Publication of Workshop Results

38th Meeting
Istanbul, May 26-28, 2006
The 38th Meeting was held very successfully in Istanbul, Turkey from May 26-28, 2006. The scientific organisation for this year’s main topic:

“Recent advances in organic acidurias”

was under the responsibility of Prof. Mübeccel Demirkol from Istanbul and Prof. Turgay Coskun from Ankara. As in the years before, the results of the workshops, which were held during the conference are published in this booklet.

List of Contents

<table>
<thead>
<tr>
<th>Title of Workshop</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral folate deficiency</td>
<td>5</td>
</tr>
<tr>
<td>Chairpersons: B. Fowler, Basel; V.T. Ramaekers, Aachen</td>
<td></td>
</tr>
<tr>
<td>Protein restriction in MMA and PA</td>
<td>17</td>
</tr>
<tr>
<td>Chairpersons: W. Sperl, Salzburg; G. Gökçay, Istanbul</td>
<td></td>
</tr>
<tr>
<td>Outcome of argininosuccinic aciduria and hyperargininemia</td>
<td>25</td>
</tr>
<tr>
<td>Chairpersons: M. Duran, Amsterdam; F. Wijburg, Amsterdam</td>
<td></td>
</tr>
<tr>
<td>Reproductive effects in IEM</td>
<td>33</td>
</tr>
<tr>
<td>Chairpersons: P. Lee, London; M. Schwarz, Dusseldorf</td>
<td></td>
</tr>
<tr>
<td>Working up mental retardation</td>
<td>41</td>
</tr>
<tr>
<td>Chairpersons: J. Zschocke, Heidelberg; R. Hennekam, London</td>
<td></td>
</tr>
</tbody>
</table>
Cerebral folate deficiency

Chairpersons:

B. Fowler, Basel
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Cerebral folate deficiency

Chairpersons: B. Fowler, Basel; V.T. Ramaekers, Aachen

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Introduction

Cerebral Folate Deficiency (CFD) can be defined as any neurological syndrome associated with low cerebrospinal fluid N^1-methyltetrahydrofolate (5MTHF), the active folate metabolite, in the presence of normal folate metabolism outside the nervous system (1,2). The underlying mechanisms of CFD involve either reduced folate transport to the brain or increased folate turnover within the central nervous system (CNS) (3).

Based on the clinical features and the age of onset, CFD can be classified into the infantile-onset CFD syndrome and a spastic-ataxic syndrome with learning disorders manifesting after the age of 1 year. Known conditions associated with CFD have been described and can be considered as secondary CFD forms, i.e. Rett syndrome, Aicardi-Goutières syndrome, Kearns-Sayre syndrome, 3-phosphoglycerate dehydrogenase deficiency, dihydopteridine reductase deficiency and aromatic amino acid decarboxylase deficiency (2,4-10).

For each child manifesting features of CFD, the differential diagnosis should include the use of antifolate drugs and systemic disorders of folate and vitamin B12 metabolism (tables I and II).

Folate metabolism and transport

Among the more than 25 structurally related folate compounds, only the reduced folate forms can function as active cofactors in cellular metabolism (4). The physiological function of the various reduced folate forms subserves the de novo synthesis of purines and thymidine, methylation of DNA, the conversion of homocysteine to methionine and the formation of the active methyl-group donor S-adenosylmethionine (SAM) (4).

5MTHF is the predominant, active folate form in plasma, known to cross the blood-brain barriers and is a precursor of the activated methyl-group donor SAM. SAM is used in more than 100 chemical reactions of methyl-group transfer.

Cellular folate transfer across the plasma membrane is accomplished by three different transport mechanisms, i.e. cellular uptake through 1) folate-receptor (FR) mediated endocytosis, and 2) the
reduced folate carrier (RFC1), and removal of excess intracellular folate by 3) an ATP-dependent folate exporter system. FR1 is mainly distributed at epithelial cells such as the choroid plexus, lung, thyroid and renal tubular cells, while FR2 is mainly located within mesenchymal derived cells, like red blood cells. Both are high-affinity proteins which function at the physiological nanomolar range of extracellular folate concentrations. RFC1 is ubiquitously distributed and represents a low-affinity folate transporting system with bidirectional transport across cellular membranes.

Intact transport mechanisms across the intestinal, placental and blood-brain barriers are crucial to achieve adequate folate stores within organs and the CNS. For passage across the blood-cerebrospinal fluid (CSF) barrier, plasma 5MTHF is bound by the folate receptor proteins, anchored to choroid epithelial cell membranes by a glycosylphosphatidylinositol (GPI) moiety. The choroid plexus is the main site of active folate transport to the CNS (2). This transport is an energy-dependent process and requires adequate ATP production which leads to an at least 1.5-fold higher concentration of 5MTHF in CSF compared to plasma (11).

Clinical phenotypes suggestive of CFD
The infantile-onset CFD syndrome has been identified in a large number of children worldwide. It is characterized biochemically by normal serum and low CSF folate levels, and clinically by the presence of at least three out of seven major clinical criteria:
1. Presentation 4 to 6 months after birth with irritability and sleeping disturbances;
2. Deceleration of head growth between 6 and 18 months;
3. Psychomotor retardation, sometimes followed by regression;
4. Cerebellar ataxia;
5. Pyramidal tract signs in the lower limbs;
6. Dyskinesias (choreoathetosis, ballism)
7. Epileptic seizures.
In this context it should be stressed that the neurological phenotype can be variable.

In a subgroup of these children autistic features can be encountered. In a minority of children central visual disturbances began to develop around the age of 3 years and progressive sensorineural hearing loss can start from the age of 6 years. Neuro-imaging was abnormal in only half of the patients and showed variable combinations of atrophy of frontotemporal regions, periventricular demyelination, or supra- and infratentorial atrophy. Intracranial calcifications are observed very rarely (1,2). Several children have now been identified who develop normally
during the first year and present after the age of 1 year with a spastic ataxic syndrome, which is accompanied by a learning disorder (12).

