Cerebral folate deficiency: Analytical tests and differential diagnosis

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Abstract

Cerebral folate deficiency is typically defined as a deficiency of the major folate species 5-methyltetrahydrofolate in the cerebrospinal fluid (CSF) in the presence of normal peripheral total folate levels. However, it should be noted that cerebral folate deficiency is also often used to describe conditions where CSF 5-MTHF is low, in the presence of low or undefined peripheral folate levels. Known defects of folate transport are deficiency of the proton coupled folate transporter, associated with systemic as well as cerebral folate deficiency, and deficiency of the folate receptor alpha, leading to an isolated cerebral folate deficiency associated with intractable seizures, developmental delay and/or regression, progressive ataxia and choreoathetoid movement disorders. Inborn errors of folate metabolism include deficiencies of the enzymes methylenetetrahydrofolate reductase, dihydrofolate reductase and 5,10-methenyltetrahydrofolate synthetase. Cerebral folate deficiency is potentially a treatable condition and so prompt recognition of these inborn errors and initiation of appropriate therapy is of paramount importance. Secondary cerebral folate deficiency may be observed in other inherited metabolic diseases, including disorders of the mitochondrial oxidative phosphorylation system, serine deficiency, and pyridoxine dependent epilepsy. Other secondary causes of cerebral folate deficiency include the effects of drugs, immune response activation, toxic insults and oxidative stress. This review describes the absorption, transport and metabolism of folate within the body; analytical methods to measure folate species in blood, plasma and CSF; inherited and acquired causes of cerebral folate deficiency; and possible treatment options in those patients found to have cerebral folate deficiency.

Conflicts of interest

SR is an Editor of the Journal of Inherited Disease and JIMD Reports.

Informed consent

Not applicable since no patients are reported in this manuscript.

Animal Rights

Not applicable since no animal studies were performed in the preparation of this manuscript.

1. Introduction

1.1 History of folate

Folic acid was discovered in 1941 as a factor, isolated from spinach leaves (folium being the Latin for leaves), required for the growth of *Streptococcus lactis* (Mitchell et al 1941). Around this time, in the 1930's and 40's, it was also found that yeast and liver extracts containing folic acid or a newly synthesised folic acid supplement could prevent anaemia caused by pregnancy, coeliac disease or malnutrition (see historical reviews of folic acid by Hoffbrand 2001 and Rosenberg 2012).

The term folate refers to a group of compounds containing a pteridine ring, a paraminobenzoic acid ring and glutamic acid (Figure 1). Folic acid, a common form of folate added to fortified foods, contains a fully oxidised pteridine ring and a single glutamic acid residue. Naturally occurring folates can exist in a variety of oxidation states (e.g. dihydro, tetrahydro-) and can also contain more than one glutamate residue (i.e. polyglutamate). Naturally occurring folates with an additional single carbon unit, such as a methyl, formyl, methylene or methenyl group attached to the N5 or N10 nitrogen atom, are frequently observed (Figure 1). These single carbon units are essential to the function of folates in one-carbon metabolism. For example, 5-methyltetrahydrofolate (5-MTHF) is essential for the methylation of homocysteine to methionine (Figure 1) and global deficiency of 5-MTHF results in elevated plasma homocysteine (Freeman et al 1975). S-Adenosyl-methionine (SAM), formed from methionine, is required for more than 100 methylation reactions in cells, including methylation of DNA, RNA, neurotransmitters, lipids, hormones and drug metabolites (Bottiglieri 2002 and 2013).

1.2 Folate absorption and metabolism

Folate absorption and metabolism is shown schematically in Figures 1 and 2. Dietary folate intake is largely from fruits and vegetables as polyglutamates, which are acted on by the enzyme folylpoly-³-glutamate carboxypeptidase (Visentin et al, 2014). This enzyme is present at the intestinal brush border and hydrolyses these polyglutamates to their monoglutamates, enabling absorption (Halsted et al 1997; Darcy-Vrillon et al 1988). Two folate transporters are expressed in the intestine - the reduced folate carrier (RFC) (Matherly and Hou 2008) and the proton-coupled folate transporter (PCFT) (Qiu et al 2006; Zhao and Goldman 2013). Defects in the gene encoding the PCFT (*SLC46A1*) have been described and are associated with systemic folate deficiency, emphasising the key role of PCFT in folate absorption and transport (Qiu et al, 2006; Zhao et al, 2017)). Defects in RFC, affecting intestinal transport of folate, have not been reported.

The RFC (encoded by *SLC19A1*) is an organic anion antiporter that exchanges 5-MTHF for other anions (inorganic or organic). It is ubiquitously expressed and is the main transport mechanism by which cells take-up folates from the systemic circulation (Matherly and Hou 2008). Specialised folate receptors (FRs) are also present on some cell types and represent a higher affinity transport system which operates at the nanomolar range (Chen et al, 2013; Zhao et al, 2011). The precise role and regulation of each of these different folate transport systems is still being elucidated. Folate uptake into the brain is mediated by the high affinity folate receptor α (FRα, encoded by *FOLR1*), which is highly expressed in the apical membrane of choroid plexus epithelial cells (Grapp et al, 2013) and PCFT (see figure 2). 5-MTHF delivery into the brain parenchyma appears to be mediated via exosomes that transport 5-MTHF-laden FRα molecules, traversing the cytosol of choroid plexus epithelial cells from the basolateral to the apical membranes, before being secreted into the CSF at the apical brush border (Grapp et al, 2013).

Folate metabolism is complex and has an essential role in nucleic acid biosynthesis, one carbon metabolism and amino acid metabolism, as illustrated in Figures 1 and 2. Dietary forms of folate are absorbed as their monoglutamates. Naturally occurring folates are generally considered to be in their reduced forms. Synthetic andunreduced forms of folate are reduced to dihydrofolate and then tetrahydrofolate by dihydrofolate reductase (DHFR). Tetrahydrofolate is then converted to 5,10-methylene-tetrahydrofolate by transfer of a carbon unit from serine in a reaction catalysed by

serine-hydroxymethyltransferase (SHMT). This reaction also produces glycine. 5,10-methylene-tetrahydrofolate can also be produced via the glycine cleavage system (GCS) (Kikuchi et al 2008; Pai et al 2015). 5,10-methylene-tetrahydrofolate is then converted to 5-methyl tetrahydrofolate (5-MTHF) by methylenetetrahydrofolate reductase (MTHFR). MTHFR is essential for the production of 5-MTHF, which in turn is essential for the remethylation of homocysteine to methionine. This cycle produces S-adenosyl methionine (SAM), which is used in numerous methylation reactions catalysed by methyltransferases, including those of nucleic acids, drug metabolites and neurotransmitters (Blom and Smulders, 2011). Other forms of folate, such as 10-formyl-tetrahydrofolate and 5,10-methylene-tetrahydrofolate, are involved in purine and pyrimidine synthesis, respectively.

1.3 Discovery of cerebral folate deficiency

Neurological disease was first linked to folate deficiency by Pincus and co-workers in 1973 (Reynolds et al, 1973) and low CSF folate levels were first reported in 1981 (Botez and Bachevalier 1981). Cerebral folate deficiency was subsequently defined by Raemakers and Blau (2004) as 'any neurological syndrome associated with a low CSF 5-MTHF, in the presence of normal folate metabolism outside the nervous system.' However, it should be noted that cerebral folate deficiency is often used to describe conditions where CSF 5-MTHF is low, regardless of the peripheral folate status. A variety of causes of cerebral folate deficiency have been described, ranging from inborn errors of metabolism including metabolic recycling defects (DHFR and MTHFR deficiencies) and disorders of folate transport (e.g. FOLR1 and PCFT defects, and mitochondrial disorders leading to impaired folate transport) to possible over-utilisation/degradation defects such as L-dopa methylation and neurodegeneration. The primary transport and metabolic defects typically present with extremely low cerebrospinal fluid (CSF) concentrations of 5-MTHF below 10 nmol/L prior to treatment.

2. Analytical methods for assessment of folate status

Folate status is usually initially assessed by total folate in serum or plasma using a folate-binding protein assay (see Sobczynska-Malefora and Harrington 2018 for review of folate assays). The advantages of this assay are that it is easily automated, inexpensive and quick to perform, but it

should be noted that it measures total folate, including folic acid, and therefore does not always accurately assess the levels of active forms of folate such as 5-MTHF. Other peripheral markers of folate status, such as red cell folate and total plasma homocysteine, are more specific and therefore may give a better indication of 'active' folate status than total folate in serum.

Methods have been described for the measurement of individual folate species in serum, red cells and CSF, typically using liquid chromatography-mass spectrometry (LC-MS) (Arning 2016; Asante et al ,2018; Kiekens et al, 2015; Meadows 2017; Nandania et al, 2018; Schittmayer et al, 2018). Such methods are not generally available in a diagnostic setting. For the diagnosis of cerebral folate deficiency (CFD), the most frequently used method is high performance liquid chromatography (HPLC) with fluorescence detection or electrochemical detection (ECD) of 5-MTHF in CSF (Akiyama et al 2015; Aylett et al 2013; Opladen et al 2006; Ormazabal et al 2006; Verbeek et al 2008). Most rely on the separation of 5-MTHF on a C18 HPLC column followed by ECD or fluorescence detection. Figure 3 shows 5-MTHF HPLC chromatograms, from CSF, performed in our laboratory.

5-MTHF is relatively stable in most CSF samples (Verbeek et al, 2008; Sobczynska-Malefora and Harrington 2018; Aylett et al, 2013) and does not typically degrade on an unrefrigerated autosampler overnight (personal observation). However, we have noted that 5-MTHF may degrade quickly in occasional CSF samples. The reason for this is not clear but could be related to oxidative catabolism due to deficiency of antioxidants such as ascorbic acid and/or increased generation of reactive oxygen species (Aylett et al, 2013). Consequently, we recommend that all CSF samples for 5-MTHF analysis should be snap frozen and stored at -70C. Repeat analysis of samples with low CSF 5-MTHF should be done on freshly thawed CSF and analysed immediately to distinguish between genuine low CSF 5-MTHF and ex vivo degradation of CSF 5-MTHF due to uncontrollable factors present in a particular sample.

There appears to be no rostro-caudal gradient for CSF 5-MTHF but CSF 5-MTHF concentrations are age-related with CSF 5-MTHF typically decreasing with age (Verbeek et al, 2008). Most laboratories have their own age-related reference ranges with broad agreement between different

laboratories (Verbeek et al, 2008; Aylett et al, 2013; Ormazabal et al, 2006). Reference ranges can vary between laboratories in different countries, possibly owing to differences in 'control' groups and other factors such as dietary differences. Therefore, it is important that each laboratory establishes its own references ranges and runs internal quality control (IQC) samples. In our laboratory, we make our own quality control (QC) samples by pooling CSF from samples with 'normal' chromatography, which are not 5-MTHF deficient. We also have a folate deficient QC (QCD) from a patient with 5,10-MTHFR deficiency. These QCs enable us to monitor our consistency of measurement over time and also make sure that we are separating 5-MTHF from other nearby peaks present in the QCD (see Figure 3). As CSF is often also taken for measurement of other analytes, such as monoamine metabolites and pterins, it makes sense to have a consistent sample collection protocol, which takes account of potential rostro-caudal gradients and stability of each of these analyses (Battlori et al, 2017).

