The Journal of Nutrition 155 (2025) 2267–2274

THE JOURNAL OF NUTRITION

journal homepage: https://jn.nutrition.org/

Nutrition and Disease

The Role of Folate Receptor α Autoantibodies in Folate Deficiency, Disease Severity, and Treatment Response in Adolescents with Major Depressive Disorder

Pascal Gloor [1], Isabelle Haeberling [2], Katharina Spanaus [3], Gerd A Kullak-Ublick [1], Gregor Berger [2,**], Michele Visentin [1,*]

[1] *Department of Clinical Pharmacology and Toxicology, University Hospital Zurich, University of Zurich, Zurich, Switzerland;* [2] *Department of Child and Adolescent Psychiatry and Psychotherapy, Psychiatric Hospital, University of Zurich, Zurich, Switzerland;* [3] *Institute of Clinical Chemistry, University Hospital Zurich, University of Zurich, Zurich, Switzerland*

A B S T R A C T

Background: Low concentrations of systemic folates have been associated with a higher risk of major depressive disorder (MDD) and more severe symptoms. Moreover, folate supplementation has been shown to increase the response to selective serotonin reuptake inhibitors (SSRIs) and serotonin norepinephrine reuptake inhibitors (SNRIs). Folates reach the brain through the choroid plexus via transcytosis mediated by the folate receptor alpha (FRα). FRα also represents the main mechanism of folate retrieval from the nascent urine. Autoantibodies against the FRα autoantibodies(FRAAs) have been found in the serum of individuals with cerebral folate deficiency.

Objectives: This study aimed to assess the role of serum FRAA titer on serum folate concentration, disease severity, and response to the SSRI/SNRI treatment in adolescents with MDD.

Methods: Serum samples at baseline obtained from the participants of a large multicenter intervention trial in moderately to severely depressed youth were analyzed. Quantification of FRAA was performed by enzyme-linked immunosorbent assay. Serum folate concentration was determined by radioligand binding assay.

Results: FRAA titer in the patients with folate deficiency (≤3.0 ng/mL) was significantly higher than that in the patients with a normal folate concentration, and a low FRAA titer was associated with a reduced risk of folate deficiency. No correlation was found between the Children's Depression Rating Scale—Revised score and the serum folate concentration or the FRAA titer. In regression analysis, the effect size of the serum folate concentration on the response to SSRI/SNRI was larger than that of the FRAA titer. The response rate to the treatment in the high-folate group was ~4 times that in the low-folate group (28.5% compared with 6.7%).

Conclusions: In conclusion, patients with high-FRAA titers carry a higher risk of folate deficiency. Moreover, the response to SSRI/SNRI treatment is less likely in patients with folate deficiency.

Keywords: autoantibodies, depression, folate, folate receptor α, FRAA

Introduction

Folates are bivalent anions, hydrophilic compounds that diffuse poorly across cell membranes. Inside the cells, folates exist in interconvertible one-carbon-substituted forms of tetrahydrofolate acting as cofactors of several biochemical reactions, including the methylation of homocysteine to methionine and of deoxyuridylate to thymidylate, and the formation of the purine ring [1,2]. Moreover, folates enhance the biosynthesis of tetrahydrobiopterin (BH_4), the cofactor for the hydroxylation of phenylalanine and tryptophan in the de novo synthesis pathway of monoamines [3–5]. Folate bioavailability depends on the correct expression, localization, and function of folate-specific transport systems that facilitate the movement of folates across

Abbreviations: CDRS-R, Children's Depression Rating Scale—Revised; CFD, cerebral folate deficiency; CSF, cerebrospinal fluid; FRα, folate receptor alpha; FRAA, folate receptor alpha autoantibodies; MDD, major depressive disorder; ROC, receiver operating characteristic; SNRI, serotonin norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; THF, tetrahydrofolate.

* Corresponding author.

** Corresponding author. *E-mail addresses:* gregor.berger@pukzh.ch (G. Berger), michele.visentin@usz.ch (M. Visentin).

https://doi.org/10.1016/j.tjnut.2025.05.012

Received 3 March 2025; Received in revised form 5 May 2025; Accepted 8 May 2025; Available online 14 May 2025

cell membranes. The proton-coupled folate transporter (*SLC46A1*), which is highly expressed on the apical brush-border membrane of the duodenum and proximal jejunum, mediates folate intestinal absorption [6,7]. The reduced folate carrier (*SLC19A1*) is the main route of folate delivery to most peripheral tissues. The transport of folates into the cerebrospinal fluid (CSF) is mainly mediated by the glycosylphosphatidylinositol-anchored protein folate receptor alpha (FRα, *FOLR1*) [8]. FRα is also highly expressed in the placenta, where it is arguably important for folate supply to the fetus [9]. Folates are essential for the development and proper function of several tissues, including the central nervous system, from the neurulation during the first 4 wk of human gestation to the maturation and maintenance of the nervous system during infancy and childhood [10–12].

Children with a nonfunctional proton-coupled folate transporter have systemic and cerebral folate deficiency (CFD; hereditary folate malabsorption), which manifests with severe hematologic deficits, neurologic developmental delays, and ultimately, seizures [11]. Children with loss-of-function mutations in the *FOLR1* gene encoding FRα are characterized by isolated CFD, psychomotor decline, and seizures [12–17]. CFD can also result from the presence of autoantibodies against FRα (FRAA) that block folate binding to the receptor and/or impair the transcytotic process thereof [18]. In fact, an inverse correlation exists between the titer of FRAA and the concentration of folates in the CSF [19,20]. Studies showed a significant association between the FRAA titer and autism spectrum disorder. In 1 study, 25 patients with early-onset low-functioning autism with or without neurologic deficits were evaluated for serum folate, CSF 5-methylTHF, and serum FRAA concentrations. In spite of normal serum folate, CSF 5-methylTHF was low in 23 of 25 patients. Elevated FRAA concentration was found in the serum of 19 of these 23 patients [21]. In another study, serum FRAA was found in 77.5% (31/40) of children with autism spectrum disorder, compared with 54.8% (23/42) of neurotypical children [22]. In a self-controlled trial, children with nonsyndromic infantile autism were treated with high-dose folinic acid if FRAA tested positive. Compared with the untreated patients with autism, a 2-y treatment decreased the initial Childhood Autism Rating Scale score from severe to moderate or mild autism, achieving complete recovery in 17 of 82 children [23].

