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Cerebral folate deficiency in adults: A heterogeneous potentially treatable condition

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ABSTRACT

Objective: To describe the phenotype and the response to folinic acid supplementation of cerebral folate deficiency (CFD) in adults, a disorder diagnosed on low 5-methyltetrahydro-folate (5MTHF) in cerebrospinal fluid (CSF), which can correspond to a inherited disorder of folate metabolism (IDFM) or to a metabolic consequence of various neurological diseases.

Methods: We conducted a retrospective study on 224 adult patients with neurological symptoms who had a 5MTHF CSF dosage, collecting their neurologic and neuroimaging data.

Results: 69 patients had CFD (CSF 5MTHF level $<$ 41 nmol/L), 25 of them had severe CFD (sCFD; $\leq$25 nmol/L) with adult onset neurological symptoms in 41%. 56% of sCFD patients had an underlying identified neurologic disorder, mainly mitochondrial diseases, hepatic encephalopathy and primary brain calcifications (no identified IDFM), the others were classified as undiagnosed. sCFD patients presented most frequently pyramidal syndrome (75%), movement disorders (56%), cerebellar syndrome (50%) and intellectual disability (46%). MRI findings mostly showed white matter abnormalities (WMA; 32%) and calcifications (12%), and were normal in 23%. The clinico-radiological phenotype of sCFD patients was not clearly different from non CFD patients in terms of manifestations frequency. However, their neurological picture was more complex with a higher number of combined neurological symptoms (4.7 ± 1.6 vs 3.4 ± 1.7, p = .01). In Magnetic Resonance Spectroscopy (MRS), Choline/Creatine (Cho/Cr) ratio was lower in sCFD patients (n = 7) compared to non-CFD patients (n = 73) (p = .005), with good sensitivity (71%) and excellent specificity (92%). Among twenty-one CFD patients treated with folinic acid, nine had a sustained improvement, all with sCFD but one (50% of sCFD patients improved). In two undiagnosed patients with extremely low 5MTHF CSF values, MRI WMA and low Cho/Cr ratios, folinic acid treatment leaded to a dramatic clinical and radiological improvement.

Conclusion: CSF 5MTHF dosage should be considered in patients with mitochondrial diseases, primary brain calcifications and unexplained complex neurological disorders especially if associated with WMA, since folinic acid supplementation in patients with sCFD is frequently efficient.

1. Introduction

Cerebral folate deficiency (CFD), defined as a low cerebral spinal fluid (CSF) concentration of 5-methyltetrahydrofolate (5MTHF), has been reported in children with various neurological disorders, associated or not to a peripheral folate deficiency [1–3]. 5MTHF participates in DNA, amino-acids, proteins and neurotransmitter synthesis and is a methyl donor in homocysteine remethylation [4]. After intestinal

Abbreviations: 5MTHF, 5-methyltetrahydro-folate; CFD, Cerebral Folate Deficiency; sCFD, severe Cerebral Folate Deficiency; Cho/Cr, Choline/Creatine; CSF, Cerebro-Spinal Fluid; FOLR1, Folate Receptor 1; FRα, Folate Receptor α; HE, Hepatic Encephalopathy; ID, Intellectual Disability; IDFM, Inherited Disorders of Folate Metabolism; KSS, Kearns-Sayre Syndrome; MRI, Magnetic Resonance Imaging; MRS, Magnetic Resonance Spectroscopy; MTHFR, Methyl-Tetra-Hydro-Folate; PBC, Primary Brain Calcifications; PGK1, Phospho-Glycerate-Kinase 1; POLG, Polymerase Gamma associated mutations; SPG 11, Spastic Paraplegia 11

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absorption folate is reduced to 7,8-dihydrofolate (DHF) then to 5,6,7,8-tetrahydrofolate (THF) by dihydrofolatereductase (DHFR). Serine can then acts as a carbon unit donor to THF via the serine-hydroxymethyltransferase (SHMT), generating 5,10-methylene-THF and glycine; and methylene-tetra-hydro-folate-reductase (MTHFR) transforms 5,10-methylene-THF in 5MTHF, the active and most stable circulating form of folate [5].

Transport of 5MTHF from blood to CSF mostly involves the Folate Receptor α (FRα) [5–7]. FRα-mediated endocytosis into CSF is ATP-dependent, allowing 5MTHF to be up to three times more concentrated in CSF than in blood [2]. PCFT (Proton-Coupled Folate Transporter) and RFC (Reduced Folate Carrier) are two other folate transporters also located in the choroid plexus epithelial cells, but have a lower affinity for folate than FRα, and are mainly active in their intestinal absorption [6,7].

