HELENIC SOCIETY FOR THE STUDY OF INBORN ERRORS OF METABOLISM

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Inborn Errors of Metabolism

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Abstracts
The availability during the last decade of technology that allows detection of a large number of inherited metabolic disorders in blood spots is an important advance but has created a number of challenges for scientists, physicians and health service planners.

These challenges include:

- the selection of which disorders to screen for and which criteria should be applied to decide this
- choosing the best setting for laboratories that perform the screening tests bearing in mind that newborn screening is much more than just a laboratory test
- deciding what is necessary for pretest counselling and how to provide this bearing in mind that screening tests for genetic disorders and may involve DNA testing as in the case of Cystic Fibrosis
- selection of cut-off points for positive cases to minimise false positive results and provision of rapid efficient confirmatory testing
- how to guarantee the provision of optimum management of treatable disorders including financial support for expensive therapies
- deciding what to do in the case of probable benign disorders
- how to maximise knowledge on treatment outcome

The way to meet these challenges will be steered by several factors including local infrastructures and circumstances, social attitudes and the nature of health care systems.

A survey of the situation in European countries within the Eurogentest project, supported by a 2007 publication (Journ Inher Metab Disease, 2007; 30:439-444) shows that the number of disorders screened for at that time ranged from 2 to 21 and was carried out in different settings such as private labs carrying out testing with separate clinical management or screening labs fully integrated within paediatric departments of University hospitals.

Whatever the laboratory setting, competent personnel trained in both the technology and the diseases, use of external quality control such as that provided by CDC or ERNDIM, moves towards accreditation awarded by a competent accrediting body to an accepted international standard, adherence to best practice guidelines where available and establishment of strong links to clinical, nursing and dietetic services are some of the steps needed to ensure the maximum quality of services in this rapidly expanding field.
[THE IMPACT OF EARLY DIAGNOSIS]

Making a diagnosis of an inherited metabolic disease (IMD) can often be difficult in view of the rarity of individual disorders and the specialist investigations required. However the biochemical disturbances associated with IMDs, if unrecongnised, can be extremely harmful. Certain conditions are amenable to treatment and if this is given urgently, before irreversible damage has occurred, the long term prognosis is considerably better. For an increasing number of IMDs newborn screening provides an opportunity to achieve this. There is compelling evidence that for Phenylketonuria, Homocystinuria, Medium Chain Acyl-CoA Dehydrogenase deficiency, Tyrosinaemia, Glutaric Aciduria Type 1 and Biotinidase deficiency, that treatment commenced shortly after birth can markedly improve the outcome for affected patients. For other disorders the clinical benefits may be less clear.

If the specificity of a screening test is poor then there will be a large numbers of false negative results, leading to considerable resource implications for both clinicians and laboratory scientists and undue anxiety for parents. Such factors must be taken into consideration. For any screening programme clinical services must be available to enable rapid treatment, family counselling and long term follow up.
Inborn errors of metabolism (IEM) share a number of features, despite their diversity of gene defects. The majority of patients present clinically with one of the phenotypes: acute or progressive encephalopathy, primary muscle disease, primary liver disease or primary renal disease. Encephalopathy is the most common clinical manifestation of IEM, and may be a presentation with lethargy/coma, or with seizures, or a combination of lethargy and seizures. When dysmorphism is associated with neurological abnormality, metabolic disorders with brain malformation should be searched, such as peroxisomal disorders, mitochondrial disorders, carbohydrate deficient glycoprotein disorders, and glutanic aciduria type 2.

The differential diagnosis of a neonate with seizures and/or hypotonia in the absence of any biochemical abnormality may be very difficult when the manifestation itself is not well defined. Treatable IEM with neonatal seizures are disorders such as: pyridoxine-dependent epilepsy, glucose transporter 1 deficiency, cerebral creatine disorders, serine biosynthesis disorders. Non-treatable IEM with neonatal seizures may be glycine encephalopathy, mitochondrial disorders, peroxisomal disorders, congenital defect of glycosylation, and molybdenum cofactor deficiency.

Especially the symptom hypotonia has to be discussed in the context of the clinical presentation. A clinical distinction should be attempted between IEM with “hypotonia” and the multiple other causes of hypotonia in the neonate, such as congenital myasthenic syndromes, syndromes as Prader-Willy syndrome, and primary neuromuscular disorders as spinal muscular atrophy with respiratory distress. The types of IEM and their mode of presentation in the newborn are discussed, with particular emphasis on those disorders that present in the newborn period with an encephalopathy.
Inborn errors of metabolism comprise a large class of genetic diseases involving disorders of metabolism. The majority is due to defects of single genes that code for enzymes that facilitate conversion of various substances (substrates) into others (products). In most of the disorders, problems arise due to accumulation of substances, which are toxic or interfere with normal function, or to the effects of reduced ability to synthesize essential compounds. Inborn errors of metabolism are now often referred to as congenital metabolic diseases or inherited metabolic diseases.

Inherited metabolic diseases often present around the time of birth or in infants and children in the acute setting. In view of the major improvements in treatment, it has become increasingly important for first-line physicians especially neonatologists and pediatricians that in order not to miss a treatable disorder to be able to initiate a simple method of clinical screening, particularly in the emergency room. We present a simplified classification of inborn errors of metabolism in three groups.

Group 1 includes inborn errors of intermediary metabolism resulting in acute or chronic intoxication. It encompasses aminoacidopathies, organic acidurias, urea cycle disorders, sugar intolerances, metal disorders and porphyrias. Clinical expression can be acute or systemic or can involve a specific organ, and can strike in the neonatal period or later and intermittently from infancy to late adulthood. Most of these disorders are treatable and require the emergency removal of the toxin by special diets, renal replacement procedures, cleansing drugs or vitamins.

Group 2 includes inborn errors of intermediary metabolism that affect the cytoplasmic and mitochondrial energetic processes. Cytoplasmic defects encompass those affecting glycolysis, glycogenesis, gluconeogenesis, hyperinsulinisms, and creatine and pentose phosphate pathways. Mitochondrial defects include respiratory chain disorders, Krebs cycle and pyruvate oxidation defects, mostly untreatable, and disorders of fatty acid oxidation and ketone bodies that are treatable.