Late-onset CFD has only been reported in one male patient with normal development until the age of 9 years when a rapidly progressive sensorineural hearing loss occurred, followed by slowly progressive neurodegeneration with cerebellar ataxia, pyramidal tract dysfunction and spinal muscular atrophy. Neuroimaging showed cerebral and cerebellar atrophy with bilateral hypodensities of the basal ganglia. A mitochondrial encephalopathy could be excluded. In the presence of normal serum folate, his CSF was xanthochromic with a high protein content. The patient’s CSF demonstrated a profoundly lowered immunoreactivity for both the FR1 and FR2 proteins. However the underlying aetiology remains to be established (13).

**Investigations in CFD**

Testing of CSF folate is recommended in children manifesting the clinical features compatible with the infantile-onset CFD syndrome, unexplained spastic-ataxic syndromes and late-onset sensorineural hearing loss followed by progressive signs of pyramidal and cerebellar dysfunction. Resembling neurological conditions with overlapping features should be excluded by appropriate investigations, like for example cerebral palsy, hereditary spastic ataxic and cerebellar syndromes, Friedreich ataxia and Angelman syndrome.

In patients with the described infantile-onset CFD syndrome and spastic ataxic syndromes normal values can be found for serum homocysteine, folate, vitamin B12, red blood cell folate concentration and methylenetetrahydrofolate reductase activity in lymphocytes or fibroblasts (2). It is essential to take care to freeze the CSF samples immediately at – 70 ° Celcius, preferably at the bedside, in order to avoid rapid degradation of the unstable 5MTHF compound. Routine CSF investigations show normal cell counts, glucose and protein concentrations in almost all patients. CSF analysis shows reduced 5MTHF concentration in all patients compared to normal controls while CSF follow-up studies of untreated patients have shown a further decrease of 5MTHF. The commonly used method of 5MTHF analysis of CSF is HPLC with electrochemical or fluorescent detection. Each laboratory has to establish its own age-related, population cut-off values. A recommendation is to measure plasma and CSF 5MTHF simultaneously in order to establish a CSF/plasma 5MTHF ratio. The currently used CSF methods only measures free 5MTHF concentrations, but the 5MTHF fraction bound to proteins is negligible.

In about half of patients with low MTHF in CSF analysis of monoamine metabolites also shows a reduced concentration of 5-hydroxyindoleacetic acid (5HIAA) in the presence of normal homovanillic acid concentrations (except for a low HVA concentration in one patient).
CSF pterin analysis shows isolated reduction of neopterin concentration in one-third of patients, while CSF biopterin can be slightly reduced. Homocysteine is not elevated in CSF in most patients and single observations have found elevated cysteine and cystathionine concentrations suggesting that homocysteine will be removed through the transulfuration pathway.

The serum in the majority of children with the infantile-onset CFD syndrome contained autoantibodies directed against the FR. These antibodies were characterized as high-affinity blocking autoantibodies against membrane-bound folate receptors that are present on the choroid plexus (15). The presence of these blocking FR autoantibodies prevents the binding of plasma 5MTHF and its subsequent transport across the blood-CSF barrier into the CSF.

FR autoantibodies have now also been identified in one child with the spastic-ataxic CFD syndrome (Ramaekers, unpublished results). Incubation of serum from patients with KB cell cultures which highly express the folate receptor on their membrane, have shown that these autoantibodies are able to block folate incorporation. At this moment research efforts focus on further characterization of these recently discovered FR autoantibodies. For these tests, it seems to be important to purify the human FR antigen derived from placental membranes.

However, in a number of patients FR autoantibodies tested negative and the underlying mechanisms for low CSF folate in these patients remain to be identified.

**Diagnosis and differential diagnosis**

Systemic depletion of the pool of metabolically active reduced folates should be considered and has been encountered among children with malnutrition, intestinal malabsorption due to coeliac disease, the use of antifolate agents like methotrexate and rarely during chronic use of anticonvulsant drugs and carbidopa (table 1). A rare form of hereditary folate malabsorption with a high rate of consanguinity has been described manifesting macrocytic anaemia, low systemic folate status together with severe neurological deficits and intracranial calcifications (16). Variants with a milder neurological phenotype have been identified. Early detection and parenteral treatment with high doses folinic acid appears to improve prognosis. The defect is localised at both the intestinal and blood-CNS barriers, but its genetic cause remains to be identified.

The amino acids serine, glycine and histidine act as the most important one-carbon donors to replenish the pool of unsubstituted tetrahydrofolates. Therefore, conditions with lowered levels of these amino acids will be expected to reduce secondarily the 5MTHF concentrations and thus SAM-mediated methylation reactions.
A de novo disorder of serine synthesis due to 3-phosphoglycerate dehydrogenase deficiency leads to a recognizable clinical entity with microcephaly, intractable seizures, feeding disturbances and severe neurological disability (7). The presence of low serine in the brain will consequently reduce the substrate for the enzyme serine hydroxymethyltransferase and reduce the production of 5,10-methylene-THF, the precursor for 5MTHF and ultimately for SAM.

Methylenetetrahydrofolate reductase deficiency (MTHFR) should always be considered because this can also lead to low 5MTHF levels associated with homocysteine accumulation manifesting with a variable phenotype from mild to severe neurological disabilities (17,18). The commonly occurring polymorphisms of the MTHFR gene, like the C677T and the A1298C mutation, are not associated with CFD. In case of suggestive neurological signs, further measurements of enzyme activity in fibroblasts should be performed for diagnostic purposes.

Known rare inborn errors of metabolism associated with secondary CFD include dihydropteridine reductase (DHPR) and aromatic L-amino acid decarboxylase deficiency (AADC)(6,8). Diagnosis depends on a high index of clinical suspicion and typical findings from CSF amino acid levels, biogenic monoamine and pterin metabolites.

Kearns-Sayre syndrome has already been described to be associated with low CSF folate (9). Several children with other mitochondrial encephalopathies were found to have low CSF folate due to failure of the active ATP-dependent folate transport across the choroid plexus.

Recent reports have described secondary CFD to occur in a number of girls with Rett syndrome and patients with a variant or classical Aicardi-Goutières syndrome (4,5).