Several clinical studies have found a higher prevalence of low serum folates in patients with major depressive disorder (MDD) than in individuals without depression [24–26]. Moreover, patients with low serum folates presented with a higher depression inventory score and suicidal behaviors [26,27]. The etiology of MDD is most likely multifactorial, with common features being alterations in brain tissue volume, connectivity, monoamine concentrations, glutamatergic metabolites, and neurogenesis [28]. The standard treatment of MDD often consists of monotherapy with selective serotonin reuptake inhibitors (SSRIs) or serotonin norepinephrine reuptake inhibitors (SNRIs) to block the reuptake of monoamines, thereby slowing their clearance from the synaptic cleft [29]. Despite their wide use, there is growing skepticism about the overall efficacy of SSRI/SNRI monotherapy. In the first level of the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial, only ~30% of patients have had a stable remission with the SSRI citalopram [30]. A recent meta-analysis over 6 randomized controlled trials provides supporting evidence of folate supplementation increasing the response to SSRI/SNRI [31]. A case-control study found CFD in 36% of patients with refractory depression. In the same study, it was shown that a 6-wk folate supplementation in patients with CFD was associated with an overall reduction of the depression inventory score at follow-up [32]. Patients with MDD who do not respond to SSRI/SNRI might be characterized by an elevated serum FRAA titer, which in turn might contribute to a suboptimal cerebral folate concentration. The aim of the present retrospective study was to evaluate the impact of serum FRAA on disease severity and response to treatment in patients with pediatric MDD.

Methods

Patients

This retrospective analysis included serum samples at baseline obtained from the participants of the Omega-3-pMDD study, a multicenter, randomized, double-blind, placebo-controlled clinical trial [33]. The original study protocol was approved by the local ethics committee (BASEC-Nr. 2016-00101) and the regulatory affairs and is registered at clinicaltrials.gov protocol No. NCT0316307. The local ethics committee (BASEC-Nr. 2023-01237) approved the measurement of folate and FRAA in the serum from patients who have signed the agreement for further use of the samples. The inclusion criteria for test subjects were consistent with those of the Omega-3-pMDD study. Eligible subjects were female or male, enrolled in a participating center (inpatient or outpatient). Subjects were aged from 8 to <18 y at the time of study entry. A primary diagnosis of MDD, as defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) criteria (fourth edition), was required, with at least moderate severity, which was measured using the German version of the Children's Depression Rating Scale—Revised (CDRS-R) [34]. In this assessment, the interviewer collected ratings on 14 depression-related symptoms from both parents and children, integrating these reports into a single composite score. The interviewer also evaluated 3 additional nonverbal symptoms through behavioral observation of the child during the interview. These individual symptom scores were summed to produce a final score that indicates the depression severity. Only patients with a CDRS-R score of 40 or higher were included in the study. Data on sociodemographic characteristics, antidepressant treatment and comorbid Attention-Deficit/Hyperactivity Disorder (ADHD) were obtained by reviewing patients' clinical records. When information was missing, either parents or adolescents were asked to provide the missing details.

No clinically significant laboratory finding in hematology, chemistry, or urine analysis was permitted, based on the judgment of the treating doctor. In addition to the Omega-3-pMDD study consent, subjects included in this analysis had to provide consent for the further use of their biological material. The exclusion criteria included withdrawal of the informed consent for the use of biological material or insufficient quantity or quality of the biological material needed for the analyses. The relevant information on the patient cohort is summarized in Table 1.

Quantification of FRAA

The ELISA for FRAA detection was performed on high-binding 96-well plates (#CLS3361, Corning COSTAR) as described previously [35], with some modifications. The plate was printed with 50 ng of recombinant human FOLR1 protein

TABLE 1
Patients' characteristics.

Variable	*N* (%) or mean (SD)
Total subjects	206 (100%)
Age (y)	15.7 (8–18)
Gender	
Male	55 (26.7%)
Female	151 (73.3%)
ADHD comorbidity	
Yes	30 (14.6%)
No	176 (85.4%)
Treatment with antidepressants[1]	
Yes	66 (32.0%)
No	140 (68.0%)
Treatment	
Antidepressant monotherapy	50 (75.8%)
SSRI/SNRI	49 (74.2%)
Others	1 (1.5%)
Combination therapy	16 (24.2%)
SSRI + neuroleptic	12 (18.2%)
Antidepressant combination	4 (6.0%)
Response to treatment[2]	
Yes	11 (16.7%)
No	55 (83.3%)

Abbreviations: ADHD, Attention-Deficit/Hyperactivity Disorder; CDRS-R, Children's Depression Rating Scale—Revised; SNRI, serotonin norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor.
[1] At the time of the recruitment.
[2] Reduction of the CDRS-R Score of >30% within 3 mo from the beginning of the pharmacologic treatment.

(#FO1-H52H1, ACROBiosystems) or with a serial dilution of human IgG (#I4506, Sigma-Aldrich) in bicarbonate buffer (2.5% w/v glycerol in 25 mM $NaHCO_3$), with a 0.106–3.375 µg/mL concentration range. The plate was sealed and incubated overnight at 4°C. Subsequently, the plate was washed 3 times with a Tris-buffered saline solution (TBS-T) (100 mM Tris, 150 mM NaCl, 0.05% (w/v) Tween-20, pH adjusted to 7.6 with HCl). Fifty µL of the serum samples diluted 1:50 in TBS-T or TBS-T only were added to the FOLR1- and to the IgG-printed wells, respectively. Both standards and samples were printed in duplicate. After a 2-h incubation at room temperature, the plate was washed 3 times with TBS-T and then incubated for 1 h at room temperature with an anti-human IgG horseradish peroxidase-linked whole antibody diluted 1:5000 in TBS-T (#A18903, ThermoFisher Scientific). Then, the plate was washed 3 times with TBS-T, followed by 1 wash with TBS (100 mM Tris, 150 mM NaCl, pH adjusted to 7.6 with HCl). The plate was incubated for 5 minutes with the SuperSignal ELISA Femto Maximum Sensitivity Substrate (ThermoFisher Scientific) and then the samples were transferred to a collection white 96-well plate. The luminescent signal was acquired with the GloMax Multi Detection System (Promega) with 10 s integration time. The values obtained from the standard were best fitted using linear regression. Repeatability (intra-assay variability, CV%) and intermediate precision (inter-assay variability, CV%) were 20.6% and 21.2%, respectively. A sample derived from a pool of neurotypical subjects and one previously tested positive using a different FRAA test were included in every run and served as negative and positive controls, respectively.

