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Review article

A diagnostic algorithm for the evaluation of early onset genetic-metabolic epileptic encephalopathies

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ABSTRACT

Early onset epileptic encephalopathies represent a struggling challenge in neurological clinical practice, mostly in infants and very young children, partly due to an unclear and still debated cathegorization. In this scenario genetic and metabolic epileptic encephalopathies play a central role, with new entries still needing an arrangement. In this Paper we present a brief overview on genes, metabolic disorders and syndromes picturing the pathogenesis of genetic and metabolic epileptic encephalopathies with onset under one year of age. These forms will be classified, according to a combined clinical and genetic-metabolic criterion, into two main groups including seizures as prominent/unique symptom and seizures associated with a syndromic phenotype. Starting from this classification we suggest a possible simplified diagnostic algorithm, discussing main decision making nodes in practical patients management. The aim of the proposed algorithm is to guide through metabolic and molecular-genetic work up and to clarify "where" and "what" to search in biochemical, electroencephalographic and neuroimaging investigations.

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1. Introduction

Early onset epileptic encephalopathies are severe diseases, occurring in the neonatal and/or the early infantile period, in which cognitive, sensory and motor development is impaired by epileptic activity itself (including both recurrent clinical seizures and prominent interictal epileptiform discharges).[1]

The relevance of metabolic disturbances and genetic factors in their pathogenesis is still underrated covering a minority but widely ranging incidence of cases (0,1 to 300 per 100.000 live births).[2,3] The most recent advances in neurobiological researches have defined the emerging sense of alterations of synaptogenesis, pruning, migration and differentiation, neurotransmitters synthesis and release, enzymes structure and functioning, membrane receptors and transporters activity in determining neuronal discharge, neuronal migration anomalies and altered intercellular signaling.[3–6]

The expanding phenotypes of several epileptic encephalopathies complicate the possibility of a systematic categorization and the development of diagnostic guidelines in patients management.[1,3,7–11] In this paper we have attempted to follow a pragmatical and simplified approach to clear out the early stages of differential diagnosis in early onset genetic-metabolic epileptic encephalopathies.

2. Diagnostic algorithm

Fig. 1 summarizes the diagnostic steps of a possible diagnostic algorithm for early onset genetic-metabolic epileptic encephalopathies. The diagnostic suspect of a genetic-metabolic epileptic encephalopathy should be considered mostly when: 1) epilepsy is drug-resistant and shows a progressively worsening clinical course[12,13]; 2) seizures are associated with septic-like symptoms (poor feeding, recurring vomiting, respiratory distress or apnea, metabolic acidosis and coma) or are induced by diet changes[12,13]; 3) seizures are linked to developmental delay or loss of priorly acquired milestones[12,13]; 4) epilepsy begins with a not otherwise explicable status epilepticus[12,13]; 5) EEG recordings show a typical and specific pattern[12,13]; 6) patient's history displays parental consanguinity and/or familial history of unexplained neonatal epilepsy or sudden infant deaths.[12,13]

Clinical presentation of early onset epileptic encephalopathies can be divided, according to the key symptomatology, into two main patterns including seizures as prominent/unique symptom or seizures associated with a syndromic phenotype (Table 1).

According to this scheme, laboratory diagnostic investigations should be clinically and electrophysiologically oriented with a rationale progressive approach (Fig. 2).

Electroencephalogram (EEG) abnormalities in early onset epileptic encephalopathies range from focal and multifocal patterns, disorganized or background slowing, generalized anomalies and periodic EEG rhythms, such as suppression-burst and hypsarrhythmia, with frequent pattern to pattern transitions.[3]

In Table 2 we have divided genetic-metabolic early onset epileptic encephalopathies, according to their EEG prominent patterns, into three clusters: forms associated with typical EEG, subtypes with predominant but multiple patterns and variants with no specific patterns.

Neuroimaging findings in epileptic encephalopathies are divided in three broad topics: anatomical or structural abnormalities, functional or metabolites anomalies and subtle or no findings (Table 4).

Magnetic resonance imaging (MRI) is important in detecting epileptogenic cortical malformations or syndromes with specific or nearly specific features.[16] Thinning of the corpus callosum, atrophy of brain tissue, cerebellar and basal ganglia symmetrical involvement and white matter anomalies are suggestive for inborn errors of metabolism although also cerebral and cerebellar dysgenesis, disorders of neuronal migration and hypoxic-ischemic encephalopathy can occasionally masquerade a metabolic defect.[4,14–16] Diffusion weighted imaging (DWI) is particularly proper in the study of white matter abnormalities.[14–16] Proton magnetic resonance spectroscopy (HMRS) should be performed when it is suspected a disease with alterations of specific measurable metabolites while functional assessments, including single photon emission computed tomography (SPECT), positron emission tomography (PET) or functional MRI, can reveal subtle cortical metabolic defects that are otherwise undetectable on conventional neuroimaging.[15,16]

3. Epileptic encephalopathies presenting with seizures as prominent/unique symptom

3.1. *Clinical evaluation and laboratory investigations*

In this group of patients seizures are the most evident and characterizing feature and other neurological symptoms include cognitive and developmental delay that follow epilepsy. Epilepsy is frequently polymorphic mainly including tonic seizures, infantile spasm or myoclonic seizures. Convulsive clinical semiology is variable among different epileptic encephalopathies and there are no pathognomonic patterns with mutual transitions between different seizure-types.[17]