Inherited disorders of folate metabolism (IDFM) can induce CFD [5]. Congenital folate malabsorption, due to a PCFT gene defect, causes megaloblastic anemia with immunodeficiency, seizures and cognitive impairment [8]. DHFR deficiency presents with megaloblastic anemia and epilepsy during infancy [9]. MTHFR deficiency can associate cognitive decline, spastic paraparesis, peripheral neuropathy and epilepsy, with leukoencephalopathy [10]. In those three diseases, CFD is usually concomitant with a low blood level of folate. Loss-of-function mutations in the FOLR1 gene (coding for FRα protein) have been described as an infantile progressive cerebellar syndrome with movement disorders, epilepsy and developmental regression, hypomyelination and cerebral atrophy on MRI. Folate in serum is normal [11–14]. Apart from those IDFM, CFD has been reported, usually in absence of peripheral deficiency, in children with genetic diseases, particularly mitochondrial disorders, (including Kearns-Sayre Syndrome (KSS) [5,15,16]), Rett syndrome, Aicardi-Goutière's syndrome [5]; but also as an idiopathic syndrome causing psychomotor retardation, hearing loss, cerebellar ataxia, pyramidal syndrome, behavioral abnormalities or epilepsy [2,19]. Some studies suggest autoimmunity might be at cause, based on the detection of serum Frα-antibody, although it remains controversial [2,5,18,20–22]. The exact cause of CFD remains unclear in numerous children with neurologic manifestations.

Treatment of CFD is the oral administration of high doses of folinic acid and has resulted in previous studies in normalization of 5MTHF in the CSF, and occasionally in a clinical and radiological improvement [13,15,23,24].

So far, CFD has only been reported as a pediatric condition, except for one case with an onset of symptoms at age 58 characterized by progressive memory loss and myoclonus, who was successfully treated with folinic acid [25].

To clarify the phenotypic spectrum of CFD in adults, we retrospectively collected clinical and radiological features from a series of patients with neurological symptoms in whom dosage of CSF 5MTH was performed.

2. Material and methods

We retrospectively collected clinical and radiological data from patients seen at the Pitié-Salpêtrière Hospital (Paris, France) who underwent 5MTHF CSF dosage between 2008 and 2015. CSF 5MTHF level was analyzed in those patients as part of the routine investigations of a non-diagnosed neurological syndrome, and, for some of them, as part of their CFD follow-up. All but three dosages were performed in the department of biochemistry at the Robert Debré hospital (JFB). Two were run at the Trousseau hospital (FM) and one at the University Children's Hospital of Zurich (Nenad Blau). CSF 5MTHF values were determined using high-pressure liquid chromatography coupled to tandem mass spectrometry in the positive mode. There is a negative correlation between normal 5MTHF upper threshold and age: normal values for 5MTHF in CSF are higher in children than in adults [26]. Extrapolating decrease of CSF 5MTHF values with increasing age in a pediatric population, we determined the cut-off value for CFD diagnosis in adults as a 5MTHF concentration strictly inferior to 41 nmol/L. This value was used in previous reports in the literature [25–28]. We chose arbitrarily the threshold of 25 nmol/L to determine a sub-group of severe CFD patients (CSF 5MTHF $<$ 25 nmol/L), on whom we mainly focused in our study, as we estimated that moderate CFD between 25 and 41 nmol/L was a biologic condition not susceptible to induce clinical manifestations. Retrospectively our arbitrary choice turned out to be relevant as almost all CFD patients who improved with folate substitution had a severe CFD (see results below), whereas moderate CFD patients did not improve. In addition to 5MTHF, 5-Hydroxyindolacetic acid (5HIAA, a serotonin metabolite) and homovanillic acid (HVA, a dopamin metabolite) were quantified in CSF. No correlation was evidenced between 5MTHF levels and HVA levels, a weak negative correlation was found between 5MTHF and HIAA levels ($r = 0.145$, $p = .036$). MRS (Magnetic Resonance Spectroscopy), performed in 100 patients, was analyzed with measures of Choline/Creatine (Cho/Cr), N-Acetyl Aspartate (NAA)/Creatine, and Choline/NAA ratios at long echo time (TR = 1500 ms, TE = 135 ms) in the white matter (centrum ovale or semi ovale). Normal ratios were determined using data of 12 age-similar controls without neurological disorder. Cho/Cr ratio, in particular, was considered as normal if ≥ 0.90.

IBM SPSS Statistics 24.0.0 was used for statistic tests, with use of the Khi-2, Fisher and Mann-Whitney tests, when appropriate. Correlations were studied using the Spearman correlation ratio.

