1 Genetic testing

In patients with ADNFLE, it would be advisable to search for pathogenic variants in *CHRNA4*, *CHRNB2*, and *CHRNA2* (though less frequently mutated than the first two

genes) through NGS panels testing. If no mutation is found, the molecular analysis of *KCNT1*, *DEPDC5*, and *NPRL3* can be performed on the basis of suggestive clinical features associated with the specific gene (e.g., early onset and psychiatric symptoms may suggest *KCNT1* involvement). MLPA should always be considered to rule out duplications and deletions in negative cases.

4.1.5. Autosomal dominant lateral temporal epilepsy

Also known as autosomal dominant partial epilepsy with auditory features (ADPEAF), autosomal dominant lateral temporal epilepsy (ADLTE) is characterized by focal seizures with distinctive auditory symptoms, associated with visual aura in a minority of patients. The onset is in adolescence-adult age and the clinical course is benign [76]. The transmission is autosomal dominant with incomplete penetrance [77]. Mutations in *LGI1* (Leucine rich, Glioma inactivated 1, OMIM *604619), encoding a glycoprotein with a leucine-rich repeats (LRR)-containing domain, have been identified in 30-50% of familial cases [76, 78], but only in 1% of sporadic cases [79]. *LGI1* deletions have been rarely described in ADLTE families. More recently, mutations in *RELN*, which encodes reelin (a secreted glycoprotein co-localizing with LGI1 in neurons) have been identified in some *LGI1*-negative families [80].

4.1.5.1 Genetic testing

NGS-based panels are useful to identify possible mutations in *LGI1* and *RELN*. Alternatively, direct sequencing of *LGI1* (mutated in the majority of cases) can be performed. *RELN* sequencing is usually not recommended because of the large number

of exons (65 exons). *LGI1* mutations can be investigated in sporadic cases as well, but the diagnostic yield is very low. Deletions and duplications should be excluded by MLPA, especially in familial cases.

4.1.6. Genetic epilepsy with febrile seizures plus

Three distinct groups of patients with genetic epilepsy with febrile seizures plus (GEFS+) may be identified on the basis of the electro-clinical presentation and the clinical course: one third of subjects have only persistent febrile seizures (lasting up to the age of 10 years), one third experience afebrile seizures during childhood, and one third may suffer from childhood absences, myoclonic-atonic epilepsy, or focal epilepsy (especially temporal epilepsy) [81]. Most GEFS+ families carry *SCN1A* mutations (predominantly missense changes). Pathogenic variants in *SCN2A* and *SCN1B* (encoding voltage-gated sodium channels) as well as in *GABRG2* and *GABRD* (encoding the subunits of GABA-A receptor) have been reported less frequently [82-84].

4.1.6.1 Genetic testing

The clinical impact of genetic testing in GEFS+ is very limited. NGS-based panels can be performed to identify *SCN1A* mutations in familial cases or pathogenic variants less commonly involved genes (*SCN2A*, *SCN1B*, *GABRG2*, and *GABRD*). However, it is important to consider that genetic tests will probably yield negative results in most patients.

4.2. Epileptic encephalopathies

Developmental epileptic encephalopathies (DEE) are a group of severe childhood-onset disorders characterized by recurrent and intractable seizures, profound alterations of cerebral electrical activity, and arrest or regression of psychomotor development. In these conditions, representing about 40% of the epilepsies in the first 3 years of life, seizures contribute per se to the development of ID [85-87]. The current classifications are still incomplete due to the complexity of these syndromes. Nonetheless, some common and distinctive disease entities can be identified on the basis of the age of onset, seizure semiology, EEG patterns, and neurological comorbidities: Ohtahara syndrome (OMIM #308350), malignant migrating partial seizures of infancy (MMPSI), West syndrome (infantile spasms), Dravet syndrome (OMIM #607208), Lennox-Gastaut syndrome. More recently, Pathogenic variants in the GABA Transporter SLC6A1 have been identified in patients with myoclonic-atonic epilepsy (MAE) (OMIM #616421) [88]. Despite the efforts, a non-neglectable number of patients with severe epileptic manifestations and cognitive disabilities remain unclassified [89]. DEE have a heterogeneous aetiology, including congenital and acquired cofactors, but recent studies have suggested that genetic factors play a pivotal role in their pathogenesis. In most cases, mutations are sporadic, namely *de novo* pathogenic variants in a single autosomal dominant gene. Autosomal recessive and X-linked DEE are less frequent (Table 3).

Genetics of DEE is complex and several factors should be considered when evaluating the single patient, including variable expressivity, incomplete penetrance, and genetic and allelic heterogeneity. Accordingly, the genotype-phenotype correlations

are not always clear-cut, since different phenotypes may be associated with a same genetic alteration and vice-versa. Therefore, patient phenotyping by means of a thorough electro-clinical evaluation is fundamental before starting genetic investigations. Indeed, these clinical data play a pivotal role in guiding the choice of the best genetic tests and significantly help the geneticist in the interpretation of the tests results. Genotype-phenotype correlations may appear substantial in selected cases (e.g., patients with Dravet syndrome caused by *SCN1A* mutations), but they are generally quite indefinite due to the genetic and allelic heterogeneity. Six large genetic and clinical DEE groups can be defined: 1) encephalopathies associated with pyridoxine-dependent seizures; 2) West syndrome/infantile spasms spectrum; 3) Dravet syndrome and related disorders; 4) continuous spike and waves during slow-wave sleep syndrome (CSWS)/Landau Kleffner syndrome spectrum; 5) encephalopathies with onset in the first year of life not included in previous groups (e.g., Ohtahara syndrome); 6) encephalopathies with onset after the first year of life (e.g., Lennox-Gastaut syndrome) (Table 3).

4.2.1 Genetic testing

The most appropriate initial step in these patients is performing a comprehensive NGS panel to investigate possible mutations in known causative genes (e.g., *SCN1A*) and a CGH-array to rule out chromosomal imbalances. In selected patients, after careful phenotyping and compatibly with available resources, WES represents a valuable diagnostic option.

4.3. Lesional epilepsies: malformations of cortical development

MCDs are a well-known cause of symptomatic epilepsy. Although a definite epileptic phenotype is recognizable in some patients with specific malformations, the type of epilepsy is usually extremely variable (especially in subjects with focal cortical dysplasia, FCD) and largely depends on the type and location of the MCD [90]. To date, mutations in more than 100 genes involved in mitosis, apoptosis, cell fate specification, cytoskeletal structure and function, neuronal migration, and membrane function have been associated with MCDs (Supplementary Table 2). In a recent study, post-mitotic causal mutations have been identified through subcloning and subsequent sequencing of subcloned DNA in 17% of patients from a large MCDs case series. Most of these variants were not detectable by common sequencing techniques [91]. According to the current classification based on the causative neuronal development alteration [92], three distinct groups of MCDs can be recognized: 1) abnormal neuronal and glial proliferation and apoptosis; 2) abnormal neuronal migration; 3) post-migrational and migrational anomalies. This classification only considers the primary pathogenic neurodevelopmental process involved in the generation of the cortical malformation, but MCDs-related genes are involved in many stages of brain development that are per se genetically and functionally interdependent. Accordingly, a new classification based on neurobiological pathways and neuroimaging has been recently proposed [93]. Four different groups of MCDs have been identified: 1) megalencephalias and FCDs; 2) tubulinopathies and lissencephaly; 3) syndromes with polymicrogyria (PMG); 4) cortical heterotopias. This classification represents the pivotal starting point to address the genetic pathways involved in MCDs-related epilepsies.

4.3.1. Megalencephaly, hemimegalencephaly, and focal cortical dysplasias

Megalencephaly indicates an abnormally increased growth of cerebral structures due to alterations of the complex processes involved in brain development. This condition is characterized by an occipito-frontal head circumference measurement 2 standard deviations above the age-related mean [94]. Recent studies have shown that megalencephaly with normal cortical imaging, megalencephaly with PMG, and dysplastic megalencephaly can all result from mutations in the same genes of the PI3K-AKT pathway [95]. In children with diffuse, symmetrical, or slightly asymmetric megalencephaly, epileptic seizures may begin at any time in childhood, with onset from the first days of life to 4-5 years [96]. Patients with dysplastic megalencephaly (including the classical hemimegalencephaly) usually have more severe intellectual and motor impairment. Epilepsy begins in the first weeks or months of life, often with epileptic spasms, and subsequently evolve into epileptic encephalopathy. In almost all patients with FCD, the lesion is detected after the onset of a focal epilepsy. Seizures may have an early onset as epileptic spasms with asymmetric features and associated focal seizures. FCDs are a frequent cause of focal epileptic status [97].

4.3.1.1 Genetic testing

In these patients, specific NGS-based panels can be performed to identify possible pathogenic variants in the PI3K-mTOR-AKT cascade.

4.3.2. Tubulinopathies and lissencephaly

According to recent studies, both lissencephaly and pachygyria-like malformations are associated with mutations in the same genes and molecular pathways, which are

involved in several distinct MCDs [98, 99]. To date, about a dozen genes involved in the pathogenesis of tubulinopathies has been identified (*DYNC1H1*, *KIF2A*, *KIF5C*, *TUBA1A*, *TUBA8*, *TUBB*, *TUBB2B*, *TUBB3* and *TUBG1*). Mutations in *LIS1* and *DCX* are the most common in lissencephaly, whereas more complex phenotypes are associated with mutations in other genes (*ARX*, *RELN*, and *VLDLR*) [93, 100]. Patients harbouring mutations in genes associated with tubulinopathies usually show severe neurodevelopmental disorders and drug-resistant seizures. In these cases, seizures usually begin as epileptic spasms, often lacking the classic hypsarrhythmia pattern on EEG. Polymorphic seizures (focal, tonic, tonic-clonic, and atypical absences) are common, with evolution towards clinical-EEG patterns of Lennox-Gastaut syndrome.

4.3.2.1 Genetic testing

It is indicated to search for mutations in the genes involved in tubulinopathies through brain malformations specific NGS-based panels.

4.3.3. Polymicrogyria

PMG indicates a condition where a large number of excessively small convolutions are observed in the cerebral cortex. The topographic distribution of this abnormal gyri is largely variable, ranging from unilateral to bilateral, and from symmetrical to asymmetrical. The perisylvian cortex is most frequently involved. According to recent research, PMGs are clinically and radiologically heterogeneous, and have a distinct pathogenesis [93]. Therefore, PMG should not be considered a single malformation but a spectrum of different phenotypes. The most recognizable clinical syndrome is bilateral perisylvian PMG [92], which is characterized by the association of oro-motor deficits, ID,

and epilepsy (focal and secondarily generalized seizures). Some patients with unilateral PMG may develop transiently intractable seizures with EEG features of continuous spike-and-wave complexes in sleep. Some CNVs have been occasionally associated with PMG, whereas deletions in 1p36.3 and 22q11.2 are quite common [93]. Although mutations in *PIK3R2* have been recently reported in patients with bilateral perisylvian syndrome, other studies suggest that it is uncommon to identify germinal mutations in isolated PMGs [90].

4.3.3.1 Genetic testing

Brain malformation NGS-based panels can be performed in these patients to identify possible pathogenic single nucleotide variants.

