Treatable newborn and infant seizures due to inborn errors of metabolism

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ABSTRACT – About 25% of seizures in the neonatal period have causes other than asphyxia, ischaemia or intracranial bleeding. Among these are primary genetic epileptic encephalopathies with sometimes poor prognosis and high mortality. In addition, some forms of neonatal infant seizures are due to inborn errors of metabolism that do not respond to common AEDs, but are amenable to specific treatment. In this situation, early recognition can allow seizure control and will prevent neurological deterioration and long-term sequelae. We review the group of inborn errors of metabolism that lead to newborn/infant seizures and epilepsy, of which the treatment with cofactors is very different to that used in typical epilepsy management.

Key words: newborn, infancy, seizures, refractory epilepsy, cofactors, vitamins, therapeutic option

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Neurometabolic diseases comprise a large group of inborn errors of metabolism (IEM) affecting the brain that have not yet been fully defined (Saudubray, 2012). These diseases are caused by dysfunction of genes that control the intermediary metabolism of carbohydrates, lipids, amino acids, vitamins, or energy metabolism. Accumulating compounds or lack of substrates can provide useful biomarkers in the diagnostic work-up for these rare disorders. Neurometabolic disorders can present with seizures or epilepsy in the newborn period regardless of the metabolic substrate affected (*table 1*). Seizures may be part of a more complex neurological presentation or be the leading, and

sometimes only, feature of the disease. The physiopathological mechanisms are often pleomorphic (*table 2*). In general, the developing brain is more susceptible to seizures than the adult brain (Arzimanoglou *et al.*, 2004). The age at onset of neurometabolic epilepsy depends on affected pathways and factors linked to the development of the nervous system in a manner that is not always obvious; during the neonatal period, childhood and adolescence, various coordinated gene expression programmes are activated and subsequently silenced as the organism grows and matures. Consequently, the effects of a pathogenic gene mutation may remain unnoticed until the mutant gene is activated

Table 1. Treatable Inborn Errors of Metabolism with seizures in the newborn and infant period.

- Non-ketotic hyperglycinaemia (NKH)
- Molybdenum cofactor (MOCOD) and isolated sulfite oxidase deficiency (ISOD)
- Urea cycle defects (UCD)
- Maple syrup urine disease (MSUD)
- Organic acidurias (OA)
- Pyridoxine-dependent epilepsy (PDE)
- Pyridoxal 5′-phosphate-dependent seizures (PNPO deficiency)
- Glut-1 transporter deficiency
- Serine deficiency
- Menkes disease
- Mitochondrial cytopathies
- Holocarboxylase synthetase deficiency
- Creatine defect disorders (GAMT)

Table 2. Physiopathology of neonatal/infant seizures in Inborn Errors of Metabolism.

- Reduced energy supply (Glut-1)
- Disturbances in neuronal membrane permeability/integrity (holocarboxylase synthetase deficiency)
- Misbalance intra/extracellular ions (OA)
- Neurotoxic compounds (UCD, OA, MOCOD)
- Energy depletion and accumulation of radicals (mitochondrial diseases)
- Neurotransmitters and amino acid imbalance (PNPO, PDE, NKH, SSADH, GABA)
- Molecular transport abnormalities (Menkes)
- Neuronal system circuit dysfunction (MOCOD)
- Substrate deficiency (serine)

OA: organic acidurias; UCD: urea cycle defects; MOCOD: molybdenum cofactor deficiency; PNPO: pyridox(am)ine 5′-phosphate oxidase; PDE: pyridoxine-dependent epilepsy; NKH: non-ketotic hyperglycinaemia; SSADH: succinate semialdehyde dehydrogenase; GABA: γ-Aminobutyric acid.

during a determined stage of development or even in adulthood. For example, the foetal brain preferably consumes products of lipid degradation, including ketone bodies. In neonates, cerebral glucose consumption is still minimal, increasing gradually during infancy and childhood until it reaches up to three times the level of consumption of the newborn. On other occasions, the function of certain defective genes is replaced by others as development progresses, causing reversible or transient clinical symptoms, such as in the case of a special subform of cytochrome c oxidase deficiency that causes severe hypotonia and weakness, the outcome of which is the restoration of muscle enzyme activity and the normalization of the hypotonia and weakness at a few years of age (Pascual, 2007).

Physiopathology

The immaturity of inhibitory systems during early brain development and its dysregulation under metabolic dysfunction play a major role in neonates and lower the seizure threshold. This is explained by the strong expression of inotropic glutamate receptors in general and an expression of receptor subunits that facilitate increased calcium influx (Pascual, 2007). The expression of GABA receptors and GABA glutamate decarboxylase is low in the newborn period. Activation of GABA receptors may be excitatory in the immature brain caused by high intracellular chloride concentrations. There is considerable evidence that alterations in GABA signalling can cause seizures, and that seizures can change GABAergic signalling (Rakhade and Jensen, 2009).

Many IEM interfere, early or late, with key functions of brain metabolism, for example, the transport and utilisation of energy substrates, the production of energy-rich phosphates, the metabolic coupling between neurons and astrocytes, the neurotransmitter signalling pathways, the autoregulation of cerebral blood flow, and the transport of substrates across the blood/brain barrier (*table 2*). In some IEM, accumulating compounds may cause direct neurotoxicity, and certain triggers, such as fever or catabolism, may precede seizure onset and encephalopathy. In these IEM, it is believed that symptoms remain latent until the accumulation of toxic products is sufficient to interfere with cell functions, for example, in urea cycle disorders or some organic acidurias. Primary or secondary disturbances in the neurotransmitter pathways with excess of excitation or lack of inhibition in the immature brain can also enhance seizure activity (*table 2*).

