National Metabolic Biochemistry Network
Best Practice Guidelines

The Biochemical Investigation of Fits and Seizures for Inherited Metabolic Disorders

Introduction

These guidelines describe the differential diagnosis of biochemical causes of severe neurological crises presenting as fits/seizures. They suggest an approach to diagnosis but do not include an exhaustive list of all possible defects.

It is useful to note that fits may well be associated with febrile disorders but intercurrent illness can also provoke a metabolic crisis in affected children. This is frequently the case for disorders of fatty acid oxidation. However it is important to appreciate that between 2 and 5% of the general population suffer from some limited form of epilepsy. Whilst there is commonly a hereditary basis, monogenic disorders together form a small subset of less than 30%. The most common group of inherited epilepsies are the channelopathies which are not further discussed in this guideline.

The Guidelines

Clinical history: take careful note of age at onset, prematurity or birth trauma, infections, intercurrent illness, therapy (especially chemotherapy or valproate), severity, EEG changes, family history. Specific signs and symptoms can be helpful in directing further testing e.g. a history of in utero hiccoughs can represent antenatal seizures; seizures post fasting may suggest hypoglycaemia; Zellweger syndrome presents with characteristic dysmorphic features and a number of lysosomal storage disorders present with cherry red spot on fundoscopy or vacuolated lymphocytes on a peripheral blood film. Be particularly aware of intractable seizures that are resistant to multiple antiepileptic drugs as the possibility of a metabolic disease will be high.

Seizures are a secondary phenomenon in many serious metabolic disturbances. Note particularly any evidence of hypoglycaemia, hypocalcaemia, metabolic acidosis or hyperammonaemia. If present then follow relevant investigation protocol – see separate guidelines.

<u>First Line Investigations</u>

These are essentially the starting point for investigating potential metabolic disturbances:-

Routine biochemistry:
Sodium, potassium and calcium (serum/plasma)
Blood gases
Ammonia (plasma)
Urate (serum/plasma)
Lactate (serum/plasma/CSF)

Metabolic Investigations:
Organic acids (urine)
Amino acids (plasma/CSF)
Acylcarnitines (bloodspot or plasma)

Second Line Investigations

When nothing helpful has been forthcoming from the initial tests but the symptoms persist and there is still concern about the possibility of a metabolic disorder, further investigations should be considered. Which disorders and tests should be considered depends on the clinical context and these investigations may also be indicated at the outset if there are specific clinical signs (see Table below). The age at presentation is a guide only - considerable overlap is observed in practice.

Please note;

- At this stage a panel of lysosomal enzymes may be tested to investigate lysosomal storage disorders. The most relevant enzymes are α-and β- mannosidase, α-fucosidosidase, α-neuraminidase (sialidosis), β-galactosidase (GM1 gangliosidosis) and β-hexosaminidase (Tay-Sachs disease). Please ensure you are clear on what is contained in the leucocyte enzyme panel as these may differ between labs and not all relevant enzymes may be included.
- The following list does not contain all the disorders that should have been detected via the first line investigations outlined above, e.g. urea cycle defects and acute organic acidurias.

For further information about the availability or sample requirements for individual tests access the Metabolic Assay Directory on the MetBioNet website (www.metbionet).