4.3.4. Cortical heterotopias

Periventricular nodular heterotopia (PNH) is the most frequent and widely described form of neuronal heterotopias. This condition is characterized by the inappropriate position of otherwise normal neurons. Focal epileptic seizures with variable age of onset represent the most common presenting symptom (up to 80-90% of patients) [101]. Pathogenic variants in the X-linked *FLNA* are responsible for the majority of cases, with female predominance. Mutations in *ARFGEF2* account for a large portion of the remaining patients, whereas other possible candidate genes are *CADM1*, *DMT1*, and *EML1* [102].

4.3.4.1 Genetic testing

NGS-based panels specifically designed to investigate genes involved in brain malformations are indicated.

4.4. Progressive myoclonus epilepsies

Progressive myoclonus epilepsies (PMEs) include several inherited neurological diseases with typical onset in the second childhood or adolescence and progressive clinical course. The common phenotype consists of the association of cortical myoclonus with epileptic seizures, dementia, and ataxia. Cortical myoclonus is the cardinal feature. It is typically intentional, action-induced, and progressively worsens over time [103, 104]. Epileptic seizures may lack in some patients with very mild phenotypes or in those who received early antiepileptic treatment for the myoclonus. The diagnosis might be delayed in cases mimicking idiopathic generalized epilepsy. However, the combination of accurate electro-clinical characterization, psychomotor assessment, neuroimaging, and instrumental studies (e.g., muscle biopsy) may provide relevant clues towards the right clinical diagnosis. Since most PMEs show an autosomal recessive inheritance, parental consanguinity should be carefully investigated. Occasionally, *de novo* variants have been identified, whereas maternally inherited mutations are responsible for PMEs in mitochondrial disorders (Table 4). According to the aetiological survey of Franceschetti et al. on PMEs in Italy [105], Unverricht-Lundborg disease (EPM1) and Lafora's disease (EPM2) are the most common disorders, followed by late-infantile and adult onset neuronal ceroid lipofuscinosis (NCL), myoclonus epilepsy with ragged-red fibers (MERRF), PME with or without renal failure (due to *SCARB2* mutations), type I or II sialidosis, Gaucher disease, type C Niemann-Pick disease, and juvenile-onset Huntington disease. Thanks to recent advances in genetic diagnosis, other rare disorders are currently identifiable: PMEs with

impaired ceramide synthesis, *KCNC1*-related myoclonus epilepsy and ataxia, and congenital generalized lipodystrophy type 2 [106, 107]. Genetic work-up should be based on the clinical presentation, including relevant associated symptoms (Figure 2). Epidemiological data should be accurately considered, giving priority to the most common disorders (EPM1 and Lafora's disease). The possibility of atypical phenotypes with extremely mild or severe clinical presentation should be considered as well [108-111]. A thorough clinical investigation is of paramount importance, since even infrequent signs may point towards a specific diagnosis: for example, ocular or peripheral nervous system involvement may be suggestive of ceroid lipofuscinosis. Eventually, skin or muscle biopsy may be useful to support the diagnosis in specific disorders.

4.4.1 Genetic testing

NGS-based panels including known genes associated with PMEs are usually indicated in these patients.

5. Treatment options and precision medicine

5.1. Antiepileptic drugs

The discovery of the genetic defects behind a specific form of epilepsy might fully or partially explain the positive or negative (paradoxical) response to specific antiepileptic drugs (AEDs). Dravet's syndrome represents a classic example. In this condition, clinical worsening has been observed in patients treated with sodium-channel blockers, which should therefore be avoided. Conversely, sodium-blocking AEDs should be considered as first line therapy in *KCNQ2*-related DEE [112].

5.2. Ketogenic diet

The effectiveness of ketogenic diet in glucose transporter deficiency syndrome (GLUT1DS) is an excellent example of how the knowledge of the genetic defect underlying an epileptic disorder may suggest a specific treatment strategy. This condition is caused by mutations in *SLC2A1* (encoding the glucose transporter GLUT1), which result in a deficient glucose transportation through the blood-brain barrier [113]. Ketogenic diet is a high-fat diet with a low carbohydrate content providing ketone bodies to the brain as an alternative energy source to glucose. Consequently, this dietetic regimen should be effective in conditions with impaired brain glucose supply. The correction of this deficiency in patients with GLUT1DS results in the improvement of both epilepsy and associated neurological symptoms (e.g., movement disorder and ID) [114]. Of course, this diet should be started as soon as possible after the diagnosis, since early treatment significantly improves long-term prognosis.

5.3. Precision medicine

Precision medicine aims to offer customized treatment for any epileptic patient, based on the involved gene and specific molecular alteration [112]. In addition to the abovementioned use of ketogenic diet in GLUT1DS, several other examples of rational therapeutic strategies in genetic epilepsies are available (Table 5).

Pyridoxine-dependent epilepsy (PDE) is caused by mutations in *PNPO*, encoding an enzyme catalyzing the biosynthesis of pyridoxal-5'-phosphate (PLP) [115]. This is the active form of pyridoxine and is necessary for several biological processes, including neurotransmitter biosynthesis. PDE is characterized by convulsive seizures

with onset right after birth, or even before. AEDs are generally not effective, but these patients respond well to high daily doses of PLP. More recently, *DEPDC5* mutations have been associated with sporadic and familial epilepsy types with a broad phenotypic spectrum [116].

DEPDC5 is a negative regulator of mTOR, a protein kinase which regulates several essential cellular processes. Abnormalities in the mTOR pathway leading to mTOR hyperactivation have been associated with many human diseases, ranging from cancer to MCDs [117]. Similarly to *DEPDC5*, *TSC1* and *TSC2* encode a complex with inhibitory activity on mTOR. Loss-of-function mutations in these genes cause tuberous sclerosis, a complex disorder characterized by drug-resistant epilepsy. These observations have pushed scientific research towards the development of mTOR inhibitors to be used in patients with epilepsy, starting from the well-known rapamycin. Consequently, these drugs have been named rapalogs and include tensirolimus, everolimus, and ridaforolimus. Other medications blocking upstream and downstream targets of mTOR pathway have been further developed (e.g., PF-4708671) [117]. In the vast majority of the studies, rapamycin exerted a positive effect on the clinical manifestations of animal models of selected epileptic disorders [99, 118]. However, further studies are needed to confirm the potentially beneficial impact of mTOR inhibitors in the treatment of specific types of epilepsy.

Most cases of Dravet syndrome are caused by heterozygous loss-of-function variants in *SCN1A*, which encodes a voltage-gated sodium channel involved in the generation and propagation of action potential [119]. In these patients, the abnormal function of SCN1A in the inhibitory neurons generates an excitation/inhibition imbalance

leading to epileptogenesis [120]. According to this pathogenic model, sodium channel blocking drugs (e.g., carbamazepine, lamotrigine, oxcarbazepine, and phenytoin) should be avoided in *SCN1A*-associated Dravet syndrome, since these drugs are usually ineffective or can actually worsen seizures [121, 122]. These patients might instead benefit from new therapeutic strategies developed in the recent years, such as antisense oligonucleotides restoring SCN1A mRNA and peptides selectively activating the Nav1.1 channel in inhibitory interneurons (e.g., Hm1) [123, 124].

The type of the genetic variant identified in a specific gene further influences the therapeutic strategy. As an example, the administration of the appropriate treatment in patients with *de novo* mutation in *GRIN2A*, encoding the N-methyl-D-aspartate (NMDA) receptor subunit NR2A, strongly depends on the functional effect of the mutation [125]. Indeed, drugs with NMDA-blocking activity (e.g., memantine and felbamate) have shown to be effective in reducing the frequency and severity of seizures in individuals carrying gain-of-function variants and new drugs which selectively block NMDA receptors containing the NR2A subunit are being developed (e.g., MPX-004 and MPX-007) [126-128]. However, the picture may be more complex in case of variants affecting the sensitivity of the NMDA receptor to these drugs [129]. Subjects harboring loss-of-function *GRIN2A* variants may instead benefit from treatment with positive allosteric modulators of NMDA receptors [130-132].

5.4. Adverse drug reactions

A large number of studies have addressed the relationship between specific genetic variations and the occurrence of adverse reactions to AEDs. In particular, adverse

cutaneous drug reactions have been associated with polymorphisms in the MHC system. The best example is the Chinese population of Han ethnicity and, more generally, the individuals of Asian-heritage carrying the HLA-B*1502 allele. These subjects are at increased risk of developing Stevens-Johnson's syndrome (SJS) and toxic epidermal necrolysis (TEN) following carbamazepine intake [133]. A similarly increased risk has been observed in the Japanese and European populations harboring the HLA-A*3101 allele [134], which represents a risk factor for the development of severe skin reactions after the administration of sodium channel blockers, such as carbamazepine. Although the available data are still insufficient to support the need of a specific genetic screening, a careful assessment of the benefits and risks should be considered when these AEDs are chosen.

5.5. Epilepsy surgery

Patients with drug-resistant epilepsy who are candidates for surgery may carry germinal or somatic pathogenic variants. In the latter case, genetic alterations involve only the surgically resected cerebral area, usually corresponding to the structural abnormality identified on MRI (e.g., FCD). Theoretically, if all mutated cells in the epileptogenic zone are removed, the post-surgical outcome should not differ from that observed in other drug-resistant focal epilepsies. However, this topic has not been specifically addressed yet and further studies are necessary. Conversely, when surgery is chosen to treat an epileptic disorder caused by germinal mutations (e.g., *SCN1A*, *DEPDC5*, etc.), the situation is more complex. To date, only anecdotal reports without long-term follow-up are available [135]. In these cases, the mutation is present in all neuronal cells and,

therefore, the resection of the epileptogenic zone per se might not be curative. Indeed, the diffuse genetic alteration might give rise to independent epileptic foci or sustain an epileptogenic network extending beyond the removed area. At the moment, the role of specific genetic tests in supporting surgical indication in patients with drug-resistant epilepsy still remains controversial.

6. Conclusion

Due to the heterogeneity of epileptic disorders with presumed genetic aetiology, the choice of the most appropriate genetic test to perform in each patient is of paramount importance to increase the diagnostic yield. This choice should be guided by several factors, including personal history, electro-clinical features, and neuroimaging findings. Cytogenetic analysis through array-CGH and, in selected cases, FISH may be helpful in syndromic patients. MLPA can be, instead, performed to rule out possible deletions of genes already associated with distinctive epileptic phenotypes. In non-syndromic patients, NGS-based tests play the primary role. A possible approach is to start with NGS-based panels expressly assembled to include the most relevant genes responsible of common epileptic disorders. In light of the recent advances achieved in NGS-based techniques and the improved interpretation of test results, WES currently represents a remarkable diagnostic tool in patients with epilepsy without a genetic diagnosis (especially if panels result negative). Of note, it should be stressed that the correct interpretation of WES data cannot overlook an accurate clinical and instrumental phenotyping of the patient, in order to optimize the diagnostic yield. Eventually, through WGS it is possible to detect CNVs, which are responsible of several specific epileptic

conditions, and sequence human genome in a unique test. The advancements in the knowledge on human genome will hopefully allow to interpret the changes in non-coding regions that are currently indecipherable, possibly explaining some unsolved cases.