General approach to inborn errors of metabolism with seizures

Though IEM are rare, differential diagnoses should be considered during routine work-up, especially for neonatal seizures (Saudubray, 2012). Unclear aetiology and therapy resistance should always prompt biochemical investigations. A detailed medical history is important, including the family pedigree, followed by a full physical and neurological examination. Some neonatal presentations of IEM may be

accompanied by true birth asphyxia as a misleading confounder. In the presence of birth asphyxia and therapy resistance of seizures over 24 hours, neonatologists should consider IEM as an underlying cause. The newborn/infant baby with seizures is an emergency and should immediately be transferred to an intensive care unit where a diagnostic algorithm should be in place. This algorithm will help to exclude the more common causes accounting for about 75% of neonatal seizures (intraventricular haemorrhage, hypoxic-ischaemic encephalopathy, hypoglycaemia, electrolyte imbalance, neonatal stroke or CNS infection), followed by an extended urgent routine laboratory work-up to unravel hallmarks of IEM with systemic neonatal decompensation (*table 3*). If this extended routine work-up is inconclusive, controlled vitamin trials and selective screening tests should be performed that may confirm the suspected disease or, at least, serve to place it within a group of metabolic disorders, that should be investigated further (Campistol, 2000; Scriver *et al.*, 2003; Pascual *et al.*, 2008). The diagnostic process is not complete until the enzymatic and genetic abnormality causing the disease has been identified.

Newborn screening by mass spectrometry is carried out in most high-income countries, but programmes vary considerably and do not cover IEM that cause neonatal seizures, aside from atypical phenylketonuria, biotinidase deficiency, some organic acidurias, and some fatty acid oxidation disorders. In some countries, it is mandatory to investigate as few as seven IEM, while it would be possible to detect more than 50 diseases by analysing the acylcarnitine- and amino acid profile of dried blood spots by mass

Table 3. Disease-specific metabolites in body fluids.

Disease	Plasma	Urine	CSF
Urea cycle defects	ammonia, amino acids	orotic acid	
Organic acidurias	acylcarnitines, amino acids	organic acids	
PDE due to Antiquitin deficiency	pipecolic acid	AASA, PA	(AASA, PA, NT, PLP)
PNPO		vanillactate	PLP, NT
ADSL		purines	
Atypical PKU	amino acids	pterins	(NT)
NKH	amino acids		amino acids
Serine deficiency	amino acids		amino acids
MSUD	amino acids, acylcarnitines	organic acids	
Mitochondriopathies	lactate, glycine		lactate
Glut-1 deficiency	glucose		glucose, lactate
Menkes	copper, ceruloplasmin		
MOCOD	uric acid	sulfocysteine	
ISOD		sulfocysteine	
Holocarboxylase synthetase deficiency	lactate, ammonia	3-OH isovaleric, methylcrotonylglycine	
GAMT deficiency	guanidino acetic acid, creatine	guanidino acetic acid	guanidino acetic acid, creatine

PDE: pyridoxine-dependent epilepsy; PNPO: pyridox(am)ine 5′-phosphate oxidase;
ADSL: adenylosuccinate lyase; PKU: phenylketonuria; NKH: non-ketotic hyperglycinaemia; MSUD: maple syrup urine disease;
MOCOD: molybdenum cofactor deficiency;
ISOD: isolated sulfite oxidase deficiency; GAMT: guanidinoacetate methyltransferase;
PA: pipecolic acid; GAA: guanidinoacetate acid; AASA: α-aminoadipic semialdehyde;
PLP: pyridoxal 5′-phosphate; NT: neurotransmitter.

spectrometry. However, diagnosing IEM still requires a high degree of clinical suspicion, as there is no particular set of tests to detect all or even most neurometabolic diseases. The most widely used tests can be performed on plasma and urine, and should ideally be performed ahead of standardized vitamin trials early in the course of disease. Still, sample collection should not delay treatment. Some IEM will necessitate the analysis of CSF. In this respect, thorough sampling and immediate freezing of CSF samples at -80° helps to avoid the need for repeated lumbar puncture, as storage at room temperature will not allow later biochemical work-up. *Table 3* lists IEM that cause neonatal seizures and their respective metabolites in body fluids. Confirmation testing should be performed on a genetic level and/or using enzyme assays with appropriate material (fibroblasts or muscle tissue). Cerebral MRI is a key investigation, but for IEM presenting with neonatal seizures, it is rarely diagnostic. The exceptions are a diagnostic pattern of brain oedema for Molybdenum cofactor deficiency, cystic white matter lesions and brain atrophy or polymicrogyria for Zellweger disease, diffusion abnormalities on diffusion weighted imaging -apparent diffusion coefficient (DWI-ADC) for maple syrup urine disease and non-ketotic hyperglycinaemia (NKH), and finally hypoplastic corpus callosum for NKH (Kanekar and Byler, 2013). Also magnetic resonance spectroscopy (MRS) may be indicative of mitochondrial disorders with a high lactate peak or Canavan disease based on a high NAA peak.

Proton MRS enables identification of an increasing number of metabolites within the central nervous system by means of a non-invasive *in situ* and *in vivo* procedure, and is especially helpful in detecting creatine deficiency syndromes.

In the case of a fatal epileptic encephalopathy of unclear aetiology, investigations using dried blood spots and fibroblast cultures, which can be obtained during the first 18 hours after death and preserved indefinitely in liquid nitrogen, may be helpful to establish a post-mortem diagnosis for further genetic counselling (Marin-Valencia *et al.*, 2010).

Treatable seizures and epilepsy in the neonatal or infant period due to inborn errors of metabolism

Of the approximately 750 metabolic diseases that are currently known, some 40-60% can lead to isolated or recurrent convulsive seizures at some point during their course. The cumulative incidence of these disorders is low (1:2,000-3,000 live births), with about 25% manifesting during the neonatal or infant period.

Early diagnosis has important clinical implications for specific treatment options (Saudubray, 2012).

For IEM that manifest in neonatal infants with metabolic crises, well established treatment regimens are in place that require enrolment of a metabolic specialist. Exchange transfusion is effective when it is necessary to eliminate toxic metabolites. Peritoneal dialysis offers a simple alternative but is not as effective as exchange transfusion or haemodialysis which allows a much higher clearance.