Group 3 involves cellular organelles and includes lysosomal, peroxisomal, glycosylation, and cholesterol synthesis defects. Some of them are treatable.

Simple clinical algorithms based upon the leading clinical and biological abnormalities will be presented and the principles of treatment for each group of disorders will be discussed.
Late onset forms of IEM presenting initially in adulthood are often unrecognized, consequently their exact prevalence is unknown. Most often they have psychiatric or neurological manifestations, including atypical psychosis or depression, unexplained coma, peripheral neuropathy, cerebellar ataxia, spastic paraparesis, dementia, movement disorders, epilepsy and multi-system diseases. Adult physicians caring for patients with IEM are also involved in the management of those with early onset forms who reach adulthood. The transfer of such patients from paediatric to adult care raises a number of medical, dietetic and social concerns.

A further important issue is the diagnosis of adult patients who had their first clinical signs in childhood but for whom the diagnosis was missed either because IEM were not considered or because the disease or its mild clinical form had not been described at that time.

Adult physicians who want to specialize in IEM are faced with the fact that with the exception of several review articles, most if not all existing books and diagnostic algorithms refer to paediatric forms of these diseases. Late onset forms of IEM always display attenuated phenotypes that, in some instances, are associated with one or more clinical manifestations that differ from the classic clinical picture described in children. Although, the limited information about adult forms of IEM makes the speciality new and quite exploratory, the diagnostic approach in adults is facilitated by the fact that the nervous system is already mature. Therefore, clinical presentations are more homogenous than in children where clinical signs usually differ depending on their stage of maturation.

The typical situation is that of a patient with an unexplained and bizarre neurological or psychiatric problem and in whom the usual aetiologies have been excluded by appropriate tests. The diagnostic approach in such a situation is always based on two questions: When to suspect an IEM? and when an IEM is suspected, what type of metabolic investigations must be performed?

Some general clinical features are highly suggestive of an IEM: when clinical signs or symptoms are fluctuating, especially when triggered by fasting, exercise, fever, catabolic circumstances or post partum; when clinical signs suggest a diffuse disease including neurological signs plus systemic signs (eyes or skin problems, organomegaly etc.) or involvement of different parts of the nervous systems (optic nerves and cerebellum; leukoencephalopathy and polyneuropathy etc.).

Metabolic diseases involving the nervous system may fall into 5 main categories, each of which display some similarities in clinical presentation, diagnostic methods and treatment strategies.

**Energy metabolism defects** include respiratory chain disorders (that can be primary or secondary as can occur in organic acidurias), pyruvate dehydrogenase deficiency, Krebs cycle deficiencies, GLU1 deficiency, β oxidation defects and disorders involving co-factors such as ETF deficiency, vitamin E deficiency, biotinidase deficiency, biotin responsive basal ganglia disease, creatine deficiency syndromes and CoQ synthesis defects. Acute manifestations are often triggered by infections and encompass Leigh syndrome, acute optic neuropathy, acute cerebellar ataxia or pseudo-strokes. Chronic presentations often involve muscles, cerebellum, basal ganglia (Parkinsonism), cortex (epilepsy, myoclonus) or the peripheral nervous system (axonal polyneuropathie). In adults, these diseases rarely involve the brain white matter and spasticparaparesis is very uncommon.
Lipid metabolism disorders include some lysosomal diseases, mainly sphingolipidoses (Krabbe’s disease, metachromatic leukodystrophies, Niemann Pick A, B and C, Fabry disease and Gaucher disease), peroxisomal disorders (adrenomyeloneuropathy, Refsum disease, disorders of pristanic acid metabolism, peroxisome biogenesis disorders), Tangier disease, and cerebrotendinous xanthomatosis. Given the huge proportion of lipids in the nervous system, these diseases can produce almost all kinds of symptoms. Leukodystrophies and demyelinating polyneuropathies are hallmarks of disorders interfering with myelin formation or maintenance. A past history of prolonged neonatal jaundiced is suggestive of disorders of cholesterol and bile acids metabolism. Splenomegaly is highly suggestive of some lipid storage diseases such as Niemann Pick C, Gaucher disease, Tangier disease and Niemann Pick A or B. Other presentations are less specific: cerebellar ataxias, dementia, psychiatric disorders, epilepsy, spastic paraparesis. The slow progression of symptoms, corresponding to progressive lipid storage, is very suggestive of these disorders.

Intoxication syndromes include porphyrias, urea cycle defects, organic acidurias and amino acidopathies. The occurrence of acute symptoms that accompany the metabolic crisis is very characteristic. However in mild adult forms, symptoms can be progressive, giving rise to leukoencephalopathies, epilepsy, psychiatric disorders, or spastic paraparesis.

Neurotransmitter metabolism disorders are mostly represented by defects in the synthesis of serotonin and dopamine. Clinical signs are related to dopamine deficiency (dystonia, Parkinsonism, oculogyric crisis), noradrenergic deficiency (ptosis, myosis, hypotension) or serotonin deficiency (sleep disturbance, dysthermia, behavioural disturbance). Dopa-responsive dystonia or Parkinsonism is highly suggestive. Diurnal fluctuations of symptoms are also characteristic with improvement in the morning and worsening during the day. Diagnosis of these disorders relies on analysis of neurotransmitter metabolism in the CSF. Cerebral folate deficiency can be added to this group because it shares several clinical features and diagnostic methods although this syndrome is still highly heterogeneous.