In the future, LC-MS methods measuring a wider variety of folate species should be more readily available in diagnostic laboratories, allowing quicker and more detailed identification of defects in folate metabolism and transport. Other measures of one-carbon metabolism in tandem with folate measurement in CSF have been described (Smith et al, 2012). Such methods will also be particularly useful in monitoring treatment, especially in metabolic defects where there is not yet a clear consensus regarding what form/route of folate and/or other supplements (cobalamin, pyridoxine etc.) should be administered.

3. Inborn errors of folate transport and metabolism

Inborn errors of folate metabolism and transport can broadly be divided into those disorders where there is a generalised systemic folate deficiency, and those disorders characterized by cerebral (central) folate deficiency (CFD) with normal peripheral folate levels. The former include defects of intestinal folate absorption and of systemically expressed enzymes. In isolated CFD, defective folate transport across the blood-brain barrier leads to CFD with normal plasma and red cell folate levels. In addition, other inborn errors of metabolism may be associated with what appears to be a secondary CFD, since the underlying pathophysiological mechanisms remain obscure. The largest

group of such disorders are primary mitochondrial disorders, i.e. inherited disorders directly or indirectly affecting the function of the oxidative phosphorylation (OXPHOS) system.

3.1 Disorders of folate transport

3.1.1 Proton-coupled folate transporter (PCFT) deficiency

Hereditary folate malabsorption (OMIM #229050) is an autosomal recessive disorder caused by biallelic mutations in the *SLC46A1* gene encoding PCFT (Qiu et al, 2006; Wolf 2007). This transporter is essential for intestinal folate absorption and transport from blood to the brain. In patients affected by PCFT deficiency, lack of folate availability for the various folate-related pathways leads to decreased levels of all folate species (Qiu et al, 2006). More than 30 affected individuals have been reported to date (Zhao et al 2017). Affected infants typically present a few months after birth with symptoms and signs attributable to both systemic and central folate deficiency. Clinical features include feeding difficulties leading to faltering growth, megaloblastic anemia, neutropaenia, thrombocytopaenia, immune deficiency, recurrent infections (especially *Pneumocystis* pneumonia), diarrhoea and mouth ulcers (Kronn and Goldman 2017). Neurological features include global developmental delay, seizures and behavioural disturbance. Neuro-imaging may show demyelination and intracranial calcifications. Biochemical investigations reveal both plasma and CSF folate and 5-MHTF deficiencies. Treatment with parenteral or high dose oral folinic acid (5-formyl-tetrahydrofolate, 5-formyl-THF) rapidly corrects blood folate levels in PCFT deficiency but CSF 5-MTHF deficiency may be more difficult to correct. Therefore, it is recommended that CSF 5-MTHF levels are monitored in PCFT deficiency (Manea et al 2018). Early treatment can prevent the neurological complications of the disease (Kronn and Goldman 2017).

3.1.2 Folate receptor alpha deficiency

Mutations in *FOLR1* (OMIM #613068) encoding FRα were first described in 2009 in 3 children with progressive movement disorder, developmental regression and seizures associated with extremely low CSF 5-MTHF levels and normal peripheral folate levels (Steinfeld et al, 2009). More than 20 affected children have now been reported in the medical literature, and their main clinical features are summarised in Table 2. Symptom onset was between 6 months and 4.5 years in all

reported cases, with a median onset of 2 years. Most affected children appeared normal at birth, although one case had congenital microcephaly. There is usually a symptom-free interval of variable duration prior to symptom onset, which has led to the suggestion that 5-MTHF transport by FRβ may compensate for FRα deficiency in early infancy (Molero-Luis et al, 2015). Typical clinical signs at presentation are tremor, ataxia, hypotonia and developmental delay (especially motor and speech), with subsequent developmental regression and intractable seizures. Seizures are most often myoclonic, but individual patients may have several seizure types including astatic, tonic, tonic-clonic and atypical absences. Recurrent episodes of status epilepticus have been reported in three affected children. Other clinical features include movement disorders (especially chorea and athetosis), acquired microcephaly and polyneuropathy. CSF 5-MTHF levels are extremely low in FRα deficiency, typically < 5 nmol/L. The highest reported value was 11 nmol/L in one of 12 cases for whom absolute values are available (Table 2). Plasma and red cell folate levels are normal. Neuroimaging reveals hypomyelination, particularly affecting subcortical white matter, together with cerebellar and/or cerebral atrophy. Treatment with oral folinic acid supplementation (at doses ranging from 0.5 to 7 mg/kg/dy) has been reported to result in improvement of seizures, neuro-imaging appearances and CSF 5-MTHF levels (Table 2). However, response to treatment is variable and is related to the duration of symptoms at the time of initiation of treatment, emphasizing the importance of early recognition and treatment of this disorder. Because of the poor response to oral folinic acid in many cases, more recent reports have advocated intermittent intravenous folinic acid therapy, in addition to daily oral dosing (Delmelle et al, 2016). Folic acid is contraindicated in this condition, as it competitively binds to FRα, further reducing 5-MTHF transport into the brain.

3.2 Disorders of folate metabolism

3.2.1 Methylenetetrahydrofolate reductase (MTHFR) deficiency

MTHFR deficiency (OMIM #236250) is the most frequent inborn error of folate metabolism, with >200 patients reported in the literature (Burda et al, 2015). In this autosomal recessive disorder, there is defective synthesis of 5-MTHF, which is the essential methyl group donor for the remethylation of homocysteine to methionine (Watkins et al, 2012). Characteristic biochemical features of severe MTHFR deficiency are hyperhomocysteinemia with low-normal plasma

methionine, and CFD with low-normal plasma total folate levels (Skovby et al 2003). Since most plasma folate assays measure folate by immunoassay, which does not specifically measure 5-MTHF (SobczyĐska-Malefora and Harrington, 2018), plasma folate is not always low in MTHFR deficiency. Clinical presentations of severe MTHFR deficiency are extremely variable, ranging from early onset in the first months of life with encephalopathy, apnoea, seizures and hydrocephalus, to later onset with neurological decline, ataxia, seizures, cerebral atrophy and delayed myelination. Vascular occlusion, psychiatric manifestations and white matter abnormalities may be observed in older children and adults (Skovby et al 2003). The limited amounts of 5-MTHF available to be transported to the brain, coupled with diminished formation within the CNS, can explain the profound CFD in MTHFR deficiency. A common genetic variant in *MTHFR*, c.677 C>T, encodes a thermolabile enzyme with lower activity at higher temperatures. Homozygosity for the c.677 C>T genotype is associated with higher plasma homocysteine and folate levels compared to controls (Watkins et al, 2012), but these individuals do not have CFD (Ormazabal et al 2011). The c.677 C>T variant has been linked to a large number of disease processes, but as this variant is present very frequently in the general population, in 10-15% of Caucasians and up to 25% of Hispanics in North America (Dean 2012), it is very difficult to prove causality, and much more likely that these are spurious associations Some authors have also suggested possible association between *MTHFR* polymorphisms and neural tube defects and cardiovascular disease (Blom et al 2011; Watkins et al 2012), but a detailed discussion of this is beyond the scope of this review.

3.2.2 Dihydrofolate reductase (DHFR) deficiency

DHFR deficiency (OMIM #613839) appears to be an extremely rare autosomal recessive disorder of folate metabolism. *DHFR* mutations were first reported in 2011 by two research groups, who described a total of 6 individuals from 3 families with CFD associated with megaloblastic anaemia (Banka et al 2011; Cario et al 2011). There do not appear to have been any further subsequent reports of this disorder in the literature. The main clinical features were severe megaloblastic anemia or pancytopenia and a complex neurological picture with acquired microcephaly, refractory seizures, intellectual disability, and white matter lesions and cerebellar and/or cerebral atrophy on neuro-imaging. Biochemically, peripheral biomarkers were normal (folate, cobalamin and homocysteine), but all cases had profound CFD. Improvement of CSF 5-MTHF levels, seizure

control and other neurological symptoms was observed after oral folinic acid supplementation (Banka et al, 2011; Cario et al 2011).

3.2.3 5,10-Methenyl-tetrahydrofolate synthase (MTHFS) deficiency

MTHFS deficiency is the most recent genetically defined disorder of folate metabolism. MTHFS (OMIM *604197) is an enzyme involved in the conversion of folinic acid (5-formyl-THF) to 5,10-methenyl-tetrahydrofolate (Figure 1). 5,10-methenyl-tetrahydrofolate is in turn converted to 5,10-methylene-tetrahydrofolate, the precursor of 5-MTHF, or 10-formyltetrahydrofolate, which is important for purine metabolism. Recently, Rodan et al. (2018) described two patients with biallelic mutations in *MTHFS*. These patients presented with microcephaly, global developmental delay, epilepsy and cerebral hypomyelination, at birth in one case and 3 months in the other. CSF 5-MTHF levels were low-normal (~40-50 nmol/L, ref range 40-150). Enzyme analysis revealed impaired MTHFS activity and highly elevated levels of 5-formyl-THF, the substrate of this enzyme, in cultured skin fibroblasts from one patient. The pathophysiology of this disorder is still not fully understood, as the CSF 5-MTHF levels are not as low as typically observed in related folate disorders with a similar clinical phenotype. Rodan et al. hypothesise that although extracellular 5-MTHF levels are not low, intracellular levels may be deficient and suggest that accumulation of intracellular 5-formyl-THF polyglutamates may inhibit synthesis of 5,10-methylene-tetrahydrofolate by SHMT and in turn lead to inhibition of 5-MTHF production. A previous case of suspected MTHFS deficiency was described by Scaglia and Blau (2013) but complete molecular characterisation was not shown. The patients described by Rodan et al. continued to deteriorate with folic acid or folinic acid supplementation, but one patient showed some improvements after oral 5-MTHF and intramuscular methylcobalamin administration. The rationale behind the B12 administration was to decrease methyl group consumption in the endogenous synthesis of methylcobalamin.