In neonates with intractable seizures, blood glucose, electrolytes, lactate, pyruvate and ammonia testing should represent the first diagnostic steps.[18] If the primary investigations exclude hypoglycemia, hypocalcemia, hypomagnesemia, elevations of lactate and ammonia, it should be a common practice a trial with the administration of vitaminic compounds (pyridoxine, pyridoxal phosphate or folinic acid - see Table 3).[18,19] Vitamin dependent epileptic encephalopathies are characterized by seizures since the first weeks of life.[18] Even if seizures are the prominent clinical feature, various signs of multisystemic involvement can be evidenced.[18] Multisystemic signs comprise abnormal fetal movements and cry, irritability, exaggerated startle responses, dystonic movements, occasional microcephaly/macrocephaly, respiratory distress, abdominal distention, bilious vomiting, hepatomegaly, hypothermia, shock and acidosis.[18,20–22] The most common seizure-types are represented by multifocal and erratic or generalized myoclonic jerks and clinical evaluation is frequently complicated by paroxysmal non epileptic symptoms.[20–22] Positive responsiveness to vitamin

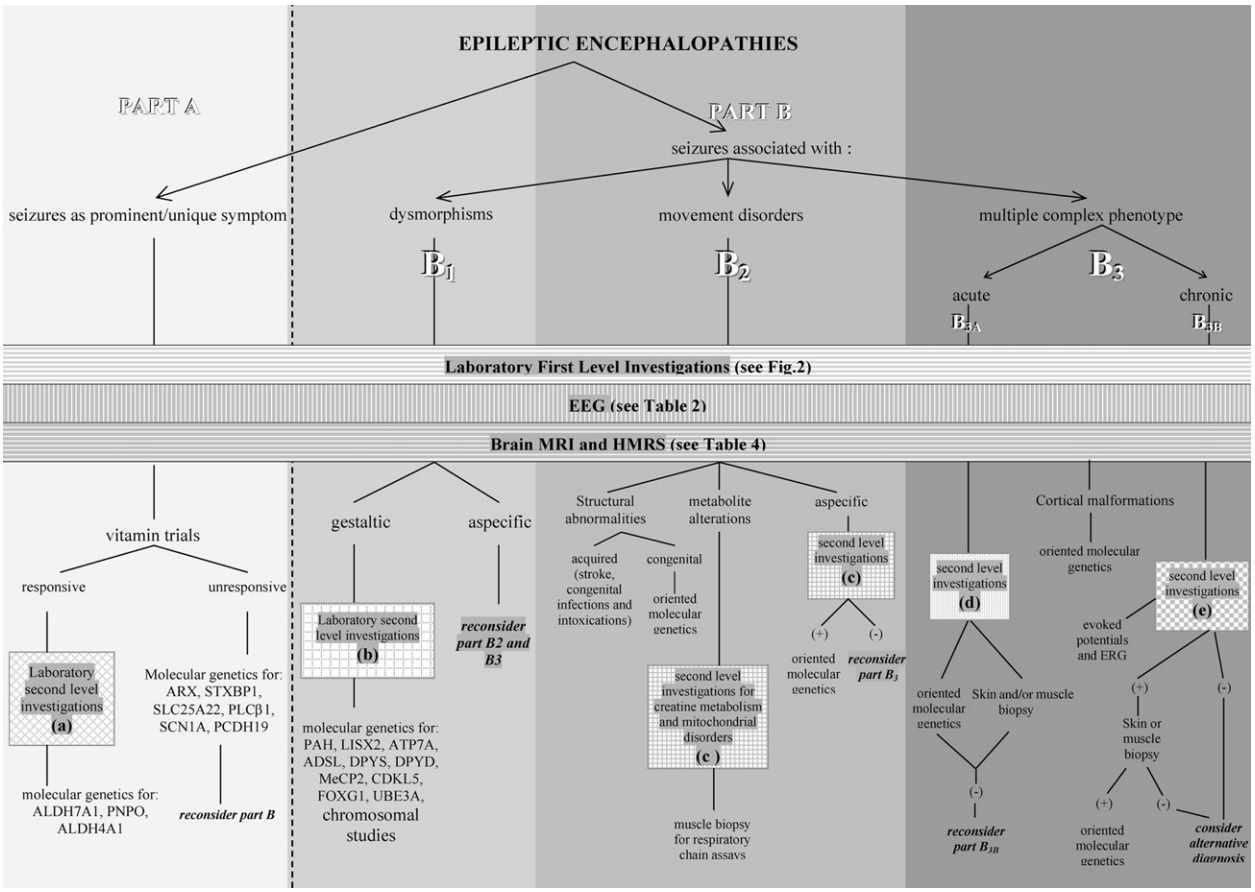

Fig. 1 – A diagnostic algorithm for children with genetic and metabolic early onset epileptic encephalopathies. Laboratory second level investigations (divided in the groups a, b, c, d and e) are listed in Fig. 2.

administration should prompt to characterize the underlying biochemical defect through cephalospinal fluid researches (pyridoxine related metabolites and folates) and the gene sequencing for aldehyde dehydrogenase 7 family member A1 (ALDH7A1) or pyridoxamine 5′-phosphate oxidase (PNPO) mutations.[19–22] Clinicians should be aware that a negative responsive trial does not completely exclude a vitamin dependent epilepsy.[19,21]

In infants and children with suggestive clinical histories and electroencephalographic patterns but unresponsive to vitamin trials other specific molecular genetic investigations should be taken into account.[23–29] Genetic forms linked to mutations of syntaxin binding protein 1 (STXBP1), solute carrier family 25 member 22 (SLC25A22), phospholipase C-β1 (PLCβ1) have a neonatal onset and show a phenotypic spectrum covering classic patterns of both Ohtahara and/or West syndrome.[23–25] Deletions of gene encoding for membrane associated guanylate kinase, WW and PDZ domain containing 2 (MAGI2) have been observed in some patient with infantile spasms even if their pathogenic role is still debated.[26,27] Children with Dravet and Dravet-like syndromes should be screened for mutations of two genes: sodium channel neuronal type 1-α subunit (SCN1A) and protocadherin 19 (PCDH19).[28,29] Their onset usually occur in the first six months of life in children with a previous normal developmental history.[28,29] Seizure-types in Dravet syndrome are multiple and heterogeneous.[28] They include prolonged generalized or unilateral clonic seizures triggered by fever, photostimulation or hot-water, myoclonic seizures, atypical absences, partial seizures or recurrent episodes of status epilepticus.[28] PCDH19 mutations usually involve females and are associated with a less severe clinical phenotype.[29]