3. Results

224 adult patients were screened for CFD. 69 patients with CFD were identified, including 25 patients with severe CFD (sCFD; Table 1). Mean age at onset was 24 years (± 21, 0–65). Pediatric onset ($<$ 15 yo) was more frequent in sCFD (13/22, 59%) than in non-severe CFD (15/40, 37.5%). Blood level of folate was available in 38/69 cases patients, and in 15/25 sCFD patients. It was normal in 36 cases, increased in one case and decreased in another case. Among the 31 CFD

Table 1
Population characteristics.

	5MTHF (nmol/L); (± SD)	Age at CFD diagnosis (y); (± SD)	Age at symptoms onset (y); (± SD)	Sex-ratio (M/F)
All patients (n = 224)	51 (± 23, 2–159)	/	21 (± 21, 0–77)	1.24
CFD (n = 69)	27 (± 11, 2–40)	43 (± 14, 13–79)	24 (± 21, 0–65)	1.87
Undiagnosed CFD (n = 37)	29 (± 12, 2–40)	40 (± 15, 13–75)	18 (± 19, 0–65)	2.08
Severe CFD (n = 25)	15 (± 8, 2–25)	40 (± 13, 13–62)	16 (± 18, 0–52)	2.12
Undiagnosed severe CFD (n = 11)	14 (± 8, 2–25)	37 (± 14, 13–61)	10 (± 14, 0–40)	2.66
Patients treated for CFD (n = 25)	19 (± 11, 2–35)	41 (± 14, 13–65)	20 (± 19, 0–35)	1.08
Non-CFD patients (n = 155)	62 (± 18, 41–159)	/	19 (± 21, 0–77)	1.04
Undiagnosed non-CFD (n = 110)	64 (± 21, 41–159)	/	17 (± 19, 0–68)	1.06

SD: Standard Deviation; M: male; F: Female. Undiagnosed: means no neurological disease (except CFD condition, if so) could explain neurological symptoms after diagnostic work up.

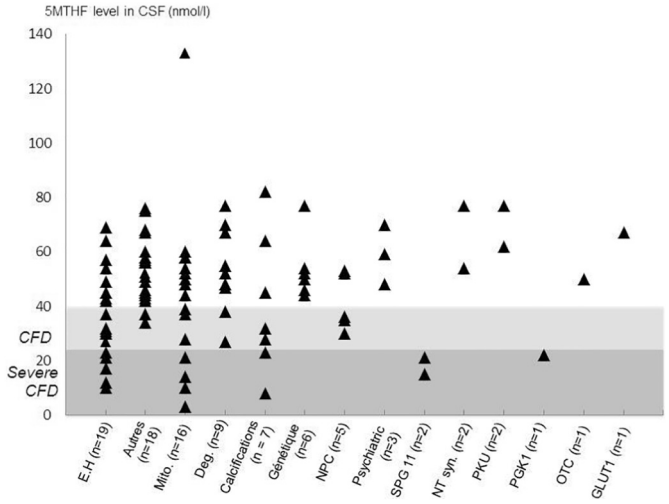

Fig. 1. Underlying diagnosis and 5MTHF level. H.E. = Hepatic Encephalopathy; Others = epileptic disorders, multiple sclerosis, chronic polyradiculopathy, post-anoxic encephalopathy, stiff Person syndrome; Mito. = mitochondriopathy; Deg. = Neurodegenerative disorders (MSA, Parkinson, PSP, FTD, ALS); PBC: Primary Brain Calcifications; Genetic = Non-metabolic genetic disorders (SPG 4, Pelizaeus-Merzbacher, SCA3, 2/4 translocation, euchromatine histone methyl transférase deficit, 22Q deletion); NPC = Niemman Pick Type C; Psychiatric = psychiatric disorders; SPG11: Spastic Paraplegia 11;; NT syn = Neurotransmitter synthesis disorders (Sepiapterin reductase deficiency & DHPR); PKU: Phenylcetonuria; PGK1: Phosphoglycerate Kinase Deficiency; OTC = OTC deficiency; GLUT1 = GLUT1 deficiency.

patients with missing folate concentration in serum, 22 had no systemic manifestation or biological abnormalities (anemia and/or elevated homocysteine) suggesting folate deficiency, one had macrocytic anemia, and no such data were available for the remaining patients (n = 8). For patients whom information on treatment was available, no medications interfering with folate metabolism, such as methotrexate, pyrimethamine or thrimethoprim, was mentioned.

3.1. Associated conditions

Among the sCFD group (n = 25), 14 (56%) had an identified diagnosis (Fig. 1). A mitochondrial disorder was diagnosed in four patients: one with Polymerase Gamma (POLG) mutations, one with KSS and two patients without a proven genetic defect but features highly suggestive of a mitochondrial disorder (including muscle biopsy). The other diagnosis included Primary Brain Calcifications (PBC, n = 2) without genetic variants identified, SPG11 mutations (n = 2), PGK1 mutations (n = 1), and Hepatic Encephalopathy (HE; n = 5). Screening for mutations of FOLR1 gene was negative in the ten tested CFD patients (including six sCFD). Nine patients were tested for Frα-antibodies, seven being positive. However it was not possible to confirm positivity in the two patients for whom another test was performed.