Analysis of total folates

Serum folate concentrations were determined using the commercially available Elecsys Folate III kit (Roche Diagnostics), a competitive binding assay using a ruthenium-labeled folate binding protein followed by electrochemiluminescence detection on a Roche Cobas e801 module (Roche Diagnostics). A WHO international standard containing 5-methylTHF, 5-formyltetrahydrofolate (5-formylTHF), and folic acid was used as reference material (#03/178, NIBSC). The detection limit of this assay is 1.2 ng/mL and the limit of quantification is 2.0 ng/mL. Day-to-day imprecision, assessed as CV%, has been found to be 1.9% and 0.6% and total imprecision 3.9% and 2.8% at 4.5 and 12.3 µg/L, respectively.

Statistical analysis

All statistical analyses and the receiver operating characteristic (ROC) curve were performed using IBM SPSS Statistics Version 29.0. Graphic representations of correlation analyses were performed with GraphPad Prism (version 10.2.0, GraphPad Software). The Forest plot of odds ratios was prepared with Excel (Microsoft Office Professional Plus 2016). Details on the statistical analyses are provided in the figure legends.

Results

FRAA and folate serum concentration

For FRAA detection, samples were measured at the same time. FRAA were detected in 100% of the samples analyzed (206/206) with a minimum value of 3.17 µg/mL to a maximum value of 24.27 µg/mL (Table 2). Total folate could be quantified in 196 out of 206 samples, with a minimum value of 2.0 ng/mL to a maximum value of 40.0 ng/mL (Table 2). For 10 samples, the serum volume was insufficient for this analysis. Folate deficiency, that is, a serum concentration at which megaloblastic anemia is more likely to appear (≤3.0 ng/mL) [36], was measured in 9.2% of the samples (18/196). The Spearman's rank for non-normal data was computed to assess the correlation between folate and FRAA concentration. There was an inverse, albeit not significant, correlation ($r = -0.103$, $P = 0.076$) between folate and FRAA concentration (Figure 1A and B). Notably, the FRAA titer in the patients with folate deficiency (≤3.0 ng/mL) was significantly higher than that in the patients with normal folate concentration (9.34 ± 3.45 compared with 7.93 ± 3.52 µg/mL, $P = 0.0197$) (Figure 1C). In line with this, patients with an FRAA concentration lower than the median value (7.28 µg/mL) had a lower risk of manifesting folate deficiency (≤3 ng/mL) than those with a FRAA concentration higher

TABLE 2
Summary statistic of folate and FRAA concentration.

Parameter	Folate (ng/mL)	FRAA (µg/mL)
N	196	206
Mean	6.89	8.20
Min–max	2.0–40.0	3.17–24.27
Median	5.80	7.28
Q1–Q3	4.0–8.80	5.76–9.25

Abbreviation: FRAA, folate receptor alpha autoantibodies.

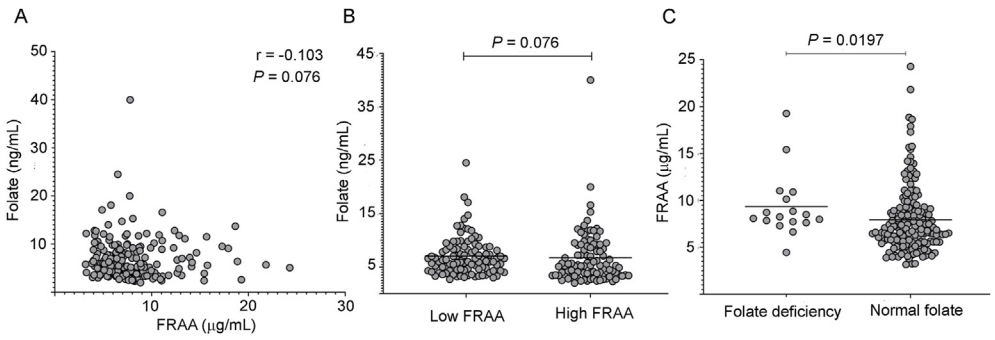

FIGURE 1. Association between FRAA and folate. Each data point represents one patient. (A) Spearman's correlation between serum total folates and FRAA titer measured in 196 subjects diagnosed with major depressive disorder (MDD). Shapiro-Wilk test for normality, $P < 0.0001$. (B) Total folate concentration in patients with high (≥ median) and low (< median) FRAA titer; median = 7.28 μg/mL. Mean values were compared by the Wilcoxon Rank Sum Test. Shapiro-Wilk test for normality, $P < 0.0001$. (C) Serum FRAA titer in patients with folate deficiency (≤3.0 ng/mL) and normal folates (>3.0 ng/mL). Mean values were compared by the Wilcoxon Rank Sum Test. Shapiro-Wilk test for normality, $P < 0.0001$. FRAA, folate receptor alpha autoantibodies.

than the median value (2/102, RR = 0.12, 95% CI: 0.028, 0.498, $P = 0.0037$). It is important to note that the very large RR observed could be influenced by the low number of events in the low FRAA group (the unexposed group), highlighting the need for cautious interpretation of the results to avoid overestimating the association. Folate and FRAA concentration were not associated with the gender. The folate serum concentration was 7.48 ± 6.03 ng/mL in male and 6.67 ± 3.52 ng/mL in female ($P = 0.945$), whereas the FRAA titer was lower in male than in female (7.29 ± 3.06 compared with 8.53 ± 3.84 μg/mL, $P = 0.066$).

