ORIGINAL ARTICLE

Folate, Vitamin B_{12}, Homocysteine, and the *MTHFR 677C→T* Polymorphism in Anxiety and Depression

The Hordaland Homocysteine Study

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Background: An association between depression and folate status has been demonstrated in clinical studies, whereas data are sparse on the relationship between depression and other components of 1-carbon metabolism such as vitamin B_{12}, homocysteine, and the methylenetetrahydrofolate reductase *677C→T* polymorphism. The relationship between anxiety and these components is less well known. This study examined the associations between folate, total homocysteine, vitamin B_{12}, and the methylenetetrahydrofolate reductase *677C→T* polymorphism, and anxiety and depression in a large population-based study.

Methods: Anxiety and depression, measured by the Hospital Anxiety and Depression Scale, were assessed in 5948 subjects aged 46 to 49 years (mean, 47.4 years) and 70 to 74 years (mean, 71.9 years) from the Hordaland Homocysteine Study cohort. By means of logistic regression models, anxiety and depression scores were examined in relation to the factors listed above.

Results: Overall, hyperhomocysteinemia (plasma total homocysteine level ≥15.0 µmol/L [≥2.02 mg/dL]) (odds ratio, 1.90; 95% confidence interval, 1.11-3.25) and *T/T* methylenetetrahydrofolate reductase genotype (odds ratio, 1.69; 95% confidence interval, 1.09-2.62), but not low plasma folate or vitamin B_{12} levels, were significantly related to depression without comorbid anxiety disorder. Plasma folate level was inversely associated with depression only in the subgroup of middle-aged women. None of the investigated parameters showed a significant relationship to anxiety.

Conclusion: Our results provide further evidence of a role of impaired 1-carbon metabolism in depression.

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HOMOCYSTEINE and the vitamins involved in 1-carbon metabolism, folate and vitamin B_{12}, have been associated with a diversity of diseases, including cardiovascular disease,[1,2] malignancies,[3] Alzheimer disease, impaired cognitive functioning,[4,5] and birth defects and pregnancy complications.[6,7] Several studies have investigated the *677C→T* polymorphism of the methylenetetrahydrofolate reductase (*MTHFR*) gene as a risk factor for these conditions, as the *C-to-T* transition causes reduced enzyme activity, and elevated plasma total homocysteine (tHcy) levels under conditions of impaired folate status.[8]

Both anxiety and depression are common symptoms or disorders with a major impact on public health.[9,10] Although a possible role of nutritional factors in the pathogenesis of neuropsychiatric disorders has long been debated,[11,12] clinical studies have shown an inverse relationship between folate status and depression.[13] Such a relationship has been inferred from studies showing increased frequency of folate deficiency among depressed patients[14-18]; more severe[18-21] and prolonged[22] depressive episodes and weaker treatment response to antidepressants in patients with low folate status[14,18,21-25]; and enhanced antidepressant response with folic acid supplementation.[26-29]

Investigations on a possible role of vitamin B_{12} status in neuropsychiatric disorders have been motivated by the central nervous system damage caused by overt or subtle vitamin B_{12} deficiency.[30,31] Data regarding the association between vitamin B_{12} status and depression are scarce.[14,18,32-34]

While some studies have demonstrated a positive relationship between depression and plasma tHcy levels[35] or the *MTHFR 677C→T* polymorphism,[36] such relationships have not been confirmed in other studies.[14,32,34,37]

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Folate, vitamin B_{12}, and homocysteine are involved in processes important for central nervous system function (**Figure 1**). Folate metabolism is linked to biopterin-dependent neurotransmitter synthesis[38] and S-adenosylmethionine–dependent methylation of biogenic amines and phospholipids in the central nervous system.[39] Homocysteine, or its metabolites such as homocysteic acid, may have a direct excitotoxic effect on the *N*-methyl-D-aspartate glutamate receptors, or may inhibit the methylation processes in the central nervous system.[39] This biochemical knowledge adds to the clinical data cited already, suggesting a possible role of 1-carbon metabolism in mental disorders, particularly in depression and dementia.[40] However, despite the extensive comorbidity between depression and anxiety,[41-43] we have found no more than 3 studies[33,44,45] addressing the possibility of impaired 1-carbon metabolism in anxiety disorders. Only one study suggests such an association, namely, between low vitamin B_{12} levels and anxiety.[33]

The present study is part of the Hordaland Health Study, which included self-administered questionnaires of anxiety and depression according to the Hospital Anxiety and Depression Scale (HADS),[46] determination of tHcy and related vitamins, and *C677T MTHFR* genotyping in approximately 6000 middle-aged and elderly subjects. The aim of the current study was to examine whether key components of the 1-carbon metabolism were associated with anxiety disorders and/or depression in this large population.

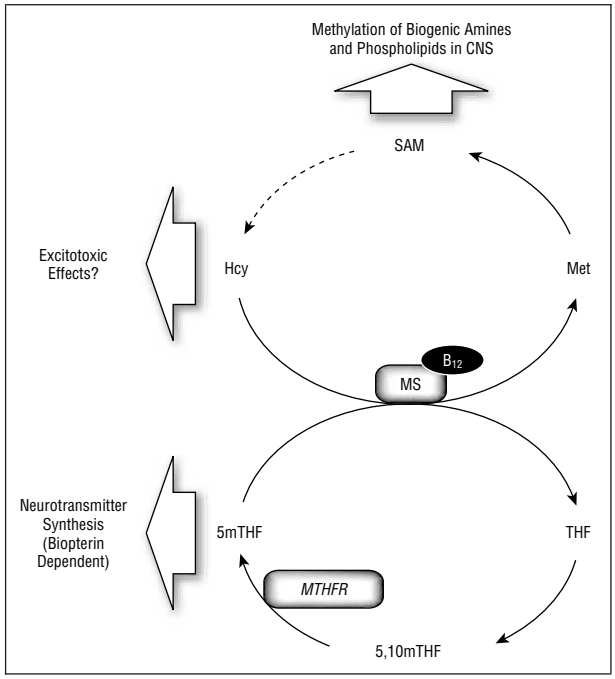

Figure 1. Components of 1-carbon metabolism and central nervous system (CNS) functions. SAM indicates S-adenosylmethionine; Hcy, homocysteine; Met, methionine; B12, vitamin B_{12}; MS, methionine synthetase; 5mTHF, 5-methyl-tetrahydrofolate; 5,10mTHF, 5,10-methylene-tetrahydrofolate; THF, tetrahydrofolate; and MTHFR, methylenetetrahydrofolate reductase.