NB: the 2 SPG11 patients are brothers.

3.2. Clinical phenotype

sCFD patients presented mostly with pyramidal tract signs (n = 18, 75%), hyperkinetic movement disorders (n = 14, 56%), cerebellar syndrome (n = 12, 50%), Intellectual Disability (ID; n = 11, 46%), cognitive decline (n = 9, 38%), peripheral neuropathy (n = 6, 25%), hearing loss (n = 6, 25%), and epilepsy (n = 6, 24%) (Table 2). Pyramidal tract signs and hearing loss were the only symptoms found more frequently in sCFD patients compared to non CFD patients (18/24 (75%) vs 63/146 (43%) p = .004, and 6/24 (25%) vs 10/146 (7%) p = .013, respectively). Three sCFD patients with hearing loss had a mitochondrial disease the other three had no identified diagnosis. Even in mitochondrial diseases, hearing loss seemed more frequent in sCFD patients (3/4, 75%) vs non CFD patients (1/9, 11%; p = .005).

Eleven patients with sCFD had no definite diagnosis (Table 2, column 4). Eight of them had a pediatric onset (71%), and shared several neurological symptoms with the three adult onset patients, but were the only one to suffer from ID (n = 6), epilepsy (n = 3), and hearing loss (n = 3). The clinical phenotype of undiagnosed sCFD patients was complex, with a mean number of combined symptoms (based on the 13 symptoms in Table 2) of 4.7 (± 1.6, 2–7) versus 3.4 (± 1.7, 1–8) for undiagnosed non CFD patients (p = .01).

3.3. MRI findings

sCFD patients presented with supra-tentorial WMA (White Matter Abnormalities; n = 8, 32%), global atrophy (n = 7, 32%), posterior fossa WMA (n = 4, 24%), basal ganglia abnormalities (n = 4 18%), and calcifications (n = 3, 12%) (Table 2). Twenty three % (n = 5) of sCFD patients had a normal MRI. Posterior fossa WMA and calcifications were the only signs found more frequently in sCFD patients compared to non CFD patients (4/17(24%) vs 5/106 (5%) p = .021, and 3/25 (12%) vs 3/136 (2%) p = .048, respectively), mainly related to a mitochondrial disease for both signs and PBC for calcifications. Interestingly, none of our non-CFD patients with a mitochondrial disease had either calcifications or posterior fossa WMA, whereas sCFD mitochondrial patients presented with such radiological features in 25% and 50% of the cases respectively.

Among our eleven patients with sCFD and no definite diagnosis, the main MRI feature was the presence of supra tentorial WMA in 8/11 (73%; 18% in non CFD undiagnosed patients, p < .001).

100 patients underwent MRS, including 7 patients with sCFD (of whom 5 were without definite diagnosis), 20 with non-severe CFD, and 73 without CFD. Cho/Cr ratio was lower in sCFD patients compared to controls (0.85 ± 0.19 vs 1.05 ± 0.13, p = .005) and compared to non-CFD patients (0.85 ± 0.19, vs 1.11 ± 0.17, p = .005). NAA/Cr and Cho/NAA ratios were similar in the three groups. In our cohort, sensitivity of low Cho/Cr for diagnosis of sCFD was 71% whereas specificity was 92% (86/93). Specificity was improved to 97% considering a lower threshold (< 0.85 instead of < 0.90). Among CFD patients (n = 27), Cho/Cr significantly correlated with 5MTHF level (r = 0.562, p = .002) (Fig. 2).

3.4. Treatment and evolution

Among the 69 CFD patients, 25 were treated by pharmacological folate, either 5MTHF, folinic acid, or the association of the two (Supplementary Table 1). There was no difference in efficiency between folinic acid (25 to 75 mg per day) and 5MTHF. 5MTHF CSF level normalized in all patients who had CSF analysis under folate supplementation (n = 11). Over the 21 patients for who details on clinical follow-up was available, improvement was reported in 13 patients, and sustained in nine, based on clinical charts. Mean initial CSF 5MTHF level in CFD patients with a sustained improvement was lower than in non-improved or transiently improved patients (12 ± 11 versus 22 ± 8, p = .041) (Fig. 3). 8/16 (50%) treated sCFD showed a sustained clinical improvement. There was a pediatric onset of symptoms in 12/19 (63%) of all treated patients and in 7/8 (88%) of persistently improved treated patients. Clinical improvement was observed mostly on gait (n = 7), movement disorders (n = 6), and behavior (n = 5). Two undiagnosed patients with extremely low 5MTHF levels in CSF (2 and 3 nmol/l), symmetrical diffuse WMA and low Cho/Cr ratios, had a dramatic and sustained improvement of both clinical and neuroimaging abnormalities (Fig. 4). Both had otherwise a negative extensive diagnostic work-up, including whole exome sequencing, and there was in particular no argument for a mitochondrial disease.

