Review Article

Genes of Early-Onset Epileptic Encephalopathies: From Genotype to Phenotype

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Abstract

Early-onset epileptic encephalopathies are severe disorders in which cognitive, sensory, and motor development is impaired by recurrent clinical seizures or prominent interictal epileptiform discharges during the neonatal or early infantile periods. They include Ohtahara syndrome, early myoclonic epileptic encephalopathy, West syndrome, Dravet syndrome, and other diseases, e.g., X-linked myoclonic seizures, spasticity and intellectual disability syndrome, idiopathic infantile epileptic-dyskinetic encephalopathy, epilepsy and mental retardation limited to females, and severe infantile multifocal epilepsy. We summarize recent updates on the genes and related clinical syndromes involved in the pathogenesis of early-onset epileptic encephalopathies: Aristaless-related homeobox (ARX), cyclin-dependent kinase-like 5 (CDKL5), syntaxin-binding protein 1 (STXBP1), solute carrier family 25 member 22 (SLC25A22), nonerythrocytic α-spectrin-1 (SPTAN1), phospholipase C β1 (PLCβ1), membrane-associated guanylate kinase inverted-2 (MAGI2), polynucleotide kinase 3′-phosphatase (PNKP), sodium channel neuronal type 1 subunit (SCN1A), protocadherin 19 (PCDH19), and pyridoxamine 5′-phosphate oxidase (PNPO).

Introduction

Early-onset epileptic encephalopathies are severe disorders in which cognitive, sensory, and motor development is impaired by recurrent clinical seizures or prominent interictal epileptiform discharges during the neonatal or the early infantile periods [1]. Several studies elucidated the pathogenic role of genetic mutations involved in the synaptogenesis, pruning, neuronal migration and differentiation, neurotransmitter synthesis and release, structures, and functions of membrane receptors and transporters [2,3].

We review the genes more frequently associated with early-onset epileptic encephalopathies and their associated phenotypes (Table 1).

Aristaless-Related Homeobox Gene (ARX, Online Mendelian Inheritance in Man Number 300382)

The Aristaless-related homeobox gene maps to Xp22.13 and includes five coding exons. The Aristaless-related homeobox protein contains the paired/Q50 homeodomain, the Aristaless preserved domain, octapeptide and acidic domains, four polyalanine tracts, and three nuclear localization sequence motifs [4].

The Aristaless-related homeobox gene acts as both a transcriptional repressor and activator in an incompletely characterized biochemical cascade, and modulates cerebral development and patterning through the regulation of differentiation, proliferation, and tangential migration of neuronal precursors and cortical interneurons [5,6].

To date, 44 different pathogenic mutations of the Aristaless-related homeobox gene in 100 families have been associated with two groups of human syndromes exhibiting highly variable phenotypes, i.e., syndromes with and without malformations [7]. The first group includes X-linked lissencephaly or hydranencephaly, associated with abnormal genitalia (Online Mendelian Inheritance in Man number 300215), and Proud syndrome (Online Mendelian Inheritance in Man number 300004). Syndromes without malformations include idiopathic Ohtahara syndrome, idiopathic West syndrome, X-linked myoclonic seizures, spasticity and intellectual disability, and idiopathic infantile epileptic-dyskinetic encephalopathy (Online Mendelian Inheritance in Man number 308350 for these four diseases), nonsyndromic X-linked mental retardation (Online Mendelian Inheritance in Man number 300419), and Partington syndrome (Online Mendelian Inheritance in Man number 309510). Premature termination or null mutations are mainly related to brain malformations (e.g., lissencephaly, hydranencephaly, and agenesis of the corpus callosum), whereas...
expansion of the polyalanine tracts is linked to syndromes without malformations and with early epileptic encephalopathies [8,9].

Expansions of the polyalanine tracts represent the most common and well-studied result of Aristaless-related homeobox gene pathologic mutations [10]. Among these, a 24-bp duplication mutation (c.429-452dup) accounts for 45% of all published cases [10-12]. Nasrallah et al. demonstrated that in these contexts, an improper folding of the Aristaless-related homeobox mutated protein produces intranuclear inclusions resulting in an increased neuronal cell-death [10]. The pathogenic mechanism of Aristaless-related homeobox gene-related epileptic encephalopathies probably involves the subsequent loss of function of cortical GABAergic interneurons, resulting in overall increased neuronal excitability [7,11,12].

