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Folate metabolism biomarkers from two randomised placebo-controlled clinical studies with paroxetine and venlafaxine

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Abstract

Objectives: Based on the hypothesis of a role for folate and vitamin B12 in major depressive disorders (MDD), we aimed at validating the association between folate pathway biomarkers and depression or antidepressant response in clinical trial populations.

Methods: We investigated serum levels erythrocyte folate and serum levels of homocysteine, vitamin B12, and folate as disease and response biomarkers for MDD in two independent randomized, placebo-controlled clinical trials, where paroxetine or venlafaxine were used as active controls, for a total of 881 patients.

Results: Significant but weak correlations between depression severity and biomarker levels could be detected in the paroxetine study for serum folate and vitamin B12, with no correlations for any biomarker in the venlafaxine study. Besides a weak association for erythrocyte folate in the venlafaxine study, no significant associations were observed between treatment response and pre-treatment levels of any of the biomarkers tested.

Conclusions: Notwithstanding the relatively large number of patients tested, we did not find consistent associations between folate biomarkers and MDD severity, or response to paroxetine and venlafaxine. Our results may be related to the particular study design or clinical population; however, our findings do not support the hypothesis of a dysfunction of one-carbon metabolism in MDD.

Keywords: antidepressant**;** folate; depression; biomarkers.

1. Introduction

The research on biomarkers for major depressive disorder (MDD) that could aid diagnosis or predict treatment response is especially focussed on blood-based biomarkers due to the ease of collection and reduced costs (Domenici and Muglia, 2007). Among the proposed mechanisms for the neurobiological basis of MDD (Otte et al., 2016), the metabolic hypothesis is based on the evidence of association between folate deficiency and disease development (Bender et al., 2017). Folate and vitamin B12 are involved in one-carbon metabolism necessary for the production of monoamine transmitters and for methylation involved in epigenetic regulations (Bottiglieri, 2009; Wan et al., 2018). High circulating homocysteine concentrations are considered indicative of folate or vitamin B12 deficiency since inadequate levels of these vitamins affect the production of S-adenosylmethionine, which is an activator of the homocysteine to cysteine conversion reaction (Selhub et al., 2008). Folate deficiency and low folate status have been linked to persistent depressive symptoms and poor antidepressant response, with several case-control studies since the 1960s showing high prevalence of folate and vitamin B12 deficiency in depression (Almeida et al., 2015; Bender et al., 2017; Petridou et al., 2016). Folate treatment has also been proposed as a therapeutic option or as a supplement to antidepressant treatment, however the results have not been encouraging (Almeida et al., 2015; Roberts et al., 2018; Schefft et al., 2017).

Wessen et al. were the first to examine the effect of standard antidepressant treatment on folate status, showing, in an open trial, that responders to desmethylimipramine had higher serum folate than non-responders (Wessen et al., 1994). By cross-sectional analysis, the same authors detected an inverse correlation with severity of depression (Wessen et al., 1994). Bottiglieri et al. showed that depressed patients with high plasma homocysteine concentration have significantly lower erythrocyte and serum folate values and monoamine metabolites, suggesting impairment in the metabolism of serotonin, dopamine and noradrenaline (Bottiglieri et al., 2000). Papakostas et al. demonstrated that lower folate levels were associated with poor response to antidepressant treatment in fluoxetine resistant MDD patients (Papakostas et al., 2004), and that MDD patients

with hypofolatemia treated with fluoxetine were more likely to experience a later onset of clinical improvement than eufolatemic patients (Papakostas et al., 2005). In a naturalistic investigation, higher levels of vitamin B12 were found to be associated to better outcome in MDD outpatients (Hintikka et al., 2003). More recently, baseline one-carbon metabolism biomarkers were tested as putative predictors for response in treatment-resistant MDD patients, with some evidence of lower levels of serum folate and vitamin B12 in remitters compared to non-remitters after electroconvulsive therapy (Maier et al., 2018), and no evidence of association in patients treated with ketamine infusion (Lundin et al., 2014).

