Review

Vincent Ramaekers*, Jeffrey M. Sequeira and Edward V. Quadros

Clinical recognition and aspects of the cerebral folate deficiency syndromes

Abstract: We characterized cerebral folate deficiency (CFD) as any neuro-psychiatric condition associated with low spinal fluid (CSF) N\(^5\)-methyltetrahydrofolate (MTHF) but normal folate status outside the central nervous system (CNS). The commonest cause underlying CFD syndromes is the presence of serum autoantibodies of the blocking type directed against folate receptor-\(\alpha\) (FR\(\alpha\)) attached to the plasma-side of choroid plexus epithelial cells. Blocking FR antibodies inhibit MTHF transport across the choroid plexus. Serum titers of FR antibodies may fluctuate significantly over time. Less frequent causes of CFD are \(\text{FOLR}-1\) mutations, mitochondrial disorders and inborn errors affecting folate metabolism. Maternal FR antibodies have been associated with neural tube defects while the presence of FR antibodies in either one or both parents increases the risk of an offspring with infantile autism. Recognizable CFD syndromes attributed to FR-antibodies in childhood are infantile-onset CFD presenting 4–6 months after birth, infantile autism with neurological deficits, and a spastic ataxic syndrome from the age of 1 year, while progressive dystonic or schizophrenic syndromes develop during adolescence. FR autoantibodies are frequently found in autism spectrum disorders, in an Aicardi-Goutiéres variant and in Rett syndrome. The heterogeneous phenotype of CFD syndromes might be determined by different ages of onset and periods when FR autoantibodies are generated with consequent CNS folate deficiency. Folate deficiency during various critical stages of fetal and infantile development affects structural and functional refinement of the brain. Awareness of CFD syndromes should lead to early detection, diagnosis and improved prognosis of these potentially treatable group of autoimmune and genetically determined conditions.

Keywords: central nervous system; choline; folate; folate receptor autoimmunity; homocysteine.

*Corresponding author: Prof. Vincent Ramaekers, Department of Pediatric Neurology and Center of Autism, University Hospital Liège, Rue de Gaillarmont, 600, 4032 Chênée, Belgium, Phone: +32 4 3679405, E-mail: vramaekers@skynet.be

Jeffrey M. Sequeira and Edward V. Quadros: Departments of Medicine/Cell Biology, SUNY-Downstate Medical Center, Brooklyn, NY, USA

Introduction

Intestinal absorption of dietary folates, composed of folic acid (oxidized folate form) and its structurally derived folate compounds, is achieved by the concerted action of the folate receptor-\(\alpha\) (FR\(\alpha\); synonymous to \(\text{FOLR-1}\)) and the proton-coupled folate transporter (PCFT) \([1–4]\). Folate uptake by cells and transport across the blood-brain and the placental-fetal barriers involves the following different mechanisms composed of specific proteins, being FR\(\alpha\)-mediated endocytotic processes, the PCFT cooperating closely with FR proteins, the reduced folate carrier-1 (RBC-1) and the ATP-dependent folate exporter \([5, 6]\). FR\(\alpha\) protein is a membrane-anchored protein and is characterized as a high-affinity low capacity folate transporter which can bind to very low folate concentrations within the nanomolar physiologic range. FR\(\alpha\) is expressed by specific epithelial cells and is responsible in cooperation with PCFT for folate passage across intestinal, placental-fetal epithelial barriers and across choroid plexus epithelial barriers to the spinal fluid compartment and from there across ependymal lining cells into neural tissues. The main folate transport to the central nervous system (CNS) occurs across the choroid plexus and is accomplished by FR\(\alpha\)-mediated endocytosis and PCFT action. FR\(\beta\) (synonymous to FR\(\beta\); FR-2 or \(\text{FOLR-2}\)) is related to FR\(\alpha\) and expressed by both mesenchymal-derived and epithelial cells. FR\(\beta\) expression has been demonstrated also in fetal epithelial cells, however, its functional contribution to folate transport among human and murine species has not been resolved fully \([5–7]\). Another important folate transporter across cell membranes is the low-affinity, high capacity transporter known as the RFC-1, which is expressed in most cells. An ATP-dependent folate exporter is known to regulate intracellular folate concentrations (Figure 1).
Vectorial folate transport by FR endocytosis across choroid plexus

Diet

F

F

F

Catabolism

DHF

Intestinal barrier

Blood-CSF barrier

Neuronal cells

RFC

FR1

FR2

DHFR

ATP

RFC

PCFT

RBC

RFC

F

PCFT

F

Figure 1 Folate transport across epithelial barriers (upper part) and causes underlying CFD (lower part).