Table 2
Clinical and MRI data in severe CFD patients.

	All		Undiagnosed patients		Mitochondriopathy	
	Severe CFD (n = 25)	Non-CFD (n = 155)	Severe CFD (n = 11)	Non-CFD (n = 110)	Severe CFD (n = 4)	Non-CFD (n = 10)
Clinical (%)						
Pyramidal Tract syndrome	75* (p = .04)	43	91* (p = .04)	45	50	67
Hyperkinetic abnormal movements	56	47	64	55	100	70
Cerebellar Syndrome	50	37	18	41	75	44
Intellectual disability	46	38	55	42	25	22
Cognitive decline	38	29	55	29	25	40
Peripheral Neuropathy	25	14	27	13	0	22
Hearing loss	25* (p = .013)	7	27 (p = .077)	8	75 (p = .052)	11
Epilepsy	24	23	27	23	25	22
Oculomotor disorder	17	18	9	17	75	11
Visual disorder	17	13	0	13	75	22
Parkinsonism	16	17	18	18	0	37.5
Psychiatric disorder	16	15	36	15	0	10
Bladder dysf.	13	22	9	15	0	22
MRI (%)						
Supratentorial WMA	32	23	73 (p < .001)	18	50	30
Atrophy	32	40	72	42	25	10
Posterior fossa WMA	24* (p = .021)	5	9	3	50 (p = .077)	0
Normal MRI	23	30	27	34	25	50
Basal Ganglia abnormalities	18	11	9	11	25	10
Calcifications	12* (p = .048)	2	0	12	25	0
Corpus callosum atrophy	10	4	9	1	0	0
Cerebellar atrophy	6	27	27	29	25	10

Comparison of clinical data and MRI data, in terms of frequency (%), of severe CFD patients versus non-CFD patients: in all patients (columns 2–3), undiagnosed patients (columns 4–5; ie, without neurological disease explaining symptoms, except CFD condition, if so), and in patients with identified mitochondrial disease (column 6–7). Dysf: dyfunction; WMA: White Matter Abnormalities.
* means that difference of frequencies is significant statistically between severe CFD patients and non CFD patients.

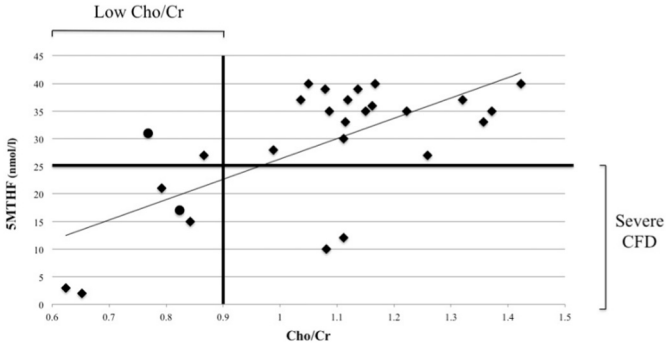

Fig. 2. Cho/Cr ratio (MRS) and 5MTHF level in 27 CFD patients. Choline and Creatine were measured in the semi-ovale center. We defined an inferior threshold of Cho/Cr of 0.90 using a sample of 12 controls (10thpercentile). Spearman correlation ratio between Cho/Cr ratio and MTHF level was 0.562 (p = .002) in CFD patients. The two ● patients had hepatic encephalopathy, a condition known to possibly display low Cho/Cr.

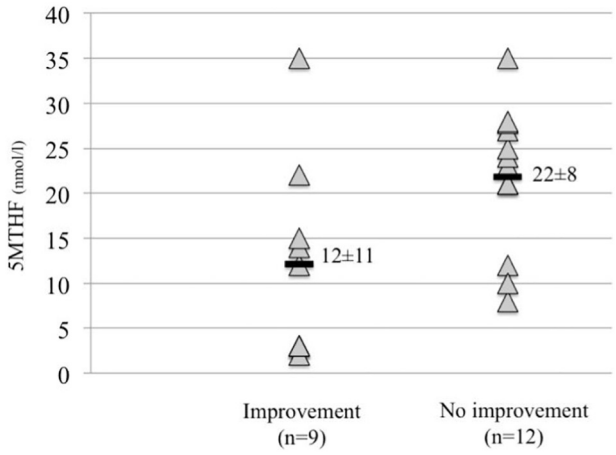

Fig. 3. Response to folinic acid substitution in 21 CFD patients according to initial CSF 5MTHF value. The initial 5MTHF value was lower in patients with sustained improvement (the black bar is the mean; p = .041).