Type II hyperprolinaemia should be considered in the differential diagnosis with Dravet syndrome (concerning frequent febrile seizures overture) and with vitamin dependent epileptic encephalopathies (owing pyridoxal phosphate de-activation with a secondary P5′P deficiency that is probably related to its pathogenesis).[30]

3.2. *The role of electroencephalography*

Some attempts, though enrolling a small amounts of patients, have been performed to define specific EEG patterns in epileptic encephalopathies related to STXBP1 and SLC25A22 genes mutations but no pathognomonic traces have been described.[23,24] Suppression-burst pattern seems to be prominent at the onset, both awake and in sleeping state, mostly when structural brain lesions are associated while the same pattern is more apparent in sleep in non structural/metabolic disorders.[23,24] Evolution into hypsarrhythmia and, then, into diffuse slow spike and waves burst pattern is frequently observed in the following stages.[23–25]

Table 1 – Clinical classification of genetic and metabolic early onset epileptic encephalopathies.

		Diseases	Pathophysiology
Epileptic encephalopathies presenting with seizures as prominent/unique symptom		Pyridoxine dependent epilepsy	Vitamin or enzymatic cofactor dependency
		Folinic acid responsive epilepsy	
		Pyridoxal-5′-phosphate responsive epilepsy	
		Dravet and Dravet-like syndrome	Channellopathy
		Ohtahara syndrome (EIEE)	Unknown/multiple etiology
		Early myoclonic encaphalopathy (EME)	
		Syntaxin binding Protein 1 (STXBP1) deficiency	Single enzyme or protein dysfunction
		Glutamate mitochondrial transporters deficiencies (SLC25A22 and SLC25A18)	
		Phospholipase C beta-1 deficiency	
		MAGI2 related epileptic encephalopathy	
		Protocadherin 19 related epileptic encephalopathy	
		Hyperprolinemia type II	Disorders of amino acids metabolism
		GABA transaminase deficiency	Neurotransmitters disorders
Epileptic encephalopathies presenting with seizures associated in a syndromic phenotype associated with:	Suggestive somatic characteristics (i.e. dysmorphic features and/or alterations of head circumference)	1p36 monosomy	Chromosomopathies
		Wolf-Hirschhorn syndrome	
		18q- syndrome	
		Angelman syndrome	
		Ring chromosome 20 syndrome	
		Down syndrome	
		Cyclin-dependent kinase-like 5 (CDKL5) deficiency	Single enzyme or protein dysfunction
		Rett Syndrome (MeCP2, CDKL5 or FOXG1-related)	
		Focal cortical dysplasia (TSC1 and TSC2)	Cerebral malformations associated disorders
		Polymicrogyria (SRPX2, KIAA1279, GPR56, PAX6, TBR2, COL18A1, RAB3GAP1, 22q11., FLN1A, ARFGEF2, LRP)	
		Subcortical band heterotopia (DCX, LIS1, trisomy 9p)	
		Periventricular nodular heterotopia (unbalanced translocation, t[1; 6][p12; p12.2)]	
		Lissencephaly (LIS1, DCX, microdeletion in 17p including LIS1 and YwaE, ARX, TUBA1A, RELN)	
		Schizencephaly (EMX2 involved in sporadic cases)	
		Early infantile epileptic encephalopathy type I (ARX-related EIEE1)	
		Miller-Dieker syndrome	
		Smith-Lemli–Opitz syndrome	
		Phenylketonuria and hyperphenylalaninemias	Disorders of amino acids metabolism
		Sulfite oxidase deficiency	Vitamin or enzymatic cofactor dependency
		Molybdenum cofactor deficiency	
		Menkes disease	
		Adenilosuccinate lyase deficiency	Purine and pyrimidine metabolism disorders
		Dihydropyriminidase and Dihydropyrimidine dehydrogenase deficiency	
	Acute multiorgan involvement	Urea cycle disorders	Endogenous toxicity
		Organic acidurias	
		Congenital disorders of glycosilation	

	Glutathione synthetase deficiency	
	Mitochondrial disorders (SUCLA2, SUCLG1)	
	Biotin metabolism disorders	Vitamin or enzymatic cofactor dependency
	Congenital glutamine deficiency	Neurotransmitters disorders
	Developmental delay, Epilepsy and Neonatal Diabetes (DEND syndrome)	Channellopathy
	Hyperinsulinism/Hyperammoniemia (HI/HA)	Single enzyme or protein dysfunction
Chronic multiorgan involvement	Mitochondrial disorders (Leigh syndrome, multiple deletion syndrome or Alpers disease, pyruvate dehydrogenase deficiency)	Energetic failure
	Lysosomal disorder (Krabbe disease)	Storage disorders
	Peroxisomal disorder (neonatal adrenoleukodystrophy, Zellweger syndrome, infantile Refsum disease, punctuate rhyzomelic chondrodysplasia)	
	Niemann-Pick disease type A and C	
	Neuronal ceroid lipofuscinosis	
	MAGI2 deletion syndrome	Single enzyme or protein dysfunction
	Serine byosynthesis disorders	Disorders of amino acids metabolism
	Nesidioblastosis	Unknown
Movement disorders	GLUT1 deficiency syndrome	Energetic failure
	Creatine deficiency syndromes (AGAT, GAMT and X-linked creatine transporter deficiency)	
	EIEE1 (ARX-related epileptic encephalopathy)	Cerebral malformations associated disorders
	4-hydroxybutyric aciduria (SSADH)	Neurotransmitters disorders