Upper part of the Figure shows intestinal folate absorption and vectorial transport across the choroid plexus and ependyme using the ATP-dependent FR1-mediated endocytosis with PCFT, and eventually neuronal folate uptake and intracellular metabolism. Lower part of the Figure shows the three known causes of impaired folate transport across choroid epithelial cells: 1. The presence of blocking FR1 auto-antibodies (shown by yellow arrows). 2. Mitochondrial dysfunction with reduced ATP generation needed by folylpolyglutamate synthetase to attach glutamate residues to folate-monoglutamate for storage. 3. Genetic FOLR-1 defects predisposing to truncated FR1 protein with functional loss or mistargeting of FR1 to membranes. DHF, dihydrofolate; DHFR, dihydrofolate reductase; F, folates; F-, folylpolyglutamate; FPGS, folylpolyglutamate synthase; FR1, folate receptor-α; GGH, γ-glutamate hydrolase representing the lysosomal enzyme for folylpolyglutamate breakdown to monoglutamate; PCFT, proton-coupled folate transporter; RBC, red blood cells; RFC, reduced folate carrier-1.

N5-Methyl-tetrahydrofolate (MTHF) is the predominant and physiological methyl-group carrying folate form in plasma and the folate form known to cross the blood-CSF barrier to enter the spinal fluid compartment and from there through a second ependymal-lining barrier into the neural tissues. MTHF transfer across the choroid plexus is an active vectorial transport mechanism resulting in at least 1.5-fold higher CSF concentrations compared to plasma levels (Figure 1). MTHF transfer across the choroid plexus is mediated by FRα anchored to plasma-facing basal membranes of choroid epithelial cells [5, 6, 8, 9]. After MTHF binding by FR proteins and cellular uptake by choroid epithelial cells through an endocytotic process, MTHF is released from its FR protein through interaction with the PCFT [1]. This is followed by MTHF delivery into the spinal fluid compartment from where MTHF is taken up by ependymal lining cells to be delivered into neural tissues.

After folate uptake by neurons, only a minor portion of assimilated folate participates in different cellular metabolic functions, while the major portion will be
stored as polyglutamate folate forms within the cytoplasm and mitochondria after attachment of multiple glutamate residues by the ATP-dependent enzyme folylpolyglutamate synthetase. Brain folate reserves have been estimated to remain adequately replete for a period of about 100 days. FRα is highly expressed in reproductive tissues and plays a major role in providing folate to the embryo and fetus.

The various reduced folate forms serve as essential co-factors in many metabolic processes [8]. After intestinal uptake, folic acid is reduced by the enzyme dihydrofolate reductase (DHFR) and part of this will be methylated to MTHF. In the brain, expression of DHFR enzyme is extremely low in humans so that the brain depends on the daily transport of MTHF and other reduced folate forms (Figure 2). Among many metabolic functions, MTHF is needed as substrate for the conversion of homocysteine to methionine by the B12-dependent enzyme methionine synthase. Through this latter enzymatic homocysteine degradation, MTHF transfers its methyl-group to methionine which enters into the adenosyl cycle and will be converted to the activated methyl-group donor S-adenosyl-methionine (SAM), used in over 100 methylation reactions. The resulting product tetrahydrofolate (THF) is recycled again to MTHF after recharging with methyl-groups originating from serine-glycine interconversion. Another folate form 10-formyl-THF is necessary for de novo purine synthesis, while 5,10-methylene-THF participates in the de novo thymidine synthesis. Thus DNA replication and formation of RNA transcripts needed for cell proliferation and maturation of the developing nervous system depends on an adequate pool of various reduced folate forms.

Moreover, epigenetic control of neuronal gene expression and transcription repression of other genes is indirectly linked to MTHF as SAM is the substrate for DNA-methyltransferase-I which enzymatically transfers a methyl-group to the CpG islands and thereby methylates gene promoter regions in order to initiate the process of gene transcription repression.

In neurologically handicapped patients, CSF measurements have detected low MTHF values while plasma and red blood cell folate, homocysteine and vitamin B12 levels were normal. This group of neurological disorders can be defined as the Cerebral Folate Deficiency (CFD) syndromes and represent different clinical phenotypes [10, 11]. The most common cause of CFD identified thus far, is the presence in serum of circulating folate receptor (FR) autoantibodies that cross react with soluble FR found in animal-derived milk (bovine, goat or camel milk), which has high structural homology with the human FR antigen [12, 13]. Less frequent causes of CFD are mitochondrial disorders and abnormalities of the FOLR-1 gene [7, 14]. This paper reviews the salient clinical features of this
new emerging group of disorders, its diagnosis and differential diagnosis, diagnostic guidelines and therapeutic strategies as well as new findings about these FR autoantibodies. We also discuss the possible reasons for the emergence of different clinical phenotypes at different ages.