Aristaless-related homeobox gene, infantile spasms, and West syndrome (early infantile epileptic encephalopathy 1)

Strømme et al. [13] demonstrated the pathogenic role of the Aristaless-related homeobox gene in an X-linked subtype of West syndrome. They reported four mutations in seven families: a GCG expansion and the 24-bp duplication (c.429-452dup), resulting in an expansion of the polyalanine tracts of the Aristaless-related homeobox gene, a missense mutation within the Aristaless-related homeobox gene homeodomain (P253L), and a truncation mutation [13]; Kato et al. also observed polyalanine expansions in a patient with sporadic cryptogenic West syndrome [14].

Subsequent observations by Strømme et al. [15] and Wohlrab et al. [16] indicated that the clinical severity of epileptic (infantile spasms and myoclonic seizures) and nonepileptic (mental retardation, dystonia, ataxia, or autism) manifestations is greater when the expansion of alanine residues is located in the first polyalanine tract.

Reish et al. [17] reported on a de novo familial duplication mutation of 27 bp, c.430-465dup, in exon 2 of the Aristaless-related homeobox gene (the same locus as the more frequent 24-bp duplication, c.429-452dup) in three affected males because of a postzygotic mosaicism, and suggested that the related reduction of the second polyalanine tract of the Aristaless-related homeobox gene beyond its natural limit of 20 amino acids resulted in a negative prognosis [17].

Aristaless-related homeobox gene and X-linked myoclonic seizures, spasticity, and intellectual disability syndrome

Scheffer et al. defined the features of “X-linked myoclonic seizures, spasticity, and intellectual disability syndrome” through linkage studies and mutational analyses of six boys with generalized spasticity, myoclonic epilepsy, and intellectual impairment. X-linked myoclonic seizures, spasticity, and intellectual disability syndrome was mainly related to a missense mutation (1058C>T/P253L) within the homeodomain of the Aristaless-related homeobox gene, and an X-linked recessive transmission was observed. The obligate female typically presented osteoendosteous hyperreflexia [18].

Aristaless-related homeobox gene and idiopathic infantile epileptic-dyskinetic encephalopathy

Guerrini et al. [19] reported on a new clinical entity, “idiopathic infantile epileptic-dyskinetic encephalopathy,” with infantile spasms, mental retardation, and generalized dystonia leading to severe quadriplegic dyskinesia. They defined an expansion from 10 to 17 GCG repeats (c.333-334 ins[GCG]7) in the Aristaless-related homeobox gene sequences encoding for the first polyalanine tract in six boys from four families [19].

More recently, Poirier et al. described a relationship between an expansion of the trinucleotide repeat GCG in the Aristaless-related homeobox gene and the presence of infantile spasms, atypical hypsarrhythmia, and dyskinetic movements in a 4-year-old boy [20].

Aristaless-related homeobox gene and Ohtahara syndrome

Kato et al. [21] demonstrated a relationship between Aristaless-related homeobox gene mutations and Ohtahara syndrome through the definition of a hemizygous, de novo 33-bp duplication in exon 2, 298-330dupGCGGCA(GCG)9, in two of three unrelated male patients. This mutation expanded the original 16 alanine residues to 27 alanine residues in the first polyalanine tract of the Aristaless-related homeobox protein. The authors suggested a possible link between the longer expansion of the polyalanine tract in Ohtahara syndrome and its earlier onset and more severe phenotype than in West syndrome [21].

Absoud et al. [22] described a rapidly progressive neurodegeneration resulting in death before age 1 year in a boy with Ohtahara syndrome, a dyskinetic movement disorder, and a shorter expansion of seven alanine residues in the Aristaless-related homeobox gene. This fatal course is not compatible with the hypothesis of a correlation between the expansion length of polyalanine tracts and clinical severity [22].