EIEE: Early infantile epileptic encephalopathy; EME: Early myoclonic encephalopathy; STXBP1: Syntaxin binding Protein 1; SLC25A22: solute carrier family 25 member 22; SLC25A18: solute carrier family 25 member 18; MAGI2: Membrane associated guanylate kinase, WW and PDZ domain containing 2; TSC1: tuberous sclerosis complex 1; CDKL5: Cyclin-dependent kinase-like 5; MeCP2: methyl CpG binding protein 2; FOXG1: Forkhead box protein G1; TSC2: Tuberous sclerosis complex 2; SRPX2: sushi-repeat containing protein, X-linked 2; GPR56: G protein-coupled receptor 56; PAX 6: Paired box gene 6; TBR2: T-box brain protein 2; COL18A1: collagen, type XVIII, alpha 1; RAB3GAP1: RAB3 GTPase activating protein subunit 1; FLN1A: filamin 1A; ARFGEF2: ADP-ribosylation factor guanine nucleotide-exchange factor 2; LRP: Leucine-responsive regulatory protein; DCX: doublecortin; LIS1: Lissencephaly-1; ARX: Aristaless-related homeobox gene; TUBA1A: tubulin, alpha 1a; RELN: reelin; EMX2: empty spiracles homolog 2; SUCLA2: succinate-CoA ligase, ADP-forming, beta subunit; SUCLG1; succinate-CoA ligase, alpha subunit; DEND: Developmental delay, Epilepsy and Neonatal Diabetes; HI/HA: Hyperinsulinism/Hyperammoniemia; GLUT1: Glucose transporter 1; GAMT: Guanidinoacetate N-methyltransferase; AGAT: Arginine–glycine amidinotransferase; SSADH: Succinic semialdehyde dehydrogenase.

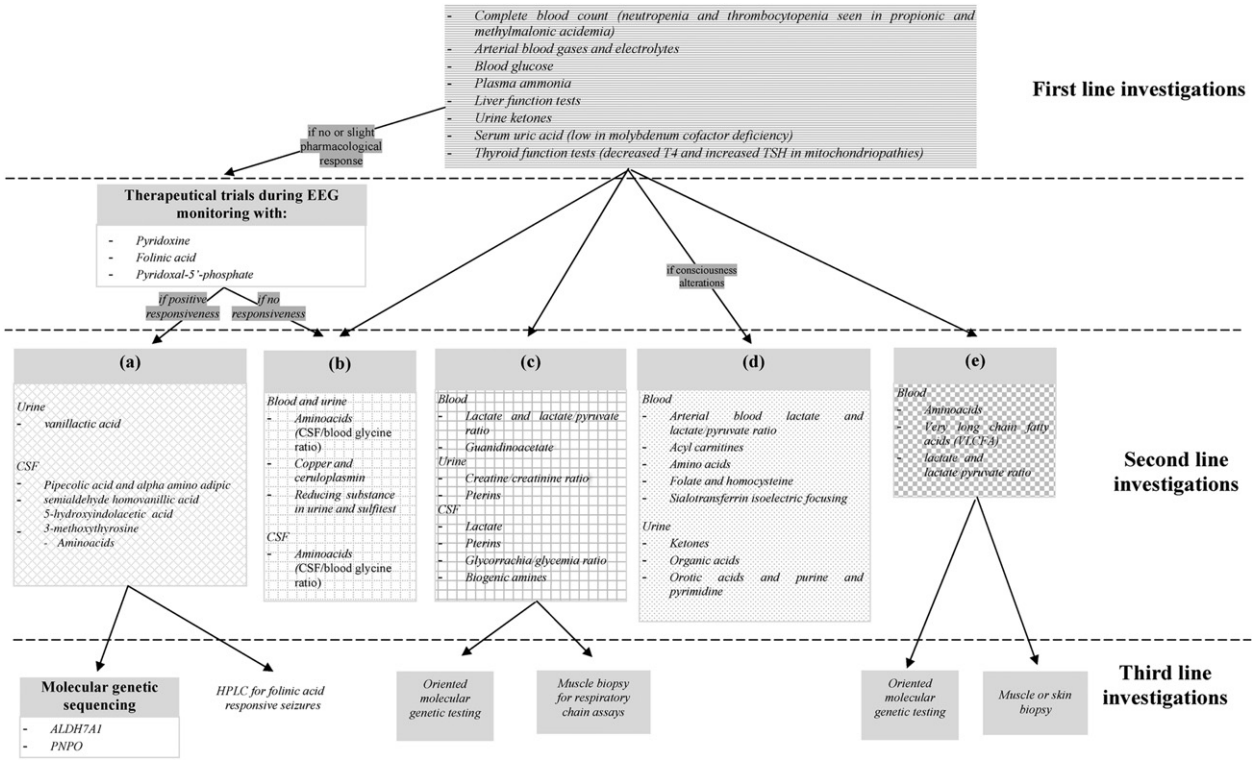

Fig. 2 – Progressive diagnostic approach to orientate laboratory investigations in early epileptic encephalopathies.