History and epidemiology

The first patients with the infantile-onset CFD associated with low CSF folate levels were reported by us in 2002 [10]. Infantile-onset CFD syndrome shows an equal gender distribution, affects all ethnic groups with an estimated prevalence between 1/4000 and 1/6000 children. However, infantile-onset CFD and other CFD syndromes are likely to be underdiagnosed. After a FOLR-1 gene defect was initially excluded, further investigation revealed that the most likely etiology of infantile CFD was the presence of postnatally acquired FR autoantibodies of the blocking type in the serum of these patients. FR autoantibodies had been discovered earlier by our colleagues at the State University of New York in the serum of mothers who had given birth to a child with a neural tube defect (NTD) [15].

Further testing for blocking FR autoantibodies in various populations indicated that as we age the immune system also changes leading to immunosenescence. We found a prevalence of blocking FR autoantibodies from <2% in the under 16 years age group increasing to a prevalence estimated in healthy adult women at 4% – 7% in Spain [16], 9%–13% in Ireland [17] and 10%–15% in the US population [15]. A low titer of this antibody in a fraction of the adult population appears to be non-pathologic. Postnatally acquired FR autoantibodies blocking folate transport to the brain have been associated with the infantile-onset cerebral folate deficiency syndrome [12], which in a number of patients manifests as low-functioning autism with neurological deficits. Thereafter, several studies associated serum FR antibodies with Rett syndrome and a variant of the Aicardi-Goutières syndrome. Mitochondrial encephalopathies have in a number of cases been associated with CFD because vectorial folate transport across the blood-brain barrier depends on adequate ATP production. The first reported FOLR-1 gene defects have only been recently identified, but the exact prevalence of genetic defects remains to be determined.

FR autoantibody assay

The two types of antibodies identified in serum of patients are: blocking antibody and binding antibody. The two antibodies can be measured by specific assays described and exert their pathological effects either by functional blocking of folate transport or by disrupting the FR by an antigen-antibody mediated inflammatory response. We have identified both IgG and IgM autoantibodies in these conditions [15–17].

Assay for blocking autoantibodies to FRα

Testing for blocking autoantibodies against FR is performed by measuring the blocking of radiolabeled folic acid binding to a known amount of purified FRα from human milk as previously described [15, 16]. Blocking autoantibodies prevent the binding of [3H]folic acid to FR and the autoantibody titer is expressed as pmol FR blocked/mL of serum. The blocking antibodies could be either IgG or IgM and this method does not identify any specific antibody type.

Assay for binding autoantibodies to FRα

An ELISA-based measurement is used to determine the presence of IgG immunoglobulins that bind to epitopes on purified apo FRα from human milk as previously described [17]. The assay only identifies IgG autoantibodies of either the blocking or binding type and does not identify IgM antibodies.

Cerebral folate deficiency syndromes associated with FR autoantibodies (Figure 3)

The clinical syndromes of CFD attributed to FR autoantibodies can be found from the prenatal period into adulthood. The different clinical phenotypes are speculated to be determined by the presence of maternal FR antibodies during pregnancy and/or the specific postnatal age at which these FR autoantibodies occur to impair CNS folate transfer.

The first paper on FR autoantibodies reported their presence in the serum of mothers with a history of neural tube defect (NTD) pregnancy [15]. These FR antibodies directed against the FRα at the placental-fetal barrier could reduce folate transfer resulting in embryonic folate deficiency that affected the normal process of neural tube closure. No consistent data described a link between the
presence of serum FR antibodies in pregnant mothers and the origin of other embryonic or fetal malformations. However, a recent paper by us demonstrated an association between the presence of FR antibodies in one or both parents having a child with infantile autism [18]. The latter finding suggested that prenatal folate depletion due to FR autoimmunity in the mother interferes with brain development and predisposes to autism. FR antibodies in the fathers are speculated to induce epigenetic DNA changes of their transmitted genome, predisposing to autism in their children. The essential role of adequate periconceptional folate levels among mothers has been shown to reduce the risk of NTD pregnancy or having a child with autism [19].