Fullston et al. described a single Aristaless-related homeobox protein truncation mutation (c.81C>G/p.Y27X) in two cousins with different clinical onsets: one with Ohtahara syndrome, and one with West syndrome [23].

More recently, Kato et al. [24] reported on two novel frameshift mutations in the terminal exon of the Aristaless-related homeobox gene (Ala524fsX534 and E536fsX672) in two members of a family with six males affected by Ohtahara syndrome. The clinical presentation appeared less severe in these patients than in patients with a 33-bp expansion in the first polyalanine tract (minor abnormalities of the genitalia and of brain structure, and delayed onset of seizures) [24].

Cyclin-Dependent Kinase-Like 5 (CDKL5, Online Mendelian Inheritance in Man Number 300203)

The cyclin-dependent kinase-like 5 gene maps to Xp22 and contains 20 coding exons. The related protein is a large, serine-threonine kinase of 1030 amino acids, including a preserved serine-threonine kinase domain in the N-terminal, and a C-terminal zone that regulates its catalytic activity and nuclear placement [25].

Cyclin-dependent kinase-like 5 is part of an incompletely characterized cascade. A recent study seems to rule out a previous hypothesis that cyclin-dependent kinase-like 5 protein could be involved in the same molecular pathways as the methyl-cytosine phosphate guanine-binding protein 2 (Mecp2) gene [26,27].

To the best of our knowledge, 53 pathologic mutations of cyclin-dependent kinase-like 5 (47 in females, and six in males) have been reported: 12 missense, four nonsense, eight splice-site, 22 deletion, and seven frameshift mutations [28-30]. No correlations between clinical severity and sites of the mutations have been demonstrated [29,30].

A severe epileptic encephalopathy (early infantile epileptic encephalopathy 2; Online Mendelian Inheritance in Man number 300672) that is characterized by intractable seizures with infantile spasms, mental retardation, and a Rett-like phenotype, is attributable to cyclin-dependent kinase-like 5 mutations [31-34]. Bahi-Buisson et al. [35] defined three clinical stages of epilepsy in 13 patients with early infantile epileptic encephalopathy 2 and cyclin-dependent kinase-like 5 mutations: (1) a first stage with early-
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<td>Myoclonic seizures Hypotonia Microcephaly Suppression burst pattern on EEG Abnormal electroretinogram Tonic spasms or tonic-clonic seizures Mental retardation Hypotonia Suppression-burst on EEG Infantile spasms with hypsarrhythmia Generalized seizures Mental retardation Spastic quadriplegia Progressive microcephaly Hypomyelination and diffuse brain atrophy on MRI</td>
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<td>Modulator of synaptic vesicle release</td>
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<td>Nonerythrocytic α-spectrin-1 (SPN1A, OMIM number 182810)</td>
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<td>Phospholipase Cβ1 (PLCB1, OMIM number 607120)</td>
<td>20p12.3</td>
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<td>Ohtahara syndrome (OMIM number 308350)</td>
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<td>West syndrome, or early infantile epileptic encephalopathy 1 (OMIM number 308350)</td>
<td>Infantile spasms</td>
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<td>Infantile spasms Mental retardation</td>
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<td>Membrane-associated guanylate kinase inverted-2 (MAGI2, OMIM number 606382)</td>
<td>7q11.23-q21.1</td>
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<td>Polymorphic seizures Microcephaly Developmental delay Behavioral disorders</td>
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<td>West syndrome, or early infantile epileptic encephalopathy 5 (OMIM number 613477)</td>
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<td>Polynucleotide kinase 3’-phosphatase (PNKP, OMIM number 605610)</td>
<td>19q13.33</td>
<td>Enzyme involved in DNA repair</td>
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<td>Polymorphic seizures Microcephaly Developmental delay Behavioral disorders</td>
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<td>Sodium channel neuronal type 1a subunit (SCN1A, OMIM number 182389)</td>
<td>2q24.3</td>
<td>Subunit of a voltage-gated sodium channel</td>
<td>Dravet syndrome (OMIM number 607208)</td>
<td>Infantile spasms Mental retardation Motor impairment</td>
<td>Sequence analysis of the entire coding region Mutation scanning of the entire coding region Linkage analysis FISH-metaphase Deletion/duplication analysis Prenatal diagnosis</td>
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</table>
Pintaudi et al. observed a presumably much more specific group of patients carrying cyclin-dependent kinase-like 5 mutations, whereas no links were evident concerning the nature and site of mutations and the X-chromosome inactivation pattern of patients [36]. No translational effects of cyclin-dependent kinase-like 5-related encephalopathy resulted from the transcriptional or posttranscriptional mechanisms, and increased levels of its protein were not detected in the cerebrospinal fluid of patients with truncation mutations involving the catalytic domain [35].