Both suppression burst and hypsarrhythmia can be recorded in vitamin dependent seizures without a clear prominence.[18–22,31,32] The intravenous administration of pyridoxine induces an aspecific EEG response that suggest but do not represent a link to diagnosis of pyridoxine-dependent epilepsy.[19]

In Dravet syndrome EEG is commonly normal at onset with the subsequent appearance of background slowing, multifocal abnormalities or photosensitivity.[33]

3.3. *The role of neuroimaging*

In this cluster of patients neuroimaging usually shows no or subtle abnormalities that do not fit the role of characterizing features. Poor data on MRI characteristics in diseases belonging to this group are reported in the literature. In Dravet syndrome focal brain atrophy, cortical dysplasia and hippocampal sclerosis have been described in some children but their cause is not clear.[34] Regarding advanced neuroimaging techniques, the demonstration of elevated gamma-aminobutyric acid (GABA) concentration at HMRS have been recently observed in a case of GABA transaminase deficiency.[35]

4. Epileptic encephalopathies presenting with seizures associated with a syndromic phenotype

4.1. *Clinical evaluation and laboratory investigations*

In this group of epileptic encephalopathies epilepsy is part of a constellation of symptoms defining a syndromic phenotype including dysmorphic features or suggestive somatic phenotypes, alterations of head circumference, acute or chronic multiorgan involvement and movement disorders (Table 1).

Even if mild facial dysmorphisms are frequently observed in metabolic epileptic encephalopathies, a gestaltic diagnosis is rarely achievable excepting for phenylketonuria, aristaless-related homeobox early infantile epileptic encephalopathy type 1 (ARX-EIEE 1), sulphite oxydase or molybdenum cofactor deficiencies and Menkes disease.[36–40] Classic phenylketonuric patients display pale skin pigmentation, blue eyes, blond hair and microcephaly.[36] ARX-EIEE 1 patients can present prominent forehead, micrognathia, pinched nasal alae and wide nasal bridge associated with abnormal genitalia.[37,38] In sulphite oxydase and molybdenum cofactor deficiencies typical facies includes puffy cheeks and small nose with a long philtrum.[39] In Menkes disease short stature, growth retardation, wormian bones, micro or brachycephaly, joint or skin laxity, hypopigmentation and steely or kinky sparse hair are common.[40]

Dysmorphisms play a weighty role in the diagnostic pathways of chromosomopathies with typical facies such as in 1p36 monosomy, Wolf-Hirschhorn syndrome, Angelman syndrome, 18 q- syndrome, ring chromosome 20 syndrome.[41] In this group, Down syndrome should be considered owing its frequently association with infantile spasms.[41]

Some epileptogenic cortical malformations are associated with peculiar syndromic phenotypes. The association of drug-resistant epilepsy with mental retardation and signs of pseudobulbar palsy may indicate a possible polymicrogyria.[42] A focal cortical dysplasia could result in intractable partial seizures.[43] The presence of specific facial features (such as high forehead, bitemporal hollowing, flattened ear helices,

Table 2 – Classification of genetic and metabolic epileptic encephalopathies based on electroencephalographic features.

Typical EEG	**Maple syrup urine disease or leucinosis, propionic acidemia**		Comb-like rhythm
	Glycine encephalopathy, holocarboxylase synthetase deficiency		Suppression-burst
	Urea cycle disorders		Low amplitude slowing
	Infantile neuronal ceroid lipofuscinoses type I		Vanishing electroencephalogram
	Tay-Sachs disease		Fast central spikes
	Alpers disease		Continuous anterior high voltage 1 to 3 per second spike-wave like activity
	Angelman syndrome		High amplitude rhythmic 2–3 Hz activity (mostly frontal) with superimposed interictal spike and sharp waves discharges, high amplitude rhythmic 4–6 Hz activity (mostly occipital), spike and sharp waves mixed with 3–4 Hz components of high amplitude, mainly posteriorly and facilitated by eye closure
Predominant but multiple patterns	*Generally*	*Disorders*	*Specifically*
	Suppression-burst or multifocal discharges	**Vitamin or enzymatic cofactor dependency:**	
		Pyridoxine dependent seizure	Suppression-burst to hypsarrithmia but also multifocal or focal abnormalities, continuous or intermitted high low delta waves with or without 1–3 Hz spikes
		Pyridoxal-5'-phosphate dependent epilepsy	Focal or multifocal abnormalities evolving to burst-suppression
		Biotin metabolism disorders	Spike and slow wave paroxysms, suppression burst
		Hyperprolinemia type II	Slow delta wave activity, suppression-burst
		Sulfite oxydase and molybdenum cofactor dependency	Background slowing, multifocal discharges, burst-suppression
		Peroxisomal disorders:	
		Neonatal adrenoleukodystrophy	Epilepsia partialis continua and posterior attenuation or slowing, suppression-burst
		Zellwegger syndrome spectrum	Multifocal spikes, bilateral slow spike and waves, suppression-burst, hypsarrhythmia
		Disorders of purine and pyrimidine	Slow background rhythm with multifocal asynchronous spikes and sharp waves, suppression-burst
		Neurotransmitters disorders	
		GABA transaminase deficiency	Suppression burst evolving into hypsarrhythmia
		Forms related to structural genes mutations:	Slow spike and waves, suppression-burst
		ARX, CDKL5, STXBP1, SLC25A22	Multifocal discharges, spike and slow wave paroxysms
	Hypsarrhythmia and multifocal or isolated focal discharges	**Disorders of amino acid metabolism**	
		Phenylketonuria and hyperphenilalaninemias	Generalized or focal paroxysms, hypsarrhythmia
		Disorders of serine biosynthesis	Background slowing, multifocal discharges, hypsarrhythmia
		HHH syndrome	Low amplitude slowing, spike-waves or slow and sharp waves, hypsarrhythmia
		Organic acidurias	Background abnormalities, focal or multifocal slow spikes and sharp waves, hypsarrhythmia
		Congenital disorders of glycosilation	Multifocal discharges, hypsarrhythmia
		Urea cycle disorders	Generalized theta-wave paroxysms, hypsarrhythmia
		Lysosomal disorders	
		Krabbe disease	Hypsarrhythmia, background slowing and progressive background disorganization
		Vitamin or enzymatic cofactor dependency	
		Menkes' disease	Multifocal high amplitude activity mixed with irregular slow waves, hypsarrhythmia
		Forms related to structural genes mutations:	Suppression burst evolving into hypsarrhythmia
		SPTAN1, PLCβ1, MAGI2	Multifocal discharges