METHODS

STUDY POPULATION

The Hordaland Health Study 1997-1999 was conducted from 1997 to 1999 as a collaboration between the National Health Screening Service, the University of Bergen (Bergen, Norway), and local health services. A subsample of the study included 2291 men and 2558 women aged 46 to 49 years (mean, 47.4 years) and 1868 men and 2470 women aged 70 to 74 years (mean, 71.9 years) who had participated in the Hordaland Homocysteine Studies in 1992 to 1993.[47] The younger age group was originally a part of the ordinary national cardiovascular risk survey program, while the older group was included to examine age effects. Participation rate was 77% of those invited. At attendance, the subjects underwent a brief physical examination (height, weight, blood pressure), and a blood sample was drawn and stored at −20°C until the biochemical analyses were performed 1 to 3 years later. A self-administered questionnaire providing information on demographic, socioeconomic, and psychosocial parameters; health behaviors; subjective health; present or former diseases; and use of medication was delivered. Among those who participated, 5948 individuals (84%) returned questionnaires with valid ratings of anxiety and depression.

The study protocol was approved by the Regional Committee for Medical Research Ethics and the Norwegian Data Inspectorate.

ASSESSMENT OF ANXIETY AND DEPRESSION

The HADS is a self-administered questionnaire consisting of 14 items, 7 for anxiety (HADS-A subscale) and 7 for depression (HADS-D subscale).[46] To avoid making individuals feel that they are being tested for mental disorders, symptoms of severe psychopathology are not included. The HADS-A contains items mainly related to restlessness and worry, and 1 item reflecting panic attacks. The HADS-D focuses mainly on the reduced pleasure response aspect (anhedonia) of depression, but also psychomotor retardation and impaired mood.

The HADS-A and HADS-D are intercorrelated, most often in the range of 0.50 to 0.60.[48] Hence, to identify more homogeneous groups with anxiety disorders or depressions, restrictions were put on the other subscale when cases were defined. Thus, anxiety disorder was defined as a HADS-A score of 8 or more restricted to a HADS-D score less than 8 to avoid comorbid disorders. Accordingly, depression was defined as a HADS-D score of 8 or more restricted to a HADS-A score less than 8. Analyses were also carried out on comorbid cases, which were defined as subjects with both subscale scores of 8 or more. To enhance the specificity of anxiety disorder and depression, analyses were carried out on cases defined to HADS-A and HADS-D scores of 11 or more, using the same restrictions as mentioned in this paragraph.

BIOCHEMICAL AND GENETIC MEASUREMENTS

Plasma tHcy was analyzed by high-performance liquid chromatography and fluorescence detection.[49] Serum folate was determined by a *Lactobacillus casei* microbiologic assay[50] and serum vitamin B_{12} by a *Lactobacillus leichmannii* microbiologic assay.[51] Both the folate and vitamin B_{12} assays were adapted to a microtiter plate format and carried out by a robotic workstation (Microlab AT plus 2; Hamilton Bonaduz AG, Bonaduz, Switzerland). The *MTHFR C677T* genotyping was performed by a real-time polymerase chain reaction as described elsewhere.[52]

STATISTICAL ANALYSES

Plasma tHcy level was divided into 4 categories (<9.0 µmol/L [<1.22 mg/dL] [reference], 9.0-11.9 µmol/L [1.22-1.61 mg/dL], 12.0-14.9 µmol/L [1.62-2.01 mg/dL], and ≥15.0 µmol/L [≥2.02 mg/dL]).[53] Plasma folate and plasma vitamin B_{12} levels

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were similarly divided into 4 categories (plasma folate: <3.80 nmol/L [<1.7 ng/mL], 3.80-4.99 nmol/L [1.7-2.2 ng/mL], 5.00-8.49 nmol/L [2.3-3.7 ng/mL], and ≥8.50 nmol/L [≥3.8 ng/mL] [reference]; plasma vitamin B_{12}: <230.0 pmol/L [<312 pg/mL], 230.0-279.9 pmol/L [312-379 pg/mL], 280.0-414.9 pmol/L [380-562 pg/mL], and ≥415.0 pmol/L [≥563 pg/mL] [reference]). Logistic regression analyses were used to estimate odds ratios (ORs) for being a case, comparing each category with the reference category of the metabolites and the *MTHFR* polymorphism. The representation of covariates as indicator variables was used to allow for assessment of nonlinear dose-response relationships, while a linear (1 *df*) representation was used to test for linear trends. Two logistic regression models were used, one with adjustment for age and sex (model 1) and one with additional adjustments for smoking status and educational level (model 2). The effect of other possible confounders, such as coffee consumption, physical exercise, body mass index, and self-reported cardiovascular disease, was examined by adding these one by one to model 2. To evaluate possible effect modification of age and sex, product terms were added separately to the models. Possible effect modification of B-vitamin supplementation and tranquilizer and antidepressant use was evaluated by stratification.

To examine whether use of B-vitamin supplements was associated with anxiety or depression, logistic regression analyses were used to estimate OR for being a case, comparing nonusers with users after adjusting for age, sex, smoking status, and educational level.

The precision of the OR estimates was expressed with 95% confidence intervals (CIs). Generalized additive logistic regression was used to provide a graphic representation of the dose-response relationship between folate, vitamin B_{12}, and tHcy and anxiety or depression. This technique is based on the generalized additive model[54] and allowed adjustment for age, sex, smoking status, and educational level.

A 2-sided $P<.05$ was chosen to indicate statistical significance. The statistical analyses were conducted with the software package SPSS 11.0 (SPSS Inc, Chicago, Ill) and S-Plus 6.0 (Insightful Corp, Seattle, Wash).

RESULTS

CHARACTERISTICS OF THE STUDY POPULATION

Table 1 summarizes some demographic characteristics and blood indexes according to age groups and sex. The prevalences of anxiety disorder and depression; use of psychoactive drugs; distribution of tHcy, vitamin B_{12}, and folate levels; and some lifestyle factors are also presented. The total numbers of subjects with anxiety disorder and depression were 622 (11.5%) and 243 (4.8%), respectively. Anxiety disorder was most prevalent among middle-aged women (15.3%) and depression among older men (7.5%).