Long-term treatment for these disorders consists of disease-specific, lifelong diets following the principle of substrate reduction, thus limiting the flux through the affected metabolic pathway. In an emergency, all the essential nutrients may alternatively be administered by parents. There are standard diets, modified for content of protein, carbohydrate or fat, in which the age-appropriate caloric and essential amino acid requirements are still satisfied. Single disease entities respond to the administration of cofactors or high-dose vitamins that have to be maintained lifelong; *i.e.* vitamin dependency rather than vitamin deficiency.

Generally, antiepileptic drugs (AEDs) are of limited effect in most cases of IEM that manifest with seizures as a hallmark of disease, especially in situations of acute decompensation (Aicardi, 1995). Early and specific treatment can prevent irreversible neurological damage (Wolf *et al.*, 2005) that occurs due to the convulsive seizures and the metabolic derangement, and may allow the patient to lead a normal life. In this article, we review IEM with seizures or epilepsy of neonatal infant onset, as well as specific treatment options.

Defects of vitamin metabolism

Pyridoxine-dependent epilepsy (PDE)

This is an autosomal recessive disease characterized by a therapeutic response to pharmacological doses of vitamin B6 (pyridoxine or PLP) and resistance to conventional AEDs. For a long time, PDE deficiency was assumed to be due to glutamic acid decarboxylase (GAD) deficiency, catalyzing the conversion of glutamate to GABA and requiring vitamin B6 as a cofactor (*table 3*) (Baxter, 1999; Toribe, 2001). Following the description of pipecolic acid as a first biomarker for PDE (Plecko *et al.*, 2000), mutations in the gene encoding α-aminoadipic semialdehyde (AASA) dehydrogenase (antiquitin) were identified as the major cause of PDE (Mills *et al.*, 2006; Stockler *et al.*, 2011). AASA dehydrogenase or antiquitin (ATQ) is encoded by the *ALDH7A1* (or *ATQ*) gene and acts in the catabolism of the essential amino acid, lysine (Mills *et al.*, 2006).

The typical manifestation of PDE is intrauterine, neonatal or infant onset with spasms and focal myoclonic, tonic or bilateral tonic-clonic seizures within hours or days after birth (Schmitt *et al.*, 2010).

Aside from this, atypical presentations with first manifestation up to 3 years of age have been described (Gospe, 2010).

The seizures are typically refractory to common anticonvulsants and may progress to status epilepticus (Dulac *et al.*, 2014). Between episodes, the infant can present with hypotonia, sleeplessness, and movement disorders, suggestive of clinical seizures but without EEG discharges, poor contact, erratic eye movements or myoclonus triggered by acoustic stimuli (Stockler *et al.*, 2011). About a third of patients show birth asphyxia or poor adaptation after birth, misleading the clinician to interpret seizures as part of hypoxic ischaemic encephalopathy.

In atypical cases, that may account for a third of cases of PDE, onset can occur later with West syndrome (Gospe, 2010) or with various seizure types up to the age of 2 years, or with neonatal seizures that may show an initial response to anticonvulsants (especially phenobarbitone) or to extremely low doses of pyridoxine. About 15% of patients with later-confirmed antiquitin deficiency show an ambiguous response to a first administration of pyridoxine (Mills *et al.*, 2014).

The EEG pattern shows asynchronous bursts of high-voltage generalised epileptiform activity, multifocal discharges, slow spike-wave complexes, burst-suppression pattern, or hypsarrhythmia in patients with infantile spasms. Neuroimaging can show non-specific findings, such as hypoplasia of the corpus callosum, cerebellar hypoplasia, cortical atrophy, hydrocephalus, white matter changes and periventricular dysmyelination, or intraventricular haemorrhage.

The response to a first dose of intravenous pyridoxine (100 mg) can be quite dramatic (in just a few minutes), with disappearance of seizures and normalisation of the EEG over 24-48 hours, but may be accompanied by severe apnoea and coma, requiring assisted ventilation. In the case of ineffectiveness, another dose of 100 mg pyridoxine might be given in sequential doses every 5-10 minutes, up to a total dose of 500 mg. If intravenous administration is not possible, pyridoxine can be given orally or enterally at equal dose, with the same risk of apnoea. A delayed response to pyridoxine is possible and treatment should be continued at 30 mg/kg/day in three single dosages for at least 3-7 days before concluding that the seizures are not responsive to pyridoxine (Gospe, 2010; Stockler *et al.*, 2011), or until ATQ deficiency has been excluded by biochemical or genetic testing (Stockler *et al.*, 2011).

In affected patients, sudden withdrawal of pyridoxine leads to seizure recurrence, generally within a period of 5-7 days, although in individual cases, seizure-free periods of up to four weeks have been described. It is recommended that pyridoxine treatment is maintained for life at a dose of 15-30 mg/kg/d, with a maximum daily dose of around 200 (to 500) mg, divided in 2-3 single dosages. Higher doses may lead to sensory or motor neuropathy, but neuropathy has also been observed in single patients on doses of around 200 mg/day (Vinus Bok, personal communication). In patients with breakthrough seizures with infections and other stressful situations, transient doubling of the pyridoxine dose is recommended. As only about 25% of PDE patients have normal cognitive outcome, despite early seizure control, additional therapeutic strategies have to be developed. A lysine-restricted diet has the potential to lower neurotoxic AASA concentrations and has become an additional treatment option for PDE (Gallagher *et al.*, 2009; Stockler *et al.*, 2011; van Karnebeek *et al.*, 2012). High-dose arginine supplementation might be an additional option, working by competitive inhibition of lysine uptake in the gut and at the blood/brain barrier (Mercimek-Mahmutoglu *et al.*, 2014).

Elevated AASA, piperideine-6-carboxylate (P6C), and pipecolic acid (PA) concentrations are found in the CSF, urine or plasma, and serve as reliable biomarkers, while secondary alterations of neurotransmitter metabolism are less consistent (Plecko *et al.*, 2005; Mills *et al.*, 2006; Plecko *et al.*, 2007; Struys *et al.*, 2012). AASA is also secondarily elevated in molybdenum cofactor deficiency, such that sulfocysteine in urine should be measured simultaneously to avoid a false diagnosis.