Although low 5MTHF in CSF appears not to be a consistent feature in all patients with Rett syndrome, an important further question concerns the exact link between the MECP2 genotype and its influence on folate transfer processes at the choroid plexus (4).

Diagnosis of infantile-onset CFD syndrome and late-onset CFD can be established based upon the salient clinical features associated with a reduced CSF folate concentration and after exclusion of disturbed folate and vitamin B12 homeostasis and metabolism outside the nervous system. The two most important mechanisms, identified so far, are the presence of serum FR autoantibodies of the blocking type and detection of mitochondrial encephalopathies. The conditions leading to secondary CFD should be considered and excluded, as indicated above.
Guidelines for diagnosis and treatment

After finding low 5MTHF values in CSF, further testing of each patient is necessary to exclude systemic folate deficiencies and secondary CFD forms. The diagnostic work-up protocol should include a complete blood count with red blood cell indices, serum and red blood cell folate, homocysteine, amino acids, lactate and pyruvate, glucose and serum gliadin antibodies. Girls with features reminiscent of Rett syndrome should undergo MECP2 gene testing. CSF analysis should include glucose, protein, cell count, lactate and pyruvate, amino acids, biogenic monoamine metabolites, pterins and 5MTHF. A CSF and serum sample can be stored for future investigations, for example FR autoantibody testing.

After diagnosis the treatment used for most patients is calcium-folinate or folinic acid in high oral doses, as this folate substance represents a stable form of a metabolically active reduced folate compound in contrast to folic acid, which is an oxidized and metabolically inactive folate form. Paradoxically, treatment with folic acid may further enhance CNS depletion of the metabolically active pool of reduced folates because folic acid has a higher FR binding affinity compared to 5MTHF and consequently competes at the FR receptor with the transport and storage of reduced folate species (19). Treatment consists of daily oral administration with 0.5-1 mg/kg body weight calcium folinate. Because side-effects of gastro-intestinal upset and an increased risk of seizures have been mentioned for calcium folinic acid, careful clinical and EEG follow-up studies should be performed after 1, 3 and 6 months (1,2). In children who do not show any clinical effect during four to six months at a folinic acid dose of 0.5-1 mg/kg/day, a lumbar puncture has to be repeated in order to monitor the change of CSF 5MTHF levels. Based upon the initial CSF levels, dose readjustments for folinic acid can be calculated.
Table I. Overview of systemic folate depletion and conditions of CFD associated with isolated 5MTHF depletion in the CNS.

<table>
<thead>
<tr>
<th>Classification of condition</th>
<th>Underlying mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systemic depletion</strong></td>
<td></td>
</tr>
<tr>
<td>Malnutrition</td>
<td>Folate deficient in the diet or food deprivation</td>
</tr>
<tr>
<td>Malabsorption</td>
<td>Reduced folate absorption in the jejunum</td>
</tr>
<tr>
<td>- Regional intestinal disorders</td>
<td>Gluten enteropathy</td>
</tr>
<tr>
<td>- Coeliac disease</td>
<td>Blocks RFC1 carrier; inhibits dihydrofolate reductase</td>
</tr>
<tr>
<td>Antifolate agents</td>
<td>Interfering with cellular folate uptake</td>
</tr>
<tr>
<td>- Chemotherapy (MTX)</td>
<td>Inhibits aromatic aminoacid decarboxylase with consequent SAM and 5MTHF overconsumption</td>
</tr>
<tr>
<td>- Anticonvulsant drugs</td>
<td></td>
</tr>
<tr>
<td>- Carbidopa</td>
<td></td>
</tr>
<tr>
<td><strong>Congenital folate malabsorption</strong></td>
<td>Hereditary factor involving folate transfer across intestinal and blood-CSF barriers</td>
</tr>
<tr>
<td><strong>Inborn errors of metabolism</strong></td>
<td>Depletion of enzymatic product 5MTHF</td>
</tr>
<tr>
<td>- MTHFR deficiency</td>
<td>Defective histidine derived one-carbon transfer</td>
</tr>
<tr>
<td>- Formiminotransferase deficiency</td>
<td></td>
</tr>
<tr>
<td><strong>Cerebral Folate Deficiency</strong></td>
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<tr>
<td>Infantile-onset CFD</td>
<td>Serum FR autoantibodies of the blocking type</td>
</tr>
<tr>
<td>Spastic ataxic syndromes</td>
<td>Serum FR autoantibodies of the blocking type</td>
</tr>
<tr>
<td>One-carbon pool deficiencies in the CNS</td>
<td>Defective serine synthesis affecting one-carbon pool</td>
</tr>
<tr>
<td>- 3-Phosphoglycerate dehydrog. def.</td>
<td></td>
</tr>
<tr>
<td><strong>Inborn errors of metabolism</strong></td>
<td>Unproven mechanism</td>
</tr>
<tr>
<td>- Dihydropteridine reductase def.</td>
<td>Overconsumption of 5MTHF and SAM</td>
</tr>
<tr>
<td>- Aromatic aminoacid decarboxy.def.</td>
<td></td>
</tr>
<tr>
<td>Kearns-Sayre syndrome</td>
<td>Disturbed active folate transport at choroids plexus</td>
</tr>
<tr>
<td>Mitochondrial diseases</td>
<td>Disturbed active folate transport at choroids plexus</td>
</tr>
<tr>
<td>Rett syndrome</td>
<td>Expression of FR pseudogenes due to MECP defect</td>
</tr>
<tr>
<td>Aicardi-Goutières syndrome</td>
<td>Variable CFD of unknown origin</td>
</tr>
</tbody>
</table>
Table II. Summary of diagnostic investigations needed for patients with a phenotype suggesting cerebral folate deficiency.