Plasma tHcy level correlated well with plasma folate value and modestly with plasma vitamin B_{12} level (Spearman coefficient $r=-0.40$, $P<.001$, and $r=-0.25$, $P<.001$, respectively), and plasma folate and plasma vitamin B_{12} levels correlated poorly ($r=0.06$, $P<.001$), as has been reported elsewhere.[55] In the *MTHFR T/T* genotype, compared with the *C/C* and *C/T* genotypes, the mean value of plasma tHcy was significantly increased (13.66 µmol/L [1.85 mg/dL] vs 10.62 µmol/L [1.44 mg/dL] and 10.98 µmol/L [1.48 mg/dL]; $P<.001$ and $P<.001$, respectively) and mean plasma folate significantly decreased (7.49 nmol/L [3.3 ng/mL] vs 8.46 nmol/L [3.7 ng/mL] and 8.15 nmol/L [3.6 ng/mL]; $P<.001$ and $P<.05$, respectively), while mean plasma vitamin B_{12} did not show significant differences between the genotypes (data not shown).

UNIVARIATE ASSOCIATIONS

The proportions of the sample with anxiety disorder or depression according to the increasing concentrations of tHcy, folate, and vitamin B_{12}, and according to *MTHFR* genotype and some lifestyle factors, are listed in **Table 2**, stratified by age and sex. The distributions indicated associations with both anxiety disorder and depression for a variety of the variables listed. Plasma tHcy and folate levels and *MTHFR* genotype all seemed associated with depression, as did educational level, smoking status, coffee consumption, use of antidepressants and tranquilizers, physical exercise, and body mass index. Associations with anxiety disorder were seen for smoking status, use of antidepressants and tranquilizers, and body mass index, but only in some strata within plasma tHcy, folate, and vitamin B_{12} levels.

HOMOCYSTEINE

Plasma levels of tHcy were not significantly associated with anxiety disorder (**Table 3**) but were significantly related to depression (**Table 4**), as demonstrated by logistic regression models adjusting for age and sex (model 1), or further adjusting for smoking status and educational level (model 2). The association with depression was, however, strongest at high plasma tHcy level (≥15.0 µmol/L [≥2.02 mg/dL]) (OR, 1.90; 95% CI, 1.11-3.25), and test for linear trend was significant only in model 1 ($P=.03$). Including folate level, vitamin B_{12} level, or *MTHFR* genotypes in the analyses did not essentially influence the estimates.

Figure 2 shows a graphic representation of the relationship between tHcy and the vitamins, and anxiety disorder and depression. A dose-response relationship was seen only between tHcy level and depression.

FOLATE AND VITAMIN B_{12}

Neither folate nor vitamin B_{12} was significantly related to anxiety disorder or depression. These results were obtained by logistic regression also after adjustments (models 1 and 2, Tables 3 and 4) and were supported by generalized additive logistic regression (Figure 2). However, the dose-response curves obtained by the latter approach suggested a weak negative relationship between folate and depression but not anxiety disorder, and the curve for the vitamin B_{12} and depression relationship was actually U-shaped (Figure 2).

The estimates did not change markedly when analyses were performed separately for the 4 age and sex strata, except for middle-aged women. In this subgroup, ORs (95% CIs) were 3.41 (1.02-11.42) and 3.08 (0.97-9.72) (model 1) and 3.15 (0.94-10.60) and 3.05 (0.96- 9.65) (model 2) for the 2 lower folate categories, respectively. The *P* for trend was .02 in both models.

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Table 1. Characteristics of the Study Population: the Hordaland Homocysteine Studies*

	No. (%)				
	Men		Women		
	Age, 46-49 y	Age, 70-74 y	Age, 46-49 y	Age, 70-74 y	Total
Anxiety disorder	127 (10.5)	63 (5.4)	248 (15.3)	184 (13.1)	622 (11.5)
Depression	54 (4.8)	89 (7.5)	35 (2.5)	65 (5.1)	243 (4.8)
Comorbid anxiety disorder and depression	75 (5.6)	40 (3.1)	103 (5.8)	92 (5.9)	310 (5.2)
Plasma tHcy, μmol/L					
<9.0	446 (29.7)	153 (11.0)	1097 (58.4)	426 (24.1)	2122 (32.5)
9.0-11.9	707 (47.1)	508 (36.5)	564 (30.0)	718 (40.6)	2497 (38.2)
12.0-14.9	241 (16.1)	430 (30.9)	150 (8.0)	402 (22.7)	1223 (28.7)
≥15.0	107 (7.1)	300 (21.6)	66 (3.5)	224 (12.7)	697 (10.7)
Plasma folate, nmol/L					
<3.80	167 (10.1)	181 (12.4)	179 (8.7)	164 (8.8)	691 (9.8)
3.80-4.99	265 (16.0)	241 (16.5)	264 (12.8)	227 (12.2)	997 (14.2)
5.00-8.49	812 (49.0)	697 (47.8)	952 (46.3)	812 (43.8)	3273 (46.6)
≥8.50	413 (24.9)	340 (23.3)	661 (32.1)	653 (35.2)	2067 (29.4)
Plasma vitamin B_{12}, pmol/L					
<230.0	111 (6.7)	201 (13.7)	181 (12.4)	205 (11.0)	699 (9.9)
230.0-279.9	250 (15.1)	227 (15.5)	241 (16.5)	274 (14.7)	1013 (14.4)
280.0-414.9	816 (49.2)	653 (44.5)	697 (47.8)	774 (41.6)	3181 (45.2)
≥415.0	481 (29.0)	385 (26.3)	340 (23.3)	607 (32.6)	2150 (30.5)
MTHFR genotype					
C/C	818 (49.2)	759 (51.7)	1034 (50.2)	897 (48.3)	3508 (49.8)
C/T	707 (42.6)	587 (40.0)	844 (41.0)	811 (43.6)	2949 (41.8)
T/T	136 (8.2)	123 (8.4)	181 (8.8)	151 (8.1)	591 (8.4)
Educational level, y					
<10	273 (16.6)	478 (34.7)	464 (22.7)	878 (53.4)	2093 (31.2)
10-12	691 (42.0)	581 (42.2)	885 (43.4)	573 (34.9)	2730 (40.7)
>12	681 (41.4)	319 (23.1)	692 (33.9)	192 (11.7)	1884 (28.1)
Current smoker	469 (30.1)	195 (13.8)	574 (30.1)	210 (11.9)	1448 (21.8)
Alcohol consumption, alcohol U/14 d†					
0	228 (14.2)	469 (36.5)	542 (28.3)	834 (57.9)	2073 (33.2)
1-14	1135 (70.9)	688 (53.6)	1306 (68.1)	586 (40.7)	3715 (59.9)
>14	238 (14.9)	127 (9.9)	70 (3.6)	21 (1.5)	456 (7.3)
Coffee consumption, cups/d					
0	128 (7.9)	92 (6.4)	204 (10.2)	127 (7.0)	551 (8.0)
1-5	1004 (61.6)	1198 (82.8)	1429 (71.2)	1571 (86.5)	5202 (74.4)
>5	497 (30.5)	156 (10.8)	375 (18.7)	119 (6.5)	1147 (16.6)
B-vitamin users	390 (29.1)	304 (22.6)	674 (38.1)	485 (29.2)	1853 (30.3)
Antidepressant users	32 (1.4)	48 (2.6)	103 (4.0)	93 (3.8)	276 (3.0)
Tranquilizer users	11 (0.5)	24 (1.3)	29 (1.1)	41 (1.7)	105 (1.1)
Physical exercise					
No or little activity	158 (9.8)	138 (10.3)	186 (9.4)	239 (9.7)	721 (11.1)
Moderate activity	696 (43.4)	728 (54.4)	937 (47.5)	1026 (41.6)	3387 (52.3)
Heavy activity	751 (46.8)	472 (35.3)	850 (43.1)	301 (12.2)	2374 (36.6)
BMI					
<20.0	23 (1.4)	30 (2.0)	105 (5.1)	106 (5.7)	264 (3.7)
20.0-24.9	598 (36.0)	535 (36.4)	1128 (54.6)	664 (35.7)	2925 (41.4)
25.0-29.9	849 (51.1)	765 (52.0)	631 (30.5)	752 (40.4)	2997 (42.5)
≥30.0	192 (11.6)	140 (9.5)	202 (9.8)	339 (18.2)	873 (12.4)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); MTHFR, methylenetetrahydrofolate reductase; tHcy, total homocysteine.