7. Expert opinion

In the last two decades, significant advances have been accomplished in molecular genetics. The discovery of new causative genes for several epileptic disorders has remarkably improved the knowledge on the epileptogenesis. In particular, the identification of the genes encoding the subunits of voltage-gated channels (sodium, potassium, and chloride channels) and the subunits of the acetylcholine and GABA receptors have provided fundamental insights into the pathogenic mechanisms underlying several epileptic disorders. Accordingly, the development of new drugs specifically targeting mutated proteins and selectively addressing pathogenic mechanisms has opened new scenarios for personalized therapeutic approaches (precision medicine). As an example, the understanding of the pathophysiology of *KCNT1*-relatd epilepsies has supported the use of quinidine in these conditions [136]. Vipocentine, an alkaloid potentiating GABA-evoked currents, has been successfully used to treat Lennox-Gastaut syndrome caused by *GABRB3* mutation [137]. The new compounds MPX-004 and MPX-007 have been developed to selectively block the NMDA receptors containing the NR2A subunit in patients with gain-of-function mutations in *GRIN2A* [128]. These improvements have also contributed to enhancing the pharmacogenomics, which represents a valuable tool for clinicians to predict the efficacy and tolerability of a specific drug in the single patient.

Notwithstanding the complexities of genetic testing in epileptic disorders, every effort should be made to achieve a definite genetic diagnosis for both clinical and research implications. Indeed, the genetic characterization of patients with epilepsy may play a pivotal role in the choice of the best therapeutic options and improve patient care. At the same time, it supports epilepsy research in the complex process of improving the understanding of the pathogenesis and natural history of epileptic disorders. The strict collaboration between the geneticist and pediatric neurologist still represents the keystone of the genetic diagnosis in epilepsy, alongside the data provided by instrumental studies, mainly EEG and brain MRI, and neuropsychological assessment.

Interdisciplinary approach and international collaboration will definitely allow to achieve further significant advances in epilepsy research in the next future. Neurologists will play a more and more important role in the diagnostic process of patients with epilepsy, fostering a needful collaboration with clinical geneticists. Indeed, the correct patient phenotyping is essential to support the geneticist in choosing the most appropriate test in the single case. Furthermore, clinical and instrumental information represent essential clues for the geneticist in the interpretation of the results of complex NGS-based tests. Knowledge integration will hopefully increase the diagnostic yield of WES and WGS, allowing clinicians to achieve a final diagnosis in several, previously unsolved cases.

A relevant step forward is further expected in the interpretation of WGS data. The advancements in the knowledge on the structure and functions of non-coding regions of human genome, the enhancement of CNVs databases, and the development of specific non-coding variant databases will represent the keystone in our capabilities of taking

advantage of all the potentialities offered by WGS in the clinical practice. The development of specific genetic variant effect prediction algorithms will possibly represent a further tool to use in the complex interpretation process.

Significant advancements in precision medicine have been made in the last few years. The understanding of new pathological mechanisms underlying several forms of epilepsy and the discovery of new causative genes made possible by NGS-based techniques are the main drivers of these progresses. In the future, each patient will hopefully be treated in a very personalized way. According to the pathogenic variant harbored, the gene involved, and the possible responses to specific medical treatments (pharmacogenomics), a customized approach will be feasible to optimize patient care and improve the clinical management. A significantly promising field might be that of DEE. In these patients, the development of molecularly targeted drugs might be helpful not only in stopping epilepsy, but also in stabilizing the progression of cognitive impairment and the frequently observed neurodegenerative features. Early treatment will possibly allow to minimize the effects of epilepsy on cognitive performances and, hopefully, partially revert the unavoidable cognitive decline.

In recent years, many efforts have been made to improve genetic diagnosis in patients with epilepsy, and many others are expected in the next few years. As the knowledge on the causes and pathomechanisms underlying epilepsy continues to grow, new scenarios concerning the treatment and optimal management of epileptic disorders will emerge. The rapid technological advances in the field of medical genetics and the improvements in clinical investigation will play the pivotal role in supporting us in the fight against epilepsy in the next future.

Funding

This paper was not funded.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

References

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

1. Striano P. Epilepsy towards the next decade: New trends and hopes in epileptology. Basel: Springer International Publishing, 2015 ***of considerable interest because it represents a precious summary of recent scientific and clinical trends in epileptic disorders*

2. Andrade DM, Minassian BA. Genetics of epilepsies. Expert Rev Neurother. 2007;7:727-34

3. Orsini A, Zara F, Striano P. Recent advances in epilepsy genetics. Neurosci Lett.

2018;667:4-9 **of considerable interest because it provides a detailed description of the most recent advances in epilepsy genetics*

4. Symonds JD, Zuberi SM, Johnson MR. Advances in epilepsy gene discovery and implications for epilepsy diagnosis and treatment. Curr Opin Neurol. 2017;30:193-9

5. Mantegazza M, Rusconi R, Scalmani P, et al. Epileptogenic ion channel mutations: from bedside to bench and, hopefully, back again. Epilepsy Res. 2010;92:1-29

6. Nicita F, De Liso P, Danti FR, et al. The genetics of monogenic idiopathic epilepsies and epileptic encephalopathies. Seizure. 2012;21:3-11

7. Perry MS, Poduri A. Two Studies, One Message: High Yield of Genetic Testing in Infants and Young Children With Severe Epilepsies. Epilepsy Curr. 2018;18:24-6

8. Bianchi A. Definition of the phenotype for genetic studies. Chapter 5. In Perinatal Brain Damage: from Pathogenesis to Neuroprotection. London-Paris, John Libbey Eurotext, 2009

9. Fisher RS, Cross JH, French JA, et al. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. Epilepsia. 2017;58:522-30 **of interest because it is the most recent operational classification in epilepsy*

10. Fisher RS, Cross JH, D'Souza C, et al. Instruction manual for the ILAE 2017 operational classification of seizure types. Epilepsia. 2017;58:531-42

11. Kwan P, Brodie MJ. Early identification of refractory epilepsy. N Engl J Med. 2000;342:314-9

12. Kwan P, Brodie MJ. Definition of refractory epilepsy: defining the indefinable? Lancet Neurol. 2010;9:27-9

13. Peljto AL, Barker-Cummings C, Vasoli VM, et al. Familial risk of epilepsy: a population-based study. Brain. 2014;137:795-805

14. Christensen J, Overgaard M, Parner ET, et al. Risk of epilepsy and autism in full and half siblings-A population-based cohort study. Epilepsia. 2016;57:2011-8

15. Bennett RL, French KS, Resta RG, et al. Standardized human pedigree nomenclature: update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Couns. 2008;17:424-33

16. Seneviratne U, Cook MJ, D'Souza WJ. Electroencephalography in the Diagnosis of Genetic Generalized Epilepsy Syndromes. Front Neurol. 2017;8:499

17. Giannakodimos S, Panayiotopoulos CP. Eyelid myoclonia with absences in adults: a clinical and video-EEG study. Epilepsia. 1996;37:36-44

18. Hrachovy RA, Frost Jr. JD. Infantile epileptic encephalopathy with hypsarrhythmia (Infantile Spasms/West syndrome). J Clin Neurophysiol. 2003;20:408-25

19. Guerrini R, Pellacani S. Benign childhood focal epilepsies. Epilepsia. 2012;53:9-18

20. Kim SH, Korff CM, Kim AJ, et al. A practical, simple, and useful method of categorizing interictal EEG features in children. Neurology. 2015;85:471-8

21. Assi L, Saklawi Y, Karam PE, et al. Treatable Genetic Metabolic Epilepsies. Curr Treat Options Neurol. 2017;19:30

22. Papetti L, Parisi P, Leuzzi V, et al. Metabolic epilepsy: an update. Brain Dev. 2013;35:827-41

23. Wolf B. The neurology of biotinidase deficiency. Mol Genet Metab. 2011;104:27-34

24. Kirkham FJ. Indications for the performance of neuroimaging in children. Handb Clin Neurol. 2016;136:1275-90

25. Bernasconi A, Cendes F, Theodore WH, et al. Recommendations for the use of structural magnetic resonance imaging in the care of patients with epilepsy: A consensus report from the International League Against Epilepsy Neuroimaging. Epilepsia. 2019;60:1054-68 ***of considerable interest because it provides useful and up-to-date recommendations for the correct use of neuroimaging in the diagnosis and management of epileptic patients*

26. Lange M, Kasper B, Bohring A, et al. 47 patients with *FLNA* associated periventricular nodular heterotopia. Orphanet J Rare Dis. 2015;10:134

27. El Achkar CM, Olson HE, Poduri A, et al. The genetics of the epilepsies. Curr Neurol Neurosci Rep. 2015;15:39

28. Epilepsy Phenome/Genome Project & Epi4K Consortium. Copy number variant analysis from exome data in 349 patients with epileptic encephalopathy. Ann Neurol. 2015;78:323-8

29. de Kovel CG, Trucks H, Helbig I, et al. Recurrent microdeletions at 15q11.2 and 16p13.11 predispose to idiopathic generalized epilepsies. Brain. 2010;133:23-32

30. Mefford HC, Muhle H, Ostertag P, et al. Genome-wide copy number variation in epilepsy: novel susceptibility loci in idiopathic generalized and focal epilepsies. PLoS Genet. 2010;6:e1000962

31. Mullen SA, Carvill GL, Bellows S, et al. Copy number variants are frequent in genetic generalized epilepsy with intellectual disability. Neurology. 2013;81:1507-14

32. Striano P, Coppola A, Paravidino R, et al. Clinical significance of rare copy number variations in epilepsy: a case-control survey using microarray-based comparative genomic hybridization. Arch Neurol. 2012;69:322-30

33. Olson H, Shen Y, Avallone J, et al. Copy number variation plays an important role in clinical epilepsy. Ann Neurol. 2014;75:943-58

34. Møller RS, Dahl HA, Helbig I. The contribution of next generation sequencing to epilepsy genetics. Expert Rev Mol Diagn. 2015;15:1531-8

35. Myers CT, Mefford HC. Advancing epilepsy genetics in the genomic era. Genome Med. 2015;7:91

36. Mefford HC. Clinical Genetic Testing in Epilepsy. Epilepsy Curr. 2015;15:197-201

37. Weber YG, Biskup S, Helbig KL, et al. The role of genetic testing in epilepsy diagnosis and management. Expert Rev Mol Diagn. 2017;17:739-50

38. Costain G, Cordeiro D, Matviychuk D, et al. Clinical Application of Targeted Next-Generation Sequencing Panels and Whole Exome Sequencing in Childhood Epilepsy. Neuroscience. 2019;418:291-310

39. Jang SS, Kim SY, Kim H, et al. Diagnostic Yield of Epilepsy Panel Testing in Patients With Seizure Onset Within the First Year of Life. Front Neurol. 2019;10:988

40. Staněk D, Laššuthová P, Štěrbová K, et al. Detection rate of causal variants in severe childhood epilepsy is highest in patients with seizure onset within the first four weeks of life. Orphanet J Rare Dis. 2018;13:71