FRAA, disease severity, and response to the treatment

The Spearman's rank for nonnormal data showed that neither the folate concentration (Figure 2A) nor FRAA titer (Figure 2B) correlated with the disease severity (CDRS-R score). At the time of the recruitment, 66 of 206 patients were under SSRI treatment. The rate of response to the treatment, defined by a reduction of the CDRS-R score of >30% within 3 mo from the beginning of the pharmacologic treatment, was 16.7% (11/66) (Table 1). Binary regression analysis showed that the effect size of the folate concentration was larger than that of the FRAA titer on the response to the treatment. For every unit increase in the folate concentration, the likelihood of response to the treatment increased by 11% ($P = 0.079$) (Figure 3). The ROC curve for folates, displaying the overall ability of predicting the response to the SSRI/SNRI treatment, defined an area under the curve of 0.62 (Figure 4). The rate of response to the treatment in the high-folate group (>Q3, 8.80 ng/mL) was ~4 times that in the low-folate group (≤Q1, 4.00 ng/mL) (28.5% compared with 6.7%) (Figure 4B).

Discussion

The presence of autoantibodies against the FRAA blocking folate binding to the receptor and/or the transcytotic process thereof has been associated with CFD [18] as well as with neural-tube defects [37,38]. In our study, the FRAA titer in the folate deficient group (plasma folate concentrations at which megaloblastic anemia is more likely to appear, that is ≤3 ng/mL (≤6.8 nmol/L) [36]), was significantly higher than that in the normal folate group, and the relative risk of folate deficiency was elevated in patients with a high titer of FRAA. These results suggest that FRAA might also play a role in folate systemic homeostasis. Normal concentrations of folate in the blood are maintained by proper dietary intake and intestinal absorption, and by efficient renal reabsorption. FRα is not expressed in the intestine, whereas it is highly expressed at the brush-border membrane of the proximal tubular cells, where it plays a major role in the retrieval of folate, which is extensively filtered by the glomerulus [9,39]. Consistently, both total body and kidney-specific Folr1 K.O. mice display lower cerebral and plasma folates [40,41]. A single bolus dose of 5-formylTHF (0.25 mg/kg) could rescue the systemic but not the cerebral concentration of folate in the total Folr1 K.O. mice [41]. Moreover, plasma folate concentrations in Folr1 K.O. mice on a normal diet were comparable with the concentrations in wild-type mice on the low-folate diet [40]. Taken together, these data suggest that FRα-mediated renal reabsorption is critical to sustain a normal systemic concentration of folate when there is an inadequate dietary intake and/or intestinal absorption. Under normal conditions, antibodies are generally retained by the glomerulus, making the interaction between FRα and FRAA at the luminal side unlikely. However, when free folates bind to FRα, several cellular processes occur, including transcytosis to the basal membrane, where FRAA could then either interact with the folate-FRα complex, obstructing the release of folates, or attach to the empty receptor that is returning to the luminal side, affecting the receptor's function in the process. The impact of a high FRAA titer on the systemic folate concentration is likely trivial in the presence of an adequate folate intake and intestinal absorption [13,14]. Conversely, a high FRAA titer might play a critical role in lowering the folate concentration in those individuals with inadequate folate intake.

Clinical studies indicated a link between folate deficiency and depression [24–26,42–44]. In most of these studies, a positive correlation between serum and/or cerebral folate concentrations and monoamine metabolism was also found [42–44]. From a biochemical standpoint, folate is important for the synthesis of tetrahydrobiopterin (BH4), a cofactor essential for the synthesis of serotonin and dopamine [3]. The prevalence of folate

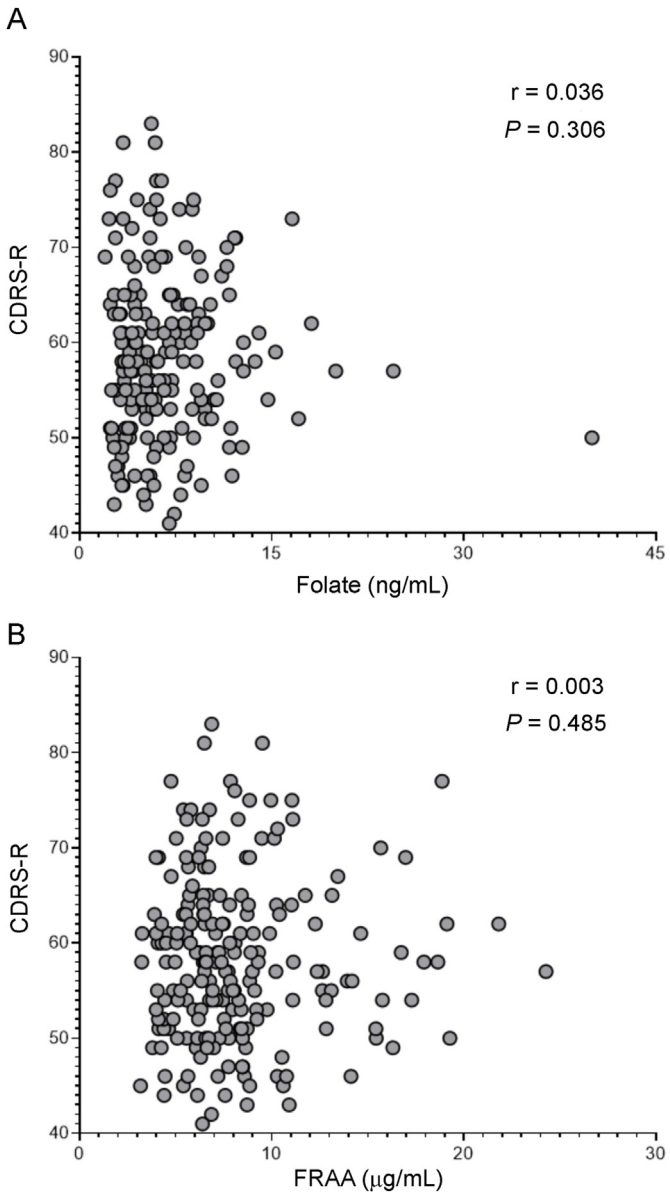

FIGURE 2. Relationship between FRAA or folate and disease severity. (A, B) Spearman's correlation between serum total folates (A, $n = 196$) or FRAA titer (B, $n = 206$) and the Children's Depression Rating Scale—Revised score (CDRS-R) in subjects diagnosed with pediatric major depressive disorder (pMDD). Shapiro-Wilk test for normality, $P < 0.0001$. Each data point represents one patient. FRAA, folate receptor alpha autoantibodies.