The data available on the relationship between MDD and other components of one-carbon metabolism such as vitamin B12, B6, homocysteine, and the methylenetetrahydrofolate reductase 677CT polymorphism suggested further exploration of these metabolites as potential markers for depressive states (Almeida et al., 2015; Bender et al., 2017; Bjelland et al., 2003; De Berardis et al., 2019). Early evidence of correlation of folate metabolism biomarkers with depression severity or antidepressant response were obtained either in naturalistic follow-up studies, where clinical and treatment variables were not controlled a priori (Hintikka et al., 2003), in open trials with no placebo group (Wessen et al., 2003; Papakostas et al., 2005), or in limited number of patients (Bottiglieri et al, 2000; Papakostas et al., 2004), warranting further investigations in studies with controlled illness and treatment variables to confirm or refute the findings (Hintikka et al., 2003). We therefore aimed to investigate the association between the folate pathway and depression or antidepressant response in controlled clinical trials, and with increased number of patients with respect to prior studies. To address this objective, we measured serum levels of homocysteine, vitamin B12, and folate, as well as erythrocyte folate, as biomarkers for disease and response in MDD using data from two independent randomized, placebo- and active-controlled studies. The purpose of the study was: i) to confirm the presence of a correlation between different biomarkers of the folate metabolism; ii) to verify whether folate biomarker levels are associated to depression

severity; iii) to verify whether any of the biomarker tested could predict response to antidepressant.

2. Materials and Methods

Clinical studies

The data were collected in two clinical trials in which the serotonin, dopamine and norepinephrine (triple) reuptake inhibitor GSK372475 was compared with the selective serotonin reuptake inhibitor paroxetine (SND103288, "Paroxetine study", clinicaltrial.gov identifier NCT00448058) or the selective serotonin and norepinephrine reuptake inhibitor venlafaxine (SND103285, "Venlafaxine study", clinicaltrial.gov identifier NCT0042064) and placebo. Both studies were 10-week randomized, multi-centre, double-blind, parallel-group, placebo- and active-controlled, flexible-dose studies in male and female outpatients (18-64 years of age) with a psychiatric diagnosis of a major depressive episode (with current episode duration of at least two weeks but less than two years), associated with major depressive disorder according to DSM-IV-TR. Patients were excluded if the illness symptoms were better accounted for by another diagnosis, or if the patient had a current diagnosis of any of the following: panic disorder, antisocial or border line personality disorder, bipolar disorder, schizophrenia, or other psychotic disorders. Previous failure to an adequate therapeutic course of two or more antidepressants and risk of suicide were considered among additional exclusion criteria. The paroxetine study population consisted of 493 male and female subjects (mean age 42.9 ± 11.19, 70% females); 166 of them were randomized to paroxetine, 171 to GSK372475, and 156 to matching placebo capsules. The venlafaxine study population consisted of 393 male and female subjects (mean age 42.6 ± 11.67, 62% females); 133 of them were randomized to venlafaxine, 134 to GSK372475, and 126 to matching placebo capsules. The adjusted mean change in HAM-D-17 from baseline at week 10 was –2.96 (90% C.I. –4.28/–1.64, $p< 0.001$) and –2.35 (90% C.I. –3.84/ –0.86, $p=0.010$) for paroxetine and venlafaxine,

respectively (Learned et al., 2012). Findings on inflammatory biomarkers of the active controls arms of the two studies have also been previously reported (Carboni et al., 2019). Both multi-centre study protocols were reviewed and approved by national, regional, or investigational centre Ethics Committees or Institutional Review Boards, and were conducted in accordance with the International Conference on Harmonisation (ICH) Good Clinical Practice guidelines, applicable country-specific requirements, and ethical principles outlined in the World Medical Association Declaration of Helsinki on the Ethical Principles for Medical Research Involving Human Subjects. Written informed consent was obtained from all subjects before their involvement in any study related procedure. Since no efficacy was seen with GSK372475, the biomarker analysis was restricted to the paroxetine, venlafaxine and placebo groups.

Biomarker measurements

Circulating biomarkers were analysed in blood samples collected at randomization (week 0) and week 10. Approximately 9 ml of blood were obtained from a forearm vein. To assess serum biomarkers, coagulation was allowed for 30 min, serum samples were separated by 10 min centrifugation at 1000g at room temperature, split into aliquots and stored at -20°C pending shipment for analysis. For erythrocyte folate measure, blood was collected in EDTA-containing tubes, completely inverted 10 times and stored at -20°C pending shipment for analysis. The analysis was performed by Quest Diagnostics. Biomarkers were analysed by ADVIA Centaur competitive immunoassay using direct chemiluminescent technology. Samples were pre-treated to release folate or vitamin B12 from endogenous binding proteins. The assay principle is based on competition of the analyte within the sample with acridinium ester-labelled analyte in the Lite Reagent for a limited amount of biotin-labelled analyte binding protein. Biotin-labelled analyte binding protein binds to avidin covalently coupled to paramagnetic particles in the Solid Phase. The mixture of specimen samples, DTT/Releasing Agent, Lite and Solid Phase reagents were incubated. The paramagnetic particles we held on to the wells with a pair of magnets during washing steps. Acid and base reagents were dispensed to initiate the chemiluminescent reaction. The

concentration of analyte in samples was determined by interpolating with a standard curve generated by a set of calibrators of known concentration.