Metal storage disorders include Wilson’s disease, neuroferritinopathy, aceruloplasminemia, PANK2 associated neurodegeneration, PLA2G6 and recently identified disorder of manganese metabolism. The hallmark of these diseases is the metal deposits that occur in the basal ganglia and that are visible on brain MRI. The main presentations are movement disorders because of the primary involvement of the basal ganglia. Treatments, when they exist, are mainly based on metal chelators.
**[BIOCHEMICAL INVESTIGATIONS OF METABOLIC DISORDERS]**

**Key Facts**

- Evidence for the presence of an inherited metabolic disease may often be derived from detailed clinical evaluation of the patient and examination of the family history.
- Important stumbling blocks in identifying an inherited metabolic disease include the fact that signs and symptoms are often non-specific, leading to initial testing to exclude routine childhood illnesses and delaying consideration of metabolic disorders.
- Even when appropriately suspected, ordering physicians may be unfamiliar with important biochemical interrelationships and the appropriate diagnostic tests to order, occasionally leading to inappropriate sample collection and storage.
- An absence of acute metabolic decompensation (e.g., hyperammonemia, hypoglycemia, overwhelming metabolic acidosis, anion gap) does not necessarily rule out an inherited metabolic disease.
- Consultation and coordination with a licensed Clinical Biochemical Genetics laboratory helps to insure that appropriate tests are ordered, the correct samples are obtained, and the limitations of the testing scheme are clearly defined prior to metabolic work-up.

**General Remarks**

Most known inherited metabolic diseases are identified via biochemical analyses of various body fluids, predominantly blood and urine, but also cerebrospinal, vitreous and even bile fluids. Concentrations of physiologically relevant metabolites in plasma or serum are generally tightly controlled, and thus increases/decreases of specific intermediates may have diagnostic relevance. Normative data for many compounds of intermediary metabolism are highly dependent upon the metabolic state at sampling, and appropriate interpretation of assay results requires knowledge of intake and other physiological data, including fasting or postprandial status or post-exercise status. Some disorders may only be identified through specific function tests (e.g., loading) that stress metabolic conditions or result in supraphysiological increases in metabolite load. Such tests have inherent risks and escalate the potential for metabolic overload and decompensation; accordingly, such tests should only be instituted by experienced clinicians in the appropriate hospital setting, and only when other diagnostic options which carry less patient risk have been exhausted.

Many inherited metabolic disorders induce the accumulation of substrates which are either metabolized via alternative processes (alternate pathways, liver biotransformation) and/or removed via excretion in the urine. Studies carried out in urine for many such diseases may be more straightforward and sensitive than plasma/serum analyses. Differences in fluid intake and urinary dilution, and their effect on metabolite concentrations, are usually accounted for by correcting urine metabolite levels with creatinine output. Urine analyses are generally less influenced by metabolic and nutritional changes, since the specimen is collected over a time period and often (but not always) there are significant differences between normal and pathological values that are readily recognized. As a general rule, a spot urine sample (morning void to enhance metabolite concentrations) is sufficient for most studies. Exceptions may occur, however, as in the case of some fatty acid oxidation disorders that frequently show urinary abnormalities only under fasting or loading conditions, or in the case of certain disorders (e.g., cystinuria, porphyrias, etc.) where a 24-hour urine collection may be required.
Laboratory investigations for inherited metabolic diseases are complex and susceptible to technical problems. To maintain acceptable standards, laboratories performing biochemical investigations for inherited metabolic diseases should process a sufficiently high number of samples to maintain diagnostic acumen, and should participate in quality assurance/quality control (QA/QC) processes. Referring physicians should bear in mind that many analyses are often qualitative and not quantitative (although the expanding implementation of tandem mass spectrometry (MS-MS) is changing this paradigm), thereby leading to a certain level of subjective interpretation. Furthermore, the conditions examined are biochemically heterogeneous in their expression, which can complicate identification of subtle abnormalities. For these reasons, diagnostic laboratories must adhere to accepted practices of internal and external QC schemes, which insure ongoing education of laboratory staff and competence in analytical performance. External schema are particularly important, providing data on accuracy and bias of results. Participation in external QC schemes (and acceptable performance) are often a requirement for external accreditation of the laboratory. ERNDIM (European Research Network for Inherited Disorders of Metabolism) offers proficiency testing for urine and blood amino acids, urine organic acids, plasma acylcarnitines and qualitative mucopolysaccharide analyses, proficiency testing for purine and pyrimidine analysis, and a more extensive menu of special assays in urine and blood, and have recently instituted a pilot program for lysosomal enzyme analysis. Depending on the country or region, laboratory accreditation may be optional or mandatory.

**Table An overview of metabolic investigations**

- Routine clinical chemistry in blood (e.g. glucose, ammonia, bloodgases etc.)
- Simple colorimetric evaluations in urine
- Amino and organic acids, carnitine and acylcarnitines
- Lactate, pyruvate, non-esterified fatty acids and ketones
- Congenital disorders of glycosylation (CDG)
- Purines and pyrimidines, orotic acid
- Sugars and polyols
- Glutathione
- Mucopolysaccharides and oligosaccharides
- Very long chain fatty and pristanic acids (peroxisomal function)
- Creatine and folate
- Sterols, bile acids and porphyrins
- Biogenic amines and pterins

**Legend to Table:** The first four categories form the core tests for any patient suspected of having an inherited metabolic disease (e.g., baseline selective screening). The remaining test groups rely more heavily on enhanced clinical suspicion.
**CSF INVESTIGATIONS FOR THE DIAGNOSIS OF NEUROMETABOLIC DISEASES**

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**Neurometabolic Diseases**

**Where to fish?**

*Know Where To Fish*

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**Prof. Ron Wevers**

Dept. of Laboratory Medicine

Radboud University Nijmegen

Medical Centre

Nijmegen, The Netherlands

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**Do not miss a treatable neurometabolic disease**

1. Cerebrospinal fluid provides a diagnostic window on the brain
2. Little CSF is obtained
   Mostly only once
   Various advanced techniques required
3. Preserve every drop (~ 60°C)
4. Cave: concentration gradient
   pH increases rapidly \( \rightarrow > 10 \)
5. Next generation sequencing: preserve DNA

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**Glucose transporter type 1**

- passive glucose transporter (facilitated diffusion)
- solute carrier family 2, member 1; SLC2A1; GLUT1
- in BBB (endothelium) and in membrane of erythrocytes
- tetramer

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**Clinical signs in Glut1 defect**

Leen W et al. Brain 2010

**Phenotype variability**

- Classical [44%] refractory epilepsy (< 2 yrs)
  developmental delay (60%)
  movement disorder
  microcephaly (44%)
- Non classical type 1 [15%] similar but no epilepsy
- Non classical type 2 paroxysmal exercise induced dyskinesia in adults