4. Discussion

We reported here clinical and radiological characteristics of CFD patients diagnosed in adulthood, focusing on 25 patients with severe CFD (sCFD). To date, this is the first time such a large cohort of adult patients tested for CFD has been studied. The large variety of recruited patients allowed us to obtain a proper picture of CFD condition in neurological diseases. However, we may have underestimated some CFD associated conditions, for which clinicians had enough diagnostic evidences not to investigate further with 5MTHF dosage in CSF. Age of symptoms onset greatly varied among patients, highlighting the fact that sCFD should not be restricted to a childhood onset condition. CFD diagnosed in adults is probably a different condition than CFD described so far in pediatric patients. Indeed, none of our patients had an inherited disorder of folate metabolism (IDFM). In addition, our undiagnosed sCFD patients did not have the phenotype described in "idiopathic" CFD children, especially due to the frequent absence of epilepsy [2,19]. The heterogeneous clinical and MRI phenotype of adult sCFD patients we observed probably reflects the various underlying causal diseases, identified (as mitochondrial diseases reported here) or not. However, our study provides useful clues to identify adult patients who should be screened for sCFD in clinical practice. We suggest that screening should be considered in the work-up of patients with an unexplained neurological disease, particularly when they have multiple symptoms, and/or diffuse symmetrical WMA on brain MRI, and/or a reduced Cho/Cr ratio on MRS. It may also be discussed in patients with a condition known to possibly result in sCFD, such as mitochondrial disorders, HE, PBC, SPG11 or PGK1. Among the nine patients that experienced sustained improvement with folinic supplementation, eight had sCFD. We thus recommend the use of a cut-off value of 25 nmol/L

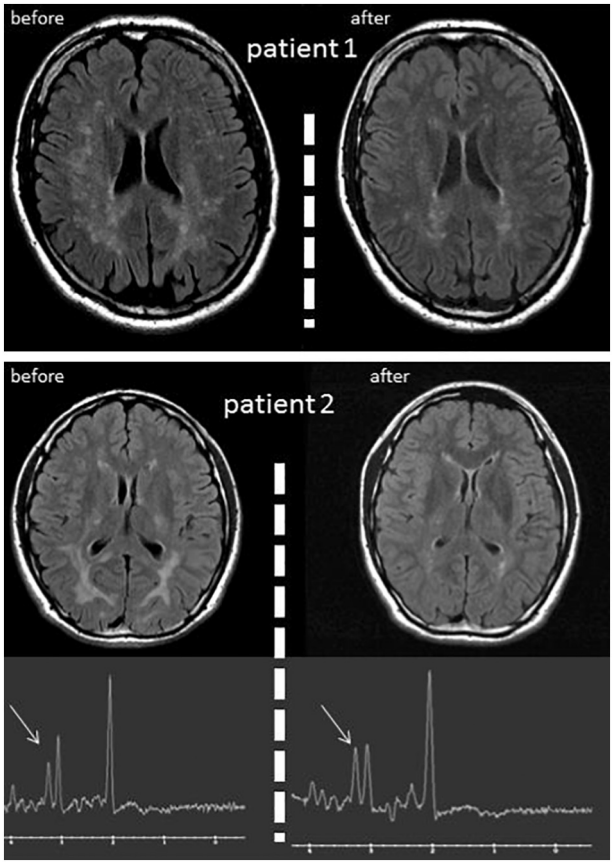

Fig. 4. Radiological improvement in two extremely severe CFD patients after folinic acid treatment. For both patient 1 and 2 (see Supplementary Table 1) who had very low CSF 5MTHF values (2 and 3 nmol/L), posterior supra-tentorial WMA was important at diagnosis of CFD before treatment. In both patients, MRI highly improved as 5MTHF normalized in CSF, associated to a sustained dramatic clinical improvement till last follow up after 3 years of treatment. In patient 2, initial low Cho/Cre ratio normalized after treatment (see white arrows). For spectroscopy: TE = 135 ms, in centrum semi-ovale.

to select patients for whom folinic acid supplementation should be tested (Fig. 5).