AASA in urine and PA in plasma decline upon treatment, but usually remain elevated while on pyridoxine. Other non-specific biochemical disturbances reported are lactic acidosis, hypoglycaemia, electrolyte disturbances, hypothyroidism, and diabetes insipidus (Mercimek-Mahmutoglu *et al.*, 2012) and may, in part, improve with pyridoxine treatment. Measurement of the enzyme deficiency in fibroblast homogenates is possible but requires a complex laboratory method. The *ALDH7A1* gene is located on chromosome 5q31. Diagnosis is confirmed by mutation analysis and, to date, more than 60 different mutations within the 18 exons of the *ALDH7A1* gene have been reported. More than half are missense mutations with an altered amino acid in the protein sequence (Stockler *et al.*, 2011), but large deletions or duplications are not detected by standard sequencing methods. If, in the presence of positive biomarkers, sequencing does not reveal point mutations, molecular testing for deletions should be performed. Expression studies are helpful in measuring residual enzyme activity of single mutations, but are beyond routine investigation.

The outcome of patients with PDE is variable and not as good as was first reported. Some patients have normal development and remain seizure-free on pyridoxine monotherapy, while other patients present incomplete seizure control and marked developmental delay. Some authors therefore distinguish three PDE phenotypes (Scharer *et al.*, 2010):
- (1) PDE with complete seizure control and normal developmental outcome;
- (2) complete seizure control and developmental delay or autism;
- (3) incomplete seizure control and developmental delay (Plecko, 2013).

Due to autosomal recessive inheritance, there is a 25% recurrence risk for subsequent offspring. Intrauterine treatment with 10 to 100 mg of pyridoxine should be considered in forthcoming pregnancies, but despite preventing seizures, it does not prevent intellectual disability (Rankin *et al.*, 2007; Bok *et al.*, 2010). Postpartum confirmation testing should be performed as soon as possible, as high-dose treatment with pyridoxine could have a proconvulsive effect in non-affected neonates (Hartmann *et al.*, 2011).

Pyridoxal 5′-phosphate-sensitive seizures or pyridox(am)ine 5′-phosphate oxidase (PNPO) deficiency

Kuo and Wang (2002), Clayton *et al.* (2003), and Mills *et al.* (2005) described a new group of newborn infants who presented with therapy-resistant seizures and early infantile epileptic encephalopathy, who were largely resistant to pyridoxine, but responded to pyridoxal 5′-phosphate (PLP), the active vitamin B6 cofactor.

Seizures usually cease within 60 minutes from first PLP administration, followed by the onset of hypotonia, and respiratory and neurological depression. Again, first administration of PLP can lead to severe apnoea such that resuscitation equipment should be at hand. Within a few days, the patient returns to normal and the seizures stay controlled, provided the therapy is maintained at 30-50 mg/kg/d, administered orally or enterally, and divided in four to six single dosages per day. PLP is only available as an oral chemical powder from naturopathic stores and has not been licensed as a drug outside of Asia. PLP should be administered immediately after being dissolved in order to prevent oxidation; due to potential liver toxicity, monitoring of transaminases is advocated even on short-term PLP trials and one patient was reported to have liver cirrhosis on long-term use (Mills *et al.*, 2014). The reported cases of PLP-dependent seizures are scarce and, except for a few features, exhibit marked overlap with those of PDE patients. Patients with PNPO deficiency are frequently born premature and have immediate signs of encephalopathy and seizures, with lactic acidosis and hypoglycaemia. The seizure semiology and EEG findings are indistinguishable from antiquitin deficiency, perhaps with more ocular, facial and other automatisms. Maternal reports of foetal seizures are frequent (more so than for PDE), and a burst-suppression pattern on EEG is more common than in PDE. In contrast to PDE, breakthrough seizures, while on PLP, are frequently observed and patients may be sensitive to the precise time intervals between PLP administration throughout the day. If left untreated, the disorder results in death or profound developmental impairment with global brain atrophy and a disturbed pattern of myelination (Ruiz *et al.*, 2008). In patients treated early, the outcome is usually much better (Stockler *et al.*, 2011; Porri *et al.*, 2014).

PNPO deficiency lacks a specific biomarker. Raised levels of glycine, threonine, and 3-methoxytyrosine, and reduction of pyridoxal 5′-phosphate concentrations in CSF are secondary phenomena of cerebral vitamin B6 deficiency and can thus also be found in other conditions (*table 3*). Recently, a patient with PNPO deficiency was found to have normal PLP levels in CSF while off vitamin B6 supplementation (Levtova *et al.*, 2013). The biomarkers of antiquitin deficiency are in the normal range in PNPO-deficient patients. The CSF biochemical profile can mimic that of aromatic L-amino acid decarboxylase deficiency (elevated vanillactate in urine, low concentration of HVA and 5-HIAA, and high concentration of L-dopa, 5-hydroxytryptophan, and 3-O-methyldopa) (Ormazábal *et al.*, 2008), but individuals with elevated concentrations of biogenic amines have been described (Porri *et al.*, 2014).

PLP-responsive seizures are caused by pyridox(am)ine 5′-phosphate oxidase deficiency, the key enzyme responsible for converting pyridoxamine 5′-phosphate and pyridoxine into pyridoxal 5′-phosphate (PLP). PLP is the only active vitamin B6 cofactor, and functions in more than 140 pyridoxal 5′-phosphate-dependent enzymatic reactions, including glutamic acid decarboxylation into GABA, lysine degradation, and serine formation. Recently, a regulatory role for PNPO in PLP recycling and its intracellular transport has been identified, partly explaining why treatment of PNPO deficiency is more difficult than that of PDE. In contrast to antiquitin deficiency, PNPO deficiency cannot be diagnosed using a specific biomarker. Low PLP in CSF is very suggestive, but has been described in several IEM (Mills *et al.*, 2005; Footitt *et al.*, 2011). Thus, a definite diagnosis can only be established by molecular analysis of the PNPO gene.

The paradigm of exclusive PLP responsiveness has been challenged recently by the description of certain mutations in the PNPO gene that were associated with

response to pyridoxine, but resistance to PLP (Mills *et al.*, 2014; Plecko *et al.*, 2014; Ware *et al.*, 2014).