41. Ko A, Youn SE, Kim SH, et al. Targeted gene panel and genotype-phenotype correlation in children with developmental and epileptic encephalopathy. Epilepsy Res. 2018;141:48-55

42. Dunn P, Albury CL, Maksemous N, et al. Next Generation Sequencing Methods for Diagnosis of Epilepsy Syndromes. Front Genet. 2018;9:20 **of considerable interest because it shows the significant advances achieved with the use of Next Generation Sequencing (NGS)-based techniques in the diagnosis of epileptic syndromes*

43. Trump N, McTague A, Brittain H, et al. Improving diagnosis and broadening the phenotypes in early-onset seizure and severe developmental delay disorders through gene panel analysis. J Med Genet. 2016;53:310-7

44. Bowdin S, Gilbert A, Bedoukian E, et al. Recommendations for the integration of genomics into clinical practice. Genet Med. 2016;18:1075-84

45. Tetreault M, Bareke E, Nadaf J, et al. Whole-exome sequencing as a diagnostic tool: current challenges and future opportunities. Expert Rev Mol Diagn. 2015;15:749-60

46. Lindy AS, Stosser MB, Butler E, et al. Diagnostic outcomes for genetic testing of 70 genes in 8565 patients with epilepsy and neurodevelopmental disorders. Epilepsia. 2018;59:1062-71

47. Bennett CA, Petrovski S, Oliver KL, et al. ExACtly zero or once: A clinically helpful guide to assessing genetic variants in mild epilepsies. Neurol Genet. 2017;3:e163. eCollection 2017 Aug

48. Richards S, Aziz N, Bale S, et al. 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. Genet Med. 2015;17:405-24

49. Dunn P, Albury CL, Maksemous N, et al. Next Generation Sequencing Methods for Diagnosis of Epilepsy Syndromes. Front Genet. 2018;9:20

50. Cheung SW, Bi W. Novel applications of array comparative genomic hybridization in molecular diagnostics. Expert Rev Mol Diagn. 2018;18:531-42

51. Monlong J, Girard SL, Meloche C, et al. Global characterization of copy number variants in epilepsy patients from whole genome sequencing. PLoS Genet. 2018;14:e1007285

52. Pirooznia M, Goes FS, Zandi PP. Whole-genome CNV analysis: advances in computational approaches. Front Genet. 2015;6:138

53. Nolan D, Carlson M. Whole Exome Sequencing in Pediatric Neurology Patients: Clinical Implications and Estimated Cost Analysis. J Child Neurol. 2016;31:887-94

54. Schwarze K, Buchanan J, Taylor JC, et al. Are whole-exome and whole-genome sequencing approaches cost-effective? A systematic reviewof the literature. Genet Med. 2018;20:1122-30

55. Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia. 2010;51:676-85

56. Arsov T, Mullen SA, Damiano JA, et al. Early onset absence epilepsy: 1 in 10 cases is caused by GLUT1 deficiency. Epilepsia. 2012;53:e204-7

57. Larsen J, Johannesen KM, Ek J, et al. The role of *SLC2A1* mutations in myoclonic astatic epilepsy and absence epilepsy, and the estimated frequency of GLUT1 deficiency syndrome. Epilepsia. 2015;56:e203-8

58. Suzuki T, Delgado-Escueta AV, Aguan K, et al. Mutations in *EFHC1* cause juvenile myoclonic epilepsy. Nat Genet. 2004;36:842-9

59. Zara F, Specchio N, Striano P, et al. Genetic testing in benign familial epilepsies of the first year of life: clinical and diagnostic significance. Epilepsia. 2013;54:425-36

60. Gardella E, Becker F, Møller RS, et al. Benign infantile seizures and paroxysmal dyskinesia caused by an *SCN8A* mutation. Ann Neurol. 2016;79:428-36

61. Grinton BE, Heron SE, Pelekanos JT, et al. Familial neonatal seizures in 36 families: Clinical and genetic features correlate with outcome. Epilepsia. 2015;56:1071-80

62. Pisano T, Numis AL, Heavin SB, et al. Early and effective treatment of *KCNQ2* encephalopathy. Epilepsia. 2015;56:685-91

63. van Vliet R, Breedveld G, de Rijk-van Andel J, et al. *PRRT2* phenotypes and penetrance of paroxysmal kinesigenic dyskinesia and infantile convulsions. Neurology. 2012;79:777-84

64. Trivisano M, Terracciano A, Milano T, et al. Mutation of *CHRNA2* in a family with benign familial infantile. Epilepsia. 2015;56:e53-7

65. Symonds JD, Zuberi SM, Stewart K, et al. Incidence and phenotypes of childhood-onset genetic epilepsies: a prospective population-based national cohort. Brain. 2019;142:2303-18

66. Chen WJ, Lin Y, Xiong ZQ, et al. Exome sequencing identifies truncating mutations in PRRT2 that cause paroxysmal kinesigenic dyskinesia. Nat Genet. 2011;43:1252-5

67. Heron SE, Grinton BE, Kivity S, et al. *PRRT2* mutations cause benign familial infantile epilepsy and infantile convulsions with choreoathetosis syndrome. Am J Hum Genet. 2012;90:152-60

68. Méneret A, Grabli D, Depienne C, et al. *PRRT2* mutations: a major cause of paroxysmal kinesigenic dyskinesia in the European population. Neurology. 2012;79:170-4

69. Lemke JR, Lal D, Reinthaler EM, et al. Mutations in *GRIN2A* cause idiopathic focal epilepsy with rolandic spikes. Nat Genet. 2013;45:1067-72

70. Licchetta L, Pippucci T, Baldassari S, et al. Sleep-related hypermotor epilepsy (SHE): Contribution of known genes in 103 patients. Seizure. 2019;74:60-4

71. Menghi V, Bisulli F, Tinuper P, et al. Sleep-related hypermotor epilepsy: prevalence, impact and management strategies. Nat Sci Sleep. 2018;10:317-26

72. Tinuper P, Bisulli F, Cross JH, et al. Definition and diagnostic criteria of sleep-related hypermotor epilepsy. Neurology. 2016;86:1834-42

73. Aridon P, Marini C, Di Resta C, et al. Increased sensitivity of the neuronal nicotinic receptor alpha 2 subunit causes familial epilepsy with nocturnal wandering and ictal fear. Am J Hum Genet. 2006;79:342-50

74. Heron SE, Smith KR, Bahlo M, et al. Missense mutations in the sodium-gated potassium channel gene KCNT1 cause severe autosomal dominant nocturnal frontal lobe epilepsy. Nat Genet. 2012;44:1188-90

75. Korenke GC, Eggert M, Thiele H, et al. Nocturnal frontal lobe epilepsy caused by a mutation in the GATOR1 complex gene *NPRL3*. Epilepsia. 2016;57:e60-3

76. Michelucci R, Pasini E, Malacrida S, et al. Low penetrance of autosomal dominant lateral temporal epilepsy in Italian families without *LGI1* mutations. Epilepsia. 2013;54:1288-97

77. Rosanoff MJ, Ottman R. Penetrance of *LGI1* mutations in autosomal dominant partial epilepsy with auditory features. Neurology. 2008;71:567-71

78. Ottman R, Winawer MR, Kalachikov S, et al. *LGI1* mutations in autosomal dominant partial epilepsy with auditory features. Neurology. 2004;62:1120-6

79. Bisulli F, Tinuper P, Scudellaro E, et al. A de novo *LGI1* mutation in sporadic partial epilepsy with auditory features. Ann Neurol. 2004;56:455-6

80. Dazzo E, Fanciulli M, Serioli E, et al. Heterozygous reelin mutations cause autosomal-dominant lateral temporal epilepsy. Am J Hum Genet. 2015;96:992-1000

81. Berkovic SF, Scheffer IE. Febrile seizures: genetics and relationship to other epilepsy syndromes. Curr Opin Neurol. 1998;11:129-34

82. Kivity S, Oliver KL, Afawi Z, et al. SCN1A clinical spectrum includes the self-limited focal epilepsies of childhood. Epilepsy Res. 2017;131:9-14

83. Bonanni P, Malcarne M, Moro F, et al. Generalized epilepsy with febrile seizures plus (GEFS+): clinical spectrum in seven Italian families unrelated to SCN1A, SCN1B, and GABRG2 gene mutations. Epilepsia. 2004;45:149-58

84. Zhang YH, Burgess R, Malone JP, et al. Genetic epilepsy with febrile seizures plus: Refining the spectrum. Neurology. 2017;89:1210-9

85. Kalser J, Cross JH. The epileptic encephalopathy jungle - from Dr West to the concepts of aetiology-related and developmental encephalopathies. Curr Opin Neurol. 2018;31:216-22

86. McTague A, Howell KB, Cross JH, et al. The genetic landscape of the epileptic encephalopathies of infancy and childhood. Lancet Neurol. 2016;15:304-16

87. Jehi L, Wyllie E, Devinsky O. Epileptic encephalopathies: Optimizing seizure control and developmental outcome. Epilepsia. 2015;56:1486-9

88. Carvill GL, McMahon JM, Schneider A, et al. Mutations in the GABA Transporter SLC6A1 Cause Epilepsy with Myoclonic-Atonic Seizures. Am J Hum Genet. 2015;96:808-15

89. Nieh SE, Sherr EH. Epileptic Encephalopathies: new genes and new pathways. Neurotherapeutics. 2014;11:796-806

90. Kuzniecky R. Epilepsy and malformations of cortical development: new developments. Curr Opin Neurol. 2015;28:151-7

91. Jamuar SS, Lam AT, Kircher M, et al. Somatic mutations in cerebral cortical malformations. N Engl J Med. 2014;371:733-43

92. Barkovich AJ, Guerrini R, Kuzniecky RI, et al. A developmental and genetic classification for malformations of cortical development: update. Brain. 2012;135:1348-69

93. Guerrini R, Dobyns WB. Malformations of cortical development: clinical features and genetic causes. Lancet Neurol. 2014;13:710-26

94. Pavone P, Praticò AD, Rizzo R, et al. A clinical review on megalencephaly: A large brain as a possible sign of cerebral impairment. Medicine (Baltimore). 2017;96:e6814

95. Rivière JB, Mirzaa GM, O'Roak BJ, et al. De novo germline and postzygotic mutations in *AKT3*, *PIK3R2* and *PIK3CA* cause a spectrum of related megalencephaly syndromes. Nat Genet. 2012;44:934-40

96. Mirzaa GM, Conway RL, Gripp KW, et al. Megalencephaly-capillary malformation (MCAP) and megalencephaly-polydactylypolymicrogyria-hydrocephalus (MPPH)

syndromes: two closely related disorders of brain overgrowth and abnormal brain and body morphogenesis. Am J Med Genet A. 2012;158A:269-91

97. Guerrini R, Filippi T. Neuronal migration disorders, genetics, and epileptogenesis. J Child Neurol. 2005;20:287-99

98. Fry AE, Cushion TD, Pilz DT. The genetics of lissencephaly. Am J Med Genet C Semin Med Genet. 2014;166C:198-210

99. Kim JK, Lee JH. Mechanistic Target of Rapamycin Pathway in Epileptic Disorders. J Korean Neurosurg Soc. 2019;62:272-87