The same authors expanded the description of clinical features of cyclin-dependent kinase-like 5-related encephalopathy in a larger series of 20 affected girls with early-onset seizures, severe hypotonia, poor eye contact, and some Rett-like signs (secondary deceleration of head growth, severe motor impairment, sleep disturbances, hand apraxia, and stereotypies). Cranial magnetic resonance imaging of these patients indicated diffuse cortical atrophy associated with hyperintensities in the white matter of the temporal lobe as a possible diagnostic marker, without prognostic significance in this disease. The authors speculated that the variability of clinical presentations in cyclin-dependent kinase-like 5-related encephalopathy resulted from the transcriptional or translational effects of cyclin-dependent kinase-like 5 mutations. No links were evident concerning the nature and site of mutations and the X-chromosome inactivation pattern of patients [36].

Various nonspecific electroencephalographic abnormalities (hypsarrhythmia, focal or multifocal activity, diffuse high-voltage sharp waves, and generalized 0 rhythm) were reported in children carrying cyclin-dependent kinase-like 5 mutations, whereas Pintaudi et al. observed a presumably much more specific pseudo-periodic trace in two girls [37]. Melani et al. reported on three electroclinical stages in six children less than 1 year of age: tonic-vibratory contractions associated with an electroderecremental event, a clonic phase related to an irregular series of sharp waves and spike slow waves, and final myoclonic jerks, linked with bilateral, rhythmic sharp waves [38].

### Table 1 (continued)

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<td>Adhesion protein</td>
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<tr>
<td>Pyridoxamine 5-prime-phosphate oxidase (PNPO, OMIM number 603287)</td>
<td>17q21.32</td>
<td>Enzyme in pyridoxine activation cascade</td>
<td>PNPO deficiency (OMIM number 610090)</td>
<td>Parental consanguinity Low Apgar scores Perinatal respiratory distress Pyridoxine-unresponsive seizures Suppression burst pattern on EEG</td>
<td>Sequence analysis of the entire coding region Carrier testing</td>
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Abbreviations:
- EEG = Electroencephalogram
- FISH = Fluorescence in situ hybridization
- MRI = Magnetic resonance imaging
- OMIM = Online Mendelian Inheritance in Man
- * Laboratories offering the reported gene testing are listed at the website www.genereviews.org.

Onset, recurrent convulsive seizures, severe hypotonia, and normal findings of interictal electroencephalograms; (2) a second stage involving epileptic encephalopathy with infantile spasms and hypsarrhythmia; and (3) a third stage with refractory tonic or myoclonic epilepsy. The authors observed that progression to the third stage was more frequent and occurred more quickly in patients with truncation mutations involving the catalytic domain [35].

The same group described a novel homozygous solute carrier family 25, member 22 gene in 25 unrelated children with early myoclonic epilepsy (early infantile epileptic encephalopathy 3, Online Mendelian Inheritance in Man number 609304). In the same study, the authors did not identify any mutation in the coding zone of the solute carrier family 25, member 22 gene in 25 unrelated children with early myoclonic encephalopathy and progressive microcephaly. They also demonstrated the prominent expression of solute carrier family 25, member 22 in cerebral regions involved in the pathogenesis of myoclonic seizures (red nuclei, substantia nigra, and olivocerebellar networks). Moreover, they speculated that an epileptic phenotype in patients carrying solute carrier family 25, member 22 mutations could be attributable to abnormal glutamate synthesis and turnover in the cytoplasm of both astrocytes and neurons, to a secondary impairment of the mitochondrial respiratory chain, or to alterations of neuronal oxygen handling [40].