SI conversion factors: To convert tHcy to milligrams per deciliter, divide by 7.397; folate to nanograms per milliliter, divide by 2.266; vitamin B_{12} to picograms per milliliter, divide by 0.738.

*There is no common sample number to be reported in the table because the number of participants varied between the different variables according to different numbers of missing data. The total number for each variable can be calculated with the numbers and percentages provided in the table.

†A unit of alcohol consisted of 12.8 g of alcohol.

THE *MTHFR 677C→T* POLYMORPHISM

Depression but not anxiety disorder was related to the *C677T MTHFR* genotype. The OR (95% CI) for depression by logistic regression was 1.69 (1.09-2.62) for the *T/T* genotype (using *C/C* as reference) in model 2 (Table 4). This estimate was essentially the same when carried out in the 4 age and sex strata (data not shown).

Including coffee consumption, physical exercise, body mass index, or self-reported cardiovascular disease in the logistic regression analyses did not significantly influence the estimates. We observed no effect

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Table 2. Relationship of Anxiety Disorder and Depression to Various Characteristics of the Study Population: the Hordaland Homocysteine Studies*

	Anxiety Disorder, %†					Depression, %†				
	Men		Women			Men		Women		
	Age 46-49 y	Age 70-74 y	Age 46-49 y	Age 70-74 y	Total (n)	Age 46-49 y	Age 70-74 y	Age 46-49 y	Age 70-74 y	Total (n)
Plasma tHcy, μmol/L										
<9.0	12.1	6.1	14.3	11.9	12.7 (212)	2.6	2.4	1.8	3.3	2.6 (39)
9.0-11.9	12.0	5.4	16.0	13.5	11.9 (227)	5.4	7.0	2.4	5.9	5.4 (96)
12.0-14.9	7.8	4.8	19.2	12.1	9.5 (87)	6.0	9.2	2.0	5.3	6.0 (53)
≥15.0	9.2	6.2	20.8	17.6	11.7 (60)	7.3	8.3	7.3	4.9	7.3 (36)
Plasma folate, nmol/L										
<3.80	4.8	8.0	14.4	14.9	10.3 (51)	7.1	8.0	5.0	9.5	7.1 (34)
3.80-4.99	10.6	7.4	17.9	15.7	13.0 (93)	5.4	8.9	4.3	3.3	5.4 (36)
5.00-8.49	13.3	4.5	15.4	11.8	11.6 (295)	4.7	7.5	2.4	5.2	4.7 (110)
≥8.50	7.8	5.1	14.5	13.5	11.4 (182)	4.2	6.5	1.3	4.6	4.2 (62)
Plasma vitamin B_{12}, pmol/L										
<230.0	7.6	6.1	20.7	12.5	12.1 (64)	5.9	12.6	1.8	3.4	5.9 (29)
230.0-279.9	9.4	3.3	12.4	16.3	10.6 (81)	5.0	8.3	2.2	3.9	5.0 (36)
280.0-414.9	11.4	7.3	15.8	12.7	12.2 (299)	4.5	5.3	2.4	5.6	4.5 (102)
≥415.0	10.7	3.4	14.0	12.3	10.8 (176)	4.9	8.0	1.3	5.5	4.9 (74)
MTHFR genotype										
C/C	10.7	5.5	15.6	12.4	11.5 (308)	5.1	7.5	2.5	5.0	5.1 (127)
C/T	10.1	4.9	15.3	14.2	11.6 (263)	4.1	6.5	2.1	4.9	4.1 (85)
T/T	12.5	8.0	13.4	11.3	11.6 (50)	7.3	12.1	3.9	6.9	7.3 (30)
Educational level, y										
<10	13.7	5.1	17.2	15.6	13.3 (202)	6.7	10.2	4.1	5.4	6.7 (94)
10-12	9.7	5.7	14.0	10.4	10.4 (221)	4.6	6.5	2.4	4.8	4.6 (92)
>12	10.2	4.5	15.0	13.8	11.3 (173)	2.9	5.2	1.6	1.4	2.9 (40)
Current smoker										
No	9.5	5.1	13.5	12.3	10.3 (417)	4.7	7.0	2.2	4.6	4.7 (177)
Yes	12.8	6.3	18.5	15.6	14.7 (152)	5.5	10.7	2.5	9.2	5.5 (51)
Alcohol consumption, alcohol U/14 d‡										
0	11.4	6.2	15.6	13.6	12.2 (189)	5.9	9.1	4.8	4.7	5.9 (85)
1-14	9.6	6.0	15.0	12.4	11.3 (330)	4.2	6.3	1.6	4.9	4.2 (113)
>14	12.4	2.0	14.3	20.0	10.1 (36)	5.0	10.7	0.0	0.0	5.0 (17)
Coffee consumption, cups/d										
0	15.1	5.5	16.0	9.4	12.4 (52)	3.7	6.8	1.5	3.3	3.7 (14)
1-5	10.5	5.5	13.9	13.3	11.2 (449)	4.9	7.0	2.7	4.7	4.9 (185)
>5	9.0	5.6	19.1	16.0	12.5 (106)	4.8	9.2	2.5	11.3	4.8 (37)
Use of B-vitamin supplements										
Nonusers	10.4	5.5	15.3	13.4	11.3 (417)	5.1	8.2	2.7	4.7	5.1 (177)
Users	10.7	5.5	15.2	12.9	12.1 (199)	4.0	4.1	2.3	5.9	4.0 (61)
Use of antidepressants										
Nonusers	10.4	5.1	14.3	12.3	10.9 (570)	4.6	7.2	2.3	4.9	4.6 (227)
Users	22.2	21.4	39.1	33.3	32.3 (53)	12.6	21.4	9.3	10.0	12.6 (16)
Use of tranquilizers										
Nonusers	10.4	5.0	15.0	12.5	11.2 597)	4.8	7.6	2.4	5.0	4.8 (240)
Users	60.0	40.0	71.4	44.4	48.1 (26)	9.7	0.0	33.3	11.8	9.7 (3)
Physical exercise										
No or little activity	9.6	6.4	20.8	12.5	12.8 (63)	6.9	18.5	4.8	9.8	10.1 (48)
Moderate activity	9.6	4.5	17.0	12.2	11.4 (294)	5.3	7.9	2.8	4.1	4.9 (119)
Heavy activity	11.4	6.4	11.6	15.2	10.9 (212)	3.8	3.3	1.6	4.5	3.0 (53)
BMI										
<20.0	30.8	17.4	23.4	20.0	21.8 (42)	3.8	9.5	4.8	1.5	3.8 (6)
20.0-24.9	10.9	6.4	15.1	13.2	12.3 (275)	4.9	8.6	2.1	5.9	4.9 (101)
25.0-29.9	10.3	4.3	14.5	13.1	10.3 (240)	4.5	6.3	2.3	4.0	4.5 (98)
>30.0	9.2	5.9	14.6	10.7	10.5 (66)	6.2	9.4	4.4	6.9	6.2 (37)