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**Genetics of Glut1 defect**

- 50% of residual Glut1 activity gives clinical signs
- Most cases have only 1 mutation
- Majority: autosomal dominant
  Exception: autosomal recessive

**Mutations Nijmegen series**

57 patients, 49 different mutations
5 known mutations
Novel:

- 43 missense
- 15 nonsense
- 13 frame shift
- 4 splice site
- 2 translation initiation codon
- 6 multiple exon deletions

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International Symposium

Inborn Errors of Metabolism

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24
Lumbar puncture Glut1 cases (n=54)

<table>
<thead>
<tr>
<th></th>
<th>Classical (n=46)</th>
<th>Non-classical (n=8)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF glucose</td>
<td>0.3 – 2.4</td>
<td>1.5 – 2.0</td>
<td>2.5 – 5.7</td>
</tr>
<tr>
<td>Glucose CSF/blood</td>
<td>0.19 – 0.52</td>
<td>0.33 – 0.47</td>
<td>0.50 – 0.60</td>
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<tr>
<td>CSF Lactate</td>
<td>0.6 – 1.5</td>
<td>0.7 – 1.1</td>
<td>1.3 – 1.9</td>
</tr>
</tbody>
</table>

Take home message
Every child with unexplained drowsiness deserves a fasting lumbar puncture to exclude this treatable condition.

Glut1 treatment
The ketogenic diet

Biogenic amine metabolism
Tyrosine Hydroxylase deficiency

Concentration gradient in CSF

Neurotransmitter metabolites

Type A: ‘Simple’ extrapyramidal movement disorder
Williamson et al. Brain 2010; 36 cases

CSF findings in Tyrosine Hydroxylase deficiency

<table>
<thead>
<tr>
<th></th>
<th>Case SD</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HVA</td>
<td>0.17</td>
<td>400 – 700</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>1.51</td>
<td>125 – 250</td>
</tr>
<tr>
<td>Ratio HVA/5-HIAA</td>
<td>0.8</td>
<td>2.0 – 4.5</td>
</tr>
<tr>
<td>MHPG</td>
<td>1.5</td>
<td>28 – 64</td>
</tr>
<tr>
<td>L-Dopa</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>3-Methoxytyrosine</td>
<td>&lt; 5</td>
<td>&lt; 5</td>
</tr>
</tbody>
</table>

Rule in TH deficient cases: CSF HVA < 50% lower reference range limit.
**Type B: Complex encephalopathy**

*Williamson et al. Brain 2010; 36 cases*

**TH deficiency**

- motor retardation
- hypokinesia
- rigidity
- truncal hypotonia
- no diurnal fluctuation

**Key features**

- complex encephalopathy
- MRI abnormalities
- insufficient clinical response to low-dose L-Dopa
- nystagmus
- rhabdomyolysis
- prematurity
- paroxysmal sweating

**Biogenic Amine Metabolism**

*Aromatic L-amino acid decarboxylase deficiency*

**Case OW, 3 months**

Length, weight, head: P10 and ↓ to microcephaly
Psychomotor retardation
Extrapyramidal signs, dystonia
Feeding problems (poor drinking)
Convulsions (↑)

**Urine organic acid profile**

*Aromatic L-amino acid decarboxylase deficiency*
**Aromatic L-Aminoacid Decarboxylase deficiency**

- motor retardation / hypokinesia
- choreothetoid movements
- truncal hypotonia

- dystonia of limbs
- oculogyric crisis
- ptosis
- excessive sweating
- temperature instability
- mental retardation
- epilepsy
- impaired sympathetic blood pressure modulation
- increased burden of parental psychiatric disease

**The clinical message**

Dopamine biosynthesis defects (TH, AADC) present in the first year of life with (extreme) truncal hypotonia as most prominent symptom. Extrapyramidal signs (tremor, dystonia) may be observed but may also occur at a later stage.

So:
Consider neurotransmitter diseases in patients with infantile hypokinesia. CSF analysis should follow according to a standardized protocol.

**Folates and the brain**

A) Low CSF folate (and normal in blood)

- Kearns-Sayre syndrome (mtDNA depletion)
- Other mitochondrial disorders
- Defect in folate binding protein (Wevers et al. JINP 1954)
- Autoantibodies to folate receptor (Rameskars et al. NEJM 2006)

B) Folate responsiveness

- Some epileptic encephalopathies
- Pyridoxine dependent seizures (AASA, defect in ALDH1A1 antitoxin)

**Folate receptor alpha**

Steinfeld et al. AJHG 2009

- 3 Patients (onset ± 2yrs)
- progressive movement disorder (ataxia, pyramidal tract signs)
- psychomotor decline
- epilepsy
- profound hypomyelination
- depletion of choline (MRS)
- folic acid therapy

CSF folate < 5 nmo/L

(ref. 43 – 159)

Blood folate normal

**Dihydrofolate reductase**

Banke S et al. AJHG 2011 (not yet online)

- 3 Patients (presentation first months of life)
- severe anemia (→ pancytopenia)
- blood smear: oval macrocytes + microcytes + hypersegmented neutrophils
- seizures (partially controlled)
- cerebral + cerebellar atrophy

**Molecular mechanism DHFR defect**

Banke S et al. AJHG 2011 (not yet on-line)

BH₃₃ is cofactor in catecholamine biosynthesis

<table>
<thead>
<tr>
<th>Reference</th>
<th>CSF</th>
<th>BH₃₃</th>
<th>HVA</th>
<th>5-HIAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13/23</td>
<td>27 – 155</td>
<td>298/298</td>
<td>198/142</td>
</tr>
<tr>
<td></td>
<td></td>
<td>324 – 1098</td>
<td>342 – 1098</td>
<td>199 – 608</td>
</tr>
</tbody>
</table>

remains low after folic acid therapy

Blood folate, methyalaminic acid, Hcye and Phe were normal.
Inborn Errors of Metabolism

Therapy in dihydrofolate reductase defect
Folic acid
- Partial response to folate
- Immediate beneficial response to folic acid
  * anemia resolved
  * seizure control partial
  * BH4 dropped further (23 → 13; rel. 27 – 105)
  * 4 yrs therapy follow up microcephaly, ataxia and severe developmental delay
  Fetal DMR expression
  Adult DMR expression