<table>
<thead>
<tr>
<th>Procedure if CFD is suspected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematology and RBC indices</td>
</tr>
<tr>
<td>Serum and RBC folate, homocysteine, amino acids</td>
</tr>
<tr>
<td>Lactate, pyruvate, glucose and serum gliadin antibodies</td>
</tr>
<tr>
<td>Consider MECP2 mutation (Rett syndrome)</td>
</tr>
<tr>
<td>Spinal tap: Glucose, lactate, pyruvate</td>
</tr>
<tr>
<td>CSF 5MTHF (sample collection critical, protein bound negligible)</td>
</tr>
<tr>
<td>Pterins and biogenic monosamines, Amino acids</td>
</tr>
<tr>
<td>Store serum and CSF</td>
</tr>
<tr>
<td>Folate receptor autoantibodies of the blocking type is still a research tool</td>
</tr>
</tbody>
</table>
After crossing the intestinal barrier with the use of RFC1, folates enter the circulation and can be transported into mesenchymal tissues, for example red blood cells (RBC) by the folate receptor 2 (FR2). After passage across choroid epithelial cells using FR1 receptor mediated endocytosis, folates will enter the CSF and enter neuronal cells by the RFC1, located at axons and dendrites. The presence of circulating FR autoantibodies in serum, indicated by the symbol (←), can bind to membrane-attached FR1 of choroid epithelial cells, block its binding site to folate and hence be responsible for reduced folate transfer into the central nervous system.
Figures 1: After crossing the intestinal barrier with the use of RFC1, folates enter the circulation. The passage across choroid epithelial cells, using FR1 receptor mediated endocytosis, is an ATP-dependent process. In Kearns-Sayre syndrome and mitochondrial encephalopathies secondary CFD will occur, because this active transport across the blood-CSF barriers will fail and the CSF/serum ratio for the crossing 5-methyltetrahydrofolate metabolite will equalize, thereby causing relative folate deficiency within the CNS.

References


Protein restriction in MMA and PA

Chairpersons:

W. Sperl, Salzburg
G. Gökçay, Istanbul
Protein restriction in MMA and PA

Chairpersons: W. Sperl, Salzburg; G. Gökçay, Istanbul

Introduction:

The amount of protein in MMA and PA recommended during the eighties and early nineties can be delineated from single case reports and studies in a limited number of patients (1, 2, 3). One study to elucidate protein requirements in MMA was published in 1985 (4). In that period the protein restriction was mostly below 1.2 g/kg bw/day of natural protein intake. A high percentage of poor outcome was noticed in both PA and MMA patients, especially in PA patients with early onset a high proportion of mortality was reported (1). Many of the patients showed poor growth and weight gain, mental retardation and finally nasogastric tube feeding was frequently used to overcome feeding problems (2, 3).

In 1998 recommendations for protein intake were discussed during a workshop on PA (Salzburg)(5). A retrospective study indicated, that a number of patients, especially early PA patients with a better outcome and lower mortality rate were given an age adapted higher amount of natural protein than in the decade before. In addition special amino acid mixtures were recommended to fulfil the protein requirement at least according to the protein requirements of normal healthy children (5). Many questions remained open. There was and is still a need for a prospective study with a well defined clinical and metabolic follow up of these patients.

8 years later, for the preparation of this workshop concerning protein restriction in PA and MMA, a questionnaire was sent out to gather protein recommendations from different metabolic centers.
Results from a questionnaire concerning protein recommendations in PA and MMA

28 participants, questionnaires from 16 centers* and 12 countries
186 MMA patients 117 PA patients

Figure 1: Distribution of the patients to different countries and centers participating in the workshop at the EMG meeting 2006 Istanbul

*Responding participants: Austria (Salzburg/Innsbruck), Belgium (Brussels), Croatia (Zagreb), Czech Republic (Prague), England (London), France (Marseille), Germany (Düsseldorf, Heidelberg, Hamburg), Italy (Florence), Poland (Warszawa), The Netherlands (Amsterdam), Turkey (Istanbul, Ankara, Sivas)

Figure 2: Age distribution of the patients with MMA and PA
**Figure 3a:** Average protein intake of MMA patients

![Average protein intake of MMA patients](image1)

**Figure 3b:** Average protein intake of PA patients

![Average protein intake of PA patients](image2)

**Figure 4a.** Protein intake in MMA (all age groups)

![Protein intake in MMA](image3)

Age Groups: 1; 0-3 months, 2; 3-6 months, 3; 6-12 months, 4; 1-4 years, 5; 4-7 years, 6; 7-11 years, 7; >11 years
Comment:

There is a decrease of the total protein amount according to the age dependent protein requirements in normal children (see figure 3 a b). Most centers use natural protein and add special amino acids to reach the recommended total protein intake. 2 centers did not offer special amino acid supplementation. The proportion of natural to special protein was in average about 2:3:1.

In figure 4 a and 4 b the average and standard deviation scores of all the protein recommendations are shown for MMA and PA patients. There is a great variance in the amount of special amino acid mixtures given by the different groups. The variance in the amount of natural protein as well as in the special protein intake is greatest in the first half year of life (Figure 4 a b). 2 centres do not use a specialized amino acid mixture and restrict protein intake. In the first three months of life, still some centers use low amounts of natural protein. Protein recommendations in PA do not differ very much from those for MMA.
Breastfeeding in MMA-PA

8/16 centers responded to have an experience in breastfeeding in MMA and also 8/16 for PA (Table 1). “Limited breast feeding on demand” was discussed during the workshop briefly. It means to start first with a bottle containing a milk with a defined amount of special protein with sufficient energy to utilize this protein but no natural protein. Thereafter the baby can continue with breast feeding on demand to get the necessary amount of natural protein by breast milk (6, 9).