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); MTHFR, methylenetetrahydrofolate reductase; tHcy, total homocysteine.

SI conversion factors: To convert tHcy to micrograms per deciliter, divide by 7.397; folate to nanograms per liter, divide by 2.266; vitamin B_{12} to picograms per milliliter, divide by 0.738.

*Sums of subjects with anxiety disorder and depression differ between the variables because of varying numbers of missing data.

†Percentages are the percentage of anxiety disorder or depression within each value of the variable.

‡A unit of alcohol consisted of 12.8 g of alcohol.

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Table 3. Odds Ratios for Having Anxiety Disorder at Different Levels of tHcy, Folate, Vitamin B_{12}, and *MTHFR* Polymorphism in Model 1 (Adjusted for Age and Sex) and Model 2 (Adjusted for Age, Sex, Smoking Status, and Educational Level): the Hordaland Homocysteine Studies

	Subjects With Anxiety Disorder, No. (%)	Model 1			Model 2		
		OR	CI	*P* Value	OR	CI	*P* Value
tHcy, µmol/L							
<9.0	212 (12.7)	1			1		
9.0-11.9	227 (11.9)	1.14	0.91-1.42		1.10	0.88-1.38	
12.0-14.9	87 (9.5)	1.01	0.75-1.36		0.96	0.71-1.29	
≥15.0	60 (11.7)	1.39	0.98-1.98		1.31	0.92-1.87	
Test for trend				.18			.36
Test for homogeneity				.24			.33
Folate, nmol/L							
<3.80	51 (10.3)	0.94	0.66-1.35		0.90	0.63-1.30	
3.80-4.99	93 (13.0)	1.27	0.95-1.69		1.21	0.90-1.62	
5.00-8.49	295 (11.6)	1.07	0.87-1.32		1.06	0.86-1.31	
≥8.50	182 (11.4)	1			1		
Test for trend				.59			.84
Test for homogeneity				.37			.47
Vitamin B_{12}, pmol/L							
<230.0	64 (12.1)	1.23	0.89-1.72		1.23	0.88-1.72	
230.0-279.9	81 (10.6)	1.10	0.82-1.48		1.07	0.80-1.44	
280.0-414.9	299 (12.2)	1.19	0.96-1.47		1.19	0.96-1.47	
≥415.0	176 (10.8)	1			1		
Test for trend				.23			.27
Test for homogeneity				.39			.39
MTHFR genotype							
C/C	308 (11.5)	1			1		
C/T	263 (11.6)	1.00	0.83-1.21		1.00	0.83-1.21	
T/T	50 (11.6)	0.95	0.67-1.34		0.95	0.67-1.35	
Test for trend				.82			.87
Test for homogeneity				.94			.95

Abbreviations: CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; tHcy, total homocysteine.
SI conversion factors: to convert tHcy to milligrams per deciliter, divide by 7.397; folate to nanograms per milliliter, divide by 2.266; vitamin B_{12} to picograms per milliliter, divide by 0.738.

modification by age or sex or by use of B vitamins, tranquilizers, or antidepressants.

There were no significant associations between levels of plasma folate, vitamin B_{12}, or tHcy or *MTHFR 677C→T* polymorphism and comorbid anxiety disorder and depression. Nonusers of B-vitamin supplements were not more prone to anxiety or depression than users.

ESTIMATES IN SUBGROUPS WITH HIGHER HADS SCORES

Elevating the cutoff for both anxiety disorder and depression to HADS-A score of 11 or more and HADS-D score of 11 or more, respectively, resulted in only minor changes in the estimates. The relationship with tHcy was weaker for anxiety disorder and stronger for depression; neither of them was significant. The OR for anxiety disorder and depression associated with folate or vitamin B_{12} was unchanged, except for the relationship between vitamin B_{12} and depression, which became significant at the lower vitamin B_{12} level with an OR (95% CI) of 2.33 (1.05-5.21) in model 1 and 2.39 (1.07-5.36) in model 2. Significant effects of *MTHFR T/T* genotype were not obtained for anxiety disorder, but they were for depression. The OR (95% CI) increased to 2.65 (1.16-6.08) in model 1 and 2.75 (1.20-6.32) in model 2.

COMMENT

We investigated the association between anxiety disorders and depression and key components of the 1-carbon metabolism in a cohort of 5948 subjects. The strongest relationship was observed between the *T/T MTHFR* genotype and depression, and the association was present for both cutoff levels of depression. Associations were observed between tHcy and depression, lowest level of vitamin B_{12} (<230.0 pmol/L [<312 pg/mL]) and depression with high cutoff (HADS-D score ≥11), and in middle aged women, between depression and folate. Only a weak relationship or no relationship was seen between anxiety disorder and tHcy, folate, or vitamin B_{12} level or *MTHFR* genotype.