Thus, consecutive vitamin trials with pyridoxine, followed by PLP, should be a standard procedure for every neonate or infant baby with therapy-resistant seizures, and biomarkers and genetic testing should be performed rigorously.

Other pyridoxine or PLP-responsive seizure disorders include neonatal hypophosphatasia (TNSALP deficiency), familial hyperphosphatasia (PIGV deficiency or Mabry syndrome), and hyperprolinaemia type 2 (P5CD deficiency) (Nunes *et al.*, 2002; Stockler *et al.*, 2011; Plecko, 2013).

Folinic acid-responsive seizures (FARS)

In 1995 (Hyland *et al.*, 1995) and 1999 (Torres *et al.*, 1999), patients were described with neonatal seizures that were resistant to phenobarbital (PB) and valproic acid (VPA), and some of them also to pyridoxine, but responded to folinic acid (3-5 mg/kg/day) after a variable period of time (3-30 days) (Torres *et al.*, 1999). Despite this, the patients suffered from developmental delay or a fatal course of disease (Nicolai *et al.*, 2006; Stockler *et al.*, 2011). The analysis of CSF biogenic amines by HPLC showed an unidentified compound that was called "peak X" which was used as a disease marker. Recently, FARS was identified to be genetically identical to ATQ deficiency, and some patients with "peak X" showed a clear response to pyridoxine, and all had positive biomarkers, such as elevated AASA and PA (Gallagher *et al.*, 2009). To date, the role of folinic acid in PDE is not understood, aside from the fact that peak X is not exactly the same as any biomarker that has so far been recognized with antiquitin deficiency. In the case of a neonate with seizures and an incomplete pyridoxine response, add-on treatment of folinic acid, at 3-5 mg/kg/d, should still be considered (Gospe, 2010; Stockler *et al.*, 2011).

Holocarboxylase synthetase deficiency

Deficient holocarboxylase synthetase results in multiple carboxylase deficiency. As biotin-dependent carboxylases are essential for neoglucogenesis and amino acid metabolism, holocarboxylase deficiency impairs energy and protein metabolism. Patients present in the newborn period with a progressive neurological deterioration. Myoclonic seizure or infantile spasms may be accompanied by a burst-suppression pattern without response to conventional AED therapy (Dulac *et al.*, 2014). MRI can demonstrate subendymal cysts, which may suggest a metabolic origin (Wilson *et al.*, 2005). Early treatment with biotin, with dosages up to 20-200 mg/d, may lead to complete resolution of the seizures and all clinical symptoms.

Metabolic acidosis with elevation of lactate and ammonia, and a specific organic aciduria profile with 3-OH isovaleric acid and methylcrotonylglycine, are characteristic diagnostic findings (Wolf, 2011).

Defects of purine and pyrimidine metabolism

Adenylosuccinate lyase (ADSL) deficiency

This disease usually presents with seizures in the first few days of life or as childhood-onset epilepsy. Although in some patients it is possible to control the seizures, it often develops into an epileptic encephalopathy (Jurecka *et al.*, 2014). Treatment with allopurinol, sodium benzoate, purine bases (*e.g.* adenine), aminoimidazole carboxamide, and D-ribose and uridine has had poor results, although in one case, D-ribose led to temporarily improved seizure control (Ciardo *et al.*, 2001; Wolf *et al.*, 2005).

Amino acidopathies

There are several inborn errors of amino acid metabolism which can be accompanied by epileptic seizures in the neonatal period or in the first months of life. The most common, and those that have the greatest impact on the nervous system, are atypical PKU due to BH4 defects, non-ketotic hyperglycinaemia, and serine deficiency (Wolf *et al.*, 2005; Saudubray, 2012).

Atypical phenylketonuria (PKU) due to BH4 defects

Hyperphenylalaninaemia is usually picked up by newborn screening. Further diagnostic work-up includes determination of pterins in urine in order to detect atypical phenylketonuria (PKU). Atypical PKU is due to disorders of tetrahydrobiopterin (BH4) metabolism, the cofactor involved in the hydroxylation of phenylalanine, tyrosine and tryptophane, which are precursors for neurotransmitter synthesis. The defect may lie in the synthesis or in the BH4 recycling process. Affected patients (1% of PKU cases) present with symptoms of neurological impairment due to neurotransmitter deficiency (catecholamines and serotonin). The disorder presents early, with seizures, microcephaly, hypothermia, developmental delay, progressive neurological impairment, breathing difficulties, parkinsonism, myoclonus, chorea, dystonia and pyramidal signs. Refractory epilepsy is common, with infantile spasms or generalised seizures. Dihydropteridine reductase deficiency can be determined by assessing enzyme activity in erythrocytes, fibroblasts and lymphocytes. Treatment is based

on dietary protein (phenylalanine) restriction and BH4, L-Dopa, 5-hydroxytryptophan, and folinic acid supplements (Blau *et al.*, 2011).

Non-ketotic hyperglycinaemia (NKH)

This is caused by a defect in the activity of the glycine degradation system; a multi-enzyme complex present in liver and brain. L-Glycine is an obligatory co-agonist of NMDA receptors and hyperglycinaemia therefore results in over-excitation of NMDA neurotransmission causing excitotoxicity and epilepsy. The typical history is a mature newborn, presenting with episodes of apnoea, hiccups (often felt by the mother before birth), dysautonomia, progressive lethargy, and coma within a few days after birth. This is accompanied by segmental and erratic myoclonus which may evolve into epileptic spasms and focal motor seizures resistant to medication. The EEG pattern deteriorates rapidly, with periods of burst suppression and progression towards hypsarrhythmia after three months (Chen *et al.*, 2001). MRI/MRS demonstrates thin or dysplastic corpus callosum, delayed myelination, and the glycine peak in the proton spectrum.