The same group described a novel homozygous solute carrier family 25, member 22 mutation (p.G236W) in the son of consanguineous Algerian parents, and tested the total loss of activity of the mutated protein in vitro. The proband manifested severe myoclonic epileptic encephalopathy beginning in the first days after birth, with hypotonia and microcephaly, a suppression-burst pattern on electroencephalogram, and an abnormal electroretinogram [41].

**Solute Carrier Family 25, Member 22 (SLC25A22, Online Mendelian Inheritance in Man Number 609302)**

The solute carrier family 25, member 22 gene maps to 11p15.5, contains nine encoding exons, and encodes for a mitochondrial glutamate/H+ symporter [39]. Molinari et al. [40] identified a missense mutation (p.Pro206Leu) in the SLC25A22 gene in four consanguineous Arab infants with early myoclonic epilepsy (early infantile epileptic encephalopathy 3, Online Mendelian Inheritance in Man number 609304). In the same study, the authors did not identify any mutation in the coding zone of the solute carrier family 25, member 22 gene in 25 unrelated children with early myoclonic encephalopathy and progressive microcephaly. They also demonstrated the prominent expression of solute carrier family 25, member 22 in cerebral regions involved in the pathogenesis of myoclonic seizures (red nuclei, substantia nigra, and olivocerebellar networks). Moreover, they speculated that an epileptic phenotype in patients carrying solute carrier family 25, member 22 mutations could be attributable to abnormal glutamate synthesis and turnover in the cytoplasm of both astrocytes and neurons, to a secondary impairment of the mitochondrial respiratory chain, or to alterations of neuronal oxygen handling [40].

The syntaxin binding protein 1 (STXBP1, Online Mendelian Inheritance in Man Number 602926)

The syntaxin binding protein 1 (STXBP1, or Munc18) gene maps to 9q34.1, and includes 20 exons. Syntaxin binding protein 1 modulates the release of synaptic vesicles through specific interactions with syntaxin A (Stx1a) and with the soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex. An open conformation of syntaxin 1A that promotes the formation of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor complex and subsequent vesicular release, and a closed conformation of syntaxin 1A that controls synaptic vesicular docking, are involved in these processes [42].

Saito et al. reported on a de novo 2.0-Mb microdeletion and four heterozygous missense mutations within the syntaxin binding protein 1 gene in five patients (two females and three males) with
Ohtahara syndrome (early infantile epileptic encephalopathy 4, Online Mendelian Inheritance in Man number 612164) [43], Hamdan et al. described two de novo truncating mutations in the region of binding with syntaxin 1A in two unrelated French-Canadian patients with early-onset seizures, mental retardation, hypotonia, abnormal gait, and tremor [44]. All mutations in both these reports were located in the hydrophobic center of the protein, and probably resulted in its misfolding. All the reported patients presented tonic spasms or tonic-clonic seizures before age 3 months, mental retardation, hypotonia, a suppression-burst pattern on electroencephalograms, and hypomyelination according to cranial magnetic resonance imaging [43,44]. More recently, Deprez et al. identified four new truncating mutations and two microdeletions that abolished syntaxin binding protein 1 gene function in six of 106 patients. None of the patients manifested a phenotype compatible with Ohtahara syndrome (one had West syndrome, whereas the others did not manifest a specific recognized epileptic syndrome), whereas profound mental retardation, ataxia, and dyskinetic movements were observed in five subjects [45]. Seven novel mutations (two frameshift mutations, three nonsense mutations, one splicing mutation, and one missense mutation) were discovered by Saitzu et al. [46] in six males and three females belonging to a larger cohort, including 29 patients with Ohtahara syndrome. The same mutations were absent in 54 patients with West syndrome. The same study demonstrated that the reported mutations produced nonsense-mediated mitochondrial RNA decay in vitro, associated with an abnormal splicing and subsequent degradation of the resultant protein [46].

In a cohort study of 86 patients with epileptic encephalopathies, Otsuka et al. defined a novel missense mutation (c.1654T>C) in the syntaxin binding protein 1 gene in a child with West syndrome and without previous Ohtahara syndrome. The same mutation was evident in a 2-year-old boy with classic Ohtahara syndrome [47].