Disorder	Supporting Clinical Signs	Biochemical Test	Gene (s) affected
Neonatal/early onset			
Aromatic amino acid decarboxylase	Mental retardation, movement disorders, hypotonia, recurrent hyperthermia, hypersalivation, bulbar symptoms, temperature instability.	Vanillactic acid/organic acids (U), Neurotransmitters (CSF)*	DDC
Asparagine synthesis defects	Microcephaly, hypotonia, severe psychomotor retardation, hyperekplexia.	Amino acids (P) (NB may not be abnormal in all patients)	ASNS
Biotinidase deficiency	Alopecia, skin rashes, hypotonia.	Biotinidase (S), organic acids (U), acyl carnitines (DBS)	BTD; HLCS
GLUT 1 deficiency	Slow head growth, microcephaly.	Glucose (CSF) (also ratio to plasma).	SLC2A1
Glutamine synthesis defects	Encephalopathy, hypotonia.	Amino acids (P), Ammonia (P).	GLUL
Glutaric acidaemia type 1	Macrocephaly, dystonia.	Organic acids (U), Acylcarnitines (DBS) (P) (NB. these are not always positive & it may be necessary to assay the enzyme in cultured fibroblasts or perform mutation analysis).	GCDH
'Classical' homocystinuria	Hypotonia, microcephaly.	Total homocysteine (P), amino acids (P)	CBS
Isolated sulphite oxidase deficiency	Lens dislocation.	Amino acids (P)(ask specifically for sulphocysteine)	SUOX
Lipoic acid synthetase deficiency	Hypotonia, progressive encephalopathy, apnoea.	Amino acids (P)(CSF) NB Paired sample required, lactate (P)	LIAS
Menkes syndrome	X-linked disorder, kinky hair, hypothermia, developmental delay.	Copper (P), caeruloplasmin (S)	ATP7A
Molybdenum cofactor deficiency	Lens dislocation.	Urate (P), Amino acids (P)(ask specifically for sulphocysteine)	MOCS1; MOCS2; GPHN
Non-ketotic hyperglycinaemia	Hypotonia, apnoea, burst suppression on EEG.	Amino acids (P) (CSF) NB Paired sample required.	GLDC; AMT; GCSH
Peroxisomal defects of β-oxidation and organelle genesis	Dysmorphism, hypotonia, liver dysfunction.	Very long chain fatty acids (VLCFA) (P)	ABCD1; ACOX1; AGPS; AGXT; AMACR; DNM1L; GNPAT; HSD17B4; PEX1; PEX2; PEX3; PEX5; PEX6; PEX7; PEX10; PEX11B; PEX12; PEX13; PEX14; PEX16; PEX19; PEX26;PHYH; SCP2
Pterin disorders	Intellectual disability, movement disorders,	Amino acids (P), pterins (U) (B), DHPR (B),	GCH1; PTS; SPR; QDPR; PCBD1

Disorder	Supporting Clinical Signs	Biochemical Test	Gene (s) affected
	hypotonia, recurrent hyperthermia, hypersalivation, bulbar symptoms.	Neurotransmitters (CSF)*	
Pyridoxal phosphate responsive seizures	Pyridoxine unresponsive but responds to pyridoxal phosphate.	Vanillactic acid/organic acids (U), Neurotransmitters (CSF)* Amino acids (CSF)	PNPO
Pyridoxine responsive seizures (Alpha-amino adipic semialdehyde dehydrogenase deficiency)	Responds to pyridoxine may take up to four weeks.	Alpha-amino adipic semialdehyde (α-AASA) (U). Neurotransmitters (CSF)*, Amino acids (CSF)	ALDH7A1
Pyridoxine responsive seizures due to congenital hypophosphatasia	May present with anti convulsant resistant seizures that respond well to pyridoxine before skeletal signs are apparent.	Alkaline phosphatase (ALP) (S) Amino acids (U), Amino acids (U) (ask specifically for phosphoethanolamine), PLP (pyridoxal 5'-phosphate)(P)	ALPL
Serine synthesis defects 3-phosphoglycerate dehydrogenase deficiency	Microcephaly, psychomotor retardation.	Amino acids (CSF)	PHGDH Consider PSAT1; PSPH
Tyrosine hydroxylase deficiency	Oculogyric crises, movement disorders, Parkinsonian symptoms, hypotonia.	Neurotransmitters (CSF)*	TH
γ-Aminobutyrate transaminase deficiency	Psychomotor retardation, hypotonia.	CSF GABA*	ABAT
Later infancy/early childhood – in addition to the above			
Cobalamin disorders		Total homocysteine (P), organic acids (U), amino acids (P)	MMACHC; HCFC1; C2orf25; MMAA; MTRR; MTR
Congenital disorders of glycosylation (CDGs)	Unusual distribution of sub cutaneous fat, strokes, ataxia, atrophy of cerebellum, clotting abnormalities, dysmorphism.	Transferrin isoforms (S) (NB will not identify all CDGs)	See reference 7.
Creatine synthesis disorders - (GAMT) Guanidinoacetate methyltransferase - (AGAT) Arginine:glycine amidinotransferase	Intellectual disability, developmental delay (particularly of speech and language), extrapyramidal symptoms (GAMT only), behavioural problems, myopathy (AGAT only)	Creatine/GAA (U)(P), brain MRS	GAMT; GATM
Creatine transporter (SLC6A8) defect	Intellectual disability, developmental delay (particularly of speech and language), behavioural	Creatine (U), brain MRS	SLC6A8