sCFD patients typically had a complex phenotype combining various neurological manifestations. Those with symptoms beginning after 15 years old were almost as numerous as pediatric onset patients. The great heterogeneity of the sCFD phenotype likely reflects that core of the observed manifestations is rather due to the underlying disorder than to the CFD per se. The only partial clinical improvement observed in some treated sCFD patients despite complete normalization of the CSF 5MTHF level is in keeping with this hypothesis. Only three symptoms were found in ≥50% of sCFD patients (pyramidal syndrome, hyperkinetic movement disorders, and cerebellar syndrome). The high prevalence of pyramidal syndrome is reminiscent of what is observed in IDFM like MTHFR deficiency [9]. However, these conditions also frequently induce peripheral neuropathy and epilepsy, which were less frequent in our sCFD patients (25% and 24% of patients respectively), probably because CFD is more profound in IDFM (< 5 nmol/l, [29]). Whereas it has not been reported in IDFM, hearing loss was found in 25% of our sCFD patients, only three of whom had a mitochondrial disease. These observations sustain the assumption that sCFD may represent a deleterious factor worsening various symptoms primarily caused by the underlying disease, and that profound CFD might even directly induce some clinical signs. This is probably why treatment efficiency was most striking in profound folate deficiency, independently of the underlying neurological diagnosis.

Brain MRI findings in sCFD were heterogeneous and highly variable, probably for the same reasons that may explain the clinical heterogeneity. WMA in the posterior fossa and calcifications were significantly associated with sCFD in comparison with non-CFD patients. Diffuse symmetrical WMA are frequently encountered in patients with CNS disorders, particularly in metabolic diseases. It is impossible to single out the role of CFD in their constitution. Nevertheless, the radiological improvement seen in two patients under sole folinic acid supplementation suggests a direct deleterious effect of very low CSF 5MTHF on brain white matter, at least in some cases. Calcifications in sCFD patients could reflect PBC-associated diseases or underlying mitochondrial disorders. However, intracranial calcifications have been described in patients with IDFM (FOLR1 deficiency [11] and congenital folate malabsorption [30]), and also with Aicardi-Goutières syndrome [31], suggesting a possible direct link with CFD. MRS has proven to be useful in diagnosis and follow-up of neuro-metabolic disorders, and is accessible in routine practice [32,33]. In our study, although on a small sample size, the Cho/Cr ratio was found to be significantly lower in sCFD, and positively correlated to the depth of the deficiency. HE is a cause of CFD potentially associated with hyperammonemia, a condition also known for inducing low choline in MRS [34]. However, hyperammonemia cannot explain MRS data in our cohort, since only 2/7 sCFD patients with low Cho/Cr ratio had HE including one with normal ammonemia. Low Cho/Cr ratio in sCFD patients might be explained by alterations of the myelination process due to CFD, resulting in a deficit of glial choline [2,13]. The fact that the Cho/Cr ratio is reduced due to choline deficiency advocates for a normal creatine level. This implies a proper biosynthesis of creatine in the liver, which needs folate for methylation of guanidinoacetate to creatine. Creatine is then transported to the brain. Normal creatine level hence supports the fact that most of the cases studied in the present work had a normal serum folate status. Even if not sufficient on its own with a sensitivity around 71%, Cho/Cr ratio appeared to be a useful non-invasive marker for sCFD diagnosis due to its high specificity (92%).

sCFD was only encountered in a few conditions in this study, namely HE, mitochondrial disorders, PGK1 mutations, SPG11 mutations, and PBC. In addition, seven patients were found to be positive for Frα-antibodies. Three mechanisms may link these conditions to low 5MTHF in CSF: (a) a default in 5MTHF cerebral metabolism, (b) a dysfunction of 5MTHF transporters and (c) an energetic impairment in its ATP-dependent transport to CSF. Folate uptake to CSF has been hypothesized to be impaired by presence of Frα-antibodies in pediatric neurological patients and adult patients with schizophrenia [2,20–22]. In our cohort, Frα-antibodies positive patients ($n = 7$ for 9 tested patients) represented a heterogeneous group of patients in terms of age of onset (3 pediatric onset, 2 adults, 2 unknown), clinical phenotype, values of CSF 5MTHF (mean 22.8, 8–41). Presence of antibodies was not confirmed in the two patients for whom a second test was performed, maybe reflecting known fluctuations of antibodies titers [21]. Three patients were treated with intravenous immunoglobulins and/or plasmatic exchanges and none showed improvement, whereas all of them responded at least transiently to folinic acid supplementation. For all those reasons, we cannot conclude regarding the contribution of auto-immunity for CFD in our cohort. In another study over 67 children with ASD (autism spectrum disorder) including five with CFD [35], Frα-antibodies were only found in 4 patients who did not have CFD, questioning the pathophysiological role of these antibodies in some populations. Regarding mitochondrial disorders, they cause an energetic failure probably responsible for the impairment of the ATP-dependent folate transport to CSF. In addition, in KSS, anatomical and physiological changes in choroid plexus might also disrupt 5MTHF uptake to CSF and can explain the elevated CSF protein level classically observed in this disease [17]. In our sCFD patients, including those without identified underlying disease, the mean CSF total protein value ($N < 0.35$) was high at 0.79 g/l ± 0.58 [0.12–2.12], suggesting that, as in KSS, a transport defect through choroid plexus may explain the cerebral folate