3.2.4 Methylenetetrahydrofolate dehydrogenase (MTHFD1) deficiency

MTHFD1 deficiency (OMIM #617780) is a rare inborn error of folate metabolism that has been reported in 7 individuals from 4 families to date (Watkins et al 2011 JMG PMID:21813566, Burda et al 2015 JIMD PMID:25633902, Ramakrishnan et al 2016 J Allergy Clin Immunol Pract

PMID:27707659). Clinical features include megaloblastic anaemia, atypical hemolytic uraemic syndrome and severe combined immune deficiency (SCID), with seizures, intellectual impairment and abnormal brain imaging in some cases. Biochemical features include elevated blood levels of homocysteine and methylmalonic acid (Watkins et al 2011 JMG), with low CSF 5-MTHF levels documented in some affected individuals (Burda et al 2015). All affected patients had biallelic mutations in *MTHFD1* encoding a trifunctional protein involved in cellular folate metabolism. The three reactions catalysed by the MTHFD1 trifunctional protein are 5,10-methylenetetrahydrofolate dehydrogenase, 5,10-methenyltetrahydrofolate cyclohydrolase and 10-formyltetrahydrofolate synthetase, which condense formate with tetrahydrofolate to provide the primary entry point of single carbons into folate-dependent one-carbon metabolism in the cytosol (Field et al 2015 PNAS PMID:25548164, Burda et al 2015). In MTHFD1 deficiency, impaired nuclear de novo dTMP biosynthesis is thought to underlie both the megaloblastic anaemia and the SCID (Field et al 2015 PNAS). Treatment of affected individuals with folinic acid supplementation (in addition to intramuscular hydroxocobalamin, betaine and prophylactic antibiotics) was associated with clinical improvement, including resolution of anaemia and improved lymphocyte function in two cases (Burda et al 2015, Ramakrishnan et al 2016).

3.3 Genetic diseases associated with CFD

3.3.1 Mitochondrial disorders.

The term primary mitochondrial disease is generally accepted to refer to a large group of heterogeneous inborn errors of metabolism that directly or indirectly affect the integrity and/or function of mitochondria, particularly the OXPHOS system (Rahman and Rahman 2018). CFD was first reported in the context of mitochondrial disease in 1983 in a young woman with Kearns-Sayre syndrome (KSS, OMIM #530000) associated with ragged-red fibre myopathy, progressive neurological decline and white matter lesions and basal ganglia calcification on computed tomography of the brain (Allen et al 1983). In the intervening 35 years CFD has been recognized as a relatively frequent occurrence in primary mitochondrial disease, although the exact prevalence remains unknown (Hyland et al 2010). CFD has most frequently been reported in patients with KSS caused by single large-scale mitochondrial DNA deletions (Pineda et al 2006; Garcia-Cazorla et al 2008; Serrano et al 2010; Broomfield et al 2015), but has also been noted in patients with other

mitochondrial disorders including complex I deficiency (not genetically defined) (Ramaekers et al 2007b; Garcia-Cazorla et al, 2008) and Alpers disease caused by biallelic *POLG* mutations (Hasselmann et al, 2010). Associated clinical features may include seizures but these are not as universal in KSS as in primary CFD disorders such as FOLR1 and DHFR deficiencies. The underlying molecular mechanisms causing CFD in mitochondrial disorders remain obscure, but it is unlikely that mitochondrial CFD is related purely to a bioenergetic failure, since CFD is not a universal feature of mitochondrial disease. In the case of KSS, an attractive hypothesis is that accumulation of deleted mtDNA molecules in the choroid plexus of affected patients leads to breach of the choroidal integrity and thus impaired 5-MTHF transport into the brain (Tanji 2000; Spector and Johanson 2010). More recently CSF FRα and 5-MTHF levels were shown to be correlated in 9 patients with KSS, providing further support for this hypothesis (Grapp et al 2013). Therapeutic response to folinic acid has been noted in KSS (Pineda et al, 2006, Quijada-Fraile et al, 2014). However, although folinic acid therapy has been reported to correct CSF 5-MTHF levels and cerebral white matter lesions, it does not appear to prevent the relentless multisystem decline associated with this disorder (Broomfield et al, 2015).

3.3.2 Serine deficiency

Three inborn errors of L-serine biosynthesis have been reported, namely deficiencies of 3-phoshoglycerate dehydrogenase (OMIM #601815), phosphoserine aminotransferase (OMIM #610992) and phosphoserine phosphatase (OMIM #614023) (Van der Crabben et al, 2013). In addition, a founder mutation in *SLC1A4* (OMIM #616657) leading to defective serine transport to the brain has been reported in the Ashkenazi Jewish population (Damseh et al, 2015). The main clinical features of serine deficiency are congenital microcephaly, postnatal growth retardation, hypertonia (progressive spastic quadriplegia), psychomotor retardation, epilepsy and hypogonadism. The link between serine and folate deficiency is that serine is transformed to glycine by SHMT2 in a reaction that adds a methylene group to THF to form 5,10-methyleneTHF. This is the primary source of one-carbon units in folate metabolism. Thus, serine deficiency leads to mild to moderate CFD and disorders of serine biosynthesis and transport should be considered in the differential diagnosis of CFD (Molero-Luis et al, 2015). Serine supplementation, together with

glycine, usually leads to improved seizure control, but has little impact on psychomotor development.

3.3.3 Dihydropteridine reductase (DHPR) deficiency

DHPR is an enzyme involved in the recycling/reduction of dihydrobiopterin to tetrahydrobiopterin, the active cofactor for aromatic amino acid (phenylalanine, tyrosine and tryptophan) hydroxylases and nitric oxide synthase. DHPR is also able to reduce the structurally related dihydrofolate to tetrahydrofolate. Patients with DHPR deficiency (OMIM #261630) are detected by the presence of hyperphenylalanaemia on newborn screening, with normal pterins and reduced DHPR activity in dried blood spots. If treated with a low phenylalanine diet alone, neurological symptoms (coarse tremor, rigidity, drooling and progressive cognitive and motor decline) develop because of neurotransmitter deficiency. Treatment therefore consists of dietary phenylalanine restriction together with L-dopa, carbidopa (a peripheral decarboxylase inhibitor) and 5-hydroxytryptophan supplementation. Progressive cerebral calcification affecting the basal ganglia and white matter has been reported to result from secondary CFD in patients with DHPR deficiency and can be prevented by folinic acid supplementation (Irons et al, 1987; Woody et al, 1989. A putative mechanisms to explain the CFD in these patients is that MTHFR enzyme activity might be redirected in the face of DHPR deficiency from 5-MTHF synthesis to the production of BH4 from qBH2, since MTHFR has similar Km values for its natural substrate 5,10-methylenetetrahydrofolate and for qBH2 (Irons et al, 1987).

3.3.4 Aromatic L-amino acid decarboxylase (AADC) deficiency

Patients with AADC deficiency (OMIM #608643) may also develop secondary CFD (Wassenberg et al, 2017). In AADC deficiency, affected patients have a severe combined deficiency of serotonin, dopamine, noradrenaline and adrenaline. More than 100 patients have been reported in the literature and clinical presentation is typically in infancy with hypotonia, global developmental delay, movement disorders (oculogyric crisis, dystonia and hypokinesia) and autonomic symptoms (Wassenberg et al, 2017). The enzyme deficiency affects the conversion of L-dopa to dopamine, leading to a build-up of L-dopa and consequently an increased reliance on folate for L-dopa

methylation. Folinic acid therapy is therefore recommended for patients with AADC deficiency if CSF 5-MTHF is low (Wassenberg et al, 2017).

3.3.5 Pyridoxine dependent epilepsy

Pyridoxine dependent epilepsy (PDE, OMIM #266100) is most commonly caused by mutations in *ALDH7A1* encoding antiquitin (α-aminoadipic semialdehyde dehydrogenase), an enzyme of the lysine degradation pathway (reviewed by Wilson et al 2019 in this issue of the *Journal of Inherited Metabolic Disease*). Antiquitin deficiency results in accumulation of ±-aminoadipic semialdehyde (AASA) and piperideine-6-carboxylate (P6C). P6C condenses with and inactivates pyridoxal phosphate, the active form of vitamin B6 (Mills et al, 2006). Most patients achieve complete seizure control on monotherapy with pyridoxine, the first dose being given in a setting where respiratory support can be provided, since apnoea may develop in B6 responsive patients. The presence of cognitive impairment in a significant majority of patients with PDE despite adequate seizure control led to the hypothesis that this was mediated by accumulating toxic intermediates in the lysine degradation pathway (Van Karnebeek et al, 2016). Current treatment protocols recommend dietary lysine restriction, together with arginine supplementation as this competes with lysine for cellular uptake, and B6 at a maximum dose of 300mg/day (to avoid peripheral neuropathy and other features of B6 toxicity). A disorder initially reported as folinic acid responsive epilepsy was subsequently shown to be allelic with PDE – both are caused by biallelic mutations in *ALDH7A1* (Gallagher et al, 2009). The precise mechanism for folinic acid responsiveness of the seizures in these patients remains obscure. Typically these patients have been treated with both pyridoxine and folinic acid and baseline levels of CSF 5-MTHF are rarely measured (Stockler et al, 2011). However, we have noticed in some older patients with PDE, that CSF 5-MTHF can fall below the reference range (unpublished observation from our laboratory - two teenage siblings with confirmed PDE had CSF 5-MTHF levels around half of the lower end of the reference range), so consideration should be given to monitoring 5-MTHF levels and supplementing with folinic acid if indicated.

4 Acquired cerebral folate deficiency
4.1 Environmental and unknown factors

To date, delineation of all neurological conditions leading to CFD is probably incomplete, as evidenced by some patients with remarkably low CSF 5-MTHF values with no genetic diagnosis. The presence of folate receptor autoantibodies in the CSF has been claimed as a cause of CFD in a wide range of conditions, ranging from an infantile onset CFD syndrome (Ramaekers et al, 2005) to schizophrenia, autistic spectrum disorder and Rett syndrome (Ramaekers et a. 2014; Frye 2013; Moretti 2008; Ramaekers et al, 2007c and 2003). These results have not been replicated in other laboratories and it is possible that in some neurological conditions, the observed mildly low CSF 5-MTHF values are an epiphenomenon with no pathophysiological implications. In the authors' experience, a relatively frequent cause of mild to moderate CFD is a suboptimal blood folate status due to inadequate intake (Perez-Dueñas et al, 2011). This common condition should be ruled out as a first step in the diagnostic investigations for CFD. For example, inappropriate folate nutrition has been associated with many neuropsychiatric disorders (Morris et al, 2002) and folate deficiency and hyperhomocysteinemia are commonly observed in psychiatric geriatric patients.

4.2 Drugs and CFD

In some cases, CFD may be secondary to medication effects. Many drugs are methylated during the normal detoxification process (Jancova et al, 2010) and this may contribute to a secondary loss of folate. 5-MTHF is required for SAM production, which is used by methyltransferases to methylate a wide range of substrates including L-dopa, a drug used to treat dopamine deficiency. Long term L-dopa/carbidopa therapy can result in secondary folate deficiency so folinic acid supplementation is recommended (Surtees and Hyland 1990). CSF 5-MTHF levels should be monitored in such patients if folate deficiency is suspected. Some antiepileptic drugs, such as phenytoin, carbamazepine, barbiturates and valproate, may reduce folate levels (Wegner et al, 1992; Morrell 2002; Opladen et al, 2010), and this should be taken into consideration when prescribing AEDs for patients with CFD and epilepsy.