100. Romaniello R, Arrigoni F, Cavallini A, et al. Brain malformations and mutations in α- and β-tubulin genes: a review of the literature and description of two new cases. Dev Med Child Neurol. 2014;56:354-60

101. Parrini E, Ramazzotti A, Dobyns WB, et al. Periventricular heterotopia: phenotypic heterogeneity and correlation with Filamin A mutations. Brain. 2006;129:1892-906

102. Kielar M, Phan Dinh Tuy F, Bizzotto S, et al. Mutations in *Eml1/EML1* lead to ectopic progenitors and neuronal heterotopia in mouse and human. Med Sci. 2014;30:1087-90

103. Shahwan A, Farrell M, Delanty N, et al. Progressive myoclonic epilepsies: a review of genetic and therapeutic aspects. Lancet Neurol. 2005;4:239-48

104. Kälviäinen R. Progressive Myoclonus Epilepsies. Semin Neurol 2015;35:293-9

105. Muona M, Berkovic SF, Dibbens LM, et al. A recurrent de novo mutation in *KCNC1* causes progressive myoclonus epilepsy. Nat Genet. 2015;47:39-46

106. Vanni N, Fruscione F, Ferlazzo E, et al. Impairment of ceramide synthesis causes a novel progressive myoclonus epilepsy. Ann Neurol. 2014;76:206-12

107. Opri R, Fabrizi GM, Cantalupo G, et al. Progressive Myoclonus Epilepsy in Congenital Generalized Lipodystrophy type 2: Report of 3 cases and literature review. Seizure. 2016;42:1-6

108. Franceschetti S, Gambardella A, Canafoglia L, et al. Clinical and genetic findings in 26 Italian patients with Lafora disease. Epilepsia. 2006;47:640-3

109. Canafoglia L, Gennaro E, Capovilla G, et al. Electroclinical presentation and genotype-phenotype relationships in patients with Unverricht-Lundborg disease carrying compound heterozygous *CSTB* point and indel mutations. Epilepsia. 2012;53:2120-7

110. Ferlazzo E, Canafoglia L, Michelucci R, et al. Mild Lafora disease: clinical, neurophysiologic, and genetic findings. Epilepsia. 2014;55:e129-33

111. Casciato S, Gambardella S, Mascia A, et al. Severe and rapidly-progressive Lafora disease associated with *NHLRC1* mutation: a case report. Int J Neurosci. 2017;127:1150-3

112. Striano P, Vari MS, Mazzocchetti C, et al. Management of genetic epilepsies: From empirical treatment to precision medicine. Pharmacol Res. 2016;107:426-9 ***of considerable interest because it provides a thorough approach to the management of genetic epilepsies, with a particular focus on the new possibilities offered by precision medicine*

113. Klepper J. GLUT1 deficiency syndrome in clinical practice. Epilepsy Research. 2012;100:272-7

114. Pearson TS, Akman C, Hinton VJ, et al. Phenotypic Spectrum of Glucose Transporter Type 1 Deficiency Syndrome (Glut1 DS). Curr Neurol Neurosci Rep. 2013;13:342

115. Baumgart A, Spiczak Sv, Verhoeven-Duif NM, et al. Atypical vitamin B6 deficiency: a rare cause of unexplained neonatal and infantile epilepsies. J Child Neurol. 2014; 29:704-7

116. Dibbens LM, de Vries B, Donatello S, et al. Mutations in *DEPDC5* cause familial focal epilepsy with variable foci. Nat Genet. 2013;45:546-51

117. Sadowski K, Kotulska-Jóźwiak K, Jóźwiak S, et al. Role of mTOR inhibitors in epilepsy treatment. Pharmacol Rep. 2015;67:636-46

118. Griffith JL, Wong M. The mTOR pathway in treatment of epilepsy: a clinical update. Future Neurol. 2018;13:49-58

119. Catterall WA. Dravet syndrome: a sodium channel interneuronopathy. Curr Opin Physiol. 2018;2:42-50

120. Sun Y, Paşca SP, Portmann T, et al. A deleterious Nav1.1 mutation selectively impairs telencephalic inhibitory neurons derived from Dravet Syndrome patients. Elife. 2016;5:e13073

121. Perucca P. Genetics of focal epilepsies: What do we know and where are we heading? Epilepsy Curr. 2018;18:356-62

122. Wirrell EC, Laux L, Donner E, et al. Optimizing the diagnosis and management of Dravet syndrome: recommendations from a North American consensus panel. Pediatr Neurol. 2017;68:18-34 e3

123. Richards KL, Milligan CJ, Richardson RJ, et al. Selective NaV1.1 activation rescues Dravet syndrome mice from seizures and premature death. Proc Natl Acad Sci USA. 2018;115:E8077-85

124. Stoke Therapeutics. Stoke Therapeutics Presents Data Showing Single Dose of ASO
Therapy Restores Normal Protein Levels in Animal Model of Genetic Epilepsy. 2018. Accessed 26 December 2018. https://www.stoketherapeutics.com/wpcontent/uploads/Stoke-PR-Dec-1-1.pdf
125. Perucca P, Perucca E. Identifying mutations in epilepsy genes: Impact on treatment selection. Epilepsy Res. 2019;152:18-30
126. Pierson TM, Yuan H, Marsh ED, et al. *GRIN2A* mutation and early-onset epileptic encephalopathy: personalized therapy with memantine. Ann Clin Transl Neurol. 2014;1:190-8
127. De Sarro G, Ongini E, Bertorelli R, et al. Excitatory amino acid neurotransmission through both NMDA and non-NMDA receptors is involved in the anticonvulsant activity of felbamate in DBA/2 mice. Eur J Pharmacol. 1994;262:11-9
128. Reif PS, Tsai MH, Helbig I, et al. Precision medicine in genetic epilepsies: break of dawn? Expert Rev Neurother. 2017;17:381-92
129. Volkmann RA, Fanger CM, Anderson DR, et al. MPX-004 and MPX-007: New Pharmacological Tools to Study the Physiology of NMDA Receptors Containing the GluN2A Subunit. PLoS One. 2016;11:e0148129
130. Zhu S, Paoletti P. Allosteric modulators of NMDA receptors: multiple sites and mechanisms. Curr Opin Pharmacol. 2015;20:14-23
131. Addis L, Virdee JK, Vidler LR, et al. Epilepsy associated *GRIN2A* mutations reduce NMDA receptor trafficking and agonist potency-molecular profiling and functional rescue. Sci Rep. 2017;7:66

132. Strehlow V, Heyne HO, Vlaskamp DRM, et al. GRIN2A-related disorders: genotype and functional consequence predict phenotype. Brain. 2019;142:80-92

133. Chung WH, Hung SI, Hong HS, et al. Medical genetics: a marker for Stevens-Johnson syndrome. Nature. 2004;428:486

134. McCormack M, Alfirevic A, Bourgeois S, et al. HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. N Engl J Med. 2011;364:1134-43

135. Baulac S, Ishida S, Marsan E, et al. Familial Focal Epilepsy with Focal Cortical Dysplasia due to DEPDC5 Mutations. Ann Neurology. 2015;77:675-83

136. Mikati MA, Jiang YH, Carboni M, et al. Quinidine in the treatment of KCNT1-positive epilepsies. Ann Neurol. 2015;78:995-9

137. Billakota S, Andresen JM, Gay BC, et al. Personalized medicine: Vinpocetine to reverse effects of *GABRB3* mutation. Epilepsia. 2019;60:2459-65

138. Mercimek-Mahmutoglu S, Cordeiro D, Cruz V, et al. Novel therapy for pyridoxine dependent epilepsy due to *ALDH7A1* genetic defect: L-arginine supplementation alternative to lysine-restricted diet. Eur J Paediatr Neurol. 2014;18:741-6

139. Platzer K, Yuan H, Schutz H, et al. *GRIN2B* encephalopathy: novel findings on phenotype, variant clustering, functional consequences and treatment aspects. J Med Genet. 2017;54:460-70

140. Ramaswami M, Gautam M, Kamb A, et al. Human potassium channel genes: Molecular cloning and functional expression. Mol Cell Neurosci. 1990;1:214-23

141. Sands TT, Balestri M, Bellini G, et al. Rapid and safe response to low-dose carbamazepine in neonatal epilepsy. Epilepsia. 2016;57:2019-30

142. Millichap JJ, Park KL, Tsuchida T, et al. *KCNQ2* encephalopathy: Features, mutational hot spots, and ezogabine treatment of 11 patients. Neurol Genet. 2016;2:e96

143. Ihara Y, Tomonoh Y, Deshimaru M, et al. Retigabine, a Kv7.2/Kv7.3-Channel Opener, Attenuates Drug-Induced Seizures in Knock-In Mice Harboring Kcnq2 Mutations. PLoS One. 2016;11:e0150095

144. Bearden D, Strong A, Ehnot J, et al. Targeted treatment of migrating partial seizures of infancy with quinidine. Ann Neurol. 2014;76:457-61

145. Yang YR, Kang DS, Lee C, et al. Primary phospholipase C and brain disorders. Adv Biol Regul. 2016;61:80-5

146. Campistol J, Plecko B. Treatable newborn and infant seizures due to inborn errors of metabolism. Epileptic Disord. 2015;17:229-42

147. Tanabe Y, Taira T, Shimotake A, et al. An adult female with proline-rich transmembrane protein 2 related paroxysmal disorders manifesting paroxysmal kinesigenic choreoathetosis and epileptic seizures. Rinsho Shinkeigaku. 2019;59:144-8

148. Chou IC, Lin SS, Lin WD, et al. Successful control with carbamazepine of family with paroxysmal kinesigenic dyskinesia of *PRRT2* mutation. Biomedicine (Taipei). 2014;4:15

149. Howell KB, McMahon JM, Carvill GL, et al. *SCN2A* encephalopathy: a major cause of epilepsy of infancy with migrating focal seizures. Neurology. 2015;85:958-66

150. Sanders SJ, Campbell AJ, Cottrell JR, et al. Progress in understanding and treating SCN2A-mediated disorders. Trends Neurosci. 2018;41:442-56

151. Dilena R, Striano P, Gennaro E, et al. Efficacy of sodium channel blockers in SCN2A early infantile epileptic encephalopathy. Brain Dev. 2017;39:345-8

152. Flor-Hirsch H, Heyman E, Livneh A, et al. Lacosamide for SCN2Arelated intractable neonatal and infantile seizures. Epileptic Disord. 2018;20:440-6

153. Hammer MF, Wagnon JL, Mefford HC, et al. *SCN8A*-related epilepsy with encephalopathy. 2016. In: GeneReviews®. University of Washington, Seattle, WA (1993-2018) (updated August 25, 2016)

154. Liu Y, Schubert J, Sonnenberg L, et al. Neuronal mechanisms of mutations in SCN8A causing epilepsy or intellectual disability. 2019. Brain;142:376-90

155. Schwantje M, Verhagen LM, van Hasselt PM, et al. Glucose transporter type 1 deficiency syndrome and the ketogenic diet. J Inherit Metab Dis. 2019

156. French JA, Lawson JA, Yapici Z, et al. Adjunctive everolimus therapy for treatment-resistant focal-onset seizures associated with tuberous sclerosis (EXIST-3): a phase 3, randomised, double-blind, placebo-controlled study. Lancet. 2016;388:2153-63

157. Okanishi T, Fujimoto A, Nishimura M, et al. The Efficacy of Everolimus for Refractory Seizures in Childhood Onset Epilepsy with Tuberous Sclerosis Complex. Brain Nerve. 2019;71:611-6

158. Canpolat M, Gumus H, Kumandas S, et al. The use of rapamycin in patients with tuberous sclerosis complex: Long-term results. Epilepsy Behav. 2018;88:357-64

159. Schubert-Bast S, Rosenow F, Klein KM, et al. The role of mTOR inhibitors in preventing epileptogenesis in patients with TSC: Current evidence and future perspectives. Epilepsy Behav. 2019;91:94-8

160. Olson HE, Poduri A, Pearl PL. Genetic forms of epilepsies and other paroxysmal disorders. Semin Neurol. 2014;34:266-79

Figure Legends

Figure 1. Diagnostic flow diagram in epileptic disorders of presumed genetic aetiology.