deficiency (≤3 ng/mL) in this study was 9.2% (18 out of 196), which is not far from the 9.5% prevalence of folate deficiency observed in the overall children population aged 11–18 in the United Kingdom between 2015 and 2019, before the implementation of mandatory folic acid fortification of flour [45] and lower than that recorded in the overall children population of similar age in developing countries such as India, where the prevalence of folate deficiency (≤3 ng/mL) is 31%–35% [46,47]. Although our analysis is limited by the lack of an age-matched control group, we lean toward the conclusion that systemic folate deficiency is not associated with a higher risk of MDD. Folate deficiency has also been associated with more severe depressive symptoms [24]. However, our study shows no

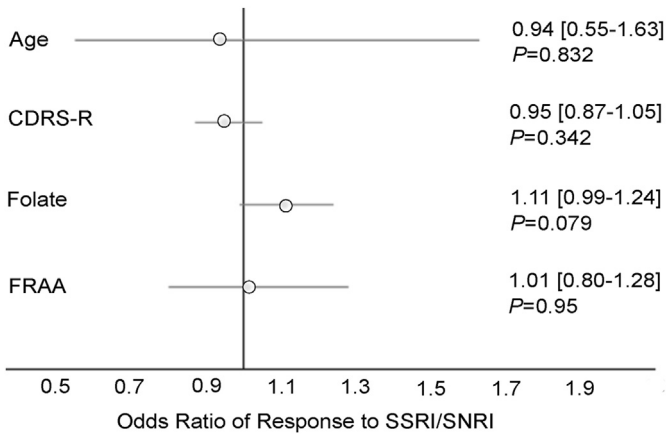

FIGURE 3. Relationship among FRAA, folate and the response to SSRI/SNRI treatment. (A) Logistic regression of the odds ratios and 95% CI of the indicated variables on the response to SSRI treatment defined as a reduction of the CDRS-R score of >30% within 3 mo from the beginning of the pharmacologic treatment. CI, confidence interval; CDRS-R, Children's Depression Rating Scale—Revised score; FRAA, folate receptor alpha autoantibodies; SNRI, serotonin norepinephrine reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor.

association between folate concentration or FRAA titer and the severity of the depressive symptoms measured per the CDRS-R score. One limitation of this study is the lack of measurement of the blocking FRAA, the fraction of autoantibodies that interfere with folate binding to the FRα. A measurement of the blocking FRAA titer will provide a more comprehensive understanding of the role of FRAA in MDD. Moreover, a longitudinal study in which the duration of the folate deficiency condition is monitored could offer valuable insights into the relationship between folate and MDD development and severity.

A meta-analysis of the clinical trials evaluating the impact of folate on the response to SSRI/SNRI concluded that folate as adjunct therapy to SSRI/SNRI improved depression scale scores, patient response, and remission rates [31]. In patients with depression who were randomly assigned to fluoxetine or fluoxetine plus folic acid (500 μg/d), the Hamilton Depression Rating Scale (HAM-D) was lower in the folic acid group than in the placebo group (8.8 compared with 11.2) [48]. Similarly, the HAM-D score was significantly lower in patients receiving fluoxetine and folic acid (10 mg/d) than those receiving fluoxetine alone (7.43 compared with 11.43) [49]. In another clinical trial, adjunctive L-methylfolate at 15 mg/d produced a response rate (HAM-D reduction of ≥50%) greater than that measured in the SSRI-only group (32.3% compared with 14.6%) [50]. In this study, we found that the rate of response to the treatment was positively associated with the systemic folate concentration. Although the overall small number of patients taking SSRI/SNRI included in this study ($n = 66$) represents a limitation, it is worth mentioning that not 1 patient with folate deficiency (≤3 ng/mL) responded to the therapy (0/6); conversely, the response rate was of 44% (4/9) in patients with a folate concentration above 10 ng/mL (22.6 nmol/L). Within the brain, FRα is highly expressed in the choroid plexus, where it binds with high affinity (Kb 1–10 nmol/L) to folate. FRα-mediated transcytosis accounts for the concentrative movement of folate from the blood into the CSF, where the concentration is up to 4-fold higher than in the plasma [8]. Once internalized, the folate-FRα complex is incorporated into

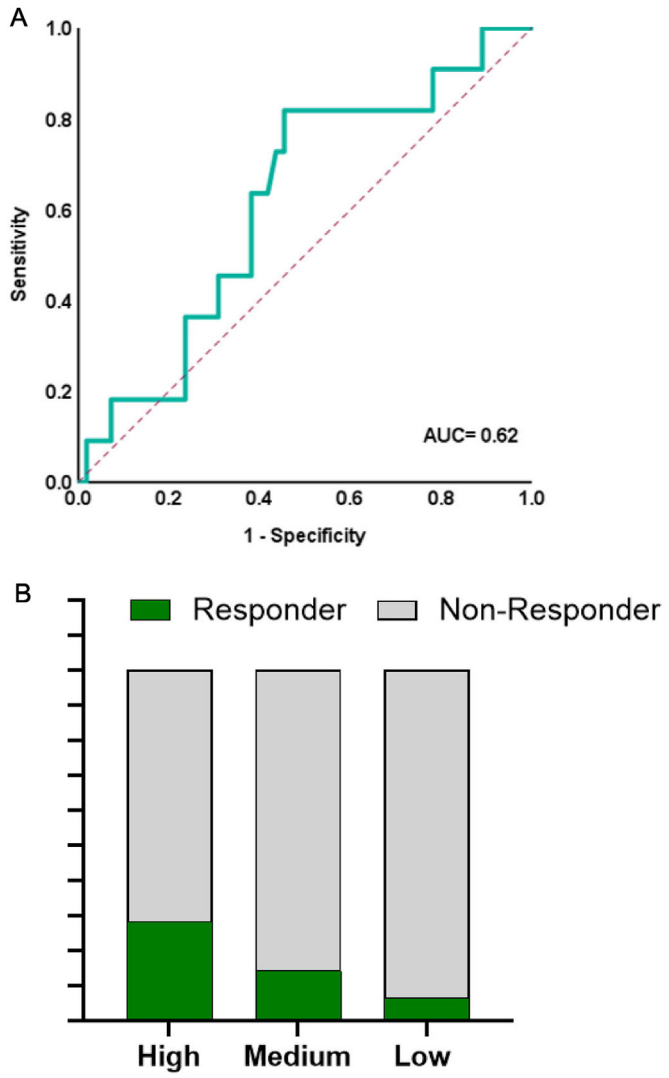

FIGURE 4. Sensitivity and specificity of serum total folate as a predictive biomarker. (A) ROC curve for serum total folate with area under the curve indicated. (B) Response rate across quartile-based categories of folate concentration. The high group includes patients with a folate concentration >8.80 ng/mL (>*Q3*). The medium group includes patients with a folate concentration between 4.0 and 8.80 ng/mL (*Q1*–*Q3*). The low group includes patients with a folate concentration <4.0 ng/mL (<*Q1*). ROC, receiver operating characteristic.