(*continued on next page*)

Table 2 (continued)

Not specific	**GLUT 1 deficiency syndrome**	Normal interictal activity associated to variable ictal patterns with slowing 2,5 to 4 Hz spike-wave discharges
	Mitochondrial disorders	Variable pattern with background slowing, multifocal spikes evolving to suppression-burst
	Niemann-Pick disease	Disorganized background activity, generalized or predominantly focal slow and sharp waves
	Creatine metabolism disorders	Multifocal spikes, hypersynchronization, slow 3 Hz waves, background slowing
	Dravet syndrome	Normal at onset, background slowing, multifocal discharges, photosensitivity

ARX: Aristaless-related homeobox; CDKL5: cyclin-dependent kinase-like 5; STXBP1: syntaxin binding protein 1; SLC25A22: solute carrier family 25 member 22; HHH: Hyperornithinemia-hyperammonemia-homocitrullinemia; SPTAN1: spectrin, alpha, non-erythrocytic 1; PLCβ1: phospholipase C-β1; MAGI2: membrane associated guanylate kinase, WW and PDZ domain containing 2.

hypertelorism, epicanthic folds, short nose with a depressed nasal root and anteverted nares, prominent lateral nasal folds, round philtrum and upper lip with a thin vermillion border, flat midface, and small chin) suggest Miller-Dieker syndrome prospecting lissencephaly at MRI.[44]

Specific genes involved in the abovementioned epileptogenic cortical malformations should be analyzed when appropriate clinical and neuroradiological data are available (Table 1).[45] Structural brain abnormalities can be also determined by alterations of metabolic microenvironment during fetal development.[4] For these reasons metabolic diseases such as pyruvate dehydrogenase deficiency, nonketotic hyperglycinemia, Glutaric aciduria type 1, Smith-Lemli Opitz syndrome, congenital disorders of glycosylation, mitochondriopathies, and peroxisome biogenesis disorders should be considered in the differential diagnosis.[4] These disorders can be investigated since prenatal age with ultrasound or fetal MRI.[4]

As a general rule, acquired microcephalies are usually linked to a metabolic dysfunction while congenital ones are due to genetic diseases.[46] Notable exceptions are represented by maternal phenylketonuria, in which the fetal brain is exposed to toxic levels of phenylalanine, and phosphoglycerate dehydrogenase deficiency in which microcephaly is congenital.[46] In infants with microcephaly or dysmorphisms it is preliminary a screening for plasma amino acids and urinary purine and pyrimidine, if clinical suspicion is detailed and no other indications from genetic consultant are obtained.[3]

Motor functions impairment is part of the concept of epileptic encephalopathy itself but in some genetic and metabolic diseases motor involvement occur together with epilepsy without being a consequence of epileptic discharges. In GLUT1 deficiency syndrome, for example, movement disorders, such as intermittent ataxia and dyskinesia, may be largely prominent over epileptic phenomena.[47] Several movement disorders are often present late in the disease but some precocious signs, such as developmental motor delay, poor coordination, dystonic posturing, rigidity or spasticity, di- or quadriplegia should be assessed even in the first year of life.[3] Motor and movement disorders should always suggest the following exams :

- urinary organic acids and, in males, creatine/creatinine ratio[3,48];
- guanidineacetate in plasma and/or urine[48];
- CSF lactate (increased in several mitochondriopathies), CSF/blood glucose ratio (under 0,35 in GLUT1 deficiency), amino acids (especially for the study of glycine and serine metabolism dysfunctions), biogenic monoamine metabolites and pterins.[3,49]

In patients with an acute multiorgan involvement, with or without septic-like symptoms, urea cycle disorders, organic acidurias, aminoacidopathies, disorders of purine and pyrimidine metabolism, biotinidase deficiency and glutathione synthetase deficiency should be ruled out.[3,50]

Signs of chronic dysfunctions/malformations in organs other than central nervous system such as liver, kidney, heart, skeleton or eye should rise the clinical suspect of storage diseases, mitochondrial defects and different forms of congenital disorders of glycosylation. It should be taken into

Table 3 – Diagnostic and therapeutic trials of treatable genetic-metabolic epileptic encephalopathies.