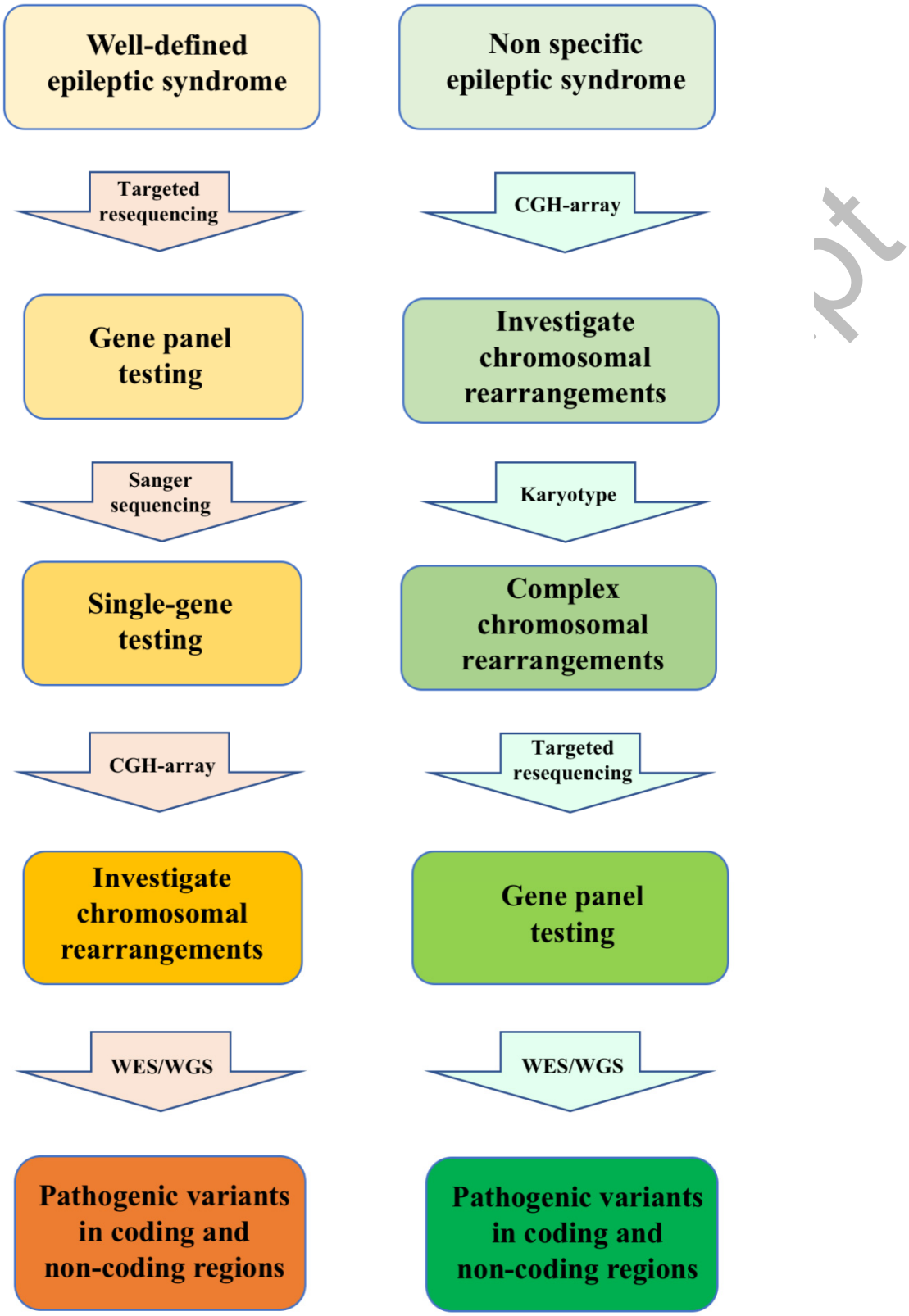
Well-defined epileptic syndrome
Non specific epileptic syndrome
Targeted resequencing
CGH-array
Gene panel testing
Investigate chromosomal rearrangements
Sanger sequencing
Karyotype
Single-gene testing
Complex chromosomal rearrangements
CGH-array
Targeted resequencing
Investigate chromosomal rearrangements
Gene panel testing
WES/WGS
WES/WGS
Pathogenic variants in coding and non-coding regions
Pathogenic variants in coding and non-coding regions

Figure 2. Flowchart for the genetic diagnosis of progressive myoclonus epilepsies.

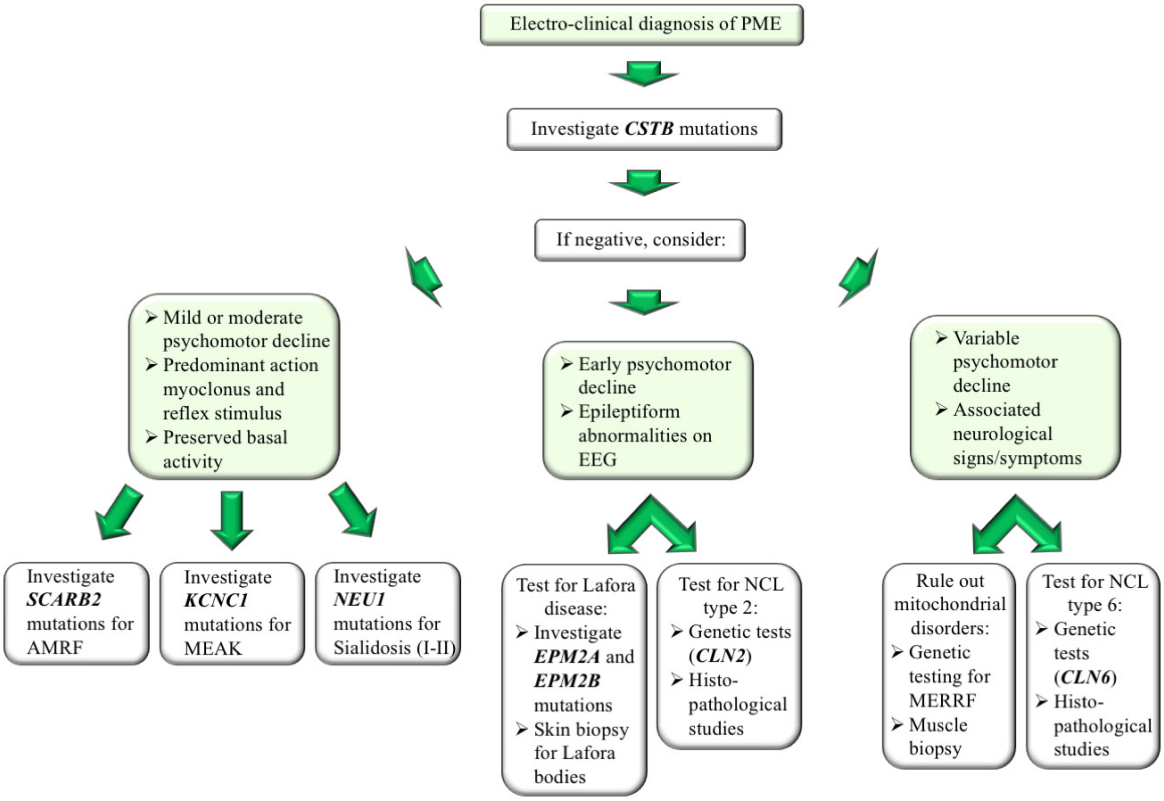

Table 1. Genetic Tests available for epilepsy patients [160].

	Technique	Description	Indication
Cytogenetic analysis	**Array-CGH**	DNA hybridization-based detection of CNVs at various loci	Epilepsy associated with DD, ASD, and/or dysmorphism
	Karyotype	Profile of all chromosomes from a single cell	Dysmorphism or multiple congenital anomalies; suspected monosomy, trisomy, or chromosomal rearrangements
	MLPA	Detects CNVs in a single gene	Suspected defect in a specific gene not detected by Sanger sequencing
	FISH	Probe hybridizing with a specific chromosomal region	Confirmation of a deletion/duplication in a specific region (e.g., 22q11)
Sequencing	**Sanger sequencing**	Direct detection of nucleotide variants in a single gene	Suspected defect in a specific gene (e.g., *SLC2A1* in GDF)
	Targeted gene panel sequencing of clinically related genes	Simultaneous detection of nucleotide variants in multiple genes	Epileptic disorders associated with many genes (e.g., EE)
	WES	Detection of nucleotide variants in coding sequences	Presumably genetic epilepsy of unknown aetiology
	WGS	Detection of nucleotide variants and CNVs in the entire genome (coding and non-coding sequences)	Presumably genetic epilepsy of unknown aetiology

ASD (autism spectrum disorder); CNVs (copy number variants); DD (developmental delay); EE (epileptic encephalopathies); FISH (fluorescent in situ hybridization); GDF (GLUT1-deficiency syndrome); MLPA (Multiplex ligation-dependent probe amplification); WES (whole exome sequencing); WGS (whole genome sequencing).

Table 2. Summary of most relevant genes associated with genetic epilepsies.