Other drugs known to deplete folate levels include anti-folate cancer drugs such as methotrexate (inhibits DHFR) (Lambie and Johnson 1985; Vezmar et al, 2009) and some antibiotics, such as trimethoprim, whose mechanism of action is via inhibition of bacterial DHFR (Lambie and Johnson 1985). Nitrous oxide (laughing gas) is used as an anaesthetic and may be abused as a recreational

drug. Long term or excessive use can result in inhibition of methionine synthase via deactivation of its cobalamin cofactor, resulting in cellular folate becoming trapped as 5-MTHF - 'the folate trap hypothesis' (Lumb et al, 1980). 5-MTHF levels may be normal or elevated, but this folate would be trapped due to the inactivity of methionine synthase and inhibition of other downstream pathways requiring different folate species.

4.3 Oxidative stress and toxic insult resulting in CFD

Aylett et al (2013) showed a positive correlation between levels of CSF 5-MTHF and those of the antioxidant ascorbic acid (vitamin C). It was hypothesised that ascorbic acid and other antioxidants may protect 5-MTHF in the CSF and some cases of CFD may be due to a deficiency of these antioxidant defences and/or an increase in oxidative stress/toxic insult (see figure 2B). Chronic immune response activation is one possible cause of increased oxidative stress and interestingly there have been some reports of CFD associated with elevated levels of the immune response activation marker, neopterin (Hasselmann et al, 2010; Papandreou et al, 2018; Blau et al, 2003). In our laboratory, we have noticed that in some CSF samples 5-MTHF degrades rapidly on the HPLC autosampler (unpublished findings) even though 5-MTHF is typically thought to be relatively stable in biological samples (Verbeek et al, 2008). This may suggest that in some samples, antioxidant defences are compromised and/or oxidising species are present in the CSF. Such findings may have clinical relevance.

5 Therapeutic aspects: different folate forms and routes of administration
5.1 Folate, folinic acid and 5-methyltetrahydrofolate

Folate is an umbrella term for several compounds including 5-MTHF, 5-formyl-THF (folinic acid), 10-formyl-THF, 5,10-methylene-THF and THF (Scaglione et al, 2014). It should be noted that the currently available formulations (folic acid, folinic acid and 5-MTHF) have different properties (Scaglione et al, 2014). In most studies reporting treatment of CFD, folinic acid has been used, with good results in many cases, under the rationale that the brain has a limited capacity to reduce folic acid. Folic acid is not an active drug since it needs to be converted into metabolically active forms by several metabolic reactions, including reduction by DHFR (Forssen et al, 2000; Scaglione et al,

2014). Genetic or acquired disturbances in folate availability may decrease synthesis of the active vitamers, and so therapy with metabolically active forms of folate is preferred.

Folinic acid is converted to THF without the need for DHFR enzymatic activity (Rajagopalan et al, 2002). 5-MTHF, which represents >90% of total plasma folate (Scaglione et al, 2014) has important advantages over other folate forms, since it is well absorbed in the intestine and its bioavailability is not affected by DHFR and MTFHR deficiencies. The use of 5-MTHF could also prevent the potential negative effects of unconverted folic acid in the brain, and it is thought to be the most efficient way to restore CSF 5-MTHF concentrations (Knowles et al, 2016).

5.2 Routes of administration

Specific therapeutic approaches have been described for each of the individual disorders of folate transport and metabolism in the relevant sections above. As documented above, oral folate therapy has been used extensively in CFD, with variable results. Because neurodevelopmental outcomes have not been normal, unless therapy is started extremely early (the best results have been observed in siblings treated at a pre- or oligosymptomatic stage), recently it has been suggested that parenteral (either intramuscular or intravenous) folinic acid or 5-MTHF administration may further improve the clinical outcome of patients with different forms of CFD. Although current experience is limited owing to the rarity of the genetic conditions leading to CFD, promising preliminary results highlight the importance of designing clinical trials to assess alternative routes of folate administration (Delmelle et al, 2016; Manea et al, 2018; Torres et al, 2015).

5.3 Adverse effects

To our knowledge, there are no publications of adverse effects of folinic acid or 5-MTHF in patients with primary CFD, other than some anecdotal observations in small cohorts of patients (Frye et al, 2013). Nevertheless, it is important to consider the possibility of adverse effects of folate therapy in the pathological conditions described above, and to perform appropriate monitoring for potential toxicity. For example, excess folic acid may act as a competitive inhibitor of DHFR activity (Reynolds et al, 2006). Another consideration is that administration of high doses of folate may lead to significant excitatory effects in neuronal cells (Morrell et al, 2002).

6. Conclusion

As can be seen from this review, folate species are vital in many metabolic pathways. Concentrations of 5-MTHF are maintained at a higher concentration in the CSF than plasma, indicating the importance of this folate species in the brain. Primary deficiencies in folate metabolism or transport can result in CFD. However, it is becoming increasingly acknowledged that CFD is also a common secondary finding in many other metabolic defects as well as being observed after toxic insults/drug therapy. Although prolonged CFD can result in a range of clinical symptoms such as white matter changes on MRI due to impaired myelination, developmental delay and seizures, it is also a potentially treatable condition. Therefore, it is important to highlight that measurement of CSF 5-MTHF is useful in a wide range of conditions, as a biomarker to monitor the condition, to understand the underlying aetiology (oxidative stress, transport impairment, metabolic defects in one carbon metabolism, increased utilization of 5-MTHF) and also to monitor treatment with folate species such as folinic acid and 5-MTHF.

References

Akiyama T, Tada H, Shiokawa T et al (2015) Total folate and 5-methyltetrahydrofolate in the cerebrospinal fluid of children: correlation and reference values. *Clin Chem Lab Med* 53(12):2009-14.

Akiyama M, Akiyama T, Kanamaru K et al (2016) Determination of CSF 5-methyltetrahydrofolate in children and its application for defects of folate transport and metabolism. *Clin Chim Acta* 460:120-5.

Al-Baradie RS, Chaudhary MW (2014) Diagnosis and management of cerebral folate deficiency. A form of folinic acid-responsive seizures. *Neurosciences (Riyadh)*. 19(4):312-6.

Allen RJ, DiMauro S, Coulter DL et al (1983) Kearns-Sayre syndrome with reduced plasma and cerebrospinal fluid folate. *Ann Neurol* 13(6): 679-682 .

Arning E, Bottiglieri T (2016) Quantitation of 5-Methyltetrahydrofolate in Cerebrospinal Fluid Using Liquid Chromatography-Electrospray Tandem Mass Spectrometry. *Methods Mol Biol* 1378:175-82.

Asante I, Pei H, Zhou E et al (2018) Simultaneous quantitation of folates, flavins and B(6) metabolites in human plasma by LC-MS/MS assay: Applications in colorectal cancer. J *Pharm Biomed Anal* 158:66-73.

Aylett SB, Neergheen V, Hargreaves IP et al (2013) Levels of 5-methyltetrahydrofolate and ascorbic acid in cerebrospinal fluid are correlated: implications for the accelerated degradation of folate by reactive oxygen species. *Neurochem Int* 63(8):750-5.

Banka S, Blom HJ, Walter J et al (2011) Identification and characterization of an inborn error of metabolism caused by dihydrofolate reductase deficiency. *Am J Hum Genet* 88(2):216-25.

Batllori M, Molero-Luis M, Ormazabal A et al (2018) Cerebrospinal fluid monoamines, pterins, and folate in patients with mitochondrial diseases: systematic review and hospital experience. *J Inherit Metab Dis*. 41(6):1147-1158.

Batllori M, Molero-Luis M, Ormazabal A et al (2017) Analysis of human cerebrospinal fluid monoamines and their cofactors by HPLC. *Nat Protoc* 12(11):2359-2375.

Becker M, Axelrod DJ, Oyesanmi O et al (2007) Hematologic problems in psychosomatic medicine. *Psychiatr Clin North Am* 30(4):739-59.

Blau N, Bonafé L, Krägeloh-Mann I et al (2003) Cerebrospinal fluid pterins and folates in Aicardi-Goutières syndrome: a new phenotype. *Neurology* 2003 61(5):642-7.

Blom HJ, Smulders Y. (2011) Overview of homocysteine and folate metabolism. With special references to cardiovascular disease and neural tube defects. *J Inherit Metab Dis* 34(1):75-81.

Botez MI, Bachevalier J (1981) The blood-brain barrier and folate deficiency. *Am J Clin Nutr*. 34(9):1725-30.

Bottiglieri T (2013) Folate, vitamin B_{12}, and S-adenosylmethionine. *Psychiatr Clin North Am* 36(1):1-13.

Bottiglieri T (2002) S-Adenosyl-L-methionine (SAMe): from the bench to the bedside--molecular basis of a pleiotrophic molecule. *Am J Clin Nutr* 76(5):1151S-7S.

Broomfield A, Sweeney MG, Woodward CE et al (2015) Paediatric single mitochondrial DNA deletion disorders: an overlapping spectrum of disease. *J Inherit Metab Dis* 38(3):445-57.

Burda P, Kuster A, Hjalmarson O, et al (2015) Characterization and review of MTHFD1 deficiency: four new patients, cellular delineation and response to folic and folinic acid treatment. *J Inherit Metab Dis* 38(5):863-72.

Burda P, Schäfer A, Suormala T et al (2015) Insights into severe 5,10-methylenetetrahydrofolate reductase deficiency: molecular genetic and enzymatic characterization of 76 patients. *Hum Mutat* 36(6):611-21.

Cario H, Bode H, Debatin KM et al (2009) Congenital null mutations of the FOLR1 gene: a progressive neurologic disease and its treatment. *Neurology* 73(24):2127-9.

Cario H, Smith DE, Blom H et al (2011). Dihydrofolate reductase deficiency due to a homozygous DHFR mutation causes megaloblastic anemia and cerebral folate deficiency leading to severe neurologic disease. *Am J Hum Genet* 88(2):226-31.

Chen C, Ke J, Zhou XE et al (2013) Structural basis for molecular recognition of folic acid by folate receptors. *Nature* 500(7463):486-9.

Chen L, Ducker GS, Lu W et al (2017). An LC-MS chemical derivatization method for the measurement of five different one-carbon states of cellular tetrahydrofolate. *Anal Bioanal Chem* 409(25):5955-5964.

Daly S, Mills JL, Molloy AM et al (1997) Minimum effective dose of folic acid for food fortification to prevent neural-tube defects. *Lancet* 350(9092):1666-9.

Damseh, N, Simonin, A, Jalas, C et al (2015) Mutations in SLC1A4, encoding the brain serine transporter, are associated with developmental delay, microcephaly and hypomyelination. *J Med Genet* 52: 541-547.

Darcy-Vrillon B, Selhub J, Rosenberg IH (1988) Analysis of sequential events in intestinal absorption of folylpolyglutamate. *Am J Physiol* 255:G361–G366.

Davidson LS, Girdwood RH (1947) Folic acid as a therapeutic agent. *Br Med J* 1(4504):587-91.

Delmelle F, Thöny B, Clapuyt P et al (2016) Neurological improvement following intravenous high-dose folinic acid for cerebral folate transporter deficiency caused by FOLR-1 mutation. *Eur J Paediatr Neurol* 2016 20(5):709-13.

Dill P, Schneider J, Weber P et al (2011) Pyridoxal phosphate-responsive seizures in a patient with cerebral folate deficiency (CFD) and congenital deafness with labyrinthine aplasia, microtia and microdontia (LAMM). *Mol Genet Metab* 104(3):362-8.