The first report on CFD syndrome was recognized and described among five patients in 2002 [10], and was later named infantile-onset CFD syndrome which manifests 4–6 months after birth and is characterized by the sequential occurrence of more than three out of seven major clinical criteria (Figure 4) [11], based on clinical findings among 20 patients. The difficulty in establishing an early diagnosis is the fact that the full-blown clinical picture does not manifest until the age of 2½ years. For this reason a high index of suspicion and good knowledge of early symptoms and signs of infantile-onset CFD is required to establish a tentative diagnosis and start treatment as soon as possible.

Figure 3  Life tree depicting the recognized CFD syndromes attributed to FR autoimmunity according to age.

Figure 4  Time sequence of clinical features of the infantile-onset CFD syndrome.
In undiagnosed cases, visual disturbances start to manifest from the age of 3 years followed by progressive visual loss, while progressive hearing loss occurs from the age of 6 years.

After excluding FOLR-1 and FOLR-2 gene defects in the majority of cases, we found serum containing FR autoantibodies of the blocking type [12]. In 55 patients tested, there was an inverse correlation between the autoantibody and the CSF folate levels (Figure 5) [13]. Antibodies are absent in mothers of children with infantile CFD attributed to FR autoantibodies. Several families are known to us where two or more siblings are affected by this condition, and investigation of six consanguineous families where parents were first-line cousins did not reveal a single monogenetic origin, and therefore, a multigenetic origin is likely for infantile-onset CFD associated with FR autoimmunity (unpublished findings).

In about half of the patients suffering from infantile-onset CFD syndrome due to FR autoimmunity, the brain MRI showed moderate fronto-temporal atrophy with signs of delayed myelination or later signs of periventricular and subcortical demyelination manifesting from the age of 18 months. In three children, slowly progressive supratentorial and infratentorial atrophy was noted [11].

As visual disturbances manifest from the age of 3 years, visual evoked potentials after flash stimuli begin to show prolonged latencies followed by reduction and extinction of P2-wave amplitudes. Progressive sensorineural hearing loss becomes manifest from the age of 6 years. Nerve conduction studies were found to be completely normal. Somatosensory evoked responses after tibial nerve stimulation may show either no response or prolonged latencies.

The second recognized CFD syndrome is a spastic-ataxic phenotype and presents after the age of 1 year, while neurodevelopment during the first year of life is normal and features of the infantile-onset syndrome are not found. In contrast to the moderate to severe mental retardation observed in the infantile-onset syndrome, cognitive functions in the spastic ataxic syndrome remain preserved [20]. Neuro-imaging of the brain and spinal cord were normal. Visual evoked potentials may show prolonged latencies. Autosomal recessive spastic paraplegia and spastic ataxia syndromes need to be excluded.

The third recognized CFD syndrome is infantile autism, frequently associated with three or more neurological deficits encountered in the infantile-onset CFD [21], but infantile autism without neurological deficits has been increasingly recognized [22]. In a group of 25 patients with infantile autism associated with neurological deficits, spinal fluid MTHF levels were lowered due to relatively high blocking serum FR autoantibody titers in almost all (24 of 25) patients. MRI of the brain and spinal cord was normal in most cases. However, in 49 patients affected only by infantile autism without neurological deficits and normal neuro-imaging, a lower proportion of 15 out of 49 patients had spinal fluid MTHF values slightly or moderately below the reference range among healthy controls and was statistically not significant. In the latter group of infantile autism patients serum blocking FR auto-antibodies were positive in about half the patients (51%) but the mean titer was much lower compared to the group of infantile autism with neurologic abnormalities (Figure 6). These results suggested that the advent of additional neurological abnormalities in autism will be induced by lower folate levels within the nervous system.

A recent study showed either blocking and/or binding FR autoantibodies in 75% of 93 tested patients with autism spectrum disorders, but CSF folate was not measured consistently among all patients [22].

Postnatal development of FR autoantibodies has been associated with all other phenotypes belonging to the autism spectrum disorders like Rett syndrome, PDD-NOS, childhood disintegrative disorder and Asperger syndrome [10–12, 23, 24].