Although the cross-sectional designof this study allows association between parameters to be assessed, causality cannot be determined. Because anxiety disorders and depression may influence dietary habits, it is conceivable that these disorders may alter homocysteine and B-vitamin status through dietary changes. Among the parameters studied, only *MTHFR* genotype is a nonmodifiable trait.

Published studies on folate status and depression, most of which are case-control studies, demonstrate stronger relationships[56] than documented in the present study.

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Table 4. Odds Ratios for Having Depression at Different Levels of tHcy, Folate, Vitamin B_{12}, and *MTHFR* Polymorphism in Model 1 (Adjusted for Age and Sex) and Model 2 (Adjusted for Age, Sex, Smoking Status, and Educational Level): the Hordaland Homocysteine Studies

	Subjects With Depression, No. (%)	Model 1			Model 2		
		OR	CI	*P* Value	OR	CI	*P* Value
tHcy, µmol/L							
<9.0	39 (2.6)	1			1		
9.0-11.9	96 (5.4)	1.80	1.20-2.72		1.76	1.17-2.66	
12.0-14.9	53 (6.0)	1.58	0.97-2.56		1.51	0.93-2.45	
>15.0	36 (7.3)	2.00	1.17-3.41		1.90	1.11-3.25	
Test for trend				.03			.06
Test for homogeneity				.03			.04
Folate, nmol/L							
<3.8	34 (7.1)	1.48	0.93-2.37		1.31	0.82-2.11	
3.8-4.9	36 (5.4)	1.12	0.71-1.77		1.03	0.65-1.63	
5.0-8.4	110 (4.7)	1.02	0.73-1.43		0.99	0.71-1.39	
>8.5	62 (4.2)	1			1		
Test for trend				.12			.32
Test for homogeneity				.36			.64
Vitamin B_{12}, pmol/L							
<230.0	29 (5.9)	1.19	0.74-1.91		1.22	0.76-1.96	
230.0-279.9	36 (5.0)	0.99	0.64-1.53		0.98	0.63-1.52	
280.0-414.9	102 (4.5)	0.88	0.63-1.22		0.89	0.64-1.24	
>414.0	74 (4.9)	1			1		
Test for trend				.55			.51
Test for homogeneity				.60			.59
MTHFR genotype							
C/C	127 (5.1)	1			1		
C/T	85 (4.1)	0.79	0.58-1.07		0.80	0.59-1.08	
T/T	30 (7.3)	1.63	1.05-2.53		1.69	1.09-2.62	
Test for trend				.48			.41
Test for homogeneity				.01			.01

Abbreviations: CI, confidence interval; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; tHcy, total homocysteine.
SI conversion factors: To convert tHcy to milligrams per deciliter, divide by 7.397; folate to nanograms per milliliter, divide by 2.266; vitamin B_{12} to picograms per milliliter, divide by 0.738.

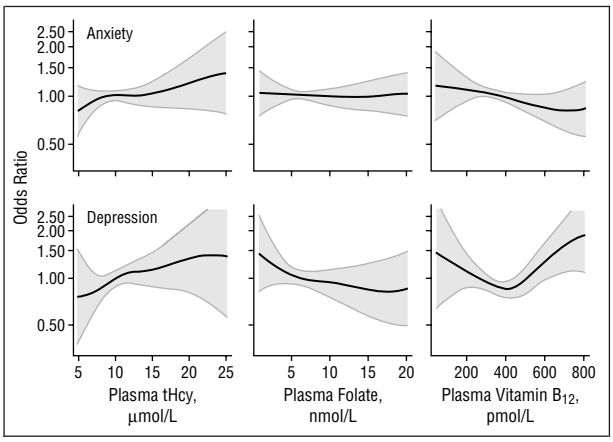

Figure 2. Dose-response relationships between plasma total homocysteine (tHcy), folate, and vitamin B_{12} levels and anxiety disorder and depression. The curves were constructed by using generalized additive regression analyses adjusting for age, sex, smoking status, and educational level. The shaded areas indicate 95% pointwise confidence intervals. To convert tHcy levels to milligrams per deciliter, divide by 7.397; folate to nanograms per milliliter, divide by 2.266; and vitamin B_{12} to picograms per milliliter, divide by 0.738.

Such case-control studies are more prone to selection bias than a population-based cross-sectional study. Although the primary participation rate of the present study was relatively high (77%), it might have been affected by mental disorders themselves, since persons with anxiety disorders and depression might be less likely to participate in the study.

The plasma levels of folate in the frozen samples were lower than would be expected in a fresh sample, in contrast to the plasma levels of vitamin B_{12} and tHcy. Others have found similar deviations of plasma folate levels in frozen samples.[57] Nevertheless, plasma folate level was correlated with tHcy level (Spearman coefficient $r=-0.40$, $P<.001$), as reported elsewhere.[55]

Serum (or plasma) folate level has been considered a less reliable measure of folate status than red blood cell folate content.[58] Hence, if red blood cell folate level had been measured, one would expect a stronger association with depression compared with our findings. However, because of substantial intermethod variation, red blood cell folate level has its shortcomings as a measure of folate deficiency as well.[59]

The HADS is a dimensional rating scale, and categorical diagnoses have to be based on research-proved cutoff levels. Optimal cutoff levels for anxiety disorders and depressive disorders are at scores of 8 or more for both subscales, resulting in sensitivities and specificities of approximately 0.80 for both HADS-A and HADS-D.[48] Higher specificity is usually obtained by raising the cutoff level, which was demonstrated by the increased OR for depression in the *MTHFR* polymorphism, tHcy level, and vitamin B_{12} level.

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The observed association between the *MTHFR T/T* genotype and depression is in accordance with results from one case-control study,[36] while another case-control study did not confirm such an association.[37] These somewhat inconsistent results of smaller studies could be explained by low statistical power due to the limited number of cases (n=71 and 32, respectively), combined with the low frequency (10%) of *MTHFR T/T* homozygosity.[8]

We found that depression was related to tHcy, folate, and vitamin B_{12} levels, but the associations were weak and/or present only in certain subgroups. This contrasts somewhat with the findings in clinical studies of impaired folate status in depressed patients,[56] and one report on the high frequency of elevated tHcy levels in depression.[18] The relatively weak associations found in the present work could be explained by better folate status and less severe depression of the subjects recruited from a general population as compared with hospitalized depressed patients.