Dyment DA, Tetreault M, Beaulieu CL et al (2015) Whole-exome sequencing broadens the phenotypic spectrum of rare pediatric epilepsy: a retrospective study. *Clin Genet* 88(1):34-40.

Ferreira P, Luco SM, Sawyer SL et al (2015) Late diagnosis of cerebral folate deficiency: Fewer seizures with folinic acid in adult siblings. *Neurol Genet* 22;2(1):e38.

Field MS, Kamynina E, Watkins D et al (2015) Human mutations in methylenetetrahydrofolate dehydrogenase 1 impair nuclear de novo thymidylate biosynthesis. *Proc Natl Acad Sci U S A*. Jan 13;112(2):400-5.

Forssen KM, Jagerstad MI, Wigertz K et al (2000) Folates and dairy products: a critical update. *J Am Coll Nutr* 19(2 Suppl), 100S–110S.

Freeman JM, Finkelstein JD, Mudd SH (1975) Folate-responsive homocystinuria and schizophrenia. A defect in methylation due to deficient 5,10-methylenetetrahydrofolate reductase activity. *N Engl J Med* 292(10):491-6.

Frye RE, Sequeira JM, Quadros EV et al (2013) Cerebral folate receptor autoantibodies in autism. spectrum disorder. *Mol Psychiatry* 18(3), 369–381.

Gallagher RC, Van Hove JL, Scharer G et al (2009) Folinic acid-responsive seizures are identical to pyridoxine-dependent epilepsy. *Ann Neurol* 65(5):550-6.

Garcia-Cazorla A, Quadros EV, Nascimento A et al (2008) Mitochondrial diseases associated with cerebral folate deficiency. *Neurology* 2008 70(16):1360-2.

Grapp M, Wrede A, Schweizer M et al (2013) Choroid plexus transcytosis and exosome shuttling deliver folate into brain parenchyma. *Nat Commun* 4:2123.

Grapp M, Just IA, Linnankivi T et al (2012) Molecular characterization of folate receptor 1 mutations delineates cerebral folate transport deficiency. *Brain* 2012 135(Pt 7):2022-31.

Halsted CH, Ling EH, Luthi-Carter R et al (1998) Folylpoly-gamma-glutamate carboxypeptidase from pig jejunum. Molecular characterization and relation to glutamate carboxypeptidase II. *J Biol Chem 273(32):20417-24.*

Hasselmann O, Blau N, Ramaekers VT et al (2010) Cerebral folate deficiency and CNS inflammatory markers in Alpers disease. *Mol Genet Metab* 99(1):58-61.

Hoffbrand, AV (2001) Historical review - The History of Folic Acid. <u>British Journal of Haematology</u> 113, 579-589.

Hyland K, Shoffner J, Heales SJ (2010) Cerebral folate deficiency. *J Inherit Metab Dis* 33(5):563-70.

Irons M, Levy HL, O'Flynn ME, et al (1987) Folinic acid therapy in treatment of dihydropteridine reductase deficiency. *J Pediatr* 110(1):61-7.

Jancova P1, Anzenbacher P, Anzenbacherova E (2010) Phase II drug metabolizing enzymes. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 154(2):103-16.

Kiekens F, Van Daele J, Blancquaert D et al (2015) A validated ultra-high-performance liquid chromatography-tandem mass spectrometry method for the selective analysis of free and total folate in plasma and red blood cells. *J Chromatogr A.* 1398:20-8.

Kikuchi G, Motokawa Y, Yoshida T et al (2008) Glycine cleavage system: reaction mechanism, physiological significance, and hyperglycinemia. *Proc Jpn Acad Ser B Phys Biol Sci* 84(7):246-63.

Kobayashi Y, Tohyama J, Akiyama T et al (2017) Severe leukoencephalopathy with cortical involvement and peripheral neuropathy due to FOLR1 deficiency. *Brain Dev* 39(3):266-270.

Knowles L, Morris AA, Walter JH (2016) Treatment with Mefolinate (5-Methyltetrahydrofolate), but Not Folic Acid or Folinic Acid, Leads to Measurable 5-Methyltetrahydrofolate in Cerebrospinal Fluid in Methylenetetrahydrofolate Reductase Deficiency. *JIMD Rep* 29:103-107.

Kronn D, Goldman ID (2017) Hereditary Folate Malabsorption. In: Adam MP, Ardinger HH, Pagon RA et al eds. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2019. 2008 Jun 17 [updated 2017 Apr 27].

Lambie DG, Johnson RH (1985) Drugs and folate metabolism. *Drugs* 30(2):145-55.

Lumb M, Deacon R, Perry J et al (1980) The effect of nitrous oxide inactivation of vitamin B12 on rat hepatic folate. Implications for the methylfolate-trap hypothesis. *Biochem J* 186(3):933-6.

Manea E, Gissen P, Pope S et al (2018) Role of Intramuscular Levofolinate Administration in the Treatment of Hereditary Folate Malabsorption: Report of Three Cases. *JIMD Rep* 2018;39:7-12.

Matherly LH, Hou Z (2008) Structure and function of the reduced folate carrier a paradigm of a major facilitator superfamily mammalian nutrient transporter. *Vitam Horm* 79:145-84.

Meadows S (2017) Multiplex Measurement of Serum Folate Vitamers by UPLC-MS/MS. *Methods Mol Biol* 1546:245-256.

Mills PB, Struys E, Jakobs C et al (2006) Mutations in antiquitin in individuals with pyridoxine-dependent seizures. *Nat Med* 12(3):307-9.

Mitchell HK, Snell EE and Williams R.J (1941) The concentration of folic acid. *Journal of the American Chemical Society* 63, 2284.

Molero-Luis M, Serrano M, O'Callaghan MM et al (2015) Clinical, etiological and therapeutic aspects of cerebral folate deficiency. *Expert Rev Neurother* 15(7):793-802.

Moretti P, Peters SU, Del Gaudio D et al (2008) Brief report: autistic symptoms, developmental regression, mental retardation, epilepsy, and dyskinesias in CNS folate deficiency. *J Autism Dev Disord* 38(6), 1170–1177.

Morrell MJ (2002) Folic Acid and Epilepsy. *Epilepsy Curr* 2(2), 31–34.

Morris MS (2002) Folate, homocysteine, and neurological function. *Nutr Clin Care* 5(3):124-32.

Nandania J, Kokkonen M, Euro L et al (2018) Simultaneous measurement of

folate cycle intermediates in different biological matrices using liquid chromatography-tandem

mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 1092:168-178.

Ohba C, Osaka H, Iai M et al (2014) Diagnostic utility of whole exome sequencing in patients

showing cerebellar and/or vermis atrophy in childhood. *Neurogenetics* 14(3-4):225-32.

Opladen T, Blau N, Ramaekers VT (2010) Effect of antiepileptic drugs and reactive

oxygen species on folate receptor 1 (FOLR1)-dependent 5-methyltetrahydrofolate

transport. *Mol Genet Metab* 101(1):48-54.

Opladen T, Ramaekers VT, Heimann G et al (2006) Analysis of

5-methyltetrahydrofolate in serum of healthy children. *Mol Genet Metab* 87(1):61-5.

Ormazabal A, García-Cazorla A, Pérez-Dueñas B et al (2006) Determination of 5-methyltetrahydrofolate in cerebrospinal fluid of paediatric patients: reference values for a paediatric

population. *Clin Chim Acta* 371(1-2):159-62.

Ormazábal A, Perez-Dueñas B, Sierra C e al (2011) Folate analysis for the

differential diagnosis of profound cerebrospinal fluid folate deficiency. *Clin*

Biochem 44(8-9):719-21.

Pai YJ, Leung KY, Savery D et al (2015) Glycine decarboxylase deficiency causes neural tube defects and features of non-ketotic hyperglycinemia in mice. *Nat Commun* 6:6388.

Papandreou A, Rahman S, Fratter C et al (2018) Spectrum of movement disorders and neurotransmitter abnormalities in paediatric POLG disease. *J Inherit Metab Dis* 41(6):1275-1283.

Pérez-Dueñas B, Ormazábal A, Toma C et al (2011) Cerebral folate deficiency syndromes in childhood: clinical, analytical, and etiologic aspects. *Arch Neurol* 68(5):615-21.

Pérez-Dueñas B, Toma C, Ormazábal A et al (2010) Progressive ataxia and myoclonic epilepsy in a patient with a homozygous mutation in the FOLR1 gene. *J Inherit Metab Dis.* 33(6):795-802.

Pineda, M, Ormazabal A, López-Gallardo E et al (2006) Cerebral folate deficiency and leukoencephalopathy caused by mitochondrial DNA deletion. *Ann Neurol* 59(2), 394–398.

Qiu A, Jansen M, Sakaris A et al (2006) Identification of an intestinal folate transporter andthe molecular basis for hereditary folate malabsorption. *Cell* 1;127(5):917-28.

Quijada-Fraile P, O'Callaghan M, Martín-Hernández E et al (2014) Follow-up of folinic acid supplementation for patients with cerebral folate deficiency and Kearns-Sayre syndrome. *Orphanet J. Rare Dis* 9(1):3.

Rahman J, Rahman S (2018) Mitochondrial medicine in the omics era. *Lancet* 391(10139):2560-2574.

Rajagopalan PT, Zhang Z, McCourt L et al (2002) Interaction of dihydrofolate reductase with methotrexate: ensemble and single-molecule kinetics. *Proc Natl Acad Sci U S A* 99(21):13481-6.

Ramaekers VT, Thöny B, Sequeira JM et al (2014) Folinic acid treatment for schizophrenia associated with folate receptor autoantibodies. *Mol Genet Metab* 113(4):307-14.

Ramaekers VT, Blau N, Sequeira JM et al (2007a) Folate receptor autoimmunity and cerebral folate deficiency in low-functioning autism with neurological deficits. *Neuropediatrics* 38(6):276-81.

Ramaekers VT, Weis J, Sequeira JM et al (2007b). Mitochondrial complex I encephalomyopathy and cerebral 5-methyltetrahydrofolate deficiency. *Neuropediatrics* 38(4):184-7.

Ramaekers VT, Sequeira JM, Artuch R et al (2007c) Folate receptor autoantibodies and spinal fluid 5-methyltetrahydrofolate deficiency in Rett syndrome. *Neuropediatrics* 38(4):179-83.

Ramaekers VT, Rothenberg SP, Sequeira JM et al (2005) Autoantibodies to folate receptors in the cerebral folate deficiency syndrome. *N Engl J Med* 352(19):1985-91.

Ramaekers VT, Blau N (2004) Cerebral folate deficiency. *Dev Med Child Neurol* 46(12):843-51.

Ramaekers VT, Hansen SI, Holm J et al (2003) Reduced folate transport to the CNS in female Rett patients. *Neurology* 61(4):506-15.