Epilepsy phenotype	Gene	OMIM	Locus
ADLTE	*LG1*	604619	10q23.33
	RELN	600514	7q22.1
ADNFLE	*CHRNA2*	118502	8p21.2
	CHRNB2	118507	1q21.3
	CHRNA4	118504	20q13.33
	KCNT1	608167	9q34.3
	DEPDC5	614191	22q12.2-q12.3
	NPRL3	600928	16p13.3
BFIS	*PRRT2*	614386	16p11.2
	SCN2A	182390	2q24.3
	KCNQ2	602235	20q13.33
	KCNQ3	602232	8q24.22
	CHRNA4	118504	20q13.33
BFIS + PKD	*PRRT2*	614386	16p11.2
	SCN8A	600702	12q13.13
BFNS	*KCNQ2*	602235	20q13.33
	KCNQ3	602232	8q24.22
BFNIS	*SCN2A*	182390	2q24.3
	KCNQ2	602235	20q13.33
EOAE	*SCL2A1*	612126	1p34.2
GEFS+	*SCN1A*	182389	2q24.3
	SCN2A	182390	2q24.3
	SCN1B	600235	19q13.11
	GABRG2	137164	5q34
	GABRD	137163	1p36.33
JME	*GABRA1*	137160	5q34
	GABRG2	137164	5q34
	GABRB3	137192	15q12
	GABRD	137163	1p36.33
	CLCN2	600570	3q27.1
	CACNB4	601949	2q23.3

	EFHC1	608815	6p12.2
Rolandic Epilepsy (atypical)	*PRRT2*	614386	16p11.2
	KCNQ2	602235	20q13.33
	KCNQ3	602232	8q24.22
	GRIN2A	138253	16p13.2
	DEPD5C	614191	22q12.2-q12.3

ADLTE (Autosomal dominant lateral temporal epilepsy); ADNFLE (Autosomal dominant nocturnal frontal lobe epilepsy); BFIS (Benign familial infantile seizures); BFNS (Benign familial neonatal seizures); BFNIS (Benign familial neonatal-infantile seizures); EOAE(Early onset absence epilepsy); GEFS+ (Generalised epilepsy with febrile seizures plus); JME (Juvenile myoclonic epilepsy); PKD (Paroxysmal kinesigenic dyskinesia).

Table 3. Most relevant causative genes in epileptic developmental encephalopathies.

Gene	OMIM	Locus	Inheritance	Epileptic syndrome - seizur
ARX	EIEE1 (#308350)	Xp21.3	XLR	West syndrome, Ohtahara syn
CDKL5	EIEE2 (#300672)	Xp22.13	XLD	Infantile spasms and Rett-like pl
SLC25A22	EIEE3 (#609304)	11p15.5	AR	West syndrome, Ohtahara syn
STXBP1	EIEE4 (#612164)	9q34.11	AD	West syndrome, Ohtahara syn
SPTAN1	EIEE5 (#613477)	9q34.11	AD	West syndrome
SCN1A, SCN9A	EIEE6 (#607208)	2q24.3	AD	Dravet syndrome
KCNQ2	EIEE7 (#613720)	20q13.33	AD	Ohtahara syndrome
ARHGEF9	EIEE8 (#300607)	Xq11.1	XLR	ID and epilepsy
PCDH19	EIEE9 (#300088)	Xq22.1	XL	Epilepsy and ID in femal
PNKP	EIEE10 (#613402)	19q13.33	AR	Microcephaly, ID, and epile
SCN2A	EIEE11 (#613721)	2q24.3	AD	Ohtahara syndrome, Dravet sy
PLCB1	EIEE12 (#613722)	20p12.3	AR	Refractory epilepsy and global psychon
SCN8A	EIEE13 (#614558)	12q13.13	AD	Dravet syndrome
KCNT1	EIEE14 (#614959)	9q34.3	AD	MMPSI
ST3GAL3	EIEE15 (#615006)	1p34.1	AR	West syndrome
TBC1D24	EIEE16 (#615338)	16p13.3	AR	Variable seizure types in the first weeks of life, then myo regression
GNAO1	EIEE17 (#615473)	16q13	AD	Abnormal EEG and seizures since the first weeks of life, and brain abnormalities
SZT2	EIEE18 (#615476)	1p34.2	AR	EIEE with dysmorphic corpus c
GABRA1	EIEE19 (#615744)	5q34	AD	Dravet syndrome
PIGA	EIEE20 (#300868)	Xp22.2	XLR	Multiple congenital anomalies-hypotonia-s
NECAP1	EIEE21 (#615833)	12p13.31	AR	DD, axial hypotonia, distal hypertonia,
SLC35A2	EIEE22 (#300896)	Xp11.23	AD	Congenital disorder of glycosylation type I
DOCK7	EIEE23 (#615859)	1p31.3	AR	EE with cortical blindnes
HCN1	EIEE24 (#615871)	5p12	AD	Dravet syndrome
SLC13A5	EIEE25 (#615905)	17p13.1	AR	Focal clonic seizures in the first d followed by polymorphic sei
KCNB1	EIEE26 (#616056)	20q13.13	AD	ID and hypsarrhythmia on I
GRIN2B	EIEE27 (#616139)	12p13.1	AD	West syndrome
WWOX	EIEE28 (#616211)	16q23.1-	AR	Refractory focal, multifocal, or generalized

		q23.2		age at onset of 2 months
AARS	EIEE29 (#616339)	16q22.1	AR	EE with hypomyelinatio
SIK1	EIEE30 (#616341)	21q22.3	AD	Early onset myoclonic encepha Ohtahara syndrome, infantile s
DNM1	EIEE31 (#616346)	9q34.11	AD	Epileptic spasms, atypical abs
KCNA2	EIEE32 (#616366)	1p13.3	AD	Polymorphic seizures, ID, and
EEF1A2	EIEE33 (#616409)	20q13.33	AD	Early infantile myoclonic sei
SLC12A5	EIEE34 (#616645)	20q13.12	AR	Migrating focal seizures
ITPA	EIEE35 (#616647)	20p13	AR	Progressive microcephaly, epi heart defects, and premature
ALG13	EIEE36 (#300884)	Xq23	XLR	Infantile spasms, Lennox–Gastaut
CASK	MICPCH (#300749); FGS4/MRXWN (#300422)	Xp11.4	XLD/XLR	Infantile spasms
KCNQ3	BFNS2 (#121201)	8q24.22	AD	Large phenotypic variability, from
GRIN1	NDHMSD (#614254); NDHMSR (#617820)	9q34.3	AD/AR	Movement disorders, polymorphic seizures since 1 year of
GRIN2A	FESD (#245570)	16p13.2	AD	Epilepsy-aphasia (Landau-Kleffner
FOXG1	Rett syndrome, congenital variant (#613454)	14q12	AD	Encephalopathy with early o epilepsy and Rett-like phenc
CHD2	EEOC (#615369)	15q26.1	AD	EE with onset in childhoo myoclonic-atonic epileps
BRAT1	NEDCAS (#618056); RMFSL (# 614498)	7p22.3	AR	DD, cerebellar signs, and seizures (rigidity, multifocal seizures, and death in
MECP2	Rett syndrome (#312750); SNE (#300673)	Xq28	XLD/XLR	Rett syndrome; severe neonatal encephalop

AD (autosomal dominant); AR (autosomal recessive); BFNS (benign familial neonatal seizures); EE (epileptic encephalopathy); EEOC (epileptic encephalopathy with childhood onset); EIEE (early infantile epileptic encephalopathy); FESD (focal epilepsy with speech disorder with or without mental retardation); FGS (FG syndrome); ID (intellectual disability); MICPCH (mental retardation and microcephaly with pontine and cerebellar hypoplasia); MMPSI (malignant migrating partial seizures of infancy); MRXWN (mental retardation x-linked with or without nystagmus); NDHMSD (neurodevelopmental disorder with or without hyperkinetic movements and seizures, autosomal dominant); NDHMSR (neurodevelopmental disorder with or without hyperkinetic movements and seizures, autosomal recessive); NEDCAS (neurodevelopmental disorder with cerebellar atrophy with or without seizures); RMFSL (rigidity and multifocal seizure syndrome, lethal neonatal); SNE (severe neonatal encephalopathy); XL (X-linked); XLR (X-linked recessive).

Table 4. Main causative genes of Progressive Myoclonus Epilepsies (PMEs).

Gene	Locus	Inheritance	Disease (OMIM #)
CSTB (EPM1A)	21q22.3	AR	PME 1A (Unverricht and Lundborg disease, #254800)
PRICKLE1	12q12	AR	PME 1B (EPM1B, #612437)
EPM2A	6q24	AR	PME 2A (Lafora disease, #254780)
EPM2B (NHLRC1)	6p22	AR	PME 2B (Lafora disease, #254780)
KCTD7	7q11.21	AR	PME 3 (EPM3, #611726)
SCARB2	4q21.1	AR	PME 4 with or without renal failure (EPM4, #254900)
GOSR2	17q21.32	AR	'North Sea' PME (EPM6, #614018)
CERS1 (LASS1)	19p13.11	AD	PME 8 (EPM8, #616230)
KCNC1	11p15.1	AD	PME associated with KCNC1 mutations (MEAK, EPM7, #616187)
MTTK	mtDNA	maternal	Myoclonic epilepsy with ragged red fibers (MERRF, #545000)
HTT	4p16.3	AD	Juvenile Huntington disease (HD, #143100)
NEU1	6q21.33	AR	Sialidosis type I-II (#256550)
CLN6	15q23	AR	Neuronal ceroid-lipofuscinosis, Kufs type, variant A (CLN4A, #204300)
NPC1	18q11.2	AR	Niemann-Pick disease, type C1 and D (NPC, #257220)
GBA	1q21	AR	Gaucher disease, type III (GDIII, #231000)
AFG3L2	18p11.21	AR/AD	Spastic ataxia with PME (SPAX5, #614487)/ Spinocerebellar ataxia 28 (SCA28, #610246)

AD (autosomal dominant); AR (autosomal recessive); EPM (epilepsy, progressive myoclonic); mtDNA (mitochondrial DNA); SCA (spinocerebellar ataxia).

Table 5. Examples of precision medicine applications in genetic epilepsies.

Epilepsy syndrome (# OMIM)	Gene	Protein function	Possible treatment	Treatment benefits
Pyridoxin-dependent epilepsy (#266100)	***ALDH7A1***	Aldehyde dehydrogenase	Pyridoxine (B6 vitamin) supplementation and low lysine diet	Reduction or resolution of seizures, limited long-term effects on cognition and development [138]
Focal epilepsy with speech disorder, with or without mental retardation (#245570)	***GRIN2A***	NMDAR subunit	NMDAR antagonists (Memantine) and dextromethorphan are potentially useful for GOF variants	Reduction of the frequency of the seizures and cessation of myoclonic jerks [125-127]
EIEE 27 (# 616139)	***GRIN2B***	NMDAR subunit	NMDAR antagonists (Memantine) and dextromethorphan are potentially useful for GOF variants	Improved awareness, behavior, and sleep [139]
EIEE 32 (# 616366)	***KCNA2***	Voltage-gated potassium channel	Potential efficacy of 4-aminopyridine (4-AP, Kv1 channels inhibitor) for GOF variants	Inhibition of Kv1.2 channel with possible benefits on epileptogenesis [140]
EIEE 7 (#613720); BFNS1 (#121200)	***KCNQ2***	Voltage-gated potassium channel	Potassium channel openers (Retigabine and Ezogabine for LOF variants), potential efficacy of sodium channel blockers (CBZ)	Decreased Seizure frequency or seizure cessation [141-143]
EIEE 14 (#614959); Nocturnal frontal lobe epilepsy (#615005)	***KCNT1***	Sodium-activated potassium channel	Potassium channel openers (Quinidine for GOF variants)	Reduction in seizure frequency and possible improvement in cognitive development [125, 144]
EIEE 12 (#613722)	***PLCB1***	Phospholipase C	Inositol	Possible improvement of enzymatic function through substrate supplementation [145]
PNP oxidase deficiency (#610090)	***PNPO***	PNP oxidase	Pyridoxal-5-phosphate	Seizure cessation and improved overall outcome [146]
Familial infantile convulsions with paroxysmal choreoatetosis (#602066); BFIS 2 (#605751)	***PRRT2***	Coregulator of synaptic transmission	CBZ	Seizure cessation and improvement of associated movement disorders [147, 148]
Dravet syndrome (#607208)	***SCN1A***	Voltage-gated sodium channel subunit	Avoid sodium channel blockers	Avoid possible worsening of seizures [121, 122]
EIEE 11 (#613721); BFIS 3 (#607745)	***SCN2A***	Voltage-gated sodium channel subunit	Favor sodium channel blockers (consider high-dose PHT) for GOF variants; avoid sodium channel blockers for LOF variants	Decrease in seizure frequency or seizure cessation [149-152]
EIEE 13 (#614558); BFIS 5 (#617080)	***SCN8A***	Voltage-gated sodium channel	Favor sodium channel blockers (consider high-dose PHT) for GOF variants;	Decrease in seizure frequency or seizure cessation [153, 154]