Disease	Screening tests	Genes	Therapeutic trial
Pxyridoxine dependent epilepsy	– trial with 1 to 5 doses of pyridoxine i.v. during continous EEG monitoring – pipecolic acid and alpha amino adipic semialdehyde dosage in CSF, plasma, urine	ALDH7A1	Pyridoxine 15 mg/kg/day
Folinic acid responsive epilepsy	Two unknown chromatographic compounds in HPLC	Allelic with ALDH7A1	Folinic acid (2,5–5 mg/day) possibly associated to pyridoxine or pyridoxal-5′-phosphate
Pyridoxal-5′-phosphate responsive epilepsy	Homovanillic acid, 5-hydroxyindolacetic acid, 3-methoxythyrosine, threonine and glycine dosage in CSF and vanillactic acid dosage in urine	PNPO	Pyridoxal-5′-phosphate (50–100 mg/kg/day) or pyridoxal-5′-phosphate (30 mg/kg/day) associated to i.v. pyridoxine (100 mg) eventually repeated in the following days till a maximum of 500 mg
Hyperprolinemia type II	Hyperprolinemia (10–15 times normal); P5C increased levels in plasma and urine; delta-1-pyrroline-3-hydroxy-carboxylate levels increased in urine; aminoaciduria; prolinuria; hydroxyprolinuria; glycinuria	ALDH4A1	Trial with pyridoxal-5′-phosphate (50–100 mg/kg/day)
Sulfite oxidase and molybdenum cofactor deficiency	Sulfocysteine and thaurin increased plasma levels; low cysteine; thiosulfate, sulfate and S-sulphocysteine elevated in urine (anion chromatography); decreased/undetectable plasma homocysteine	SUOX, MOCS1 and 2	Cyclic Pyranopterin Monophospate (80–160 μg/kg) Low protein intake
Biotin metabolism disorders	Urinary organic acids testing; ketolactic acidosis; enzyme activity testing	BTD, HLCS or MCS	Biotin (5–20 mg/day)
Serine byosynthesis disorders	Reduced serine (and glycine) in plasma and CSF; low 5MTHF in CSF	PHGDH, PSPH	L-serine (400–600 mg/kg/day) with or without glycine (200–300 mg/kg/day)
Glut1 deficiency syndrome	Hypoglycorrchia (<40 mg/dL or <2,2 mM); glycorrachia/glycemia ratio alterations (0,33 + 0,01); low CSF lactate (<1,3 mmol/L); functional studies of 3-O-methyl-D-glucose transport across red cells membranes	GLUT1	Ketogenic diet (attempted trials with methylxanthine, barbiturates, caffein and lipoic acids with poor results)
Creatine deficiency syndromes	Creatin/creatinine ratio in urine; GAA in plasma and urine	AGAT	Creatine Monohydrate (300–400 mg/kg/day) associated to possible reduction in arginine intake (15–25 mg/kg/day) or arginine and glycine supplementation
		GAMT	Creatine monohydrate (300–400 mg/kg/day) according to the age
		SLC6A8	Creatine monohydrate (250–750 mg/kg/day) in subjects with residual enzymatic activity and in female carriers eventually associated to arginine and glycine supplementation

Table 4 – MRI features of genetic- metabolic early onset epileptic encephalopathies.

Prominent corpus callosum involvement	EIEE1 (ARX-related), organic acidopathies, purine and pyrimidine metabolism disorders, D-2-hydroxyglutaric acidemia
Disorders of cortical development and malformations	*Cortical malformations due to structural genes mutations*: lissencephaly, pachigiria/polymicrogyria, subcortical band heterotopia, periventricular nodular heterotopia, focal cortical dysplasia, schizencephaly *Metabolic diseases associated with cortical malformations:* congenital glutamine deficiency, D-2-hydroxyglutaric acidemia, Smith-Lemli Opitz syndrome, Zellwegger syndrome
Cortical atrophy	Pyridoxine dependent epilepsy, pyridoxal 5-phosphate dependent epilepsy, folinic acid reponsive epilepsy, biotin metabolism disorders, Dravet and Dravet-like syndromes, CPSI, NAGS, OTC, ASL, classic citrullinaemia, arginase deficiency, HHH syndrome, EIEE2 (CDKL5 related), EIEE3 (SLC25A22 related), EIEE4 (STXBP1 related), EIEE5 (SPTAN1 related), fumaric aciduria, 2-methyl-3-hydroxybutyril-CoA dehydrogenase deficiency, Glutathione synthetase deficiency, Adenilosuccinate lyase deficiency, dihydropyriminidase deficiency, dihydropyrimidine dehydrogenase deficiency, Tay Sachs/Sandhoff disease (GM2 gangliosidosis type I and II), Niemann-Pick type A, Infantile Neuronal ceroidolipofuscinosis, creatine transporter deficiency, Menkes disease
Cerebellar involvement	Niemann-Pick type C, Glutathione synthetase deficiency, Adenilosuccinate lyase deficiency, Congenital disorders of glycosilation
Basal ganglia involvement	SSADH deficiency, Methylmalonic acidemia, Propionic acidemia, GAMT deficiency, AGAT deficiency,
White matter abnormalities	Sulfite oxidase and molybdenum cofactor deficiency, mitochondrial disorders, GABA transaminase deficiency, glycine encephalopathy, serine byosynthesis disorders, organic acidopathies, phenylketonuria, purine and pyrimidine metabolism disorders, Tay-Sachs disease, Krabbe disease, Niemann-Pick type C, peroxysomal disorders, biotin metabolism disorders, D-2-hydroxyglutaric acidemia, fumaric aciduria, Adenilosuccinate lyase deficiency, EIEE2 (CDKL5 related), creatine transporter deficiency
Functional MRI changes	GLUT1 deficiency syndrome
Metabolites distortions	Creatine metabolism disorders, Pyruvate dehydrogenase deficiency,
Not specifically characterizing features, no abnormalities	Hyperprolinemia type II, perinatal hypophosphatasia, nesidioblastosis, HI/HA syndrome, DEND syndrome, Rett syndrome (MeCP2 related), Angelman syndrome, PCDH19 related epileptic encephalopathy, congenital disorders of glycosilation, Dravet syndrome