Two sibs with unexplained neurological disease
1. NMR spectroscopy of CSF + urine, 2. preserve DNA
Cerebellar Ataxia with elevated CSF Free Stalic Acid
5 Patients (adult onset)
- cerebellar ataxia
- peripheral neuropathy
- cognitive decline
- movement changes
- cerebellar atrophy
- white matter affected (cerebellum periventricular region, periventricular level)

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Other treatable and easily missed neurometabolic disease
A) DEND
Developmental delay, epilepsy, neonatal diabetes
KCNJ11: A7P sensitive K-channel subunit
Treatment: Sulfonylurea

B) HI/Ha
Hyperinsulinism with Hyperammonemia
Dominant inheritance
Gain of function mutation in glutamate dehydrogenase
Treatment: Diazoxide

CSF techniques in neurometabolic disease
- Glucose
- Glut
- Lactate
- Pyruvate
- MA, 5-HIAA
- Serine and glycine
- HVA
- Homocysteine
- Neurons (TH, AADC)
- GABA
- Gly
- Glycine
- Serine
- Aspartate
- ammonia
- lactate
- pyruvate
- leucine
- isoleucine
- valine
- phenylalanine
- tyrosine
- homeostatic
- transport
- vasopressin
- kinin
- melatonin
- cortisol
- adrenal gland
- neuroendocrine
- hypothalamus
- pituitary gland
- pineal gland
- posterior pituitary
- anterior pituitary
- hypotalamus
- thalamus
- hypothalamus
- limbic system
- basal ganglia
- cerebellum
- brainstem
- spinal cord
- meninges
- blood-brain barrier
- cerebral spinal fluid
- urine
- blood
- sweat
- saliva
- cerebrospinal fluid
- urine
- blood
- sweat
- saliva
- cerebrospinal fluid
- urine
- blood
- sweat
- saliva

To apply NMR in CSF
in two sibs with unexplained neurological disease

REMAIN SHARP!
BLIJF SCHERP!

Do not miss a treatable neurometabolic disease!
Biopsies are a very important source of relevant information which can facilitate diagnostic processes. This is especially true in lysosomal storage diseases; examples include bone marrow analysis in acid sphingomyelinase deficiency, Niemann-Pick disease type C, and Gaucher disease, including disorders leading to development of Gaucher-like cells.

Skin biopsies are very useful for diagnosis of neuronal ceroid lipofuscinosis. For review of archived biopsy samples (liver biopsies), which may have been taken based on a suspicion of acid lipase deficiency (CESD type), there are several procedures using lysosomal markers, either luminal (cathepsin D) or membrane (LAMP1 and LAMP2), which work very well in standard routine paraffin sections.

Deficiency of lysosomal membrane protein (LAMP2 in Danon disease) can be screened using peripheral blood smears since leukocytes are rich in the LRO (lysosome related organelles) system which shares many components with classic degradative lysosomes. In all these examples, the pathologist’s effort is oriented to reaching diagnosis (“pathology before diagnosis”).

It should be stressed that pathology may also participate in unraveling the real nature of the already diagnosed disorders, many of which, despite clear definitions, are still not adequately understood. This should be, for contrast called “pathology after diagnosis”. Few examples in this sense will be given. One of the prerequisites for effective diagnostic pathology is close collaboration of pathologists with clinicians, biochemists, and molecular geneticists.
[THE ROLE OF MRI AND MRS IN NEUROMETABOLIC DISORDERS]

A couple of hundred different childhood and adolescent onset metabolic disorders are already known, most of them are genetically determined and many affect the metabolism of brain cells (neurometabolic disorders). Early diagnosis is important especially in those instances when treatment is possible. The majority of neurometabolic disorders has comparable clinical symptoms and characteristic neuroradiological features. Neurometabolic disorders can roughly be divided into grey matter and white matter disorders:

Grey matter disorders usually present with progressive loss of cognitive, motor as well as visual functions and often go along with epileptic seizures. The generalized cerebral and cerebellar brain atrophy is a characteristic finding in magnetic resonance imaging (MRI). The group of neuronal ceroid-lipofuscinosis is the prototype of grey matter disorders.

White matter disorders also called leukencephalopathies or leukodystrophies usually manifest with motor impairment. Seizures and cognitive impairment will follow later in the course. They exclusively or predominantly affect the white matter and its main component, the myelin. Thus, characteristic findings on MRI are hypomyelination, demyelination or dysmyelination defects.

Classical leukodystrophies include lysosomal storage disorders like metachromatic leukodystrophy and Krabbe disease, peroxisomal disorders like X-linked adrenoleukodystrophy and Zellweger syndrome, disorders of mitochondrial dysfunction like Leigh disease and MELAS and disorders of amino and organic acid metabolism like Canavan disease.

Besides long-term known neurometabolic disorders more than half of patients with a suspected neurometabolic disorder are unclear cases. Due to the impressive advances in the field of physics and biotechnology in recent years solving these unclear cases has improved. Especially, classical MR imaging as well as multiparametric MR investigations including localized proton MR spectroscopy (MRS), diffusion tensor imaging, and quantitative MR mapping of magnetization transfer have proved to be a powerful tool for characterization of cerebral metabolic abnormalities.
**GENETIC STUDIES: THE IMPACT OF NEW TECHNOLOGIES**

Technological advances in genetic studies during the last few years have opened up possibilities to rapidly generate large-scale sequencing data from different organisms at a reasonable cost. This new generation of non-Sanger-based sequencing technologies has delivered on its promise of sequencing DNA at unprecedented speed, thereby enabling impressive scientific achievements and novel biological applications, esp. in human genetics. The first signs of what might revolutionize the sequencing market appeared in 2005 with the landmark publication of the sequencing-by-synthesis technology developed by 454 Life Sciences and the multiplex polony sequencing protocol of George Church’s lab. Both groups used a strategy that greatly reduces the necessary reaction volume while dramatically extending the number of sequencing reactions. The strategy entailed arraying several hundred thousand sequencing templates in either picotiter plates or agarose thin layers, so that these sequences could be analyzed in parallel a huge increase compared to the maximum of 96 sequencing templates on a contemporary Sanger capillary sequencer. The sequencing-by-synthesis technology, which uses pyrosequencing for readout, initially started with a read length of 100 bp, which after 16 months on the market had increased to 250 bp. Recent developments have raised the mark again to more than 400 bp, approaching today’s Sanger sequencing read length of ~750 bp. Besides read length, the number of sequencing reads (or sequence tags) that can be produced in a single instrument run for a given cost is another important factor.