Statistical analysis

The distribution of each biomarker was explored. In order to achieve normality on the analysis scale, plasma folate, homocysteine and vitamin B12 were log-transformed prior to inclusion in statistical models; erythrocyte folate and HAM-D total score (used as endpoint for depression severity) were not transformed. Multivariate mixed model analyses were used to measure the correlation between biomarkers at baseline and week 10, including biomarkers as dependent variables, and including the fixed effects of endpoint by clinical site and endpoint by gender interactions. In other words, all biomarkers of interest were included together as dependent variables in the same model and assumed to jointly follow a multivariate normal distribution (after log-transformation where applicable). To this joint multivariate normal distribution is given a covariance matrix, which is estimated by the model and used to derive correlation estimates. The same multivariate analysis was also performed to assess the correlation between changes from baseline in biomarkers at week 10, with the additional inclusion of baseline effects. Analogous bivariate models were used to assess correlation between HAM-D and each biomarker (i.e. separate analyses performed for each marker included with HAM-D as two dependent variables in the model) at baseline, week 10, and for the change from baseline at week 10. The impact of biomarker baseline values on the change in HAM-D at week 10 was also assessed using an analysis of covariance with the change in HAM-D as the dependent variable and adjusted for clinical site, gender and HAM-D baseline. Kruskal-Wallis or Mann-Whitney tests were used to analyse difference in biomarker values between groups. All testing was done at a nominal significance level of 0.05. Statistical analyses were performed using the SAS software (SAS Institute, Cary NC).

3. Results

Biomarker study population

We assessed the level of folate metabolism biomarker including vitamin B12, homocysteine and folate in serum, consistent with previous investigations. For folate, we additionally assessed erythrocyte folate since it can more accurately reflect tissue stores than serum folate and, unlike serum folate, it is not influenced by previous folate intake (De Bruyn et al., 2014). Biomarkers data were available for 489 out of 493 patients for the paroxetine study, and for 392 out of 393 patients for the venlafaxine study. HAM-D level at baseline were 22.97 (SE 4.66) for the paroxetine study and 23.70 (SE 5.06) for the venlafaxine study (two-tailed p-value 0.0252). The mean level of biomarkers at baseline were 23.31 ng/mL (SD 15.04) for serum folate, 879.39 ng/mL (SD 333.19) for erythrocyte folate, 252.13 pg/mL (SD 128.92) for vitamin B12 and 10.45 µmol/L (SD 3.61) for homocysteine in the venlafaxine study. For the paroxetine study, the mean levels were 28.32 ng/mL (SD 19.96) for serum folate, 938.24 ng/mL (SD 330.40) for erythrocyte folate, 268.99 pg/mL (SD 155.43) for vitamin B12 and 10.06 µmol/L (SD 6.56) for homocysteine. The biomarker levels did not significantly differ within each treatment arm of the individual study (data not shown), however there were minor but statistically significantly differences for serum folate ($p<0.001$), erythrocyte folate ($p= 0.0019$) and homocysteine ($p<0.0001$) between the two studies, possibly reflecting differences between the two clinical populations, warranting an independent analysis of the two trials. Erythrocyte folate levels were higher in women in both studies ($p<0.001$), whereas serum folate was slightly higher in women ($p=0.009$) and homocysteine higher in men ($p< 0.0001$) only in the paroxetine study. Biomarker levels were also available for 340 and 251 subjects who completed the paroxetine or venlafaxine study at week 10, respectively.

-Association between biomarkers

We analysed the correlation between biomarkers both at week 0 and at week 10 for each separate study. At baseline, positive association could be seen for serum folate, erythrocyte folate and

vitamin B12; however, levels of correlation were relatively low (Table 1). Negative correlations were found between homocysteine and the other biomarkers, with the strongest correlation found for serum folate and homocysteine ($r=-0.42$, $p<0.001$). Finally, we assessed the magnitude of the correlation between biomarker changes to test if biomarkers (and by inference the metabolic process they reflect) change in a co-ordinated manner with antidepressant treatment. As shown in Table 1 (bottom part), only weak correlations between biomarker changes at week 10 were found.