Aside from the neonatal form, an atypical presentation with an extrapyramidal movement disorder has been described. NKH is diagnosed by an increased ratio of CSF to plasma glycine >0.04 with some correlation to phenotype. It is recommended to analyse organic acid levels in urine to exclude ketotic hyperglycinaemia (associated with organic aciduria), in which the increase in plasma glycine (normal in CSF) occurs due to inhibition of the hepatic glycine degradation system and the accumulation of toxic organic acids (propionic, methylmalonic and isovaleric aciduria). Recently, elevated plasma glycine has been described in a subgroup of mitochondriopathies related to iron-sulfur cluster defects and is accompanied by elevated lactate levels in plasma and CSF. Enzymatic testing for NKH warrants a liver biopsy and has been largely replaced by molecular analysis of the three respective genes involved. Most neonatal forms are caused by P, T or H protein defects (Van Hove *et al.*, 2005; Korman *et al.*, 2006; Kanno *et al.,* 2007).

Prenatal diagnosis is possible by molecular analysis of chorionic villi. As glycine is a non-essential amino acid, dietary restriction is not effective. Sodium benzoate has shown some effect in reducing seizure frequency and glycine levels in plasma, but cannot alter the poor long-term prognosis. Strychnine, dextromethorphan (an NMDA receptor antagonist), diazepam, methionine or choline supplements have also been used, but with poor results (Chien *et al.*, 2004). The response to AEDs is very limited and VPA must not be used as it further inhibits glycine metabolism. Transient

forms of NKH and a milder course associated with the A802V mutation have been described (Dinopoulos *et al.*, 2005), and for this reason we include NKH within the group of treatable disorders. Thus, counselling of parents regarding the prognosis of their affected child should be based on the full genotype.

Serine deficiency

This autosomal recessive disease usually presents with congenital or early-onset secondary microcephaly and refractory seizures during the first few months of life, which may evolve into West syndrome. Infants appear unhappy, irritable and hypertonic. More recently, a juvenile form with mental decline, absence of seizures, and behavioural problems has been described (Tabatabaie *et al.*, 2010). Defects have been described in all three steps of serine formation, with a deficiency of the enzyme 3-phosphoglycerate dehydrogenase due to a defect of the *PHGDH* gene being the most common. L-serine is an essential component for D-serine and glycine synthesis and is an important ligand to NMDA receptors, however, conversely, it functions as an inhibitory neurotransmitter in the brainstem. In infantile forms, cranial MRI can give a clue to diagnosis with delayed myelination followed by significant cerebral atrophy. Determination of low plasma serine, or more markedly low CSF serine establish the diagnosis. Patients usually respond favourably to early oral administration of L-serine supplements, at 500-700 mg/kg/d, eventually together with glycine (200-300 mg/kg/d) and folinic acid. Intrauterine treatment seems to prevent development of the clinical phenotype (van der Crabben *et al.*, 2013). Neu-Laxova syndrome is a heterogenous metabolic disorder, leading to prenatal or early postnatal lethality, due to defects in L-serine biosynthesis (Acuna-Hidalgo *et al.*, 2014).

Leucinosis or maple syrup urine disease

The severe form of this disease presents during the first week of life with poor feeding, maple syrup odour in organic fluid, dystonia, generalized seizures and coma, and a burst-suppression EEG pattern .The patients may present with neutropenia, thrombocytopenia, or pancytopenia.

Diffusion-weighted imaging (DWI) shows a characteristic pattern of bilateral symmetric restricted diffusion within the myelinated areas in the posterior limb of the internal capsule, centrum semiovale, corona radiata, corticospinal tract, thalami, posterior aspect of the mid brain, pons, middle cerebellar peduncle, medulla, and cerebellar white matter, attributed to intramyelinic oedema (Cavalleri *et al.*, 2002).

The symptoms are mainly due to the accumulation of leucine (isoleucine and valine), which at first does not cause changes on routine laboratory analysis. Metabolic acidosis with an increase in the anion gap, ketonuria, ketoacidosis, moderate hypocalcaemia, hyperlactataemia, and hypo- or hyperglycaemia only arise with the accumulation of 2-oxo-isocaproate, 2-oxoisovalerate and 2-oxomethylvalerate, the breakdown products of the three branched-chain amino acids (Scriver *et al.*, 2003; Saudubray, 2012). Diagnosis is based on detecting the characteristic profile of elevated branched-chain amino acids, as well as L-alloisoleucine in the plasma amino acid and urine organic acid tests. Acute-phase treatment must be aggressive, with peritoneal dialysis or exchange transfusion, to rapidly eliminate the accumulated branched-chain amino acids, especially leucine, whilst energy must be supplied in the form of glucose and lipids by a central venous line. Subsequently, patients require a lifelong protein-restricted diet and supplementation with a precursor-free amino acid mixture (Chen *et al.*, 2004).

Organic acidurias

These are biochemical disorders of intermediary metabolism which affect different metabolic pathways of amino acids, fatty acids, ketogenesis, ketolysis, pyruvate, carbohydrates or the Krebs cycle. Some organic acidurias of neonatal onset, such as *propionic aciduria, holocarboxylase synthetase deficiency* or *isovaleric aciduria*, cause acute systemic decompensation with severe metabolic acidosis. More than 80 organic acidurias have been reported, and most of them involve the CNS. The variable clinical expression, in part, depends on the type of enzyme deficiency, age at onset, accumulated metabolites, and triggering factors. In the acute stage, patients present with vomiting and deterioration in their general condition, with progressive lethargy and coma, associated with metabolic acidosis. Myoclonic and other types of seizures are often observed in the acute stage, but as in propionic acidaemia, can also occur during the long-term course of disease (Haberlandt *et al.*, 2009). Pancytopenia, metabolic acidosis and ketonuria can appear during decompensation leading to the assumption of sepsis of unknown origin. The diagnosis is established by means of quantitative analysis of plasma amino acids and acylcarnitines, as well as urinary organic acid. Again, early diagnosis and specific treatment are essential to improve seizure management and prevent irreversible long-term sequelae. Some organic acidurias have been included in newborn screening programmes in several European countries.