EIEE 1: Early infantile epileptic encephalopathy type 1; ARX: Aristaless-related homeobox; CPSI: Carbamyl phosphate synthase I; NAGS: N-acetyl glutamate synthetase; OTC: Ornithine transcarbamylase; ASL: Argininosuccinic acid lyase; HHH: Hyperornithinemia-hyperammonemia-homocitrullinemia; EIEE 2: Early infantile epileptic encephalopathy type 2; CDKL5: cyclin-dependent kinase-like 5; EIEE 3: Early infantile epileptic encephalopathy type 3; SLC25A22: solute carrier family 25 member 22; EIEE 4: Early infantile epileptic encephalopathy type 4; STXBP1: syntaxin binding protein 1; EIEE 5: Early infantile epileptic encephalopathy type 5; SPTAN1 : Non-erythrocytic alpha-spectrin-1; SSADH: Succinic semialdehyde dehydrogenase; GAMT: Guanidinoacetate N-methyltransferase; AGAT: Arginine–glycine amidinotransferase; GABA: gamma-aminobutyric acid; GLUT1: Glucose transporter 1; HI/HA: Hyperinsulinism–hyperammonaemia; DEND: developmental delay, epilepsy and diabetes; MeCP2: methyl CpG binding protein 2; PCDH19: protocadherin 19.

account that chronic symptoms can follow an acute metabolic derangement.[3,51]

In the case of an associated chronic multiorgan involvement a laboratory diagnosis may be extremely disappointing. Lactate, pyruvate, very long chain fatty acids, copper, ceruloplasmine, and sialotransferrin isoelectrofocusing represent, respectively, the first steps for the diagnosis of mitochondrial and peroxysomal diseases, Menkes diseases and congenital glycosylation disorders.[3]

Muscular or cutaneous biopsies and related histochemical/biochemical assays should be performed only in selected cases (mitochondriopathies or few types of lipid storage diseases).[3,52]

4.2. *The role of electroencephalography*

An unusual pattern reminding the one of "prolonged" generalized tonic-clonic seizures have been reported in a recent study on six children under one year of age with CDKL5-related epileptic encephalopathy.[53] Three electroclinical stages were described: tonic-vibratory contractions associated with an electrodecremental event, a clonic phase related to an irregular series of sharp waves and spike slow waves and final myoclonic jerks linked to bilateral rhythmic sharp waves.[53]

In metabolic epileptic encephalopathies typical EEG are substantially restricted to glycine encephalopathy, that is characterized by suppression-burst pattern sometimes evolving into hypsarrhythmia while hypsarrhythmia is quite typical in phenylketonuria and Menkes disease.[36,40,54,55] In maple syrup urine disease (or leucinosis) and in propionic acidemia a comb-like-rhythm pattern is frequently observed.[2,3,13] Urea cycle disorders associates to low amplitude slowing probably due to toxic compounds while X-linked creatine transporter deficiency shows diffuse multifocal spikes and hypersyncronization.[2,3,13] In other metabolic disease EEG findings varies according to the type of seizures: burst-suppression and multifocal spikes get together with peroxysomal and mitochondrial disorders while hypsarrhythmia and multifocal or isolated focal discharges turn up in disorders of amino acids metabolism, in several organic acidurias and in congenital disorders of glycosylation.[2,3,13] In biotinidase deficiency fast central spikes, burst-suppression or multifocal recordings with background slowing have been described.[56] In Alpers disease continuous anterior high voltage spike-waves are documented.[57] In all storage diseases and in Glut-1 deficiency syndrome progressive background slowing is typical leading to an essential EEG vanishing in neuronal ceroid lipofuscinosis.[2,3,13]

4.3. *The role of neuroimaging*

Early onset epileptic encephalopathies presenting a syndromic phenotype are often characterized by both specific and aspecific neuroradiological findings that are listed in Table 4.

Signs of cortical and subcortical brain tissues distress can be observed in children with acute onset of seizures and a symmetrical cerebral involvement, particularly concerning basal ganglia, is suggestive for a metabolic dysfunction (for instance, mitochondriopathies or some organic acidurias).[4,14,16]

In children with a chronic multiorgan disease and peculiar dysmorphisms or alterations of head circumference, neuroimaging evaluation should look for epileptogenic brain malformations or posterior fossa abnormalities, cortical atrophy, callosal dysgenesis/absence, or white matter lesions.[4,14–16] Angio MRI can be an useful tool in characterizing peculiar patterns such as vascular distortions, like the ones observed in Menkes diseases.[40]

HMRS is crucial for diagnostic approach when it is observed a creatine peak reduction or absence (disorders of creatine metabolism), lactate or glycine elevation (mitochondriopathies and glycine encephalopathy).[14–16] HMRS studies are also useful in evaluating the effect of ketogenic diet and creatine supplementation in 3-phosphoglycerate dehydrogenase deficiency and cerebral creatine deficiency.[14–16] An expanding subject concerns functional neuroimaging with PET, revealing evident or subtle metabolic dysfunctions of selected and diffuse cortical areas especially in GLUT1 deficiency syndrome but also in many idiopathic epileptic encephalopathies.[15]

5. Conclusions

To date no evidence-based data provide guidelines in developing diagnostic pathways for patients with early onset epileptic encephalopathies owing the wide number of diseases, their unclear pathomechanisms and clinical heterogeneity. The difficulty to build up a common shared route to diagnosis represent an important restriction to the development of "tailored" therapies.

The algorithm we propose can be used as a general model in daily clinical practice but it has some limitations that should be taken into account:

1) it is hard to characterize an epileptic encephalopathy just at its onset; pathognomonic signs develop in a progressive perspective and lack in early stages;
2) there is an important overlapping in the two outlined clinical clusters (epileptic encephalopathies presenting with seizures as unique symptom and epileptic encephalopathies presenting with seizures associated with a syndromic phenotype). Their key symptoms are often present in different periods, even in the same patients. In this context our classification can be used as a differential criterion only if clinicians are able to underline a clear prominence of one of them;
3) these two clinical groups include very heterogeneous diseases (as an example two markedly different epilepsies, such as Ohtahara and Dravet syndrome, are inserted in the same cluster).

Further collaborative studies are required to test the validity of the suggested diagnostic nodes in large populations of children and to draw up applicable and stardardized protocols.

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