A new CFD syndrome has been recognized among five patients presenting from adolescence to adulthood. Dystonia at onset was localized and during its course extended to generalized dystonia with subsequent development of bradykinesia and a pyramidal syndrome. Inconsistent

![Figure 5](image-url)  
**Figure 5** Correlation between blocking FR autoantibody titer and CSF MTHF level in 55 patients with cerebral folate deficiency (open circles). Filled circles represent non-CFD patients with normal levels of folate in CSF who tested negative for the autoantibody (reprinted from Dev Med Child Neurol).
of their MECP-2 genotype, 14 out of 33 Rett patients (42%) from Germany, Spain and Portugal were found to have lowered CSF MTHF levels. In the 14 Rett patients with lowered CSF folate, the presence of serum blocking FR antibodies could explain disturbed folate transport to the brain in eight of the patients resulting in a low CSF/serum folate ratio. However, in six Rett patients with low CSF folate, FR antibody testing was negative and the CSF/serum folate remained normal so that an increased folate turnover was suspected to result from increased utilization or catabolism [23].

One important observation from these studies was that in the clinical Rett phenotype without known genetic defects (MECP-2, CDKL5, FOXG1) a search for CFD and FR autoimmunity should be initiated. Our findings suggested a correlation of milk consumption with the presence of these FR antibodies in Rett syndrome [23].

**CFD associated with mitochondrial disorders and FOLR-1 gene defects**

Much rarer underlying causes of CFD are mitochondrial disorders, mitochondrial DNA depletion syndrome (Alpers type), Kearns-Sayre syndrome and genetic anomalies of the FOLR-1 gene [7, 14, 29–34].

Patients affected by FOLR-1 gene mutations exhibit a strikingly similar clinical phenotype to that observed in CFD due to FRα autoantibodies. With the exception of a single patient, all suffered from frequent epileptic seizures [7, 34, 35]. All children with reported FOLR-1 mutations had very low CSF MTHF concentrations below 10 nM and needed much higher folinic acid doses for treatment. Molecular characterization of FOLR-1 gene abnormalities identified on both alleles nonsense and missense mutations, a duplication in one patient and one splicing error in one patient, all of which lead to functional loss of FOLR-1 protein due to either protein instability, reduced membrane expression of truncated FOLR-1 proteins, or mistargetting of mutated FOLR-1 proteins [33]. None of the reported patients had signs of embryonic malformations or neural tube defects. This raises the question as to why these FOLR-1 mutations did not have any effect on embryonic and fetal development. Alternate mechanisms operating for folate transport in vivo to provide adequate maternal folate to the fetus is a potential explanation.

Previous gene knock-out experiments in mice had shown that nullizygous FOLR-1 pups had severe embryonic malformations or died in utero, while nullizygous FOLR-2 pups survived and were normal [35], confirming...
the predominant role of FOLR-1 in providing adequate folate to the embryo in the mouse.

De Marco provided evidence for genetic associations between molecular variations of the FOLR-1 gene and NTD in humans [36]. In addition, immunohistochemical and mRNA studies have confirmed the expression of FOLR-1, PCFT and the RFC-1 at the human placental-fetal barrier during the first trimester of pregnancy and at term where their concerted action is necessary to maintain vectorial folate transport to the embryo and fetus [6].

To explain the absence of embryonic malformations and late-onset of clinical features beyond 2 years among their patients with FOLR-1 gene mutations, Steinfeld et al. suggested that folate transport in humans may differ from that in the mouse and that FOLR-2 may play a role in transporting folate to the fetus.

It was suggested that FOLR-2 expression in utero and during the first postnatal 2 years among subjects with FOLR-1 homozygous mutations provided sufficient compensatory protection against folate depletion [7].

However, such speculation by Steinfeld et al. has not been substantiated with experimental data.

Finally, one report described low spinal fluid MTHF levels and improvement after folic acid intramuscularly in a child suffering from the H-ABC syndrome, or better known as Hypomyelination with Atrophy of the Basal Ganglia and Cerebellum. The serum was not analyzed for the presence of FR antibodies [37].

Diagnostic investigations and differential diagnosis

In the diagnostic work-up process of a suspected CFD syndrome, we strongly recommend all diagnostic guidelines as outlined in Table 1 to be performed. Moreover, causes of systemic folate deficiency and the use of anti-folate drugs should be considered and excluded in each patient [38] (Table 2).

If on the basis of history, clinical examination and additional investigations the patient may suffer from one of the aforementioned CFD syndromes, a lumbar puncture is necessary to measure MTHF metabolites in addition to the determination of serum FR antibodies of the blocking and binding type. After finding low CSF MTHF values, the most common underlying cause of CFD syndrome are FR antibodies of the blocking type which are able to inhibit the binding site for MTHF and prevent its transfer across the choroid plexus to the CNS [12]. In the infantile-onset CFD syndrome blocking FR antibodies belong to the IgG1, IgG3 or IgG4 isotype while often combinations of the IgG1 with IgG3 or the IgG1 with IgG4 antibodies are encountered in the same individual [13].