Defects in the urea cycle and related disorders causing hyperammonaemia

Disorders of ureagenesis, due to primary enzyme defects of the urea cycle as well as secondary inhibition by organic acids, bring about hyperammonaemia. The enzyme defects in the urea cycle may be distinguished by their characteristic plasma amino acid profile and the presence or absence of urinary orotic acid (Saudubray, 2012). Age at onset and clinical signs are variable and one third of cases presents in the neonatal period with symptoms of toxic encephalopathy. Initial signs include poor feeding, vomiting, alterations in muscle tone, lethargy, generalised or focal seizures, and, if not treated adequately, coma, brain oedema and death. Hyperammonaemia is an emergency and it is mandatory to stop protein intake and provide adequate caloric supply in order to avoid or limit long-term sequelae, such as refractory seizures and neurological deterioration (Campistol, 2000). In argininosuccinic acidaemia, a more chronic presentation with trichorrhexis nodosa, irritability, rigidity, and refractory seizures is common. Thus, plasma ammonia should also be determined for more chronic presentations and requires immediate processing in order to avoid preanalytical errors. The use of valproate should be avoided in this patient group as it interferes with ammonia detoxification. Phenobarbitone or levetiracetam appears to be safe.

Inborn errors of metabolism affecting energetic substrates

Mitochondrial cytopathies

Epilepsy is a frequent manifestation of mitochondrial diseases, illustrating that energy depletion and the accumulation of radicals lowers the seizure threshold. Some mitochondrial diseases manifest during the neonatal/infant period, with seizures being one of the most frequent symptoms. In most early-onset cases, primary lactic acidosis is a leading finding and necessitates the exclusion of secondary lactate elevation due to organic acidurias. Determination of lactate in CSF is especially important and analysis of plasma amino acids helps to identify a group of mitochondrial cofactor defects based on simultaneously elevated glycine concentrations. MRI may show bilateral changes of the basal ganglia or white matter changes. MRS may reveal elevated lactate peaks. Mitochondriopathies have the unique constellation of Mendelian, as well as maternal inheritance through mitochondrial DNA (mtDNA). The majority of mitochondriopathies are of nuclear origin and present with a more non-specific type of encephalomyopathy. In exceptional cases of

recognizable syndromes, diagnosis may be confirmed by primary molecular analysis of mtDNA or nuclear DNA, while in most cases with a more non-specific presentation, a muscle biopsy and analysis of the oxphos enzymes is still necessary. One exception to this is Alpers disease, or hepatocerebral neurodegeneration, which is caused by autosomal recessive mutations of the *POLG1* gene, required for the replication of mtDNA (Wolf *et al.*, 2009).

Impaired mitochondrial glutamate transport has recently been reported as a cause of early myoclonic encephalopathy (Wolf and Smeitink, 2002; Molinari *et al.*, 2005). In all mitochondriopathies, the use of sodium valproate should be avoided due to the risk of hepatic failure. Some patients may benefit from supplementation of thiamine, riboflavin, L-carnitine or coenzmye Q10, as well as a slowly introduced fat-enriched diet (1:1 to 3:1 fat to carbohydrates plus protein, in grams).

Glucose transporter type 1 deficiency

Patients usually appear normal at birth or with some non-specified symptoms, such as abnormal ocular or generalized movements, and present with therapy-resistant focal motor seizures from their first months of life. In addition, especially before meals or at times of fasting, patients may exhibit an episodic movement disorder with associated eye-rolling movements. About 60% of infants develop acquired microcephaly. Beyond infancy, secondary generalization of seizures predominates and more recently, Glut-1 deficiency has been described in a cohort with atypical absence epilepsy (Suls *et al.*, 2009) The EEG may be completely normal and pre-prandial recording should be considered in order to unravel altered background activity or focal discharges (De Vivo *et al.*, 2002).

Glut-1 deficiency is generally caused by sporadic haploinsufficiency of the blood/brain barrier passive glucose transporter, Glut-1 (*SLC2A1* gene), although in milder cases, the disease can also be inherited as an autosomal dominant trait (Pascual *et al.*, 2004; Pascual, 2007). Over 100 different missense mutations have been described so far and about 10% of patients carry deletions, undetected by Sanger sequencing (Leen *et al.*, 2013).

First reports suggested that a CSF/blood glucose ratio <0.4 confirms the diagnosis, but more recently, the use of absolute CSF glucose values below 40 (to 60) mg/dl, with respect to age-related normal values, were considered to be more precise. Cranial MRI is usually normal or may show non-specific changes, as delayed myelination or brain atrophy. A ketogenic diet is the only known effective treatment and seizures disappear in the majority of patients (De Vivo *et al.*, 2002; Pascual *et al.*, 2008). The usual recommended ratio of fat to non-fat intake in grams is 3:1, and can be increased to 4:1.

Ketosis can be controlled by measurement of urine or blood ketones, and patients need to be followed by a dietician with a sick day protocol in place (Klepper and Leiendecker, 2013).

Creatine metabolism disorders

Creatine is essential for the central nervous system; it is derived from food or endogenous synthesis and is taken up by the brain through an X-linked creatine transporter protein (CRTR). In humans, two inborn errors of creatine biosynthesis, *arginine-glycine amidinotransferase* (AGAT) and *guanidinoacetate methyltransferase* (GAMT), and one affecting the transporter, CRTR, are known. All the defects are characterized by absence or decrease of creatine in the brain, as measured by *in vivo* proton MRS (1H-MRS). The clinical presentation of GAMT deficiency is characterized by arrest or regression of psychomotor development with infantile seizures (myoclonic or tonic seizures with apnoea, generalised tonic-clonic seizures, focal seizures with secondary generalization or drop attacks, or febrile seizures). Severe epilepsy has been reported in some cases associated with derangement of mental status (Stöckler *et al.*, 2007; Leuzzi *et al.*, 2013).

Biochemical findings include increased guanidinoacetate (GAA) in plasma, urine and also CSF, with a reduced concentration of creatine in urine or plasma. Confirmation of the diagnosis by enzymatic and molecular studies is possible (Carducci *et al.*, 2000; Alessandri *et al.*, 2004). The pathogenic role of the increased levels of GAA in the central nervous system, specifically related to the epileptogenic effect, as well as basal ganglia toxicity, is well known (Leuzzi *et al.*, 2013).