Table 1: Breastfeeding experience of workshop participants in MMA and PA patients

<table>
<thead>
<tr>
<th></th>
<th>Screened (n)</th>
<th>Non-screened (n)</th>
<th>Affected sibling (n)</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMA (Sucking from breast/expressed breastmilk)</td>
<td>1/1</td>
<td>9/6</td>
<td>1/-</td>
<td>18</td>
</tr>
<tr>
<td>PA (Sucking from breast/expressed breastmilk)</td>
<td>1/3</td>
<td>5/4</td>
<td>3/1</td>
<td>14</td>
</tr>
</tbody>
</table>

Consensus from the workshop participants:

1. The average protein requirements of our survey reflect the mostly used protein amounts but cannot be taken as a recommendation. There is no real consensus concerning protein recommendation, the experience is very heterogeneous!
2. The major protein intake should be the natural protein. Special amino acid mixtures can be added up to reach the total protein requirement according to age (e.g. RDA) (but mind: quality of protein!!).
3. Gold standard for natural protein is breast milk: there is some experience in a few centers with „limited breast feeding on demand“.
4. A successful unwell regimen is important. Useful guidelines are published by Dixon MA, Leonard JV (1992) (7).
Further remarks and open questions from the workshop participants:

1. What you restrict you have to measure! Amino acid monitoring is important (mind depletion e.g. isoleucine), measure prealbumin.

2. Growth, weight, neurologic development are still the most valuable follow up parameters. There is no consensus for metabolic control parameters.

3. Although there is a difference between PA and MMA (renal problems, neurologic outcome, growth) in most of the centers the same protein regimen is used.

4. There is some uncertainty concerning the energy requirement. Do we need more energy for protein anabolism?

Outlook:

1. Exact requirement of isoleucine, methionine, valine, threonine has to be determined in PA and MMA patients.

2. Professionally developed guidelines could be helpful, also a patient registry.

3. Prospective outcome studies with clinical, biochemical, molecular and dietary data are necessary and are under way (see Hörster F et al ).
**Figure 5:** Development of guidelines for PA and MMA are

![Diagram](image)

**Figure 6:** EMG Istanbul 2006 may be the bridge from experience to evidence

![Diagram](image)

References:

Outcome of argininosuccinic aciduria and hyperargininemia

Chairpersons:

M. Duran, Amsterdam
F. Wijburg, Amsterdam
Outcome of argininosuccinic aciduria and hyperargininemia

Chairpersons: M. Duran, Amsterdam; F. Wijburg, Amsterdam

Introduction
Argininosuccinate lyase deficiency (ASL deficiency, argininosuccinic aciduria, OMIM207900) is an autosomal recessive inborn error caused by a deficiency of the enzyme argininosuccinate lyase. Argininosuccinate lyase catalyses the cleavage of argininosuccinate into arginine and fumarate, an essential step in the urea cycle. ASL is expressed in many tissues for synthesis of arginine. Arginine is the substrate for NO and creatine synthesis.
The chemical hallmark of ASL deficiency is an increased level of argininosuccinate (ASA) in CSF, plasma and urine in combination with decreased concentration of arginine. In addition, in most patients recurrent episodes of hyperammonemia can be detected. Clinically the disease appears to be very variable ranging from neonatal onset with severe hyperammonemia to absence of clinical disease in others. The workshop was focussed on the following aspects:

1. diagnostic procedures
2. clinical symptoms and outcome
3. the value of neonatal screening for ASL deficiency
4. therapeutic approach to ASL deficiency

1. Diagnostic procedures
The diagnosis of ASL deficiency is suggested by the detection of argininosuccinate (ASA) in plasma, urine or CSF of an affected patient. It is clear that in some patients the levels of ASA are only slightly increased in plasma with much more pronounced levels in CSF. There is also great variability with respect to the levels of ASA in plasma between different patients. Some patients always have very high plasma levels of ASA, in others the levels are less increased. Reference plasma levels of ASA are less than 1 µmol/L. ASL deficient patients may have ASA levels between 10 and 1000 µmol/L with only a small intra-individual variation (Figure 1: ASA levels in time in 5 ASL deficient patients). It appears that there is a correlation between ASA levels and the clinical presentation of the disorder, with the highest levels of ASA to be found in the patients who suffer from recurrent episodes of (severe) hyperammonemia and the less increased levels to be found in patients who present with psychomotor retardation only, without hyperammonemia.
Most of the reported patients had virtually no residual enzyme activity; hence the variation in plasma ASA levels is not explained.

**Figure 1**

ASA may be transformed into anhydrides which can be found in plasma and CSF of affected patients. Both ninhydrin-positive and -negative anhydrides have been described; hence it is difficult to make dietary balance studies using an amino acid analyzer. Tandem MS should be able to detect all forms of ASA, although the characterization of all anhydrides by this technique has yet not been achieved.

Arginine levels may be low in plasma of patients with ASL deficiency; however normal values have been reported in many patients.

**2. Clinical symptoms and outcome**

Several distinct clinical forms of ASL deficiency occur:

1. a neonatal form with feeding difficulties, seizures and early death, always accompanied by hyperammonemia
2. an infantile from with recurrent vomiting, coma, convulsions and mental retardation, most often these patients also suffer from recurrent hyperammonemia
3. a chronic from with mental retardation, seizures and intermittent ataxia. Some of these patients, who may present only with mental retardation, can have fully normal ammonia levels even when challenged with a protein loading
4. a form without clinical symptoms

A questionnaire on ASL deficiency was sent to the participants of the workshop. Data on 59 patients could be collected. Eight patients were detected by newborn screening, one by prenatal
diagnosis. In 5 patients the diagnosis was made immediately after birth because of an affected sib. In 45 patients the diagnosis was made because of clinical disease (32 male and 13 female patients). No explanation was found for the male preponderance in this large group of ASL deficient patients. It is hardly conceivable that affected females die in utero, as all reported patients were free of symptoms at delivery.

Most patients presented in the neonatal period (Figure 2). All of these patients presented with hyperammonemia. However, in some patients the diagnosis was made later in life. Some of these patients presented with only mental retardation and never experienced an episode of hyperammonemia. Data on treatment could be obtained for 41 patients (see figure 3). Almost all patients received protein restriction and arginine medication.

An unexplained entity in ASL deficiency is the occurrence of recurrent electrolyte disturbances. Of the 45 patients who were diagnosed because of clinical disease, recurrent electrolyte disturbances were reported in 15 patients. These electrolyte disturbances consist of low plasma sodium and potassium levels. This appears not to be related to the treatment (sodiumbenzoate or phenylbutyrate). Probably, renal tubular disease can be a consequence of ASL deficiency. The possibility of renal and potassium losses during the excretion of ASA, which is a tricarboxylic acid, has not been explained in detail.