**Nonerythrocytic α-Spectrin-1 (SPTAN1, Online Mendelian Inheritance in Man Number 182810)**

The nonerythrocytic α-spectrin-1 gene maps to 9q33-q34, and encodes for a filamentous cytoskeletal protein that regulates the stability of axonal structure [48].

Saitzu et al. [48] recently described two heterozygous nonerythrocytic α-spectrin-1 gene mutations in three unrelated patients, previously reported by Tohyama et al. [49], with drug-resistant seizures, hypsarrhythmia, mental retardation, spastic quadriplegia, and progressive microcephaly (early infantile epileptic encephalopathy 5, Online Mendelian Inheritance in Man number 613477). Magnetic resonance imaging indicated hypomyelination and diffuse brain atrophy [48,49]. One patient demonstrated a 2.25-Mb microdeletion, encompassing both the syntaxin binding protein 1 and nonerythrocytic α-spectrin-1 genes, that impaired spectrin heterodimer stability. That patient manifested a milder phenotype, with controlled seizures and no significant structural brain anomalies. The authors speculated that this phenotype could result from syntaxin binding protein 1 haploinsufficiency, whereas the effects on myelination were attributable to the haploinsufficiency of nonerythrocytic α-spectrin-1 [43,46].

**Phospholipase Cβ1 (PLCβ1, Online Mendelian Inheritance in Man Number 607120)**

The phospholipase Cβ1 (PLCβ1) gene maps to 20p12.3, and encodes for an enzyme that is involved in cellular signaling through the production of inositol 1,4,5 trisphosphate and diacylglycerol from phosphatidylinositol 4,5-bisphosphate. Kurian et al. [50] described a homozygous loss-of-function 0.5-Mb deletion, including the promoter and exons 1, 2, and 3 of phospholipase Cβ1, in a single male infant who developed tonic seizures and then infantile spasms. The same authors excluded a linkage to the phospholipase Cβ1 gene in 12 consanguineous families of other children with infantile spasms [50].

**Membrane-Associated Guanylate Kinase Inverted-2 Gene (MAGI2, Online Mendelian Inheritance in Man Number 606382)**

Membrane-associated guanylate kinase inverted-2 maps to 7q11.23-q21, and encodes for a scaffolding enzyme interacting with different presynaptic and postsynaptic receptors (including the N-methyl-D-aspartic acid receptor) [51].

Marshall et al. described a hemizygous 1.4-Mb deletion encompassing membrane-associated guanylate kinase inverted-2 gene in 15 of 16 patients with infantile spasms, whereas the same mutation was absent in 11 of 12 controls without a history of seizures [51].

Röthlisberger et al. recently reported that in children with infantile spasms, membrane-associated guanylate kinase inverted-2 is not necessarily involved in the hemizygous 1.4-Mb deletion, and thus further studies are required to clarify this finding [52].

**Polynucleotide Kinase 3′-Phosphatase (PNK) Gene (Mendelian Inheritance in Man Number 605610)**

The polynucleotide kinase 3′-phosphatase gene encodes for an enzyme that is involved in DNA repair networks and maps to 19q13.33. Through a genome-wide linkage analysis in seven consanguineous families, Shen et al. associated mutations in this gene with an autosomal recessive syndrome involving early-onset, drug-resistant seizures, microcephaly, developmental delay, and behavioral disorders [53].

**Sodium Channel Neuronal Type 1α Subunit (SCN1A, Online Mendelian Inheritance in Man Number 182389)**

Voltage-gated sodium channels present a large, multimeric α-subunit and two smaller, auxiliary β-subunits. The sodium channel neuronal type 1α subunit belongs to a family of nine genes encoding for α subunits. The sodium channel neuronal type 1α subunit maps to 2q24.3, and is expressed in nine isoforms (Na1.1-Na1.19) [54].

More than 600 sodium channel neuronal type 1α subunit mutations have been discovered [55]. In sodium voltage channels, they can promote both a gain of function, resulting in increased neuronal seizure susceptibility, and a loss of function, with subsequent decreased inhibitory activity of GABAergic interneurons [56].