In addition, among patients suffering from autism spectrum disorders, FR antibodies of the binding type have been found and are thought to form complexes with FR antigen at the blood-brain barrier followed by complement binding with loss of function of FR mediated folate uptake and transport capacity across the blood-brain barriers. Spinal fluid examination did not show the presence of intrathecal FR antibodies of the blocking or binding type in any of the CFD syndromes [12, 22].

The differential diagnosis of the infantile-onset CFD syndrome includes a number of conditions characterized by a combination of similar features like irritability and insomnia, deceleration of head growth with microcephaly, psychomotor retardation and regression, hypotonia and ataxia, pyramidal deficits, dyskinesias and epilepsy.

One of the conditions most closely resembling the infantile-onset CFD syndrome is Rett syndrome in girls. The differential diagnosis should consider also neuromuscular disorders, mitochondrial encephalopathies of early onset, the congenital syndrome of protein glycosylation type Ia, disorders of pterin metabolism, infantile neuronal ceroid lipofuscinosis type 1, arigininemia, Menkes disease and some neurogenetic syndromes like the Allan-Herndon-Dudley syndrome, Marineso-Sjögren syndrome, Pelizaeus-Merzbacher syndrome and an X-linked ophilagrenin 1 gene defect. Further investigations including neurophysiologic studies, neuro-imaging and appropriate laboratory testing as indicated in Table 1 can help to distinguish these disorders.

One pitfall in the diagnosis of these FR autoimmune mediated CFD syndromes is the finding of serum negative for FR antibodies even if a lumbar puncture shows lowered MTHF values in spinal fluid. The reason may lie in the large fluctuations in FR antibody titer observed over time which may show one or two peaks of high titers alternating with low or even absent FR antibodies during an interval of 6 weeks (Figure 7). However, the presence of an inversion of the normal CSF to serum MTHF ratio (normal ≥1.5) may indicate that blocking FR antibodies have been complexed to FRα sites on the choroid epithelial cells after clearance of circulating FR antibodies from serum. The fluctuating patterns of FR antibodies over time have been demonstrated now for infantile-onset CFD, infantile autism with neurological deficits and infantile autism spectrum disorders. After intervention with a
Full ophthalmologic and auditory examination
Neuro-imaging
Electrophysiology (EEG, evoked potentials; if necessary EMG and nerve conduction studies)
Laboratory investigations
Hematology and red blood cell (RBC) indices
Serum and RBC folate, homocysteine, amino acids (tryptophane, phenylalanine, serine, glycine, arginine)
Serum N'-methyl-tetrahydrofolate (MTHF)
Leucocyte or fibroblast enzyme activity for Methylene-tetrahydrofolate reductase
Plasma lactate, pyruvate, ammonia and glucose
Serum copper and ceruloplasmin
Serum CK levels
Plasma TSH and T3, T4 levels
Serum gliadin antibodies
Exclude vitamin B2, B6 and B12 deficiencies
Spinal tap for CSF
Glucose, lactate, pyruvate
CSF MTHF, pterins and biogenic mono-amines
Amino acids (tryptophan, serine, glycine)
Determination of CSF: serum MTHF ratio (normal ratio being ≥1.5)
Serum FOLR-1 antibodies of the blocking and binding type
Genetic analysis of the FOLR-1 gene (consider PCFT analysis based on clinical phenotype)
Consider MECP2 mutation for Rett syndrome (or mutations of CDKL5 and FOXG1 genes)
In case of megaloblastic anemia:
  – Consider DHFR gene alterations
  – Consider PCFT mutations (congenital folate malabsorption)
In case of low HVA, SHIAA, elevated L-dopa and 5-OH-tryptophane:
  – Consider aromatic amino acid decarboxylase deficiency
In case of high phenylalanine, low HVA, SHIAA, MTHF and elevation of neopterin, dihydrobiopterin and low tetrahydrobiopterin:
  – Consider dihydropteridin reductase deficiency
In selected instances:
  – Sialotransferrin analysis in plasma (CDG Ia syndrome)
  – Urinary amino- and organic acids (mevalonic aciduria)
  – Urinary screening for purine- and pyrimidine disorders (adenylosuccinase deficiency, dihydropyrimidine dehydrogenase deficiency)

| Table 1 | Guidelines of diagnostic and differential diagnostic investigations for CFD. |

Milk-free diet, FR antibodies showed significant down-regulation over time in most CFD patients.