Some antidepressant effect by folic acid supplementation has been reported in randomized clinical trials.[26-29] The largest (n=127) of these trials[26] showed, however, a significant beneficial effect only in women. The lack of significant results in men could be due to the small sample size or an insufficient dosage. In contrast, in our study, use of vitamin B supplements was not associated with a lower prevalence of depression. Notably, most of the B-vitamin supplements in Norway at the time of recruitment did not contain folic acid, or only in relatively low doses of 100 µg.

Associations between folate, vitamin B_{12}, and tHcy levels or the *MTHFR 677C→T* polymorphism and anxiety disorders have seldom been reported so far; this may be due to reporting or publication bias of negative findings. We found only a weak, nonsignificant relationship between plasma tHcy level and anxiety disorder, and no associations between any of the index variables and comorbid depression and anxiety disorder. Hence, our data suggest that impaired 1-carbon metabolism is related to the subgroup of depression without comorbid anxiety disorder. One possible explanation is that they have a different cause. Further subgrouping to obtain more specific categories, eg, unipolar vs bipolar depression and lifetime or recurrent depression, was not possible when HADS was used in this cross-sectional design. Such analyses of subgroups should be carried out in future studies.

In summary, results from this large population-based study suggest a role of impaired 1-carbon metabolism in depression without comorbid anxiety disorder. This conclusion is supported by increased risk among subjects with the *MTHFR T/T* genotype, which has an effect on folate distribution and thereby tHcy level. The observation that the *T/T* genotype confers increased risk suggests that altered B-vitamin status may be a risk factor for, rather than the result of, depression. However, our results are preliminary and there is a need for prospective studies. Furthermore, a dose-response relationship between B vitamins, metabolic markers, and clearly defined subgroups of depression should be investigated. More adequately sized double-blind randomized trials are warranted as well.

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REFERENCES

1. Refsum H, Ueland PM, Nygard O, Vollset SE. Homocysteine and cardiovascular disease. *Annu Rev Med.* 1998;49:31-62.
2. Homocysteine Studies Collaboration. Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA.* 2002;288:2015-2022.
3. Kim YI. Folate and cancer prevention: a new medical application of folate beyond hyperhomocysteinemia and neural tube defects. *Nutr Rev.* 1999;57:314-321.
4. Clarke R, Smith AD, Jobst KA, Refsum H, Sutton L, Ueland PM. Folate, vitamin B_{12}, and serum total homocysteine levels in confirmed Alzheimer disease. *Arch Neurol.* 1998;55:1449-1455.
5. Selhub J, Bagley LC, Miller J, Rosenberg IH. B vitamins, homocysteine, and neurocognitive function in the elderly. *Am J Clin Nutr.* 2000;71(suppl):614S-620S.
6. Scholl TO, Johnson WG. Folic acid: influence on the outcome of pregnancy. *Am J Clin Nutr.* 2000;71(5, suppl):1295S-1303S.
7. Nelen WL. Hyperhomocysteinaemia and human reproduction. *Clin Chem Lab Med.* 2001;39:758-763.
8. Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. Biological and clinical implications of the MTHFR C677T polymorphism. *Trends Pharmacol Sci.* 2001; 22:195-201.
9. Greenberg PE, Sisitsky T, Kessler RC, Finkelstein SN, Berndt ER, Davidson JR, Ballenger JC, Fyer AJ. The economic burden of anxiety disorders in the 1990s. *J Clin Psychiatry.* 1999;60:427-435.
10. Murray CJ, Lopez AD. Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. *Lancet.* 1997;349:1436-1442.
11. Fernstrom JD. Can nutrient supplements modify brain function? *Am J Clin Nutr.* 2000;71(6, suppl):1669S-1675S.
12. Petrie WM, Ban TA. Vitamins in psychiatry: do they have a role? *Drugs.* 1985; 30:58-65.
13. Bottiglieri T, Crellin R, Reynolds EH. Folate and neuropsychiatry. In: Bailey LB, ed. *Folate in Health and Disease.* New York, NY: Marcel Dekker; 1995:435-462.
14. Fava M, Borus JS, Alpert JE, Nierenberg AA, Rosenbaum JF, Bottiglieri T. Folate, vitamin B12, and homocysteine in major depressive disorder. *Am J Psychiatry.* 1997;154:426-428.
15. Bottiglieri T, Hyland K, Laundy M, Godfrey P, Carney MW, Toone BK, Reynolds EH. Folate deficiency, biopterin and monoamine metabolism in depression. *Psychol Med.* 1992;22:871-876.
16. Carney MW. Serum folate values in 423 psychiatric patients. *BMJ.* 1967;4:512-516.
17. Ghadirian A, Anath J, Engelsmann F. Folic acid deficiency and depression. *Psychosomatics.* 1980;21:926-929.
18. Reynolds EH, Preece JM, Bailey J, Coppen A. Folate deficiency in depressive illness. *Br J Psychiatry.* 1970;117:287-292.
19. Wilkinson AM, Anderson DN, Abou-Saleh MT, Wesson M, Blair JA, Farrar G, Leeming RJ. 5-Methyltetrahydrofolate level in the serum of depressed subjects and its relationship to the outcome of ECT. *J Affect Disord.* 1994;32:163-168.
20. Wesson VA, Levitt AJ, Joffe RT. Change in folate status with antidepressant treatment. *Psychiatry Res.* 1994;53:313-322.
21. Abou-Saleh MT, Coppen A. The biology of folate in depression: implications for nutritional hypotheses of the psychoses. *J Psychiatr Res.* 1986;20:91-101.
22. Levitt AJ, Joffe RT. Folate, B12, and life course of depressive illness. *Biol Psychiatry.* 1989;25:867-872.
23. Coppen A, Abou-Saleh MT. Plasma folate and affective morbidity during long-term lithium therapy. *Br J Psychiatry.* 1982;141:87-89.
24. Carney MW, Chary TK, Laundy M, Bottiglieri T, Chanarin I, Reynolds EH, Toone B. Red cell folate concentrations in psychiatric patients. *J Affect Disord.* 1990; 19:207-213.