Two epileptic syndromes have been linked to sodium channel neuronal type 1α subunit mutations: genetic epilepsy with febrile seizure plus (previously known as “generalized epilepsy with febrile seizure plus”) and Dravet syndrome (this eponym assembles “severe myoclonic epilepsy of infancy” and “severe myoclonic epilepsy of infancy-borderline”) [57].

Generalized epilepsy with febrile seizure plus is an autosomal dominant disorder characterized by febrile seizures occurring beyond their common ages and subsequent generalized or partial epilepsies. Mutations of the sodium channel neuronal type 1α subunit account for the 10% of patients with generalized epilepsy with febrile seizure plus. Patients who manifest generalized epilepsy with febrile seizure plus present with milder clinical severity and usually normal psychomotor development [58].
Dravet syndrome (Online Mendelian Inheritance in Man number 607208) is an early-onset, intractable epileptic encephalopathy with polymorphic clinical manifestations, including prolonged generalized or unilateral clonic seizures triggered by fever, photo stimulation, or hot water, myoclonic seizures, atypical absences, and partial seizures. Developmental milestones are usually normal before the onset of seizures, but are gradually impaired by recurrent epileptic episodes, resulting in mental delay, spasticity, or ataxia [59].

About 85% of patients with Dravet syndrome manifest sodium channel neuronal type 1z subunit mutations. In contrast with generalized epilepsy with febrile seizure plus, mutations of the sodium channel neuronal type 1z subunit in Dravet syndrome are mainly de novo, and more commonly arise from the paternal chromosome [60]. Neurologic impairment is generally greater with truncation mutations than with missense/nonsense or frameshift mutations, but impairment can be also influenced by mutations in modulator genes other than the sodium channel neuronal type 1z subunit (“genetic modifiers”) [61], Martin et al. [62], Ohmori et al. [63], and Singh et al. [64] suggested that the sodium channel neuronal type 8z subunit (SCN8A), the calcium channel voltage-dependent beta subunit (CACNB4), and the sodium channel neuronal type 9x subunit (SCN9A) as possible candidates for this role [62-64]. Harkin et al. [65] enlarged the spectrum of clinical phenotypes of sodium channel neuronal type 1z subunit gene mutations to cryptogenic generalized and focal epilepsies other than Dravet syndrome in a study of 188 patients with seizures occurring before age 2 years. They also defined a new “severe infantile multifocal epilepsy” that was distinguished from Dravet syndrome by a later onset of cognitive decline, a lack of absence and myoclonic seizures, and a lack of generalized spike and wave activity on electroencephalograms [65].

**Protocadherin 19 (PCDH19, Online Mendelian Inheritance in Man Number 300460)**

The protocadherin 19 gene maps to Xq22, and encodes for a transmembrane protein that controls calcium-dependent cell-cell adhesion. Protocadherin 19 may be involved in specific synaptic connections and transmissions [66].

Dibbens et al. reported on protocadherin 19 mutations in seven families with “epilepsy and mental retardation limited to females” (Online Mendelian Inheritance in Man number 300088) [67]. Hines et al. described two protocadherin 19 mutations in three cases of female mental retardation, whereas they excluded a pathogenic role of protocadherin 19 in Rett syndrome and autistic spectrum disorder [68].

Epilepsy and mental retardation limited to females is characterized by an onset of seizures between ages 6 and 36 months, a combination of febrile and afebrile seizures, and variable psychomotor and cognitive impairment. The typical prominent expression in females, although protocadherin 19 gene is on the X chromosome, has been explained through two possible mechanisms: the existence of compensatory factors in males with mutated protocadherin 19 (e.g., the protocadherin 19Y gene), and the formation of tissue mosaicism with protocadherin 19-positive and protocadherin 19-negative cells and subsequent altered interactions between the two cellular populations [67,68].