Two autosomal recessively transmitted inborn errors of metabolism frequently associated with secondary CSF MTHF depletion are due to deficient enzyme activity of aromatic amino acid decarboxylase [39] and dihydropteridin reductase deficiency [40, 41]. Simultaneous CSF measurement of mono-amine metabolites (L-Dopa, 3-O-methyl-dopa, homovanillic acid, 5-hydroxy-tryptophan and 5-hydroxy-indole acetic acid) and pterine metabolites show typical abnormal profiles for each of these two disorders. A third disorder of de novo serine synthesis, due to D-glycerate dehydrogenase deficiency, is associated with brain serine deficiency with consequent MTHF deficiency in spinal fluid because serine serves as a one-carbon pool donor in the CNS necessary for re-methylation of THF to MTHF [42].

With the finding of megaloblastic anemia or pancytopenia and homocysteinemia, plasma and red cell MTHF should be determined and inborn errors of folate metabolism should be excluded such as congenital folate malabsorption due to genetic defects of the PCFT, MTHFR reductase deficiency and DHFR deficiency [1, 43–45]. The latter group of genetic folate disorders cannot be classified as CFD syndromes since plasma and red blood cell folate metabolite profiles will be low with aberrant folate form profiles. Therefore, MTHF metabolites should be quantified in serum for which age-dependent reference values in normal children have been established [46].

**MR spectroscopic findings in CFD syndromes**

MR spectroscopy in CFD associated with FR autoantibodies may show loss of choline and inositol peaks
within the white matter. Similar MR spectroscopy studies among CFD patients associated with *FOLR-1* mutations and very low MTHF levels, could also confirm hypomyelination with low choline and inositol peaks of white matter particularly within parieto-occipital regions [7].
to myelin with methylated MBP due to cationic charge differences at the arginine 107 position. Using SAM as substrate, the addition of one or two methyl groups to arginine residues in position 107 of MBP is catalyzed by the MBP-specific protein methylase 1 (SAM: protein-arginine N-methyltransferase, EC 2.1.1.230). Consequently, myelin destabilization due to cationic charge alterations of unmethylated MBP molecules explains the inability to form compact myelin sheaths [47]. This explains delayed progression of myelination and later-onset of myelin disintegration in CFD syndromes as confirmed by brain MRI sequences at different ages and histopathological changes described as subacute combined degeneration of lateral, posterior and anterior columns within the spinal cord [48].

One hypothesis explaining the loss of choline in white matter and spinal fluid suggested that in the case of CNS folate deficiency the reduced methyl-transfer processes leading to homocysteine degradation and production of the universal methyl-donor SAM are replaced by alternative metabolic pathways mobilizing choline from white matter and neuronal cell membranes containing phosphatidyl-choline and inositol. The liberated choline will be oxidized in two steps to betaine (i.e., tri-methyl-glycine) which is used by the enzyme betaine-homocysteine S-methyltransferase for homocysteine conversion to methionine and subsequent production of the universal methyl-donor SAM. The consequent higher choline consumption due to CNS folate deficiency will deplete physiologic choline reserves and perturb normal choline metabolism essential for neurodevelopment, since choline and its derivatives serve as components of structural lipoproteins, membrane lipids and as a precursor of the neurotransmitter acetylcholine. Folinic acid supplementation has resulted in remyelination and reversal of choline and inositol peaks to normal on MR spectroscopy [7].

Figure 7 Fluctuating FR autoantibodies of the blocking type in children with infantile-onset CFD syndrome and infantile autism associated with neurological deficits.
Therapeutic strategies

After identification of FR antibodies, the treatment of choice is the administration of high doses folinic acid according to a previously described protocol [11, 38]. In treated patients a second spinal tap after 6–12 months could show normalization of MTHF levels in CSF. Treatment is started at a daily dose of 0.5–1 mg/kg body weight, divided into two equal dosages. Depending on the clinical response, daily doses could be titrated up to 2–3 mg/kg/day. If therapeutic folinic acid intervention fails to show a positive response after 6 months, MTHF should be measured again in CSF in order to adapt the folinic acid dose.

In children having CFD due to FOLR-1 mutations higher folinic acid doses up to 3–5 mg/kg/day were necessary to improve outcome and correct CSF MTHF levels [7].

The use of these high oral folinic acid doses will increase serum MTHF values significantly so that the low-affinity high capacity folate transporter, i.e., the RBC1 will be able to transport folinic acid and any MTHF formed, across the blood-CSF barrier and circumvent blocked FRα passage due to FR antibodies or FOLR-1 mutations with loss of function [11].

An animal milk-free diet has been shown to downregulate the FR autoantibody titters [13].