exosomes that are released into the CSF, cross the ependymal cell layer, and distribute in the brain parenchyma [8]. Folate supplementation effectively raises plasma folate concentrations to concentrations exceeding 100 ng/mL (340 nmol/L) [51–53] and normalizes the CSF concentration in children with FRAA-associated CFD [18] and in those without a functioning FRα [13]. Thus, incorporating folate during the SSRI/SNRI therapy may benefit patients with borderline folate deficiency by readily correcting the possible suboptimal folate CSF concentration.

Acknowledgments

We thank Robert Cabrera (Baylor College of Medicine) and Magdalena Rausch (University of Zurich) for helping in the optimization of the FRAA test.

Author contributions

The authors' responsibilities were as follows – MV, GB: conception and design; PG, IH, KS: methodology and data acquisition; PG, IH, GB, MV: data interpretation; PG, MV: writing of the initial draft; IH, KS, GAK-U, GB: revision and editing; and all authors: read and approved the final manuscript.

Conflict of interest

MV reports a relationship with Aprofol AG that includes: consulting or advisory and equity or stocks. All other authors report no conflicts of interest.

Funding

This work was supported by the University Hospital of Zurich. The statements, opinions, and data presented in this work reflect solely the authors' view.

Data Availability

Data in the manuscript, codebook, and analytic code will be made available upon request.

References

[1] M. Scotti, L. Stella, E.J. Shearer, P.J. Stover, Modeling cellular compartmentation in one-carbon metabolism, Wiley Interdiscip. Rev. Syst. Biol. Med. 5 (3) (2013) 343–365, https://doi.org/10.1002/wsbm.1209.
[2] M.S. Field, E. Kamynina, J. Chon, P.J. Stover, Nuclear folate metabolism, Annu. Rev. Nutr. 38 (2018) 219–243, https://doi.org/10.1146/annurev-nutr-071714-034441.
[3] A. Coppen, C. Swade, S.A. Jones, R.A. Armstrong, J.A. Blair, R.J. Leeming, Depression and tetrahydrobiopterin: the folate connection, J. Affect. Disord. 16 (2–3) (1989) 103–107, https://doi.org/10.1016/0165-0327(89)90062-1.
[4] C.G. Hamon, J.A. Blair, P.A. Barford, The effect of tetrahydrofolate on tetrahydrobiopterin metabolism, J. Ment. Defic. Res. 30 (Pt 2) (1986) 179–183, https://doi.org/10.1111/j.1365-2788.1986.tb01310.x.
[5] K. Chalupsky, D. Kracun, I. Kanchev, K. Bertram, A. Gorlach, Folic acid promotes recycling of tetrahydrobiopterin and protects against hypoxia-induced pulmonary hypertension by recoupling endothelial nitric oxide synthase, Antioxid. Redox Signal. 23 (14) (2015) 1076–1091, https://doi.org/10.1089/ars.2015.6329.
[6] M. Visentin, N. Diop-Bove, R. Zhao, I.D. Goldman, The intestinal absorption of folates, Annu. Rev. Physiol. 76 (2014) 251–274, https://doi.org/10.1146/annurev-physiol-020911-153251.
[7] A. Qiu, M. Jansen, A. Sakaris, S.H. Min, S. Chattopadhyay, E. Tsai, et al., Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption, Cell 127 (5) (2006) 917–928, https://doi.org/10.1016/j.cell.2006.09.041.
[8] M. Grapp, A. Wrede, M. Schweizer, S. Huwel, H.J. Galla, N. Snaidero, et al., Choroid plexus transcytosis and exosome shuttling deliver folate into brain parenchyma, Nat. Commun. 4 (2013) 2123, https://doi.org/10.1038/ncomms3123.
[9] R. Zhao, N. Diop-Bove, M. Visentin, I.D. Goldman, Mechanisms of membrane transport of folates into cells and across epithelia, Annu. Rev. Nutr. 31 (2011) 177–201, https://doi.org/10.1146/annurev-nutr-072610-145133.
[10] J.B. Wallingford, L.A. Niswander, G.M. Shaw, R.H. Finnell, The continuing challenge of understanding, preventing, and treating neural tube defects, Science 339 (6123) (2013) 1222002, https://doi.org/10.1126/science.1222002.
[11] I.D. Goldman, Hereditary Folate Malabsorption, in: GeneReviews, 2008.
[12] I.D. Goldman, FOLR1-Related Cerebral Folate Transport Deficiency, in: GeneReviews, 2024.
[13] R. Steinfeld, M. Grapp, R. Kraetzner, S. Dreha-Kulaczewski, G. Helms, P. Dechent, et al., Folate receptor alpha defect causes cerebral folate transport deficiency: a treatable neurodegenerative disorder associated with disturbed myelin metabolism, Am. J. Hum. Genet. 85 (3) (2009) 354–363, https://doi.org/10.1016/j.ajhg.2009.08.005.