Depienne et al. [69] demonstrated mutations of protocadherin 19 in 12 sodium channel neuronal type 1z subunit gene-negative patients with Dravet syndrome. A hemizygous deletion in a male and nine point mutations (four missense and five truncating mutations) in 11 females were reported, and a cellular mosaicism was demonstrated in the fibroblasts of the affected male. Protocadherin 19-positive Dravet syndrome differs from the sodium channel neuronal type 1z subunit gene-positive form because of minor variability in seizures (myoclonic jerks and atypical absences are relatively rare) [69].

More recently, Marini et al. [70] reported on various mutations in 13 probands (seven with a clinical diagnosis of Dravet syndrome, and six with focal epilepsy) in a cohort of 117 females with both febrile seizures and different epileptic phenotypes. Eleven of these 13 patients presented with mental retardation. Mutation types were variable (six missense, two truncating, three frameshift, and two splicing), and a familial transmission was demonstrated in three cases [70].

Depienne et al. also demonstrated that protocadherin 19 mutations can be found in epileptic families without an associated history of mental retardation. In the same study, based on a cohort of 150 patients, a strong sensitivity to fever of the affected subjects and 15 novel point mutations and three deletions were reported [71].

**Pyridoxine 5-Prime-Phosphate Oxidase (PNPO, Online Mendelian Inheritance in Man Number 603287)**

Pyridoxine 5-prime-phosphate oxidase is an enzyme that produces pyridoxal 5-prime-phosphate in the pathway of activation of pyridoxine (an important cofactor in neurotransmitter synthesis). Its encoding gene maps to 17q21.32, and contains seven exons [72].

Mills et al. [72] defined homozygous missense, splice site, and stop codon pyridoxamine 5-prime-phosphate oxidase gene mutations in five preterm infants from three families with parental consanguinity, low Apgar scores, perinatal respiratory distress resulting in intubation, pyridoxine-unresponsive seizures in the first hours after birth, and suppression bursts on electroencephalograms. All but one patient studied by Mills et al. [72] died during the neonatal period. The only survivor, who was treated with pyridoxal 5-prime-phosphate, manifested marked acquired microcephaly, severe developmental delay, central hypotonia, painful dystonic spasms, and persistent seizures by age 2 years [72].

**The Role of Genetic Workups in the Diagnostic Approach to Early-Onset Epileptic Encephalopathies**

The diagnostic workup of early-onset epileptic encephalopathies remains a challenge because of frequent difficulties in defining etiologies. Acquired structural abnormalities, such as hypoxic-ischemic insults and isolated cortical malformations, represent the most common causes of epileptic encephalopathy in infancy, and they should be excluded in the first diagnostic steps [73].

Specific tests for the abovementioned genes should always be performed:

- In newborns or infants with epileptic spasms, myoclonic seizures, and other drug-resistant seizures, or with status epilepticus not otherwise explicable;
- In children with a progressively worsening clinical course, including developmental delay or a loss of previously achieved milestones;
- In children with seizures associated with movement disorders, dysmorphism, alterations of head circumference, or abnormal genitalia;
- In children with a clinical history compatible with classic age-related epileptic syndromes (e.g., Ohtahara syndrome or West syndrome) or other specific epileptic encephalopathies, such as Dravet syndrome;
In children with specific electroencephalographic patterns, including suppression bursts or hypsarrhythmia; and

In children with suggestive abnormalities on cranial magnetic resonance imaging, including cortical malformations such as lissencephaly, callosal dysgenesis, hypomyelination, or cerebral atrophy.

Chromosomal abnormalities, including 1p36 monosomy, Wolf-Hirschhorn syndrome, 18q syndrome, Angelman syndrome, Ring chromosome 20 syndrome, and Down syndrome, should be suspected if their typical phenotypes are present [74]. A complete risk assessment and genetic counseling. Moreover, all these increasing number of gene mutations have been related to their

Conclusions

Genetic knowledge about early epileptic encephalopathies has revolutionized the diagnostic approach to these disorders, and an increasing number of gene mutations have been related to their pathogenesis. In the future, a more detailed classification of epileptic encephalopathic genotypes will improve the accuracy of risk assessment and genetic counseling. Moreover, all these developments could yield unexpected therapeutic applications such as gene therapy or antiepileptic drugs “tailored” to the specific patient through specific genetic markers or targets.

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