Disorder	Supporting Clinical Signs	Biochemical Test	Gene (s) affected
	problems.		
Gaucher type 2	Hepatosplenomegaly	Chitotriosidase (P) (nonspecific), β-glucosidase (L)	GBA
2-hydroxyglutaric aciduria (D-2OH glutaric aciduria, L-2OH glutaric aciduria and D/L-2OH glutaric aciduria	Psychomotor developmental delay (see reference 2 for further detail between types).	Organic acids (U) (NB specific chiral studies needed to differentiate between D, L and D/L forms).	D2HGDH; IDH2 (D form) L2HGDH (L form) SLC25A1 (D/L form)
Hyperprolinaemia Type II	Primary generalised seizures of varying severity. May be associated with intellectual disability.	Amino acids (P) (U), organic acids (U)	ALDH4A1
Neuronal Ceroid Lipofucinoses CLN 1,2 (Batten's Disease)	Visual loss, retinitis pigmentosa, dementia.	CLN1 leucocyte palmitoyl protein thioesterase (PPT) CLN2 leucocyte tripeptidyl peptidase I (TPP) (L), (B) EDTA can also be useful to look for vacuolated lymphocytes.	PPT1; TPP1 MFSD8; CLN8; CTSD; CTSF; CLN5; CLN6; CLN3; GRN; DNAJC5; CLN9
Niemann-Pick disease type C	Hepatosplenomegaly. supranuclear, ophthalmoplegia.	Oxysterols (P), filipin staining and/or cholesterol esterification (fibroblasts).	NPC1
Purine and pyrimidine disorders	Psychomotor retardation, cerebellar hypoplasia, microcephaly, feeding difficulties.	Organic acids (U), purines and pyrimidines (U) (P)	ADSL; ATIC; PRPS1; DPYD; DPYS; UPB1; DGUOK; RRM2B
Respiratory chain defects	Hypotonia, ptosis. Brain stem abnormalities.	Lactate (P) (CSF) NB lactate accumulation can be confined to CNS (i.e. elevated CSF lactate), Respiratory chain enzymes/histology (muscle biopsy).	See reference 6.
Succinic semialdehyde dehydrogenase deficiency (4-OH butyric aciduria)	Dev delay, early-onset hypotonia, late-onset expressive language impairment, hyporeflexia, ataxia.	Organic acids (U)	ALDH5A1
Later childhood – in addition to the above			
Acute porphyrias	Presentation usually after puberty, acute abdomen, psychosis.	PBG (U)	HMBS; PPOX; ALAD; CPOX
CLN3 (Juvenile Battens Disease)	Visual loss, retinitis pigmentosa, dementia.		CLN3
Disorders of folate metabolism		Discuss with your specialist laboratory – see metabolic assay directory.	SLC46A1; FOL1R; DHFR; MTHFR

Disorder	Supporting Clinical Signs	Biochemical Test	Gene (s) affected
Gaucher disease type 3	Hepatosplenomegaly, dystonia	Chitotriosidase (P) (nonspecific), β-glucosidase (L)	GBA
Lafora disease	Intellectual decline and early death.	Demonstration of storage material in tissue biopsy.	NHLRC1; EPM2A

(B) - Blood, (L) - Leucocytes, (P) - Plasma, (S) - Serum, (U) - Urine

* Before sample collection and storage refer to CSF instruction sheet from The Neurometabolic Unit, National Hospital for Neurology and Neurosurgery, London UK. A copy is available from the MetBioNet website: http://www.metbio.net/docs/MetBio-Guideline-TABA411857-30-01-2016.pdf

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Further reading:

1. Physician's Guide to the Diagnosis, Treatment and Follow-Up of Metabolic Diseases. N. Blau, M.Duran, K.M. Gibson and C. Dionisi-Vici Ed.s. 2014 Springer

2. Inborn Metabolic Diseases, Diagnosis and Treatment, 6th edition. J.M. Saudubray, M R Baumgartner and J.H. Walter Eds 2016 Springer

3. Noebels JL. The Inherited Epilepsies, in The Metabolic and Molecular Bases of Inherited Disease. Ed Scriver CR et al. 8th ed. 2001 pp 5807-5832.

4. Bernard BS and Lowenstein DH. Mechanisms of Disease; Epilepsy. NEJM 2003,349;13,1257-66.

5. Rahman S, Footitt EJ, Varadkar S, Clayton PT. Inborn errors of metabolism causing epilepsy. Developmental Medicine & Child Neurology 2012, 55: 23-36

6. GeneReviews® [Internet]. Mitochondrial Disorders Overview Pagon RA, Adam MP, Ardinger HH, et al., editors. Last Update: August 14, 2014. https://www.ncbi.nlm.nih.gov/books/NBK1224/

7. Jaeken J, Péanne R. What is new in CDG? J Inherit Metab Dis (2017) 40:569-586.

Version Number 05

Disclaimer

These are laboratory guidelines reflecting current best practice in specialist metabolic laboratories the UK. They are not evidence based but reflect expert opinion. The network cannot accept any responsibility for use of these guidelines.