[14] M.T. Papadopoulou, E. Dalpa, M. Portokalas, I. Katsanika, K. Tirothoulaki, M. Spilioti, et al., Cerebral folate deficiency in two siblings caused by biallelic variants including a novel mutation of FOLR1 gene: intrafamilial heterogeneity following early treatment and the role of ketogenic diet, JIMD Rep 60 (1) (2021) 3–9, https://doi.org/10.1002/jmd2.12206.
[15] A. Potic, S. Perrier, T. Radovic, S. Gavrilovic, J. Ostojic, L.T. Tran, et al., Hypomyelination caused by a novel homozygous pathogenic variant in FOLR1: complete clinical and radiological recovery with oral folinic acid therapy and review of the literature, Orphanet. J. Rare. Dis. 18 (1) (2023) 187, https://doi.org/10.1186/s13023-023-02802-6.
[16] Y. Kobayashi, J. Tohyama, T. Akiyama, S. Magara, H. Kawashima, N. Akasaka, et al., Severe leukoencephalopathy with cortical involvement and peripheral neuropathy due to FOLR1 deficiency, Brain Dev 39 (3) (2017) 266–270, https://doi.org/10.1016/j.braindev.2016.09.011.
[17] S. Brunetti, L. Malerba, L. Giordano, E. Parrini, R. Guerrini, G. Palumbo, et al., Cerebral folate transporter deficiency syndrome in three siblings: why genetic testing for developmental and epileptic encephalopathies should be performed early and include the FOLR1 gene, Am. J. Med. Genet. A 185 (8) (2021) 2526–2531, https://doi.org/10.1002/ajmg.a.62345.
[18] V.T. Ramaekers, S.P. Rothenberg, J.M. Sequeira, T. Opladen, N. Blau, E.V. Quadros, et al., Autoantibodies to folate receptors in the cerebral folate deficiency syndrome, N. Engl. J. Med. 352 (19) (2005) 1985–1991, https://doi.org/10.1056/NEJMoa043160.
[19] V.T. Ramaekers, E.V. Quadros, Cerebral folate deficiency syndrome: early diagnosis, intervention and treatment strategies, Nutrients 14 (15) (2022) 3096, https://doi.org/10.3390/nu14153096.
[20] J.M. Sequeira, V.T. Ramaekers, E.V. Quadros, The diagnostic utility of folate receptor autoantibodies in blood, Clin. Chem. Lab. Med. 51 (3) (2013) 545–554, https://doi.org/10.1515/cclm-2012-0577.
[21] V.T. Ramaekers, N. Blau, J.M. Sequeira, M.C. Nassogne, E.V. Quadros, Folate receptor autoimmunity and cerebral folate deficiency in low-functioning autism with neurological deficits, Neuropediatrics 38 (6) (2007) 276–281, https://doi.org/10.1055/s-2008-1065354.
[22] J. Zhou, A. Liu, F. He, Y. Jin, S. Zhou, R. Xu, et al., High prevalence of serum folate receptor autoantibodies in children with autism spectrum disorders, Biomarkers 23 (7) (2018) 622–624, https://doi.org/10.1080/1354750X.2018.1458152.
[23] V.T. Ramaekers, J.M. Sequeira, M. DiDuca, G. Vrancken, A. Thomas, C. Philippe, et al., Improving outcome in infantile autism with folate receptor autoimmunity and nutritional derangements: a self-controlled trial, Autism, Res. Treat. 2019 (2019) 7486431, https://doi.org/10.1155/2019/7486431.
[24] A. Bender, K.E. Hagan, N. Kingston, The association of folate and depression: a meta-analysis, J. Psychiatr. Res. 95 (2017) 9–18, https://doi.org/10.1016/j.jpsychires.2017.07.019.
[25] M. Khosravi, G. Sotoudeh, M. Amini, F. Raisi, A. Mansoori, M. Hosseinzadeh, The relationship between dietary patterns and depression mediated by serum levels of Folate and vitamin B12, BMC Psychiatry 20 (1) (2020) 63, https://doi.org/10.1186/s12888-020-2455-2.
[26] E.H. Reynolds, J.M. Preece, J. Bailey, A. Coppen, Folate deficiency in depressive illness, Br. J. Psychiatry 117 (538) (1970) 287–292.
[27] J.M. Kim, H.Y. Kim, H.J. Lee, J.W. Kim, H.J. Kang, S.W. Kim, et al., Prediction of suicidality according to serum folate levels in depressive patients receiving stepwise pharmacotherapy, Front. Psychiatry. 12 (2021) 747228, https://doi.org/10.3389/fpsyt.2021.747228.
[28] J.L. Price, W.C. Drevets, Neurocircuitry of mood disorders, Neuropsychopharmacology 35 (1) (2010) 192–216, https://doi.org/10.1038/npp.2009.104.
[29] J.B. Weilburg, An overview of SSRI and SNRI therapies for depression, Manag. Care. 13 (6 Suppl Depression) (2004) 25–33.
[30] M.H. Trivedi, A.J. Rush, S.R. Wisniewski, A.A. Nierenberg, D. Warden, L. Ritz, et al., Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice, Am. J. Psychiatr. 163 (1) (2006) 28–40, https://doi.org/10.1176/appi.ajp.163.1.28.
[31] R. Altaf, I. Gonzalez, K. Rubino, E.C. Nemec 2nd, Folate as adjunct therapy to SSRI/SNRI for major depressive disorder: systematic review & meta-analysis, Complement. Ther. Med. 61 (2021) 102770, https://doi.org/10.1016/j.ctim.2021.102770.
[32] L.A. Pan, P. Martin, T. Zimmer, A.M. Segreti, S. Kassiff, B.W. McKain, et al., Neurometabolic disorders: potentially treatable abnormalities in patients with treatment-refractory depression and suicidal behavior, Am. J. Psychiat. 174 (1) (2017) 42–50, https://doi.org/10.1176/appi.ajp.2016.15111500.
[33] I. Haberling, G. Berger, K. Schmeck, U. Held, S. Walitza, Omega-3 fatty acids as a treatment for pediatric depression. a phase III, 36 weeks, multi-center, double-blind, placebo-controlled randomized superiority study, Front. Psychiatry. 10 (2019) 863, https://doi.org/10.3389/fpsyt.2019.00863.