Parameters used for follow-up in patients with ASL deficiency were also reported by the workshop participants. As could be expected, plasma amino acids were used as a parameter in all reported patients, almost always in combination with measurement of plasma ammonia values. Urinary orotic acid was measured in approximately 50% of the patients during follow up.

The outcome, as reported in the 45 patients detected because of clinical disease, showed that 41 of the patients were alive. There is a huge variation in the estimated IQ/DQ in the surviving patients. However, it was clear that most of the patients suffered from severe mental retardation. In contrast, outcome appeared to be favourable with a normal mental development in some.
It may well be that these patients with a normal development belong to the group of patients with type 4 (see above) clinical form of ASL deficiency. In the 6 patients diagnosed either prenatally (n=1) or because of an affected sib (n=5), 5 were reported to suffer from psychomotor retardation, despite initiation of therapy immediately after birth.

3. The value of neonatal screening for ASL deficiency
The value of including ASL deficiency in neonatal screening programs is, at least, debatable. During the workshop, the Austrian experience was presented and discussed. A long term follow-up outcome study on 9 patients with ASL deficiency revealed that no metabolic decompensations occurred in these patients and that neurological examination in these patients, medium age 10.3 years, was unremarkable in all. In this respect it should be noted that the genotypes of these Austrian patients were entirely different from all previously published genotypes of severely affected ASL deficient patients.

It was concluded that neonatal screening for ASL deficiency will result in the detection of patients with the ‘type 4’ clinical presentation of ASL deficiency (patients with no clinical symptoms at all). Of course the workshop participants agreed that neonatal screening will also detect patients who are at risk for, or already suffering from, severe hyperammonemia. It is unclear, however how many patients with a very benign form of ASL deficiency will be picked up during neonatal screening programs. Therefore, caution for including ASL deficiency in neonatal screening programs was suggested. Caution for inclusion in neonatal screening programs is also warranted because of the relatively poor results of therapy in ASL deficiency (see below).

4. Therapeutic approach to ASL deficiency
There was major concern on the use of high doses of arginine in patients with ASL deficiency. Although it has been suggested in literature that high dose arginine therapy does not appear to be harmful in patients with ASL deficiency, it was recognised that no studies on the effects of different doses of arginine in ASL deficiency have been performed. As it is recognised that ASA concentrations may be high in CSF compared to plasma, and because the accumulating argininosuccinate will be neurotoxic, doubts were expressed about the safety of high doses of arginine in ASL deficient patients. High doses of arginine may well result in increased levels of ASA in CSF increasing the potential neurotoxic effects. There might well be a limited efflux of certain dicarboxylic acids, such as dicarboxylic acids derived from ASA, from the CNS. These
dicarboxylic acids may become neurotoxic at high concentrations (as has been studied in models for glutaric aciduria type 1).

In addition, it has recently been suggested that high doses of arginine in patients with inborn errors, such as ASL deficiency may result in increased levels of guanidinoacetate in the CNS. As guanidinoacetate is also a potential neurotoxic agent, high doses of arginine may be harmful. Finally, it cannot be judged whether treatment with high doses of arginine will compromise the delicate balance of NO synthesis, thereby causing side effects.

As a result of the discussion on the above mentioned items, the members of the workshop decided that it is no evidence for the need for high doses of arginine in patients with ASL deficiency and that these high doses may indeed be harmful. Therefore, moderate supplementation doses (e.g. 100 – 150 mg/kg) may be more advisable.

**Conclusions**

ASL deficiency has a broad clinical spectrum, ranging from severe neonatal onset to a much milder disease of even no clinical symptoms at all. However, most patients present with acute neurological deterioration with hyperammonemia in the neonatal period. Some patients present later in life with mental retardation, in some cases without any hyperammonemia. Finally, in others ASL deficiency may be present without any clinical signs and symptoms and therefore only results in biochemical abnormalities.

Neonatal screening for ASL deficiency is seriously hampered by the lack of correlation between biochemical abnormalities and clinical disease and by the fact that treatment in patients with ASL deficiency remained, until now, unsatisfactory. As high doses of arginine (> 400 mg/kg/day) may actually be deleterious in patients with ASL deficiency, it is advised to refrain from high doses and supplement patients with ASL deficiency with moderate arginine doses (e.g. 100 – 150 mg/kg/day). However, as no studies on the biochemical or clinical effects of different doses of arginine have been reported, there is an urgent need for properly designed studies to try and obtain data on the appropriate dose of arginine in these patients.
References


Reproductive effects in IEM

Chairpersons:

P. Lee, London
M. Schwarz, Dusseldorf
Reproductive effects in IEM

Chairpersons: P. Lee, London; M. Schwarz, Dusseldorf

Introduction:
An earlier EMG-workshop in Amsterdam 2001 had already focussed on the issue of pregnancy in different Inherited Metabolic Diseases (IMD). Since then only a few new research articles, case reports or abstracts had been published on this issue of pregnancy in IMD. Therefore we decided to focus our workshop on the question:

How prepared are females with IMDs for reproduction?
To answer this question, we identified the following fields of interest:

Growth & Pubertal development
- Childhood
- examples of delayed puberty in IMD
- management

Fertility
- assessment
- examples in IMDs
- management
- genetic counselling

Pregnancy in IMDs
- Teratogenicity
- early loss
- maternal health

Results:
1) Growth and Pubertal Development
For this first section, the following suggestions for diagnosis, treatment and counselling for different age groups were made by the workshop participants:
Childhood:
- discuss with child early (5/6yrs) as they are receptive and less embarrassed at that age
- use group discussion format especially for teenagers
- counselling by paediatrician and gynaecologist together

Delayed Puberty:
- clinical assessment (Tanner stage)
- anticipate when expected (galactosaemia, glycogenoses)
  Management at the age of about 10 years: bone age (+/- DEXA), gonadotrophins, pelvic ultrasound, consider thyroid/pituitary dysfunction, consider puberty induction (bone age, individual, not too late)

2) Fertility
For this second section, the following suggestions for diagnosis, treatment and counselling for different age groups were made by the workshop participants:
- Genetic counseling for the couple, by geneticist / metabolic physician
- Assessment should be considered after 6 months of unprotected intercourse: menstrual cycle, gonadotrophins, prolactin, cervical mucus, daily temperature, ultrasound of the ovaries, assess partner and consider non IMD causes

3) Examples of infertility
An overview on this issue was given by Martin Schwarz, Adult Metabolic Clinic, Dep. of Gastroenterology, Hepatology und Infectiology, University Hospital Düsseldorf, Germany.