		subunit	avoid sodium channel blockers for LOF variants	
GLUT1 deficiency I and II (#606777; #612126)	***SLC2A1***	Glucose transporter	Ketogenic diet	Decrease or cessation of epileptic seizures [155]
Tuberous sclerosis complex (#191100; # 613254)	***TSC1, TSC2***	Inhibitor regulators of mTOR pathway	Rapamicin, everolimus, and similar	Decreased seizure frequency or cessation of seizures [156-159]
FCD type II (#607341)	***mTOR, TSC1, TSC2***	mTOR pathway effectors/regulators	Everolimus and other mTOR inhibitors	Decreased seizure frequency or cessation of seizures [156, 157]

BFIS (benign familial infantile seizures); BFNS (benign familial neonatal seizures); CBZ (carbamazepine); EIEE (early infantile epileptic encephalopathy); FCD (focal cortical dysplasia); GOF (gain-of-function); LOF (loss-of-function); LTG (lamotrigine); NMDAR (N-methyl-D-aspartate receptor); PHT (phenytoin); PNP (pyridoxine 5-prime-phosphate).

Supplementary Material

Supplementary Table 1. Phenotyping checklist in epileptic patients.

EEG (electroencephalogram); IQ (intelligence quotient).

Task	Relevant fields to cover
Epilepsy features	Onset Febrile seizures Type of seizures Triggering factors Status Epilepticus Drug response
Personal History	Pregnancy, delivery, and birth history Psychomotor Development milestones Neurological co-morbidities Non-neurological co-morbidities
Familial History	Three-generation pedigree
Physical and Neurological examination	General clinical assessment Face pictures Neurological examination IQ evaluation
Instrumental Study	EEG Neuroimaging

Supplementary Table 2. Most common genes associated with malformations of cortical development (MCD).

Gene	Inheritance	MCD	Associated clinical condition (OMIM #)
PIK3R2	AD, dn	MEG	Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 1 (MPPH1, #603387)
AKT3	AD, dn	MEG	Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 2 (MPPH2, #615937)
CCND2	AD	MEG	Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 3 (MPPH3, #615938)
EZH2	AD	MEG	Weaver syndrome (WVS, #277590)
PIK3CA	AD, dn	MEG	Megalencephaly-capillary malformation-polymicrogyria syndrome (MCAP, #602501); Congenital lipomatous overgrowth, vascular malformations, epidermal nevi, skeletal/spinal abnormalities (CLOVE syndrome, #612918)
ATP6V0A2	Ar	COB	Cutis laxa, autosomal recessive, type IIA (ARCL2A, #219200); Wrinkly skin syndrome (WSS, #278250)
B3GALNT2	Ar	COB, PMG	Muscular dystrophy-dystroglycanopathy type A11 (MDDGA11, #615181)
POMGNT2	Ar	COB	Muscular dystrophy-dystroglycanopathy, type A8 (MDDGA8, #614830)

POMGNT1	Ar	COB, PMG	Muscular dystrophy-dystroglycanopathy, type A3 (MDDGA3, #253280)
FKRP	Ar	COB	Muscular dystrophy-dystroglycanopathy, type A5 (MDDGA5, #613153)
FKTN	Ar	COB, PMG	Muscular dystrophy-dystroglycanopathy, type A4 (MDDGA4, #253800)
B3GNT1	Ar	COB, NH	Muscular dystrophy-dystroglycanopathy, type A13 (MDDGA13, #615287)
ISPD	Ar	COB	Muscular dystrophy-dystroglycanopathy, type A7 (MDDGA7, #614643)
LAMB1	Ar	COB, SBH	Lissencephaly 5 (LIS5, #615191)
POMK	Ar	COB	Muscular dystrophy-dystroglycanopathy, type A12 (MDDGA12, #615249)
LARGE	Ar	COB	Muscular dystrophy-dystroglycanopathy, type A6 (MDDGA6, #613154)
POMT1	Ar	COB, PMG	Muscular dystrophy-dystroglycanopathy, type A1 (MDDGA1, #236670)
POMT2	Ar	COB, PMG	Muscular dystrophy-dystroglycanopathy, type A2 (MDDGA2, #613150)
TMEM5	Ar	COB	Muscular dystrophy-dystroglycanopathy, type A10 (MDDGA10, #615041)
ACTB	AD, dn	LIS, PG, SBH,	Baraitser-Winter syndrome type 1 (BRWS1, #243310)
ACTG1	AD, dn	LIS, PG	Baraitser-Winter syndrome type 2 (BRWS2, #614583)
ARX	XLR	LIS, AG, PG	Lissencephaly X-linked 2 (LISX2, #300215)
RELN	Ar	LIS	Lissencephaly 2 (LIS2, Norman-roberts syndrome, #257320)
VLDLR	Ar	LIS	Cerebellar hypoplasia and mental retardation with or without quadrupedal locomotion 1 (CAMRQ1, #224050)
PAFAH1B1	AD, dn	AG, LIS, PG, SBH	Lissencephaly 1 (LIS1, #607432); Miller-Dieker lissencephaly syndrome (MDLS, #247200)
DYNC1H1	AD, dn	NH, PG, PMG	Mental retardation AD 13 with neuronal migration defects (MRD13, #614563)
KIF5C	AD, dn	LIS, PMG	Cortical dysplasia, complex, with other brain malformations 2 (CDCBM2, #615282)
TUBA1A	AD, dn	AG, LIS, PG, PMG	Lissencephaly 3 (LIS3, #611603)
TUBB	AD, dn	LIS, PMG, SBH	Cortical dysplasia, complex, with other brain malformations 6 (CDCBM6, # 615771)
TUBB2B	AD, dn	LIS, PMG, FCD	Cortical dysplasia, complex, with other brain malformations 7 (CDCBM7, # 610031)
WDR62	Ar	LIS, PG, PMG	Microcephaly 2, primary, autosomal recessive, with or without cortical malformations (MCPH2, #604317)
LAMC3	Ar	PG, PMG	Cortical malformations, occipital (OCCM, #614115)
SNAP29	Ar	PG, PMG	Cerebral dysgenesis, neuropathy, ichthyosis, palmoplantar keratoderma syndrome (CEDNIK syndrome, #609528)
GMPPB	Ar	PMG	Muscular dystrophy-dystroglycanopathy, type A14 (MDDGA14, # 615350)
COL18A1	Ar	PMG, SBH	Knobloch syndrome type1 (KNO1, #267750)
NDE1	Ar	LIS/PG	Lissencephaly 4 (LIS4, #614019)/Microhydranencephaly (#605013)
KIAA1279	Ar	PG, PMG	Goldberg-Shprintzen megacolon syndrome (GOSHS, #609460)
NSDHL	XLD, XLR	PG, PMG	CK syndrome (CKS, #300831)
OCLN	Ar	LIS, PG, PMG	Pseudo-TORCH syndrome 1 (PTORCH1, #251290)
PAX6	AD, dn	PMG	Aniridia 1 (AN1, #106210)
RAB3GAP1	Ar	PMG	Warburg micro syndrome type 1 (WARBM1, #600118)
RAB3GAP2	Ar	PMG	Warburg micro syndrome type 2 (WARBM2, #614225)
RAB18	Ar	PMG	Warburg micro syndrome type 3 (WARBM3, #614222)
TBC1D20	Ar	PMG	Warburg micro syndrome type 4 (WARBM4, #615663)
RTTN	Ar	PMG	Microcephaly, short stature, and polymicrogyria with seizures (MSSP, #614833)
TUBA8	Ar	PMG	Cortical dysplasia, complex, with other brain malformations 8 (CDCBM8, #613180)
TUBB3	AD, dn	LIS, PMG	Cortical dysplasia, complex, with other brain malformations 1 (CDCBM1, #614039)
LAMA2	Ar	PMG	Muscular dystrophy, congenital, merosin-deficient, type 1A (MDC1A, #607855)
GPR56	Ar	PMG	Polymicrogyria, bilateral frontoparietal (BFPP, #606854), Polymicrogyria, bilateral perisylvian (BPPR, #615752)

SRPX2	XLR	PMG	Rolandic epilepsy, mental retardation, and speech dyspraxia (RESDX, #300643)
GPSM2	Ar	FCED, PMG, NH	Chudley-McCullogh syndrome (CMCS, #604213)
ARFGEF2	Ar	PNH	Periventricular heterotopia with microcephaly, autosomal recessive (ARPHM, #608097)
DCHS1	Ar	PG, PNH, SBH	Van Maldergem syndrome type 1 (VMLDS1, #601390)
EML1	Ar	PMG, SBH	Band heterotopia (BH, #600348)
ERMARD	AD, dn	PNH	Periventricular nodular heterotopia 6 (PVNH6, #615544)
FAT4	Ar	PNH	Van Maldergem syndrome type 2 (VMLDS2, #615546)
FLNA	XLD	PNH	Heterotopia, periventricular, type 1 (PVNH1, #300049)
MEF2C	AD, dn	PNH	Mental retardation, autosomal dominant, type 20 (MRD20,#613443)
TUBG1	AD, dn	AG, LIS, PG, SBH	Cortical dysplasia, complex, with other brain malformations 4 (CDCBM4, #615412)
KIF2A	AD, dn	AG, LIS, PG, SBH	Cortical dysplasia, complex, with other brain malformations 3 (CDCBM3, #615411)
DCX	XLR/XLD	AG, LIS, PG, SBH	Lissencephaly X-linked (LISX1, #300067), Subcortical laminal heterotopia, X-linked (SCLH, #300067)
CNTNAP2	Ar	FCD	Cortical dysplasia-focal epilepsy syndrome (CDFES, #610042)

AG (agyria); Ar (autosomal recessive); COB (cobblestone malformation); dn (de novo); FCED (focal cerebellar dysplasia); FCD (focal cortical dysplasia); LIS (lissencephaly); MCD (malformation of cortical development); MEG (megalencephaly); NH (nodular heterotopias); PG (pahygyria); PMG (polymicrogyria); PNH (periventricular nodular heterotopias); SBH (Subcortical band heterotopias); XLD (X-linked dominant); XLR (X-linked recessive).