Association between biomarker levels and depression severity during the study

Consistent with our previous investigation on protein biomarkers on the same clinical studies (Carboni et al., 2019), we used the HAM-D scores at different timepoints to investigate the correlation between depression symptom severity and levels of each biomarker. For each study, we tested the correlation between biomarker level and HAM-D score both at baseline (i.e. week 0) and at week 10, to check if any correlation was affected by antidepressant treatment (Table 2a). Additionally, we tested for correlation between changes in biomarkers levels and in HAM-D-score, to identify potential surrogate biomarkers of response (Table 2a, right column). In the paroxetine study, a few suggestive results could be detected for vitamin B12 at baseline and serum folate at week 10, which were not observed in the venlafaxine study. No correlations were observed for erythrocyte folate and homocysteine either pre- or post-dose. However, these associations may be false positives due to multiple testing, since the association is very weak and data inspection does not suggest any strong relationship between biomarker levels and severity of depressive symptoms.

Biomarkers for prediction of treatment response

Subsequently, we analysed whether associations existed between biomarker values at baseline or at week 10 and changes in HAM-D. Apart from a weak association between baseline erythrocyte folate and response in the venlafaxine clinical population (not replicated in the paroxetine study), no significant associations were observed between pre- and post-treatment of serum biomarkers levels and active treatment control or placebo response (Table 2b). Accordingly, when testing for

baseline biomarker level differences between responders and non-responders in the two clinical trials, we could only find a weak, and non-statistically significant, signal in the two studies for erythrocyte folate, which was 924.70 (SD 322.00) in responders versus 988.89 (SD 321.30) in non-responders (p=0.088) in the paroxetine study, and 870.22 (SD 319.84) in responders versus 966.82 (SD 380.78) in non-responders (p=0.054) in the venlafaxine study. As above, the weak association identified may well be ascribed to multiple testing.

4. Discussion

The objective of this study was to investigate whether one-carbon metabolism biomarkers were associated to MDD severity and with response to antidepressant treatment. Previous findings reported associations between folate biomarkers and MDD (Bender et al., 2017; Møllehave et al., 2017; Nguyen et al., 2017). Moreover, low folate and high homocysteine levels were reported to be associated with poor response to antidepressant treatment (Papakostas et al., 2005, 2004) and with remission after electroconvulsive therapy (Maier et al., 2018). Hintikka et al. (Hintikka et al., 2003) found a correlation between the levels of vitamin B12, but not of folate, and antidepressant response. The lack of a significant association with erythrocyte folate levels in part agrees with the finding of a significant, but small effect size association between depression and folate from the most recent and largest meta-analysis available (Bender et al., 2017). In this study, as expected, we found an inverse correlation between serum homocysteine and vitamin B12 and folate levels at baseline (Selhub et al., 2008). However, we did not find consistent associations between folate biomarker levels and MDD severity, and, apart from a weak and non-significant association between baseline erythrocyte folate and response, we could not detect any correlation between baseline biomarkers and response, neither for active treatment (paroxetine or venlafaxine) nor for placebo. Therefore, our study does not support previous results, which demonstrated an association between high homocysteine levels and depressive symptoms (Bottiglieri et al., 2000), and associations between high vitamin B12 or folate levels and better treatment outcome (Hintikka et al., 2003; Papakostas et al., 2004). Our work is based on a biomarker investigation in a double-

blind placebo-controlled study on a fairly homogeneous cohort. Previous investigations on the association between baseline one-carbon metabolism and response to commonly used antidepressant were conducted however in MDD open trials in outpatients, with the largest study being a naturalistic six-months follow-up investigation conducted in a cohort of 115 mixed treatment patients (Hintikka et al., 2003), two to three times smaller than our investigation. Additional differences in the study design, including specific antidepressant response timepoints and biomarker assays may explain the lack of replication of some literature findings. Furthermore, available evidence suggests that the effect size for the reduction in serum folate level in MDD patients, if true, is modest, thus making replication more difficult.

Our study has a number of potential limitations, such as the lack of control for potential confounders such as cigarette smoking, BMI, comorbid diseases or concomitant treatments. Furthermore, the results here reported are not adjusted for multiple testing and might be partly false positives. Another limitation of our investigation is that strict inclusion criteria were applied (Learned et al., 2012), thus it is to be expected that the population characteristics may not entirely represent an heterogeneous clinical population of MDD patients. On the other hands, the analysis of biomarkers in clinical study cohort might provide more consistent clinical standards with respect to investigations conducted on general patient population. In conclusion, we were unable to detect correlations of folate metabolites with depressive symptoms, and in particular with antidepressant response, in spite of the relatively large number of samples and patients tested compared with previous investigations, weakens the hypothesis of a role of one-carbon metabolism in antidepressant response.