Dealing with this issue it is important to consider that infertility is a frequent finding in the whole population, not only in woman with IMD. It is estimated that infertility affects about 14% of reproductive-age women and about 5 million couples in the United States.

Causes of female infertility in the whole population are
- Ovulatory disorders (25 %)
- Endometriosis (15 %)
- Pelvic adhesions (12 %)
- Tubal blockage (11 %)
- Other tubal abnormalities (11 %)
- Hyperprolactinaemia (7 %)

The main infertility diagnoses in a study of 2198 infertile couples in Canada were:
- unexplained infertility (26 %)
- male factor infertility (24 %)
- tubal disease (23 %)
- ovulation disorders (18 %)
- endometriosis (7 %)
- other (luteal phase, cervical or uterine defects, 2 %)

To get an overview on possible specific problems in IMD, a Medline search was performed for (in)fertility, pregnancy and inborn error of metabolism/metabolic disease. Most hits were for congenital adrenal hyperplasia, some for homocysteine, and no specific systematic or narrative review or original article was found that specifically addresses this issue.

In an overview on pregnancy and IMD some years ago Walter identified as most important in this field the following:

**Examples of Infertility**

<table>
<thead>
<tr>
<th>Galactosaemia</th>
<th>Hypergonadotrophic hypogonadism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocystinuria</td>
<td>Very early fetal loss</td>
</tr>
<tr>
<td>Less specific effects on fertility</td>
<td>Significant neurological or physical handicap</td>
</tr>
</tbody>
</table>
4) Metabolic risk factors of multifactorial birth defects

A short lecture on his own research on Down-Syndrome and its possible association with the homocysteine / folate metabolism was given by Prof. Generoso Andria, Department of Pediatrics, Federico II University, Naples, Italy

<table>
<thead>
<tr>
<th>Multifactorial Trait</th>
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<tbody>
<tr>
<td>Genetic Factors</td>
</tr>
<tr>
<td>Homocysteine / folate metabolism</td>
</tr>
<tr>
<td>Myoinositol</td>
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<tr>
<td>Chromosomal abnormalities</td>
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</table>

In a homogeneous population from Campania, Italy, he compared mothers of children with Down-Syndrome versus controls investigating the allele/genotype frequencies and risk of Down-Syndrome. A significant increase in odds ratios was found for 2 polymorphisms.

- **MTHFR 1298 C** (vs A) OR 1.46 (1.02-2.10)
- **MTHFR 1298 CC** (vs AA) OR 2.29 (1.06-4.96)
- **RFC1 80 G** (vs A) OR 1.48 (1.05-2.10)
- **RFC1 80 GG** (vs AA) OR 2.05 (1.03-4.07)

In conclusion, the MTHFR A1298C and RFC1 A80G polymorphisms seem to be genetic risk factors for Down-Syndrome.

5) Pregnancy in IEM: Teratogenicity

An overview on teratogenicity in IMD was given by Prof Roberto Cerone, University Dept. of Pediatrics, Genova, Italy
The first and main question in this field is: Is PKU the only IMD with implications for the fetus? A large number of maternal IMD seem to be benign for the fetus:

- Histidinaemia
- Hyperprolinaemia
- MSUD
- organic acidurias
- urea cycle disorders

At the moment, clear evidence of harm to the fetus besides PKU does not seem apparent for any other IMD. But, in the Italian experience, maternal tyrosinaemia type II (untreated with tyrosine levels > 500 µmol/l) was observed that appeared to have an adverse effect on the fetus (microcephaly & MRI alterations in 2 offspring). A Portuguese experience was reported about maternal glutaric aciduria type I with abnormal brain scans of the offspring picked up on newborn screening. These observations are anecdotal and not yet published.

6) Maternal health during pregnancy

Nutritional problems for the mother in the maternal PKU and the role of nutrition in pregnancy with PKU and birth defects was reviewed by Dr Ewa Kostyk, Clinical Geneticist, Krakow, Poland.

Most of our knowledge on this issue has been generated by the long-term, multicentre and international “Maternal Phenylketonuria Collaborative Study”. A large amount of data was collected about the influence of blood Phe levels, maternal weight gain, and nutrient intakes during pregnancy on the rate of microcephaly and congenital heart disease (CHD) in the offspring. Barriers to successful dietary control among pregnant women with PKU were identified, as for example:

- Mother’s IQ (poor understanding of the role of early initiation of the diet)
- Mother’s young age (unplanned pregnancies)
- Treatment costs (private insurers refuse to pay for medical foods)
- Obstetricians’ knowledge about maternal PKU

An overview is provided by the Supplement to Pediatrics published in 2003 with an extensive summary of all results of this large international study.
7) Conclusion

Overall, there are surprisingly few cases of pregnancies in women with IMDs reported in the literature, apart from phenylketonuria. Yet, many metabolic clinicians do have their own personal experiences. Unless these are collated into a registry, it is very hard to establish guidelines that are anything more than expert-based best practice. Increasing numbers of females with IMDs are growing up into the reproductive age range and it is essential for their welfare that more information is available to allow clinicians to manage them and their offspring properly. Perhaps this is an area that the metabolic community as a whole needs to work towards over the next few years?
References

1) Infertility and puberty in general

2) Reproductive effects and pregnancy in IMD
   - Levy HL. Reproductive effects of maternal metabolic disorders: implications for pediatrics and obstetrics.