Ramakrishnan KA, Pengelly RJ, Gao Y et al (2016) Precision Molecular Diagnosis Defines Specific Therapy in Combined Immunodeficiency with Megaloblastic Anemia Secondary to MTHFD1 Deficiency *J Allergy Clin Immunol Pract* 4(6):1160-1166.

Reynolds EH, Rothfeld P, Pincus JH (1973) Neurological disease associated with folate deficiency. 2(5863):398-400.

Reynolds E (2006) Vitamin B12, folic acid, and the nervous system. *Lancet Neurol* 5(11):949-60.

Rodan LH, Qi W, Ducker GS et al (2018) 5,10-methenyltetrahydrofolate synthetase deficiency causes a neurometabolic disorder associated with microcephaly, epilepsy, and cerebral hypomyelination. *Mol Genet Metab* 125(1-2):118-126.

Rosenberg IH (2012) A history of the isolation and identification of folic acid (folate). *Ann Nutr Metab* 61(3):231-5.

Scaglia F, Blau N (2013) Disorders of Folate Metabolism and Transport, Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 167–178

Scaglione F, Panzavolta G (2014) Folate, folic acid and 5-methyltetrahydrofolate are not the same thing Xenobiotica 44(5), 480–488.

Schittmayer M, Birner-Gruenberger R, Zamboni N (2018) Quantification of Cellular Folate Species by LC-MS after Stabilization by Derivatization. *Anal Chem* 90(12):7349-7356.

Serrano, M. García-Silva MT, Martin-Hernandez E et al (2010) Kearns–Sayre syndrome: cerebral folate deficiency, MRI findings and new cerebrospinal fluid biochemical features. *Mitochondrion* 10(5):429–432.

Skovby F (2003) Disorders of sulfur amino acids. In: Blau N, Duran M, Blaskovics ME, Gibson KM, Eds. *Physician's Guide to the Laboratory Diagnosis of Metabolic Diseases (2nd edition)* 243–260.

Smith DE, Smulders YM, Blom HJ et al (2012) Determinants of the essential one-carbon metabolism metabolites, homocysteine, S-adenosylmethionine, S-adenosylhomocysteine and folate, in cerebrospinal fluid. *Clin Chem Lab Med* 50(9):1641-7.

Smith I, Hyland K, Kendall B (1985) Clinical role of pteridine therapy in tetrahydrobiopterin deficiency. *J Inherit Metab Dis* 8 Suppl 1:39-45.

Sobczyńska-Malefora A, Harrington DJ (2018) Laboratory assessment of folate (vitamin B(9)) status. *J Clin Pathol* 71(11):949-956.

Spector R, Johanson CE (2010) Choroid plexus failure in the Kearns-Sayre syndrome. *Cerebrospinal Fluid Res* 7:14.

Steinfeld R, Grapp M, Kraetzner R et al (2009) Folate receptor alpha defect causes cerebral folate transport deficiency: a treatable neurodegenerative disorder associated with disturbed myelin metabolism. *Am J Hum Genet* 85(3):354-63.

Stockler S, Plecko B, Gospe SM Jr et al (2011) Pyridoxine dependent epilepsy and antiquitin deficiency: clinical and molecular characteristics and recommendations for diagnosis, treatment and follow-up. *Mol Genet Metab* 104(1-2):48-60.

Surtees R, Hyland K (1990) L-3,4-dihydroxyphenylalanine (levodopa) lowers central nervous system S-adenosylmethionine concentrations in humans. *J Neurol Neurosurg Psychiatry* 53(7):569-72.

Tabassum S, AlAsmari A, AlSaman AA (2019) Widening the phenotypic spectrum – Non epileptic presentation of folate transporter deficiency. *J Clin Neurosci* 59:341-344.

Tanji K, Schon EA, DiMauro S et al (2000) Kearns-Sayre syndrome: oncocytic transformation of choroids plexus epithelium. *J Neurol Sci* 178(1), 29–36.

Toelle SP, Wille D, Schmitt B et al (2014) Sensory stimulus-sensitive drop attacks and basal ganglia calcification: new findings in a patient with FOLR1 deficiency. *Epileptic Disord* 16(1):88-92.

Torres A, Newton SA, Crompton B et al (2015) CSF 5-Methyltetrahydrofolate Serial Monitoring to Guide Treatment of Congenital Folate Malabsorption Due to Proton-Coupled Folate Transporter (PCFT) Deficiency. *JIMD Rep* 24:91-6.

Van der Crabben SN, Verhoeven-Duif NM, Brilstra EH et al (2013) An update on serine deficiency disorders. *J Inherit Metab Dis* 36(4):613-9.

Van Karnebeek CD, Tiebout SA, Niermeijer J et al (2016) Pyridoxine-Dependent Epilepsy: An Expanding Clinical Spectrum. *Pediatr Neurol.* 59:6-12.

Verbeek MM, Blom AM, Wevers RA et al (2008) Technical and biochemical factors affecting cerebrospinal fluid 5-MTHF, biopterin and neopterin concentrations. *Mol Genet Metab* 95(3):127-32.

Vezmar S, Schüsseler P, Becker A et al (2009) Methotrexate-associated alterations of the folate and methyl-transfer pathway in the CSF of ALL patients with and without symptoms of neurotoxicity. *Pediatr Blood Cancer* 52(1):26-32.

Visentin M, Diop-Bove N, Zhao R et al (2014) The intestinal absorption of folates. *Annu Rev Physiol* 76:251-74.

Wassenberg T, Molero-Luis M, Jeltsch K et al (2017) Consensus guideline for the diagnosis and treatment of aromatic l-amino acid decarboxylase (AADC) deficiency. *Orphanet J Rare Dis* 12(1):12.

Watkins D, Schwartzentruber JA, Ganesh J et al (2011) Novel inborn error of folate metabolism: identification by exome capture and sequencing of mutations in the MTHFD1 gene in a single proband. *J Med Genet.* 2011 Sep;48(9):590-2.

Watkins D, Rosenblatt DS (2012) Update and new concepts in vitamin responsive disorders of folate transport and metabolism. *J Inherit Metab Dis* 35(4):665-70.

Wegner C, Nau H (1992) Alteration of embryonic folate metabolism by valproic acid during organogenesis: implications for mechanism of teratogenesis. *Neurology* 42 (4 Suppl 5), 17–24.

Wilson et al (2019) in this issue of the Journal of Inherited Metabolic Disease

Wolf G (2007) Identification of proton-coupled high-affinity human intestinal folate transporter mutated in human hereditary familial folate malabsorption. *Nutr Rev* 65(12 Pt 1):554-7.

Woody RC, Brewster MA, Glasier C (1989) Progressive intracranial calcification in dihydropteridine reductase deficiency prior to folinic acid therapy. *Neurology* 39(5):673-5.

Zhao R, Aluri S, Goldman ID (2017) The proton-coupled folate transporter (PCFT-SLC46A1) and the syndrome of systemic and cerebral folate deficiency of infancy: Hereditary folate malabsorption. *Mol Aspects Med* 53:57-72.

Zhao R, Goldman ID (2013) Folate and thiamine transporters mediated by facilitative carriers (SLC19A1-3 and SLC46A1) and folate receptors. *Mol Aspects Med* 34(2-3):373-85.

Zhao R, Diop-Bove N, Visentin M et al (2011) Mechanisms of membrane transport of folates into cells and across epithelia. *Annu Rev Nutr* 31:177-201.

Figure 1. Folate structures and Folate/One-carbon metabolism.

See text for description.

Key: DHF - dihydrofolate; DHFR - dihydrofolate reductase; DHPR - dihydropteridine reductase; THF - tetrahydrofolate; MS - methionine synthase; MTHFR - methylenetetrahydrofolate reductase; 5-MTHF - 5-methyl-tetrahydrofolate; SAM - S-adenosyl methionine; SAH - S-adenosyl homocysteine; CBS - cystathionine beta-synthase; B6 - pyridoxal phosphate dependent enzyme; B12 - cobalamin dependent reaction.

Figure 2A and B. Folate transport, mitochondrial metabolism and oxidative stress

A. Folate species are absorbed in the gut via PCFT and are then converted to the main transport form, 5-MTHF. 5-MTHF crosses the blood brain barrier via the choroid plexus. Both vesicle-mediated folate receptor (FR) transport and PCFT are required for this process to work optimally. The main dietary and supplementary forms of folate are shown, as are drugs such as methotrexate and trimethoprim, which inhibit folate synthesis. It is important to note that there is a concentration gradient between the blood and CSF, meaning 5-MTHF transport into the brain is energy dependent. See text for more details.

B. Oxidative stress has been proposed as a mechanism that could cause cerebral folate deficiency (see Aylett et al 2013). Oxidative phosphorylation (OXPHOS) within the mitochondria can result in electron leakage and production of oxidising species such as hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-). Antioxidants, such as ascorbate (ASC) and glutathione (GSH), scavenge these oxidants and protect the cellular components from oxidation. Oxidative stress can be caused as a result of increased oxidants (for example from defective oxidative phosphorylation or free

transition metal ions (Fe^{2+})) or impaired antioxidant defences, In conditions of oxidative stress, labile folate species could be oxidized, resulting in CFD (Aylett et al. 2013). It should be noted that the mitochondria have their own enzyme isoforms for some steps of folate metabolism and NAD(P)H produced during folate metabolism can be used to recycle ascorbate and glutathione.

Key: DHFR – dihydrofolate reductase; SHMT1 – cytosolic serine hydroxymethyltransferase; SHMT2 – mitochondrial serine-hydroxymethyltransferase; MTHFR – methylenetetrahydrofolate reductase; MTHFS – methenyl-tetrahydrofolate synthase; MTHFD1 – cytosolic methylenetetrahydrofolate dehydrogenase; MTHFD1L – mitochondrial methylenetetrahydrofolate dehydrogenase 1-like; mitochondrial methylenetetrahydrofolate dehydrogenase 2; FR – folate receptor; PCFT – proton-coupled folate transporter; RFC – reduced folate carrier;

Figure 3 5-MTHF chromatograms

HPLC chromatograms from our laboratory showing a prominent and well resolved 5-MTHF peak in 'control' CSF and no peak in a deficient control CSF from a patient with MTHFR deficiency. This highlights the importance of running 'normal' and disease quality controls in analytical methods.

Table 1

Overview of conditions that can lead to cerebral folate deficiency

Table 2

Reported cases with *FOLR1* mutations

Table 1. Overview of conditions that can lead to cerebral folate deficiency.