The effect of a milk-free diet can be explained by the high molecular mimicry between its soluble FR antigen and the human FRα antigen to which specific serum FR antibodies are generated. The serum FR antibodies have shown higher affinity for the soluble FR antigens contained in animal milk – like bovine, goat and camel milk. For this reason we speculate that exposure to soluble FR antigen from animal milk triggers the immune response within the gut to generate specific FR antibodies in genetically predisposed individuals. These FR antibodies formed against soluble milk-derived FR antigen, cross-react with the human FRα antigen exposed at the plasma-side of choroid epithelial cells at the blood-CSF barrier and other epithelial surfaces such as the reproductive system. New trials using intravenous immunoglobulins, steroids and immunosuppressive drugs still await further critical assessment.

Outcome

As soon as the diagnosis of a specific CFD syndrome has been confirmed by appropriate investigations (CSF analysis and serum FR autoantibodies), treatment should be started as soon as possible, because the longer the interval between onset of first clinical signs and symptoms and start of the therapy, the poorer the prognosis will be. In three children with unexplained irritability, hypotonia and motor delay and infantile-onset of seizures in two of these infants, low CSF folate or serum FR antibodies were identified prior to the age of 1 year. This was followed by prompt intervention with folinic acid. Follow-up of these children could demonstrate full clinical recovery with full seizure-control and lack of any neurocognitive deficits in later years. Diagnosis and folinic acid treatment beyond the age of 1 year, will increase the risk of neurologic deficits and mental retardation.

Conclusions

Early detection and treatment of FR autoimmunity is expected to prevent neurodevelopmental disorders due to CNS folate deficiency. FR autoantibody screening studies should be recommended and validated in the population to determine the incidence of FR autoimmunity, health risks and strategies for prevention. Similar genetic screening methods for FOLR-1 mutations can be performed in the general population to determine their incidence. In addition, parental counseling and testing for FR antibodies before a planned pregnancy should be assessed with the goal to reduce the incidence of neural tube defects and autism spectrum disorders. Preliminary studies have suggested that even a number of healthy individuals possess low FR antibody titers. Our findings of fluctuating FR antibodies in CFD syndrome might also be present during pregnancy of clinically healthy mothers and represent a major issue which can be resolved by repeated serum testing.

Future studies should also address the association of FR autoimmunity with neuro-psychiatric conditions in the general population to develop strategies of folate supplementation that could decrease the risk of fetal folate deficiency during pregnancy, postnatal-onset infantile CFD and the emergence of CFD syndromes during adolescence and adulthood. FR autoimmunity and CFD syndromes need to be considered particularly among patients suffering from unexplained neuropsychiatric conditions (psychomotor retardation, spastic ataxia, dystonia, psychotic and schizophrenic syndromes) which appear unresponsive to conventional intervention and drug treatment.

Acknowledgments: This research has been supported by the Fonds National de la Recherche Scientifique Belgium (FNRS; No. 3.4.540.09.F) and contributions from patients to the Quadros Scientific Fund.
Conflict of interest statement

Authors’ conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

References


Vincent Ramaekers is Professor of Child Neurology and Director of the Centre for Autism at the University Hospital, Liège, Belgium. He is also Professor at the Medical Faculty of Aachen University. His main scientific interests and contributions are novel neurometabolic syndromes, vitamin-responsive disorders and autism spectrum disorders. He contributed to the discovery of the clinical phenotype, etiology and treatment of a new emerging group of disorders, now known as the ‘cerebral folate deficiency syndromes’. His research is conducted in close collaboration with Prof. Edward Quadros and Jeffrey Sequeira at SUNY Downstate, New York and Prof. Beat Thöny at Zurich University.

Jeffrey Sequeira is a Senior Research Scientist in the laboratory of Prof. Quadros at SUNY Downstate, Brooklyn, NY, USA. His research encompasses both folate and vitamin B12. He has made significant contributions towards our understanding of the role of folate and folate receptor antibodies in fetal development and in cerebral folate deficiency syndromes.
Edward Quadros is a Research Professor in the Departments of Medicine and Cell Biology, SUNY Downstate, Brooklyn, New York. He has made seminal contributions to our understanding of absorption, transport, cellular uptake and intracellular metabolism of vitamin $B_12$. His current work aims to define the genes and pathways involved in the CNS pathology associated with $B_12$ deficiency. His research in folate metabolism is aimed at understanding the role of folate in brain development and function and of folate receptor autoantibodies in the pathology of neuro-developmental disorders and autism spectrum disorders.