With a single run of a Roche (454) GS20 instrument, the analysis of 13 Mb of sequence from the nuclear genome of a 28,000-year-old mammoth became possible, thereby paving the way for the even more challenging project of deciphering the Neanderthal genome. Next-generation sequencing has also created applications that are immediately relevant to the medical field. In cancer genetics, for example, specific cancer alleles can now be detected in tissues through ultra-deep sequencing of genomic DNA, in instances where previous Sanger-based trails have failed. Short read length, initially deemed a major drawback of next-generation sequencing, becomes a blessing when the Sanger-based 700-bp read length is traded for a much larger number of sequence reads. As cancer genetics does not follow the path of Mendelian inheritance, laser-capture microdissection must be used for enrichment of the alleles of interest and targeted by sequencing of PCR products and/or amplicon sequencing while avoiding the traditional cloning and PCR biases. Despite having already enabled a plethora of studies using next-generation sequencing, scientists and engineers who are working on this technology—and the companies that commercialize the applications—still have a long to-do list of improvements. High on the list is cost reduction: a reduction of 1–2 orders of magnitude is needed to deliver on the promise of personal genomics, which targets a cost of $1,000 for the resequencing of a human genome.
Phenylketonuria (PKU) is the most prevalent clinically significant inborn error in amino acid metabolism. Untreated, PKU causes severe mental retardation, epilepsy, behavioural problems, and sometimes eczema like issues. After its discovery by Følling and elucidation of the biochemical background, the dietary treatment was invented by Woolf and Bickel, while the finding of the bacterial inhibition assay by Guthrie enabled neonatal screening. Due to this all, PKU was the first recognized entity in which mental retardation could be explained biochemically, could be treated, in which not a medicine but a diet was ‘the drug of choice’, and for which screening on a nationwide basis became possible.

Due to the combined effect of neonatal screening and treatment, however, PKU is now a biochemical rather than a clinical diagnosis. Today, neonatal screening programs identify PKU in almost all European countries. Initiation of a phenylalanine-restricted diet very soon after birth prevents most—but not all—of the neuropsychological complications. At present, we do not know what the reason is for this still suboptimal outcome, but it may relate to poor control that is common, especially in adolescent and adult years. Due to the fact that the outcome looked fine, that means mental retardation can be clearly prevented, PKU has not raised much interest for many years. Most energy was directed to maternal PKU. This is the condition in which the fetus is damaged by the PKU of its mother when not treated well enough. Optimal treatment consists of very stringent restriction of natural protein with the amino acid supplement starting clearly before conception to start in an optimal environment.

Now, it is clear that neurocognitive outcome can still be improved, and probably quality of life, nutritional condition, and psychosocial outcome as well. Therefore, new treatment options are being developed, one of them is tetrahydrobiopterin (BH₄), the cofactor of the lacking enzyme phenylalanine hydroxylase (PAH), that stimulates PAH activity in about 20-30% of patients by working as a chaperone protein. Using BH₄ in BH₄ responsive PKU patients can decrease the severity of the social burden of the diet, and may improve outcome when metabolic control can be improved. However, BH₄ only works in patients in whom there is at least some rest capacity of the PAH, or, in other words, the PAH enzyme is not completely disintegrated. Other treatment strategies, partly in development, include phenylalanine ammonia lyase, large neutral amino acids, glycomacropeptide and gene therapy.

This paper gives an overview of the history of PKU, the challenges of today and the possibilities of the (near) future in the 21st century.
Over the last three decades, better understanding, earlier diagnosis and improved treatment options for patients with inborn errors of metabolism have had a great impact on the overall outcome of affected individuals and their life quality. Cornerstone of the treatment of the majority of Disorders of the Intermediary Metabolism has been – and still is – individualised diet alongside provision of deficient products, and / or supplementation of vitamins and co factors where appropriate.

The management of inborn errors of metabolism are challenging as these Orphan diseases usually are complex disorders, multisystemic and require individually tailored treatment. The latter might vary depending on patients’ day to day conditions, as particularly inborn errors of intermediary metabolism can give raise to acute on chronic intoxication. An essential role for sailing affected patients through acute crisis plays the emergency regime, ensuring strict calorie support in times of illnesses to avoid catabolism. This plays a particular role in aminoacidopathies, organic acidurias, urea cycle disorders eg that might additionally require emergency reduction of toxins by modified diets, extracorporal elimination and / or use of specific drugs.

According to the patients’ needs, dietary longterm management may need adjustments over time not only because of age related changes in requirements but as a result of evolving complications; suchlike in patients with Methylmalonic aciduria developing chronic renal failure. Other challenges are balancing out adequate protein intake and avoiding chronic catabolism in patients needing a strict very low protein diet.

More recent developments such like organ / cell transplantation, use of small molecules and enzyme replacement therapy, or substrate reduction and chaperone assisted therapy, had a great impact on successful treatment responses in some IEM. At the same time, we have had to accept, that there are (still) a considerable amount of IEM diagnosis with unsatisfactory response to any intervention and for some disorders, even improving patients’ biochemistry does not necessarily result in a clinical favourable response.

Management of these complex, lifelong and multisystemic disorders often require coordinated, multidisciplinary approach involving several subspecialists. Patients’ and families needs, expectations and limitations might play an additional important factor for the success of any intervention.

Early diagnosis and therefore timely start of appropriate therapy has been proven to improve outcome of patients affected by IEM. A well accepted example is MCAD deficiency for which newborn screening results in changing a potential life threatening condition to a well treatable disorder.

There are a few inborn errors of metabolism that are even amenable for treatment in utero, as shown for 3-phosphoglycerate dehydrogenase deficiency.