	Defect	Clinical signs	Typical CSF 5-MTHF levels and other key biochemical changes	Treatment	Key references
Primary causes of CFD					
	MTHFR	Megaloblastic anaemia or pancytopaenia, seizures, hydrocephalus, ataxia	Generally very low (<10nmol/L) Elevated plasma total homocysteine and low methionine	Betaine, folinic acid or 5-methyltetrahydroflate	Skovby 2003
	DHFR	Megaloblastic anaemia or pancytopaenia, microcephaly,	Generally very low (<10nmol/L)	Folinic acid	Banka et al 2011; Cario et al 2011

	seizures, white matter changes	Low CSF BH4 and low/normal CSF monoamine metabolites		
MTHFS	Epilepsy, white matter changes, microcephaly	Low normal	5-methyltetrahydrofolate and intramuscular methylcobalamin	Rodan et al 2018
FOLR1	White matter changes, developmental delay	Generally very low (<10nmol/L)	Folinic acid	Steinfeld et al 2009; Perez-Duenas et al 2010
Secondary causes of CFD				
Mitochondrial disorders	Pigmentatory retinopathy, seizures, white matter changes, ataxia	Variable - very low to normal	Folinic acid	Garcia-Cazorla et al 2008; Batllori et al

				2018
Serine deficiency	Microcephaly, growth retardation, hypertonia, epilepsy, hypogonadism	Mild to moderate CFD	Oral l-serine	Van der Crebban et al 2013; Damseh et al 2015
DHPR deficiency	Features of dopamine and serotonin deficiencies - dystonia, truncal hypotonia, temperature instability, disturbed intestinal mobility	Low-Normal	Folinic acid	Irons et al 1987; Smith et al 1985;
AADC deficiency	Features of dopamine and serotonin deficiencies - dystonia, truncal hypotonia, temperature instability, disturbed intestinal mobility	Low-Normal	Folinic acid	Wassenberg et al 2017
Antibodies to folate receptor	Schizophrenia, autism spectrum disorder.	Variable - low to normal	Folinic acid	Raemakers et al. 2005

Disorders with low CSF 5-MTHF and low peripheral total folate				
PCFT1 deficiency	White matter changes, seizures, developmental delay chronic diarrhoea, hypogammaglobulinaemia, recurrent infections, neurological disturbances	Very low (<10nmol/L)	Intramuscular folinic acid or 5-methyltetrahydrofolate	Qiu et al. 2006
Nutritional deficiency	Megaoloblastic anaemia or pancytopaenia	Variable	Better diet/folate supplements	
Environmental/Drugs such as Methorexate, L-dopa, antifolates	Various	Variable	Folic acid or folinic acid	Daly et al 1997; Becker et al 2007; Surtees and Hyland

1990.

Table 2: Reported cases with *FOLR1* mutations

Patient *	Sex	Ethnicity	Age at onset	Phenotype	MRI appearances	CSF 5-MTHF levels (nmol/L)†	Mutations	Treatment regimen	Response to treatment	Reference
1 (P9 in Grapp et al)	M	German	2.5y	Tremor, ataxia, GDD, motor regression, hypotonia, apathy, autism. Myoclonic-astatic Sz from 3.3y	Hypomyelination, cerebral/cerebellar atrophy, diffuse T2-hyperintensity of PV and SC WM	1.4 (43-159)	c.352C>T; (p.Gln118*) and c.525C>A; (p.Cys175*)	Folinic acid: oral 0.5mg/kg/dy increasing to 5mg/kg/dy	Marked clinical improvement (sitting unsupported, walking with support) and ↓Sz frequency	Steinfeld et al 2009; Cario et al 2009; Grapp et al 2012
2 (sib of 1, P10 in Grapp et al)	F	German	2.5y	Mild truncal ataxia, tremor of limbs, normal cognitive development	Mild cerebellar atrophy only	<5 (43-159)	c.352C>T; (p.Gln118*) and c.525C>A; (p.Cys175*)	Folinic acid: oral 22.5mg/kg/dy	Almost complete normalisation of neurology within 6m	Steinfeld et al 2009; Cario et al 2009; Grapp et al 2012
3 (P8 in Grapp et al)	F	Italian	2y	Speech delay, ataxia, tremor, spasticity, developmental regression, myoclonic-astatic Sz	Hypomyelination, cerebellar atrophy, focal T2-hyperintense WM lesions	<5 (43-159)	Homozygous c.130_147dup; (p.Lys44_Pro49 dup)	Folinic acid (0ral, dose not stated)	Clinical improvement	Steinfeld et al 2009; Grapp et al 2012

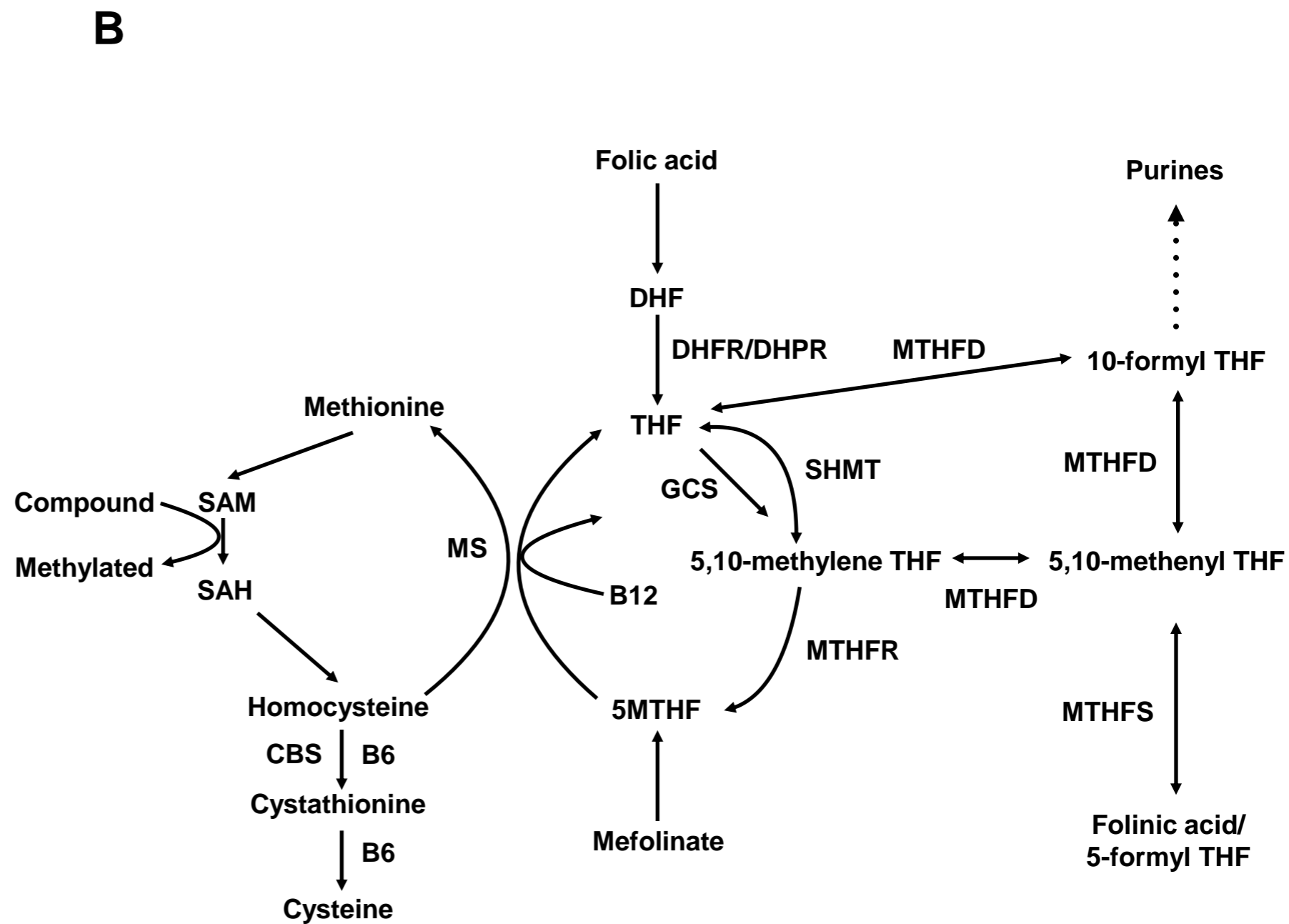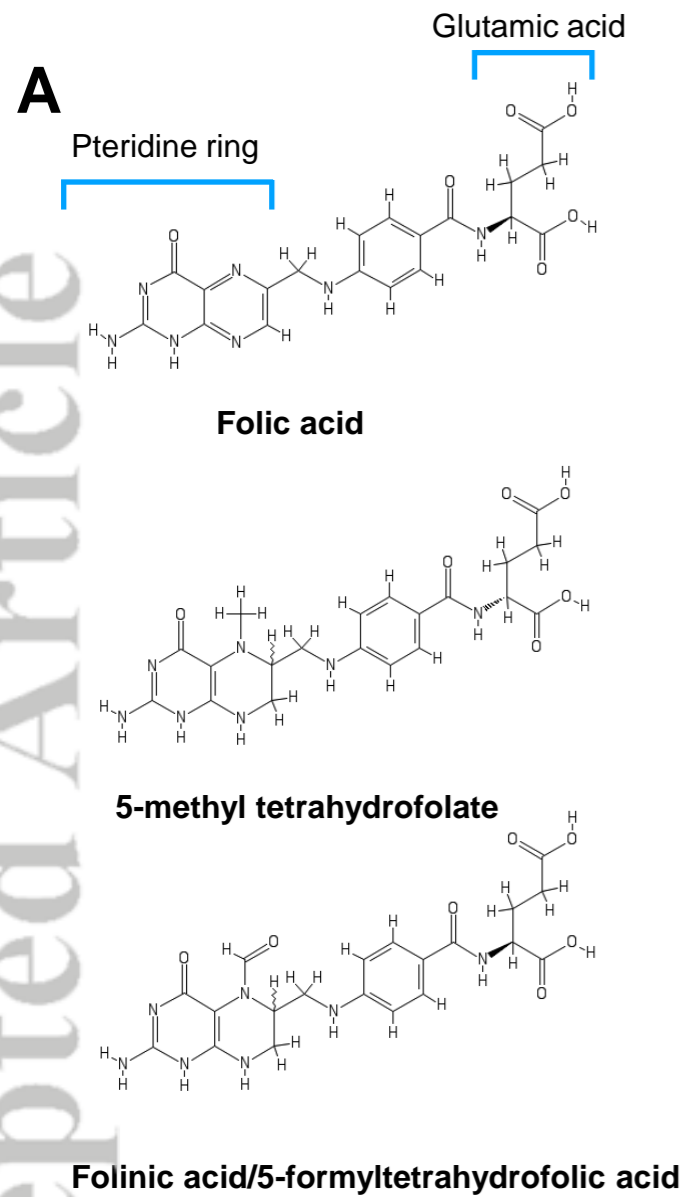|										
4 (P11 in Grapp et al)	M	Gambian	2nd year of life	Ataxia, chorea, progressive myoclonic epilepsy	Diffuse abnormal signal in cerebral WM, cerebellar atrophy	2 (48-127)	Homozygous c.313T>C; (p.Cys105Arg)	Folinic acid: oral 30mg/dy	Complete Sz control within 1m. Alive at 7y	Perez-Duenas et al 2010; Grapp et al 2012
5	M	Turkish	6m	DD, profound hypotonia, ataxia from 2y, epilepsy from 6y (myoclonic Sz and drop attacks), recurrent respiratory arrests at 8y; [patient also has LAMM – probably unrelated]	Abnormal myelination in frontal and parietal regions	<3 (63-111)	Homozygous c.610C>T; (p.Arg204*)	Folinic acid 3mg/kg/dy with pyridoxal phosphate 20mg/kg/dy	Sz free on 2 AEDs. Alive at 8.5y	Dill et al 2011
6 (P1)	F	Finnish	1y	Motor DD, developmental regression, hypotonia, ataxia, athetosis, Sz (myoclonic, tonic, psychomotor, atypical absences), recurrent status epilepticus	Hypomyelination, cerebral/cerebellar atrophy, T2-hyperintense WM lesions	<40 ¥	Homozygous c.506G>A; (p.Cys169Tyr)	Not stated	Not stated	Grapp et al 2012
7 (P2)	M	Azerbaijani	22m	GDD and regression, hypotonia, ataxia, myoclonic Sz, autistic features	Delayed myelination, cerebellar atrophy, T2-hyperintensity of PV and SC WM	<40 ¥	Homozygous c.195C>G; (p.Cys65Trp)	Not stated	Not stated	Grapp et al 2012
8 (P3)	F	Finnish	Birth	Congenital microcephaly, ataxia, hypotonia,	Hypomyelination, cerebral/cerebellar atrophy	<40 ¥	Homozygous c.506G>A; (p.Cys169Tyr)	Not stated	Not stated	Grapp et al 2012

