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Methylfolate as Adjunctive Treatment in Major Depression

To the Editor: As the principal investigator of the only previous placebo-controlled trial of methylfolate as an adjunctive treatment in major depression 22 years ago (1), I appreciated the new trial by Papakostas et al. (2) in the December 2012 issue confirming the efficacy of the vitamin in some resistant depression.

Important differences between the two trials raise interesting questions. Our study was for depressed patients with borderline or definite folate deficiency (red blood cell folate levels <200 pg/mL), but Papakostas et al. did not mention the folate status of their patients. I presume that many would not have been folate deficient. We also used 15 mg of methylfolate, but our trial was for 6 months and demonstrated increasing efficacy at 3 and 6 months in contrast to the 60-day study by Papakostas et al.

I first reported in 1967 the beneficial effect of the vitamin on mood and some aspects of cognitive and social function in an open trial of folic acid, 5 mg/day for 1 to 3 years, in folate-deficient patients with epilepsy (3). At the Medical Research Council Neuropsychiatry Research Unit, we then showed not only that folate deficiency was common in depression, as had been reported by Carney (4), but that the deficiency was associated with a poor response to antidepressant therapy (5). I subsequently collaborated with Carney and colleagues in demonstrating that depression was the most common reversible neuropsychiatric manifestation of folate-deficient megaloblastic anemia; in confirming that *S*-adenosylmethionine had antidepressant properties, thus implying that methylation is a key to understanding mood (6); and in identifying a subgroup of patients with depression, high plasma homocysteine levels, folate deficiency, and impaired neurotransmitter metabolism. This culminated in our positive trial of methylfolate, the transport form of folate into the nervous system, as adjunctive therapy in depression (1).

A crucial question for the future is to what extent the antidepressant properties of methylfolate depend on the folate status of the patient. Our own pilot observations suggest that methylfolate may have antidepressant properties as monotherapy, irrespective of folate status, but that responders show a greater rise in red cell folate levels than nonresponders (7). An important clue is the mood-elevating properties of nitrous oxide. This euphoriant effect is probably related to the instantaneous inactivation of methionine synthase, leading to an acute rise in methylfolate in the brain (7). Finally, methylation in the nervous system is a key not just to the biology of mood but to other aspects of cognitive function, including dementia. After 45 years, it is time for academic departments of psychiatry to invest more in this nonpharmaceutical approach to mental illness.

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Up-Regulation of *NOTCH4* Gene Expression in Bipolar Disorder: Future Studies

To the Editor: In the December 2012 issue, Dieset et al. (1) reported significant up-regulation of *NOTCH4* gene expression in whole blood in patients with bipolar disorder relative to healthy comparison subjects, and they identified several single-nucleotide polymorphisms (SNPs) that were significantly associated with *NOTCH4* expression. This is a nice piece of research, and their findings have encouraged future research on the molecular mechanisms of *NOTCH4* in bipolar disorder. However, several lines of their study data await further validation.

First, we are curious about whether the altered *NOTCH4* expression that was observed in patients was caused by changes in the genetic background in bipolar disorder (related to the pathogenesis of the illness) or was just an outcome of the patients' physiological conditions. This is an important issue, but was inconclusive in the article, although the authors conducted the analyses adjusting for a range of confounders. A plausible solution to this problem is the use of an intermediate group: the healthy siblings of patients. These populations shared numerous genetic risk factors with the clinical patients but did

TABLE 1. Association of the *NOTCH4* Single-Nucleotide Polymorphisms (SNPs) With Bipolar Disorder in Samples Reported by the Psychiatric GWAS Consortium (7,481 Case Subjects and 8,250 Comparison Subjects)

				Analysis		
SNP	Position	Allele	Frequency	p	Odds Ratio	SE
rs510321	32302370	A	0.1885	0.077	1.055	0.030
rs389703	32307217	C	0.8115	0.083	0.954	0.027
rs365053	32303966	G	0.1967	0.066	1.052	0.028
rs404890	32306845	A	0.2951	0.953	0.999	0.024
rs3134926	32308125	C	0.7869	0.050	0.950	0.026
rs415929	32297010	C	0.2869	0.750	0.992	0.026
rs9267873	32307330	C	0.5738	0.196	0.969	0.025

not have any psychiatric illnesses. If the *NOTCH4* expression was also elevated in the healthy siblings of bipolar patients relative to healthy comparison subjects, we could conclude that up-regulation of *NOTCH4* expression may be related to the genetic mechanism in bipolar disorder and is likely involved in the pathophysiology of bipolar illness. However, because such data were absent in the Dieset et al. study, we are cautious about drawing any conclusions before further investigations.

Another issue we are concerned with is the analyses of SNPs in or around *NOTCH4* and their associations with gene expression. Dieset et al. observed significant effects of the SNPs on *NOTCH4* expression only in healthy individuals but not in bipolar patients, although the effect went in the same direction. Are these significantly associated SNPs authentic genetic risk factors for bipolar disorder? We examined their associations with bipolar disorder in the case-control samples of European ancestries published by the Psychiatric GWAS Consortium (2), which are the largest samples so far. However, none of these SNPs were significant (Table 1), suggesting that they are not risk variants for bipolar disorder even if they show strong associations with *NOTCH4* expression in healthy individuals. On the other hand, if the increased *NOTCH4* expression in patients was related to genetic mechanisms in bipolar disorder, the gene may harbor unidentified SNPs that are significantly associated with up-regulated *NOTCH4* expression in patients. We think these SNPs may be the authentic risk genetic variants for bipolar disorder, and we call for future studies.

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Response to Li and Su Letter

TO THE EDITOR: We thank Drs. Li and Su for the positive comments and the interest shown in our work, and indeed, we hope that our study will encourage future research on molecular mechanisms of *NOTCH4* in bipolar disorder and related disorders.

We appreciate the opportunity to comment on the issues raised by Drs. Li and Su. First, as noted in the article, this is a case-control study and we cannot offer any certain explanation as to why we observed an increase in *NOTCH4* expression in patients with bipolar disorder. Although we controlled for a range of potential environmental factors, other environmental factors or even epigenetic mechanisms that we have not taken into account might be influencing *NOTCH4* expression. As stated in the article, we call for longitudinal studies investigating *NOTCH4* activity in relation to disease state and trait.

Second, Drs. Li and Su claim that we observed significant effects of the SNPs on *NOTCH4* expression in comparison subjects only. This is not correct. The effects presented in Table 3 in the article are for the sample as a whole and not just for the healthy comparison subjects. Drs. Li and Su correctly point out that none of the SNPs associated with *NOTCH4* expression have been reported to show significant associations with bipolar disorder in other large-scale samples. In fact, we also reported that we did not find any association between SNPs and diagnosis, nor did we find any significant interaction effect of SNP by diagnosis on *NOTCH4* expression levels. We would like to repeat that our primary aim was to investigate *NOTCH4* mRNA levels and that our sample was probably too small to detect any true genetic effects on diagnosis.

Finally, we agree with Drs. Li and Su that the *NOTCH4* gene might harbor unidentified SNPs that might influence the expression of *NOTCH4* in patients, and one possible direction for future studies might be deep sequencing of the *NOTCH4* gene.

In light of evidence provided by our group as well as by others, the *NOTCH4* gene has emerged as an interesting area for further research concerning disease mechanisms in bipolar disorder.

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