Although there has been major progress in the treatment of many inborn errors of intermediary metabolism, there is still a lack of generally accepted treatment protocols and guidelines.

Efforts suchlike reviewing the natural outcome of the diseases, comparing current treatment strategies - their successes and failures - and longitudinal analysis of treatment efficacy need to be continued and supported.
[VITAMIN RESPONSIVE DISORDERS]

Treatment with vitamins is one of the most powerful therapies available to clinicians working with patients with metabolic disorders. There are many reasons why a child may need treatment with a specific vitamin. They include maternal vitamin deficiency, dietary insufficiency, malabsorption, specific disorders of uptake of the vitamin from the diet or its transport to tissues, disorders of vitamin metabolism, disorders of a specific enzyme dependent upon a vitamin-derived co-factor, and accumulation of metabolites that inactivate a vitamin-derived cofactor. Clues to diagnosis may come from the history (e.g. the maternal diet, the child’s diet), specific examination findings (e.g. skin and hair changes in biotinidase deficiency, high output cardiac failure in thiamine deficiency) or first line laboratory investigations (e.g. macrocytic anaemia in some B12 and folate disorders, lactic acidosis in biotin and thiamine disorders).

It is important to be aware of the specific metabolite abnormalities that can occur in vitamin deficiency states (e.g. organic acid profiles in biotinidase deficiency and (potentially riboflavin-responsive) multiple acyl-CoA dehydrogenase deficiency, hyperhomocystinaemia in B12 and folate disorders). Sometimes the clinical situation demands a trial of vitamin therapy before metabolic results are available (e.g. neonatal epileptic encephalopathy that might respond to pyridoxine or pyridoxal phosphate). It is important, however, to collect samples prior to therapy if at all possible.

Some biochemical tests will still produce abnormal results on treatment (e.g. urinary alpha-aminoadipic semialdehyde in antiquitin deficiency); others may not (e.g. CSF pyridoxal phosphate in PNPO deficiency).

This presentation will focus on the disorders for which treatment with a vitamin is dramatically effective because these are the disorders that we must not miss. Cases will be described where there was a dramatic response to vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 (nicotinamide), vitamin B6 (pyridoxine or pyridoxal phosphate), vitamin B7 (biotin), vitamin B9 (folic acid or folinic acid) vitamin B12 (hydroxycobalamin) vitamin E and vitamin K.

The use of a multivitamin cocktail has been advocated in patients with lactic acidosis of uncertain cause (probably a mitochondrial respiratory chain disorder) and in children with acute metabolic decompensation due to a known metabolic disorder (such as an organic acidemia). Anecdotal evidence suggests that such treatment can sometimes be effective but further work is required to determine indications for this treatment.

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[ROLE OF THE DIETITIAN IN IMD]

Many children with inherited metabolic disorders (IMD) are dependent on dietary treatment. It is often reported that any kind of diet therapy is difficult to adhere to; it can affect child and family life quality as well as potentially cause psychological difficulties for the child. The main focus of this presentation will concentrate on some of the latest dietary and service developments that aim to improve the effectiveness, safety, child acceptance, dietary adherence and quality of diet therapy as well as some national and international initiatives being taken by IMD dietitians. Some of the improvements in dietetic practice may not always be headline grabbing, but steady progress is being made which hopefully help many families with a child with IMD in the future.