				developmental regression, Sz (myoclonic, tonic, tonic-clonic), aggressive behaviour					
9 (P4)	F	Finnish	Infancy	Nystagmus, gait abnormality, ataxia, tremor, hypotonia, status epilepticus (2 epsiodes), developmental regression,	Delayed myelination, cerebral/cerebellar atrophy, T2-hyperintensity in corticospinal tracts	<40 ¥	Homozygous c.506G>A; (p.Cys169Tyr)	Not stated	Not stated	Grapp et al 2012
10 (P5)	F	Finnish	2y	Ataxia, mainly myoclonic Sz, developmental regression, aggressive behaviour	Irregular myelination, cerebellar atrophy	<40 ¥	c.506G>A; (p.Cys169Tyr)and c.665A>G; (p.Asn222Ser)	Not stated	Not stated	Grapp et al 2012
11 (P6)	M	Finnish	1.5y	Motor DD, developmental regression, Sz (myoclonic, tonic, atonic and psychomotor), hypotonia, ataxia, athetoid movements	Hypomyelination, cerebellar atrophy, T2-hyperintense WM lesions	<40 ¥	Homozygous c.506G>A; (p.Cys169Tyr)	Not stated	Not stated	Grapp et al 2012
12 (P7)	F	Turkish	2y	GDD and regression, status epilepticus, tonic-clonic and short myoclonic Sz, ataxia, tremor	Hypomyelination, cerebral/cerebellar atrophy, thin corpus callosum	<40 ¥	Homozygous g.3576T>G (splice)	Not stated	Not stated	Grapp et al 2012

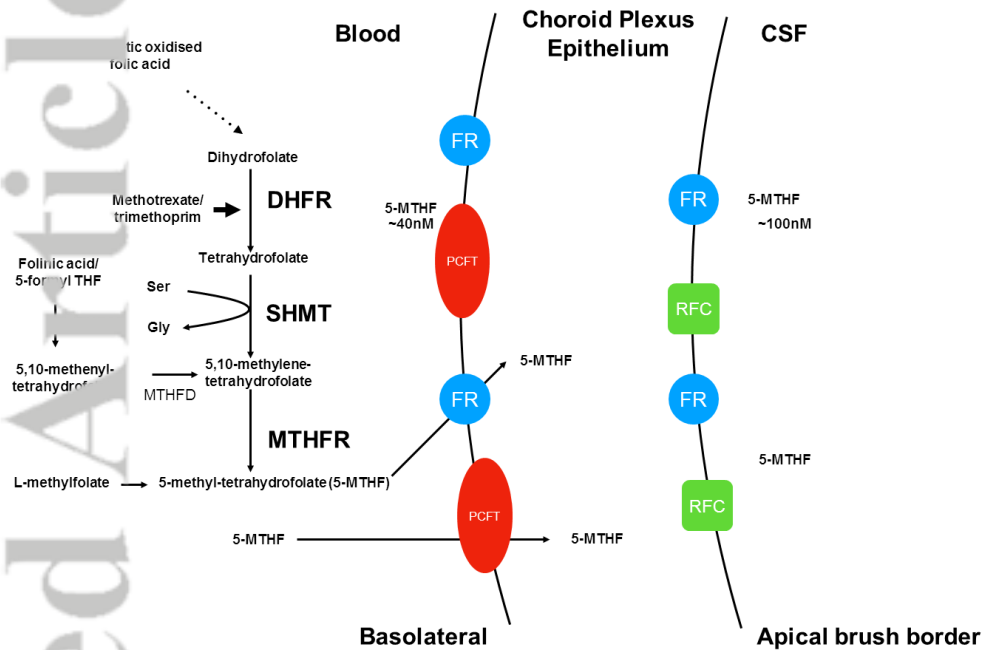|										
13	M	Ghanaian	2.5y	Hyperactive behaviour, ataxia, ID, Sz from 4y (myoclonic-tonic drop attacks)	Generalised hypomyelination, cerebellar atrophy; bilateral BG calcification on CT	Below lower limit of detection (41-117)	Homozygous c.610C>T; (p.Arg204*)	Folinic acid: oral 4.5mg/kg/dy plus 9mg iv every 1-2wk, increasing to oral 5.6mg/kg/dy with 120mg iv/wk	Improvements in irritability, gait ataxia, behaviour, attention, stereotypes, but persistent myoclonic-tonic drop attacks until on highest dose	Toelle et al 2014
14	M	Saudi	4.5y	Intractable Sz (drop attacks, myoclonic jerks), GDD, ASD	Hypomyelination (SC WM hyperintensity), cerebellar atrophy	7 (40-128)	Homozygous c.398C>A; (p.Pro133His)	Folinic acid: oral 0.75mg/kg/dy increasing to 1.7mg/kg/dy	Sz free, marked improvement in gait and social interaction	Al-Baradie et al 2014
15 (sib of 14)	F	Saudi	4y	Drop attacks, GDD	Hypomyelination (SC WM, basal ganglia, internal capsule), cerebellar atrophy, MRS normal	11 (40-150)	Homozygous c.398C>A; (p.Pro133His)	Folinic acid: oral 2mg/kg/dy	Sz free, marked improvement in development	Al-Baradie et al 2014
16	M	Japanese	1y	Ataxia, intention tremor, slurred speech, GDD, ID	Cerebellar atrophy, cerebral WM atrophy, calcification of SC WM	ND	c.374G>T; (p.Arg125Leu) and c.466T>G; (p.Trp156Gly)	Not reported	Not reported	Ohba et al 2014
17 (sib of 16)	F	Japanese	2y	Ataxia, intention tremor, slurred speech, GDD, ID	Cerebellar atrophy, calcification of basal ganglia and SC WM	ND	c.374G>T; (p.Arg125Leu) and c.466T>G; (p.Trp156Gly)	Not reported	Not reported	Ohba et al 2014
18	F	Belgian	9m	Initial GDD and challenging	Diffuse hypomyelination;	<1 (53-182)	c.332G>T; (p.Glu108Ter)	Folinic acid: oral 2 then 5	Clinical condition	Delmelle et al 2016

								then 7mg/kg/dy, then iv 6mg/kg/dy for 24h followed by 12mg/kg/dy for 48h, then monthly 20-25mg/kg over 24h	and c.373C>T; (p.Arg125Cys)	stabilised with iv dosing – halted regression, improved eye contact, better Sz control	
19 (sib of 18)	F	Belgian	<31m	GDD then developmental stasis, myoclonic and tonic Sz, ataxia	Hypomyelination	<1 (53-182)	c.332G>T; (p.Glu108Ter) and c.373C>T; (p.Arg125Cys)		Dramatic improvement with iv dosing – Sz stopped, regained walking, improved myelination on MRI	Delmelle et al 2016
20	F	Italian	22m	Myoclonic Sz, ataxia, developmental regression, later generalized tonic-clonic Sz	ND	ND	Homozygous c.128A>G; (p.His43Arg)	Folinic acid: oral 2mg/kg/dy	Marked improvement in Sz, ↓AED doses. Alive at 33y	Dyment et al 2015; Ferreira et al 2016
21 (sib of 20)	F	Italian	18m	Progressive ataxia, developmental regression, myoclonic Sz evolving to generalized tonic-clonic Sz	At 25y - extensive PV and deep cerebral WM changes with frontal lobe and cerebellar atrophy	<10 (40-120)	Homozygous c.128A>G; (p.His43Arg)	Folinic acid: oral 2mg/kg/dy	Marked improvement in Sz, ↓AED doses. Alive at 28y	Dyment et al 2015; Ferreira et al 2016
22	F	Russian	1.5y	Refractory Sz, developmental regression, progressive ataxia,	Symmetrical T2W WM hyperintensity, cerebellar atrophy; MRS ↓ frontal	0.5 (42-81)	Homozygous c.466T>G; (p.Trp156Gly)	Folinic acid: oral 2mg/kg/dy then im	Amelioration of Sz (stopped AEDs), development	Kobayashi et al 2017 (also Akiyama et al 2016

Note: the first partial row at top shows: "behaviour, at 3.5y neuro-developmental regression, ataxia, chorea, severe myoclonic epilepsy" | "MRS ↓ WM choline" | | "and c.373C>T; (p.Arg125Cys)"

| | | | | polyneuropathy | choline peak | | | 4mg/kg/dy then iv 4mg/kg/dy | and MRI WM changes. Alive at 8.5y | |
| 23 | F | Saudi | 2y | Intention tremor, speech delay, ID | Symmetrical WM hyperintensities, normal MRS | ND | Homozygous c.665A>G; (p.Asn222Ser) | Folinic acid: oral 25mg/dy then 25mg bd | No significant clinical improvement. Alive at 9.5y | Tabassum et al 2019 |

Key: * number in brackets refers to identification number in original publication; † age and laboratory-specific reference ranges given in brackets after patient's value; ¥ precise value not stated; AEDs antiepileptic drugs; BG basal ganglia; CT computed tomography; DD developmental delay; dy day; GDD global developmental delay; ID intellectual disability; LAMM congenital deafness syndrome with labyrinthine aplasia, microtia and microdontia; m months; ND not determined; PV periventricular; SC subcortical; Sz seizures; T2W T2-weighted; wk week; WM white matter; y years

A

Glutamic acid

Pteridine ring

Folic acid

5-methyl tetrahydrofolate

Folinic acid/5-formyltetrahydrofolic acid

B

Folic acid

DHF

DHFR/DHPR

MTHFD

10-formyl THF

Purines

THF

Methionine

Compound — SAM

Methylated

SAH

MS

GCS

SHMT

B12

MTHFD

5,10-methylene THF

5,10-methenyl THF

MTHFD

Homocysteine

MTHFR

5MTHF

MTHFS

CBS | B6

Cystathionine

Mefolinate

Folinic acid/
5-formyl THF

B6

Cysteine