[34] F. Keller, J. Grieb, M. Ernst, N. Sprober, J.M. Fegert, M. Kolch, Children's Depression Rating Scale-Revised (CDRS-R): development of a German version and psychometric properties in a clinical sample, Z. Kinder. Jugendpsychiatr. Psychother. 39 (3) (2011) 179–185, https://doi.org/10.1024/1422-4917/a000090.
[35] N. Yang, L. Wang, R.H. Finnell, Z. Li, L. Jin, L. Zhang, et al., Levels of folate receptor autoantibodies in maternal and cord blood and risk of neural tube defects in a Chinese population, Birth Defects Res. A Clin. Mol. Teratol. 106 (8) (2016) 685–695, https://doi.org/10.1002/bdra.23517.
[36] Guideline WHO, Optimal Serum and Red Blood Cell Folate Concentrations in Women of Reproductive Age for Prevention of Neural Tube Defects, World Health Organization, 2015. https://www.ncbi.nlm.nih.gov/books/NBK294192/.
[37] S.P. Rothenberg, M.P. da Costa, J.M. Sequeira, J. Cracco, J.L. Roberts, J. Weedon, et al., Autoantibodies against folate receptors in women with a pregnancy complicated by a neural-tube defect, N. Engl. J. Med. 350 (2) (2004) 134–142, https://doi.org/10.1056/NEJMoa031145.
[38] R.M. Cabrera, G.M. Shaw, J.L. Ballard, S.L. Carmichael, W. Yang, E.J. Lammer, et al., Autoantibodies to folate receptor during pregnancy and neural tube defect risk, J. Reprod. Immunol. 79 (1) (2008) 85–92, https://doi.org/10.1016/j.jri.2008.08.002.
[39] S.L. Samodelov, Z. Gai, G.A. Kullak-Ublick, M. Visentin, Renal reabsorption of folates: pharmacological and toxicological snapshots, Nutrients 11 (10) (2019) 2353, https://doi.org/10.3390/nu11102353.
[40] H. Birn, O. Spiegelstein, E.I. Christensen, R.H. Finnell, Renal tubular reabsorption of folate mediated by folate binding protein 1, J. Am. Soc. Nephrol. 16 (3) (2005) 608–615, https://doi.org/10.1681/ASN.2004080711.
[41] C. Alam, S. Aufreiter, C.J. Georgiou, M.T. Hoque, R.H. Finnell, D.L. O'Connor, et al., Upregulation of reduced folate carrier by vitamin D enhances brain folate uptake in mice lacking folate receptor alpha, Proc. Natl. Acad. Sci. USA. 116 (35) (2019) 17531–17540, https://doi.org/10.1073/pnas.1907077116.
[42] M.I. Botez, S.N. Young, Effects of anticonvulsant treatment and low levels of folate and thiamine on amine metabolites in cerebrospinal fluid, Brain 114 (Pt 1A) (1991) 333–348.
[43] T. Bottiglieri, K. Hyland, M. Laundy, P. Godfrey, M.W. Carney, B.K. Toone, et al., Folate deficiency, biopterin and monoamine metabolism in depression, Psychol. Med. 22 (4) (1992) 871–876, https://doi.org/10.1017/s0033291700038447.
[44] R. Surtees, S. Heales, A. Bowron, Association of cerebrospinal fluid deficiency of 5-methyltetrahydrofolate, but not S-adenosylmethionine, with reduced concentrations of the acid metabolites of 5-hydroxytryptamine and dopamine, Clin. Sci. (Lond). 86 (6) (1994) 697–702, https://doi.org/10.1042/cs0860697.
[45] K.S. Jones, D. Collins, S.R. Meadows, A. Koulman, P. Page, National Diet and Nutrition Survey data reveal a decline in folate status in the United Kingdom population between 2008 and 2019, Am. J. Clin. Nutr. 118 (6) (2023) 1182–1191, https://doi.org/10.1016/j.ajcnut.2023.10.006.
[46] T. Shalini, R. Pullakhandam, S. Ghosh, B. Kulkarni, H. Rajkumar, H.S. Sachdev, et al., Prevalence of vitamin B(12) and folate deficiencies in Indian children and adolescents, Nutrients 15 (13) (2023) 3026, https://doi.org/10.3390/nu15133026.
[47] U. Kapil, N. Sareen, Prevalence of ferritin, folate and vitamin B12 deficiencies amongst children in 5-18 years of age in Delhi, Indian J. Pediatr. 81 (3) (2014) 312, https://doi.org/10.1007/s12098-013-1091-y.
[48] A. Coppen, J. Bailey, Enhancement of the antidepressant action of fluoxetine by folic acid: a randomised, placebo controlled trial, J. Affect. Disord. 60 (2) (2000) 121–130, https://doi.org/10.1016/s0165-0327(00)00153-1.
[49] G. Resler, R. Lavie, J. Campos, S. Mata, M. Urbina, A. Garcia, et al., Effect of folic acid combined with fluoxetine in patients with major depression on plasma homocysteine and vitamin B12, and serotonin levels in lymphocytes, Neuroimmunomodulation 15 (3) (2008) 145–152, https://doi.org/10.1159/000151527.
[50] G.I. Papakostas, R.C. Shelton, J.M. Zajecka, B. Etemad, K. Rickels, A. Clain, et al., L-methylfolate as adjunctive therapy for SSRI-resistant major depression: results of two randomized, double-blind, parallel-sequential trials, Am. J. Psychiatry. 169 (12) (2012) 1267–1274, https://doi.org/10.1176/appi.ajp.2012.11071114.

[51] J.E. Alpert, D. Mischoulon, G.E. Rubenstein, K. Bottonari, A.A. Nierenberg, M. Fava, Folinic acid (Leucovorin) as an adjunctive treatment for SSRI-refractory depression, Ann. Clin. Psychiatry. 14 (1) (2002) 33–38, https://doi.org/10.1023/a:1015271927517.

[52] P.O. Greiner, J. Zittoun, J. Marquet, J.M. Cheron, Pharmacokinetics of (-)-folinic acid after oral and intravenous administration of the racemate, Br. J. Clin. Pharmacol. 28 (3) (1989) 289–295, https://doi.org/10.1111/j.1365-2125.1989.tb05429.x.

[53] G.A. Kullak-Ublick, C. Gubler, K. Spanaus, M.G. Ismair, T. Claro da Silva, A. Jetter, No major effects of vitamin D3 (1,25 dihydroxyvitamin D3) on absorption and pharmacokinetics of folic acid and fexofenadine in healthy volunteers, Eur. J. Clin. Pharmacol. 72 (7) (2016) 797–805, https://doi.org/10.1007/s00228-016-2050-0.