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Declaration of interests

LC, EI, BD, ER, RA, ED and SML were employees of GlaxoSmithKline at the time of the study. ED was an employee of F. Hoffmann-La Roche until 2015 and later served as a consultant to Roche and Pierre-Fabre in the area of genetic biomarkers. ER was an employee of Takeda until March 2020. RA is currently an employee of Takeda; SML is an employee of Indivior; EI is an employee of Leadiant.

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Table 1. Correlation between biomarkers at baseline and week 10

Correlation between biomarkers at baseline (n = 881)			
	Serum folate	Erythrocyte folate	Vitamin B12
Erythrocyte folate	0.29, **p<0.001**		
Vitamin B12	0.13, **p<0.001**	0.18, **p<0.001**	
Homocysteine	-0.43, **p<0.001**	-0.24, **p<0.001**	-0.21, **p<0.001**
Correlations between changes in biomarkers at week 10, paroxetine study (n = 340)			
	Serum folate	Erythrocyte folate	Vitamin B12
Erythrocyte folate	0.009, p=0.114		
Vitamin B12	0.17, **p=0.003**	0.00, p=0.972	
Homocysteine	-0.14, **p=0.016**	-0.10, *p=0.074*	-0.15, **p=0.005**
Correlations between changes in biomarkers at week 10, venlafaxine study (n = 251)			
	Serum folate	Erythrocyte folate	Vitamin B12
Erythrocyte folate	0.11, p=0.119		
Vitamin B12	0.16, **p=0.014**	0.01, p=0.902	
Homocysteine	-0.11, *p=0.089*	0.00, p=0.980	-0.08, p=0.258

Bold: p<0.05; italics: 0.05<p<0.1

Caption: Results from multivariate mixed model analyses (biomarkers included as dependent variables) adjusted for clinical site, gender and baseline (change at week 10 analysis only).

Table 2a. Correlations between HAM-D and biomarkers at baseline, at week 10, and between changes in HAM-D and changes in biomarkers at week 10, in paroxetine and venlafaxine studies

Biomarker	Baseline	Week 10	Change at w10
Paroxetine study	n = 489	n = 340	
Serum folate	-0.03, p=0.540	0.12, **p=0.043**	0.10, p=0.100
Erythrocyte folate	0.00, p=0.966.	0.06, p=0.282	0.05, p=0.369
Vitamin B12	0.09, **p=0.044**	0.07, p=0.206	0.07, p=0.230
Homocysteine	0.01, p=0.783	-0.08, p=0.180	-0.09, p=0.122
Venlafaxine study	n = 392	n = 251	
Serum folate	-0.02, p=0.644	0.05, p=0.441	0.11, p=0.119
Erythrocyte folate	-0.02, p=679	0.07, p=0.313	0.01, p=0.865
Vitamin B12	0.05, p=0.381	0.00, p=0.955	-0.01, p=0.863
Homocysteine	-0.02, p=0.578	0.03, p=0.688	0.05, p=0.461

Bold: p<0.05; italics: 0.05<p<0.1

Table 2b. Significance of baseline biomarkers in the analysis of change in HAM-D at week 10 in paroxetine and venlafaxine studies

Biomarker	Overall model	Placebo only	Antidepressant only
Paroxetine study	n = 340	n = 114	n = 127 (paroxetine)
Serum folate	0.235	0.982	0.901
Erythrocyte folate	0.272	0.732	0.934
Vitamin B12	0.413	0.333	0.926
Homocysteine	0.668	0.249	0.799
Venlafaxine study	n = 251	n = 91	n = 91 (venlafaxine)
Serum folate	0.302	0.239	*0.082*
Erythrocyte folate	**0.039**	0.744	0.315
Vitamin B12	0.260	0.444	0.470
Homocysteine	0.520	0.391	0.618

Bold: $p<0.05$; italics: $0.05<p<0.1$

Caption a) Results from bivariate mixed model analyses (each biomarker and HAM-D included as dependent variables) adjusted for clinical site, gender, and baseline (for change at week 10 analysis only); b) Type III p-values from analysis of covariance adjusted for clinical site, gender and HAM-D baseline