3) Examples for disease specific effects
Working up mental retardation

Chairpersons:

J. Zschocke, Heidelberg
R. Hennekam, London
Working up mental retardation

Chairpersons: J. Zschocke, Heidelberg; R. Hennekam, London

Introduction
Mental retardation is a developmental disability that first appears in children under the age of 18. It is defined as an intellectual functioning level (as measured by standard tests for intelligence quotient) that is well below average, in conjunction with significant limitations in daily living as expressed in conceptual, social, and practical adaptive skills. In some children, mental retardation is accompanied by specific additional symptoms or signs (“mental retardation plus”) that may be diagnostically relevant.

Several studies have assessed the diagnostic sensitivity and specificity of various laboratory investigations in children with mental retardation (see references). Although useful in clinical practice, the resulting recommendations do not always take into consideration the specific clinical findings in the individual patient. At the EMG-workshop we took a slightly different approach to diagnostic algorithms in affected children. Rather then asking "which tests should we perform" we discussed "which common conditions should not be missed" - which disorders may be suspected and should be confirmed or excluded in view of the clinical findings in the individual patient. To highlight the principle we concentrated on a few constellations of “mental retardation plus”.

General considerations
There is general agreement that a complete neurologic and dysmorphologic examination including a formal IQ test should be carried out in all children as part of the clinical assessment. Furthermore, in all patients, irrespective of their level of functioning, chromosome analysis is useful. An MRI scan should be carried out in all children with neurologic symptoms (including macrocephaly and microcephaly) and IQ <70 in whom no obvious diagnosis is found.

Testing for Fra(X) syndrome is indicated in all males with unexplained mental retardation that do not show microcephaly. There is no single opinion on biochemical tests that should be performed, and other additional investigations also depend on relevant clinical findings and the differential diagnosis in the individual case.
**MR and tall stature**

*Important diagnoses*
- Consider familial tall stature + coincidental MR
- Fra(X) syndrome
- Sotos syndrome (mild MR / no MR)
- Klinefelter syndrome (mild MR / no MR)
- Lujan syndrome (Marfan-like phenotype + MR)
- Homocystinuria

*Important tests*
- Chromosome analysis
- Molecular test for Fra(X) syndrome (not in children with microcephaly)
- Brand reaction / amino-acids (urine)
- X-ray hand (bone age)
- Specific molecular test (if indicated, i.e. Sotos)

**MR and macrocephaly**

*Important Diagnoses*
- Consider familial macrocephaly and coincidental MR
- Canavan syndrome
- Alexander syndrome
- Fra(X) syndrome
- Gorlin syndrome (basal naevus cell carcinoma)
- Sotos syndrome
- Cowden syndrome
- Neurofibromatosis Type I
- Sanfilippo syndrome
- Consider skeletal dysplasia

*Important tests*
- Chromosome analysis
- Molecular test for Fra(X) syndrome
- MRI scan of the brain
- X-rays of hand / skull / chest
- Organic acids (urine)
- MPS / Oligosaccharides (urine)
- Specific DNA tests if indicated (i.e. PTEN; Sotos)
**MR and microcephaly**

Microcephaly is not a good handle for the differential diagnosis!

*Diagnoses that should not be missed:*

- Maternal PKU
- Congenital infection
- Chromosome abnormality
- Consider craniosynostosis

*Important tests*

- Maternal phenylalanine
- TORCH (timing!)
- Chromosome analysis
- Test for telomeric microdeletions
- Skull x-ray
- MRI brain

**MR and obesity**

*Important Diagnoses*

- Consider familial obesity and coincidental MR
- Prader-Willi syndrome
- Fra(X) syndrome
- Chromosome anomalies
- Bardet-Biedl syndrome
- Cohen syndrome
- Albright syndrome (small stature, skeletal symptoms)
- Alström syndrome (often no MRI)

*Important tests*

- Chromosome analysis
- Test for telomeric microdeletions
- Consider specific microdeletion studies (e.g. del 1p36, del 2q37, del 6q21, del 8p23, del 9q34, del 11p13, del 13q34, del 22q13.3)
- Methylation studies chromosom 15q1.1 (Prader-Willi syndrome)
- Molecular test for Fra(X) syndrome
- Electroretinogram, ERG (>1yr; usually >3yr; Bardet-Biedl syndrome, Cohen syndrome)
- Specific DNA tests if indicated (i.e. Alström syndrome; Albright syndrome; Cohen syndrome)
MR and organomegaly

Important Diagnoses

- Lysosomal disorders (see next slide)
- Costello syndrome
- Haematological disease/leukaemia with coincidental MR
- Consider skeletal dysplasia with small thorax

Hepatomegaly without significant splenomegaly

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Clinical features</th>
<th>Investigations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen storage disease</td>
<td>Hepatocellular dysfunction, Tubulopathy, large kidneys, short stature, skeletal myopathy, hypoglycaemia, ↑↑↑ triglycerides, ↑ urate, ↑ lactate</td>
<td>Metabolic tests, enzyme studies</td>
</tr>
<tr>
<td>Disorders of gluconeogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycosylation disorders (CDG, e.g. type Ib)</td>
<td>Hepatomegaly, hepatocellular dysfunction, protein losing enteropathy, multi-system disease</td>
<td>Transferrin electrophoresis</td>
</tr>
</tbody>
</table>

Hepatomegaly with splenomegaly

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Clinical features</th>
<th>Investigations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mukopolysaccharidoses</td>
<td>Progressive disease cours, coarse features, dysostosis multiplex, corneal clouding, neurological abnormalities</td>
<td>Glycosaminoglycans (urine)</td>
</tr>
<tr>
<td>Sphingolipidoses</td>
<td>Progressive neurological abnormalities, epilepsy, ataxia, spasticity</td>
<td>Oligosaccharide (urine)</td>
</tr>
</tbody>
</table>

Conclusions

Isolated symptoms are often useful as diagnostic handles although in practice combinations of symptoms are used. Which disorders are common depends heavily on age, gender, and study population. An algorithm for the diagnostic work-up of patients with mental retardation is provided in figure 1.
Figure 1: Algorithm for the diagnostic work-up of patients with mental retardation

References


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