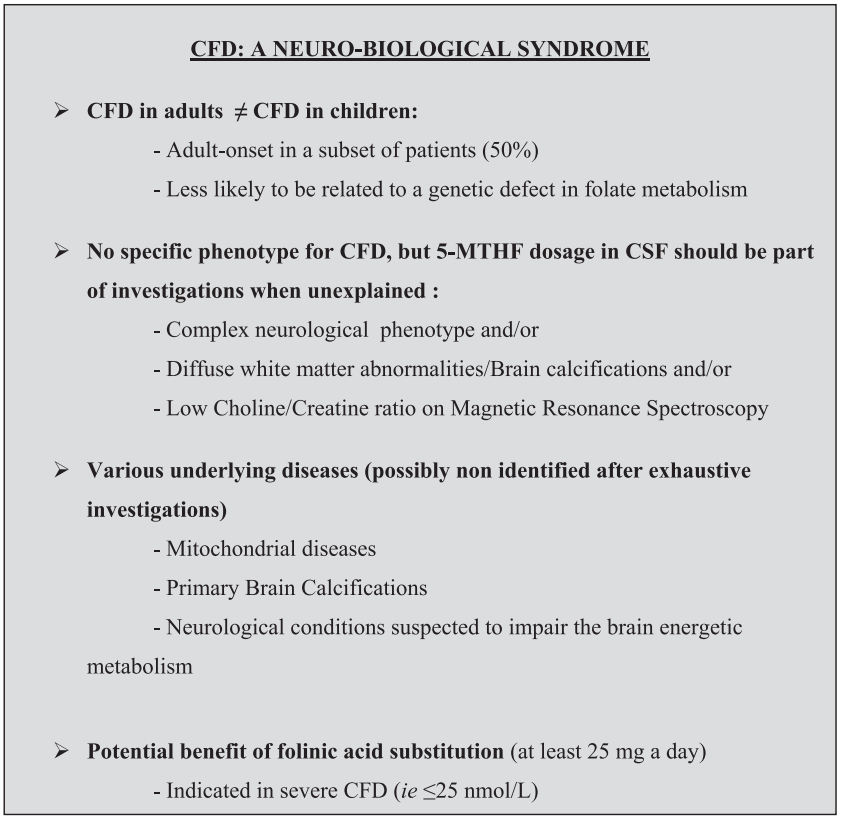

Fig. 5. CFD in adults.

deficiency. Also, mitochondrial diseases may induce cerebral oxidative stress, which could accelerate 5MTHF catabolism [36]. Our findings suggest that, severe CFD might be indicative of a mitochondrial disease, thus should prompt clinicians to further investigate this hypothesis. In HE, CFD cannot be solely explained by the liver role in folate metabolism, because none of our HE patients had reduced folate in plasma. Alteration of the Krebs cycle has been shown in HE, and could participate, via an energetic defect, to CFD [37]. PGK1 is a rare X-linked disorder, classically responsible for hemolytic anemia and myopathy; yet our patient had an unusual but reported mitochondriopathy-like clinical picture with mental retardation, parkinsonism, retinitis pigmentosa and stroke-like episodes [38]. An energy insufficiency might then explain its association with sCFD, especially as PGK deficiency not only regulates glycolysis but has also been associated with mitochondrial changes [39]. The two patients with SPG11 mutations (a disorder presenting with mental retardation, spastic paraparesis, motor peripheral neuropathy, and thin corpus callosum [40]) did not receive folate supplementation. Interestingly, spatacsin, the mutated protein in SPG11 is partly expressed in mitochondrias, where it might act as a receptor or a transporter, echoing findings in other hereditary spastic paraplegias and highlighting a potential mitochondrial involvement [41,42]. Hence, energetic defect might here again be the underlying physiopathologic link with CFD.

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Ethics approval and consent to participate

Authors declare that this work received all necessary ethical approvals and that patients involved all consented to participate.

Consent for publication

Not applicable (manuscript contains no individual person's data).

Availability of data and material

Please contact author for data requests.

Potential conflicts of Interest

Authors declare no financial or non-financial competing interests in relation with this manuscript.

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Author contributions

MM and YN were involved in conception and design of the research project, in analysis and interpretation of the data, and writing of the first draft of the manuscript. YN, CL, FS and ER were involved in the acquisition of clinical data and JFB and FM were involved in the acquisition of biochemical data. All authors read and approved the final manuscript.

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