The aim of treatment in GAMT deficiency is to increase creatine levels and decrease GAA accumulation levels. The supplementation with creatine monohydrate (400 mg/kd /day) and dietary restriction of arginine (15 mg/kg/day) and ornitine aspartate supplementation (500 mg/kg/day) is useful. With early treatment, epilepsy can be responsive and, of course, mental status and movement disorders may also improve (Mercimek-Mahmutoglu *et al.*, 2006). In AGAT and CRTR deficiency the phenotype is much more unspecific with autism, impaired speech development and epilepsy, that in AGAT deficiency might not even be prominent.

Menkes disease

The disease can begin in the neonatal period with congenital skull fractures and seizures occurring during the first few months of life. The early phase is dominated by frequent focal clonic seizures with multifocal

discharges, which, over weeks, evolve into infantile spasms with modified hypsarrhythmia (Sfaello *et al.*, 2000 ; Bahi-Buisson *et al.*, 2006). The diagnosis is suggested by additional typical findings of kinky hair, cutis laxa, and bladder diverticles. Menkes disease is caused by mutations in the ATPase 7 gene, a ubiquitous copper transporter encoded on the X-chromosome and located in the trans-Golgi network, that is particularly active in the intestine, from which most of the dietary copper is absorbed (Prasad *et al.*, 2011). The impairment of copper transport leads to dysfunction of several copper-dependent enzymes, such as mitochondrial cytochrome c oxidase, lysyl oxidase, superoxide dismutase, dopamine-beta-hydroxylase, and tyrosinase (Matsuo *et al.*, 2005). This causes elevated plasma lactate due to complex IV deficiency (cytochrome c oxidase), alteration in the molecular bridges of collagen (catalysed by lysyl oxidase) causing hair abnormalities (pili torti, trichorrhexis nodosa, moniletrix and, eventually alopecia), vascular alterations (elongated vessels, tortuosity of intracranial vessels and subdural haematoma), and Purkinje cell degeneration in the cerebellum and hypothermia. The serum copper and cerulopasmin are low and the homovanillic acid: vanillylmandelic acid ratio in urine is high. The diagnosis is confirmed by copper uptake studies in fibroblasts or molecular analysis of the *ATP7A* gene. Early treatment with subcutaneous injections of copper-histidine (50-100 micrograms/kg/d) ameliorates some of the symptoms of the disease.

Molybdenum cofactor deficiency (MOCOD) and isolated sulfite oxidase deficiency (ISOD)

These two entities have identical clinical presentations and show bilateral myoclonic or tonic-clonic seizures within days after birth that may evolve into status epilepticus. In some patients, seizures may be controlled by conventional AEDs. In 75% of patients, subtle dysmorphic features with elongated face, small nose, and puffy cheeks were described (Johnson and Duran, 2001). As with many IEM, these seizures are resistant to common anticonvulsants, at least during the initial stage of disease. Many patients show additional signs of encephalopathy, truncal hypotonia, and brisk reflexes and their condition may be mistaken for hypoxic ischaemic encephalopathy. EEG records show multifocal spike-wave activity or a burst-suppression pattern. The MRI may give a clue to diagnosis, revealing generalised brain oedema in the early stage, and a distinctive pattern of widespread restricted diffusion involving the cortex at the depths of sulci, followed by extended cystic changes of the white matter and global brain atrophy (Stence *et al.*, 2013). Few patients have shown association with different primary brain malformation. Beyond infancy, a number of ophthalmologic complications have been encountered with disorders, such as lens subluxation, optic atrophy, or nystagmus.

The underlying pathomechanism is directly related to sulfite toxicity, with a neuro-excitatory action on the nervous system. Though molybdenum is a cofactor of xanthin oxidoreductase and aldehyde oxidase, as well as sulfite oxidase, it is impairment of the latter enzyme that causes the CNS phenotype in MOCOD, as well as in ISOD. Impaired oxidation of xanthin leads to decreased uric acid in plasma in most patients with MOCOD, but of course, not in those with ISOD. Elevated sulfite in urine occurs in both disorders, but commercially available test sticks have given both false-negative and false-positive results (*table 3*). Thus, determination of urinary S-sulfocysteine is the actual gold standard to test for both disorders. Recently, elevation of AASA, the biomarker for antiquitin deficiency, has been found elevated in patients with MOCOD and ISOD, which is most likely due to secondary inhibition of AASA dehydrogenase by accumulating sulfite. ISOD, as well as MOCOD, follows autosomal recessive traits and can be confirmed by respective enzyme studies in cultured fibroblasts. While ISOD is encoded by the *SUOX* gene, MOCOD can be caused by defects in either of the three genes involved in its synthesis. About two thirds of MOCOD patients have defects in the *MOCS 1* gene, encoding the first precursor (cPMP) in the MoCo biosynthetic pathway, and are designated MOCOD type A patients. This prevalent type A patient group may be amenable to intravenous treatment with synthetic cPMP, which should be installed as soon as biochemical results suggest MOCOD, even if genotyping is pending (Veldman *et al.*, 2010). For MOCOD type B patients, harbouring mutations in the *MOCS2* gene or, in some cases, the *GEPH* gene, treatment is purely symptomatic. Dextromethorphan, an NMDA receptor antagonist, as well as dietary restriction of sulphur-containing methionine, has shown some benefit in individual patients, as has pyridoxine in those patients with elevated AASA. □

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TEST YOURSELF

(1) Prenatal or postnatal hiccup is more common in which of the following?
A. MSUD
B. Serine deficiency
C. Non-ketotic hyperglycinaemia
D. Atypical PKU
E. MOCO

(2) In a newborn with refractory epileptic spasms and focal myoclonic, tonic or bilateral tonic-clonic seizures of unknown cause, within hours or days after birth, a treatment trial should be initiated with which of the following?
A. Phenobarbital
B. Pyridoxine
C. Tiamine
D. Dextrometorphan
E. Serine

(3) Which one of the following IEM does not start with seizures in the early newborn period?
A. Urea cycle defects
B. Maple syrup urine disease
C. Pyridoxine-dependent epilepsy
D. Pyridoxal 5'-phosphate-dependent seizures
E. Glut-1 transporter deficiency

Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".