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Lysosomal storage disorders (LSD’s) comprise a group of inborn errors of metabolism that are caused by deficient activity of a lysosomal enzyme. Lysosomes are cellular compartments with an acidic pH containing many hydrolases. These hydrolases can degrade biological macromolecules that have entered the lysosome through various pathways, such as endocytosis, autophagy or direct transport. Deficiency of one of these hydrolases results in accumulation of its substrate and precursors or other compounds that are derived from this substrate. The cause for the deficient activity is a mutation in the encoding DNA-fragment in most instances, but a mutation in a transport protein can also underlie the defective enzyme activity. The principle of therapy relies on the restoration of defective degradation of accumulated substrates. The earliest effective treatment of a subset of LSD’s has been allogeneic hematopoietic stem cell transplantation (HSCT). This modality is still the treatment of choice for a narrowly defined group of LSD’s. A major step forward has been accomplished in the early nineties, when effective enzyme replacement therapy (ERT) was developed for treatment of Gaucher disease. Gaucher disease is caused by deficient activity of glucocerebrosidase, which gives rise to a heterogeneous disorder characterized by hepatosplenomegaly, cytopenia, bone disease and in severe subtypes central nervous system involvement. ERT works through cellular uptake by receptor-mediated endocytosis of purified enzyme, which is administered to the bloodstream, with subsequent delivery to the lysosome. Within the acidic milieu of the lysosome, the enzyme is capable of degrading the accumulated substrate. Alternative approaches are the use of small molecules that can either stabilize abnormally folded enzymes (chaperone-therapy) or diminish the production of new glycosphingolipids. For this last approach, called substrate reduction therapy (SRT), residual enzyme activity or sufficient alternative degradation pathways need to be present to result in a net decrease in storage material. Over the last ten years, ERT has been developed for Gaucher disease, Fabry disease, MPS I, MPS VI, Pompe disease (glycogenosis II) and MPS II. For some other lysosomal storage disorders ERT is in (pre)clinical phase of development. Several patients suffer from involvement of the central nervous system. This is the case, for example, in the rare neuronopathic Gaucher phenotypes and the severe Hurler phenotype (MPS I). HSCT can sometimes be efficacious in these instances, usually only in slowly progressive subtypes with minimal brain damage before the transplantation. ERT is not suitable to treat the central nervous system involvement, as a result of the blood-brain barrier. Currently, trials are being performed to study the potential benefit of direct delivery of enzyme to the brain by intrathecal administration. The small molecules that are used for SRT may be more suitable to cross the blood-brain barrier, but only limited data are currently available to support this. Miglustat (N-butyl-deoxynojirimycin, an iminosugar) is approved for the treatment of mild to moderate type 1 Gaucher disease and new agents are being developed. The inhibition of the formation of glucosylceramide, the first step in the production of complex glycosphingolipids, implies that miglustat or similar compounds may also be potentially efficacious in the treatment of other glycosphingolipidoses. Chaperone-therapy is currently investigated as an oral treatment for Fabry disease. Clearly, chaperones can only function when the tertiary structure of the enzyme is distorted, but at the same time the catalytic domain is preserved. This means that only for patients with certain missense mutations this approach may work. Perhaps combination therapies may have a place in the future as well. Clinical effects of treatment are variable. Gaucher disease is a successful example where ERT and SRT have shown to be able to reverse disease manifestations. For Fabry disease, the effects are less clear and perhaps timing of therapy, i.e. before irreversible complications have occurred, is more important here. Optimal dosing schedules have also not been fully delineated. As mentioned before, neurological manifestations are difficult to treat. In addition, other compartments can be difficult to target such as bone. In the mucopolysaccharidoses, skeletal pathology is a major feature and effects of ERT on bone symptoms are minimal. The high costs of ERT as well as the burden of intravenous therapy warrant a robust evaluation of effects. For this, collaborative efforts through the installation of independent disease registries are needed.
For patients with inborn errors of metabolism (IEM), certain organs are particularly susceptible to metabolic insult. Accordingly, both whole-organ and cell-based interventions can improve long-term prognosis. Successful liver transplantation has been reported in urea cycle disorders, tyrosinemia type I, maple syrup urine disease (MSUD), some porphyrias, organic acidemias, glycogen storage disorders, hyperoxaluria type I, and selected mitochondrial disorders; combined liver-kidney transplantation is particularly beneficial in some (e.g., methylmalonic acidemia, hyperoxaluria, Fabry disease), while isolated kidney transplantation may be necessary in nephropathic cystinosis and Fabry disease. Benefits of these procedures include improved metabolic control, somatic growth, and quality of life, yet chronic immunosuppression and donor organ shortages remain problematic. This has led to the concept of domino transplantation, in which the genetically altered organ is provided to a recipient who becomes chimeric. The success of organ transplantation in IEM suggests that cellular transplantation alone may be valuable. Hematopoietic stem cell transplantation has been employed in storage disorders including Hurler, Gaucher, Faber, and Wolman diseases, while hepatocyte transplantation (HTx) has shown utility in human ornithine transcarbamylase (OTC) deficiency, glycogen storage disorders, infantile Refsum disease and Crigler–Najjar syndrome type 1. Preclinical characterization of HTx in murine models is expanding, with preliminary reports in Pahenuz mice (phenylketonuria), Agt-/- mice (hyperoxaluria type I), and Bckdh-/- mice (intermediate MSUD). Results have been promising despite the challenges of efficient cell expansion and engraftment advantage for transplanted cells. Despite these limitations, cell-based therapeutics hold continuing promise for long-term treatment of IEM.
After 1881 when Waren Tay at The London Hospital in Whitechapel described in what turned out to be the first lysosomal disease to be clinico-pathologically annotated and, like Bernard Sachs in New York, noted its familial nature and tragic consequences, heroic efforts have been to treat this disorder. Classical Tay-Sachs disease causes rapidly progressive visual, motor and cognitive impairment in young infants: it is incurable and thus remains a paradigmatic neurodegenerative condition which, with justification, can be viewed as emblematic of our scholarly community of metabolism in the clinical and laboratory sciences.

Tay-Sachs and Sandhoff diseases are caused by defects in the α- and ι-subunits of β-N-acetylhexosaminidases (Hex), respectively; deficiency of the specific activator protein also causes GM2 gangliosidosis. Since their identification 22 years ago, mutations in any of the cognate genes induce neurodegenerative disease with lysosomal accumulation of glycosphingolipids, principally GM2 ganglioside. Injury to neurones and glia is associated with neuro-inflammation, cellular malfunction and neuronal injury. Not only have the molecular structures of the ι-hexosaminidases been solved, but genetically modified mice and cats with hex deficiency have been characterized. Animals harbouring mutations in the common ι-subunit of Hex, die prematurely with severe neurological disease: as authentic experimental models of human GM2 gangliosidoses, they provide invaluable resources for therapeutic research.

As a result of its high gene frequency in the Ashkenazim and the demand for prevention, Tay-Sachs disease is the paradigm of successful genetic and biochemical screening – involving as it has, diverse interventions favoured by each of the various communities within this high-risk ethnic group. Thanks to screening in enlightened countries, Tay-Sachs disease is now very rare in the Ashkenazim; but patients with Tay-Sachs and Sandhoff diseases remain a formidable challenge and continue to be diagnosed (often after unconscionable delays), principally in non-Jews.

Over many years, diverse stratagems for treating Tay-Sachs and related diseases (GM2 gangliosidoses) have been explored – these include disappointing studies of systemic, as well as intracranial delivery of various macromolecular hexosaminidase formulations to overcome the blood-brain barrier. The remarkable effect of enteral N-butyldeoxynojirimycin, which enters nervous tissue and prolongs the lifespan of Sandhoff mice, led to human trials of this iminosugar as a substrate inhibitor in human GM2 gangliosidoses; unfortunately it appears not to be sufficiently active to justify further clinical testing. The identification of pyrimethamine, a licensed anti-protozoal agent for long-term use which enters the brain, as a weak inhibitor with in vitro activity as a pharmacological chaperone of ι-hexosaminidases, has been a signal discovery - and one underpinned by molecular modelling and structural studies. Trials with this agent, designed to improve folding and enhance intracellular delivery of nascent variant hexosaminidases to the lysosome, are underway in attenuated forms of GM2 gangliosidosis. Although the stratagem holds promise for some late-onset patients, it has no place in infants and others with mutant enzymes that do not interact with the chaperone molecule and which remain inactive in situ.

We have obtained salutary effects by gene transfer: this addresses the cellular pathology and strikingly improves neurological outcome and survival in animal models. An international consortium to produce and systematically test rAAV vector systems for clinical use has been established. Delivery of therapeutic vector and the design of ethical and informative clinical trials are the principal obstacles. Wide support will be critical in this devastating disease - hence participation by committed patient advocates and European experts will be needed, as well as greatly welcomed.