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25. Abou-Saleh MT, Coppen A. Serum and red blood cell folate in depression. *Acta Psychiatr Scand.* 1989;80:78-82.
26. Coppen A, Bailey J. Enhancement of the antidepressant action of fluoxetine by folic acid: a randomised, placebo controlled trial. *J Affect Disord.* 2000;60:121-130.
27. Passeri M, Cucinotta D, Abate G, Senin U, Ventura A, Stramba Badiale M, Diana R, La Greca P, Le Grazie C. Oral 5′-methyltetrahydrofolic acid in senile organic mental disorders with depression: results of a double-blind multicenter study. *Aging.* 1993;5:63-71.
28. Coppen A, Chaudhry S, Swade C. Folic acid enhances lithium prophylaxis. *J Affect Disord.* 1986;10:9-13.
29. Godfrey PS, Toone BK, Carney MW, Flynn TG, Bottiglieri T, Laundy M, Chanarin I, Reynolds EH. Enhancement of recovery from psychiatric illness by methylfolate. *Lancet.* 1990;336:392-395.
30. Allen RH, Stabler SP, Savage DG, Lindenbaum J. Metabolic abnormalities in cobalamin (vitamin B12) and folate deficiency. *FASEB J.* 1993;7:1344-1353.
31. Lindenbaum J, Healton EB, Savage DG, Brust JC, Garrett TJ, Podell ER, Marcell PD, Stabler SP, Allen RH. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N Engl J Med.* 1988;318:1720-1728.
32. Penninx BW, Guralnik JM, Ferrucci L, Fried LP, Allen RH, Stabler SP. Vitamin B_{12} deficiency and depression in physically disabled older women: epidemiologic evidence from the Women's Health and Aging Study. *Am J Psychiatry.* 2000;157:715-721.
33. Baldewicz TT, Goodkin K, Blaney NT, Shor-Posner G, Kumar M, Wilkie FL, Baum MK, Eisdorfer C. Cobalamin level is related to self-reported and clinically rated mood and to syndromal depression in bereaved HIV-1$^+$ and HIV-1$^-$ homosexual men. *J Psychosom Res.* 2000;48:177-185.
34. Tiemeier H, Van Tuijl HR, Hofman A, Meijer J, Kiliaan AJ, Breteler MM. Vitamin B12, folate, and homocysteine in depression: the Rotterdam Study. *Am J Psychiatry.* 2002;159:2099-2101.
35. Bottiglieri T, Laundy M, Crellin R, Toone BK, Carney MW, Reynolds EH. Homocysteine, folate, methylation, and monoamine metabolism in depression. *J Neurol Neurosurg Psychiatry.* 2000;69:228-232.
36. Arinami T, Yamada N, Yamakawa-Kobayashi K, Hamaguchi H, Toru M. Methylenetetrahydrofolate reductase variant and schizophrenia/depression. *Am J Med Genet.* 1997;74:526-528.
37. Kunugi H, Fukuda R, Hattori M, Kato T, Tatsumi M, Sakai T, Hirose T, Nanko S. C677T polymorphism in methylenetetrahydrofolate reductase gene and psychoses. *Mol Psychiatry.* 1998;3:435-437.
38. van Praag HM. Depression. *Lancet.* 1982;2:1259-1283.
39. Bottiglieri T, Hyland K, Reynolds EH. The clinical potential of ademetionine (S-adenosylmethionine) in neurological disorders. *Drugs.* 1994;48:137-152.
40. Reynolds EH. Folic acid, ageing, depression, and dementia. *BMJ.* 2002;324:1512-1515.
41. Kessler RC, Nelson CB, McGonagle KA, Liu J, Swartz M, Blazer DG. Comorbidity of *DSM-III-R* major depressive disorder in the general population: results from the US National Comorbidity Survey. *Br J Psychiatry Suppl.* June 1996;(30):17-30.
42. Mezzich JE, Ahn CW, Fabrega HJ, Pilkonis PA. Patterns of psychiatric comorbidity in a large population presenting for care In: Maser JD, Cloninger CR, eds. *Comorbidity of Mood and Anxiety Disorders.* Washington, DC: American Psychiatric Press; 1990:189-204.
43. Sartorius N, Ustun TB, Lecrubier Y, Wittchen HU. Depression comorbid with anxiety: results from the WHO study on psychological disorders in primary health care. *Br J Psychiatry Suppl.* June 1996;(30):38-43.
44. Edeh J, Toone BK. Antiepileptic therapy, folate deficiency, and psychiatric morbidity: a general practice survey. *Epilepsia.* 1985;26:434-440.
45. Hermesh H, Weizman A, Shahar A, Munitz H. Vitamin B12 and folic acid serum levels in obsessive compulsive disorder. *Acta Psychiatr Scand.* 1988;78:8-10.
46. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand.* 1983;67:361-370.
47. Ueland PM, Nygard O, Vollset SE, Refsum H. The Hordaland Homocysteine Studies. *Lipids.* 2001;36(suppl):S33-S39.
48. Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale: an updated literature review. *J Psychosom Res.* 2002;52:69-77.
49. Fiskerstrand T, Refsum H, Kvalheim G, Ueland PM. Homocysteine and other thiols in plasma and urine: automated determination and sample stability. *Clin Chem.* 1993;39:263-271.
50. Molloy AM, Scott JM. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. *Methods Enzymol.* 1997;281:43-53.
51. Kelleher BP, Broin SD. Microbiological assay for vitamin B12 performed in 96-well microtitre plates. *J Clin Pathol.* 1991;44:592-595.
52. Ulvik A, Ueland PM. Single nucleotide polymorphism (SNP) genotyping in unprocessed whole blood and serum by real-time PCR: application to SNPs affecting homocysteine and folate metabolism. *Clin Chem.* 2001;47:2050-2053.
53. Vollset SE, Refsum H, Tverdal A, Nygard O, Nordrehaug JE, Tell GS, Ueland PM. Plasma total homocysteine and cardiovascular and noncardiovascular mortality: the Hordaland Homocysteine Study. *Am J Clin Nutr.* 2001;74:130-136.
54. Hastie T, Tibshirani R. *Generalized Additive Models.* London, England: Chapman & Hall; 1990.
55. Guttormsen AB, Ueland PM, Nesthus I, Nygard O, Schneede J, Vollset SE, Refsum H. Determinants and vitamin responsiveness of intermediate hyperhomocysteinemia ($\geq$40 micromol/liter): the Hordaland Homocysteine Study. *J Clin Invest.* 1996;98:2174-2183.
56. Alpert JE, Mischoulon D, Nierenberg AA, Fava M. Nutrition and depression: focus on folate. *Nutrition.* 2000;16:544-546.
57. Ocke MC, Schrijver J, Obermann-de Boer GL, Bloemberg BP, Haenen GR, Kromhout D. Stability of blood (pro)vitamins during four years of storage at –20 degrees C: consequences for epidemiologic research. *J Clin Epidemiol.* 1995;48:1077-1085.
58. Zittoun J, Zittoun R. Modern clinical testing strategies in cobalamin and folate deficiency. *Semin Hematol.* 1999;36:35-46.
59. Gunter EW, Bowman BA, Caudill SP, Twite DB, Adams MJ, Sampson EJ. Results of an international round robin for serum and whole-blood folate. *Clin Chem.* 1996;42:1689-1694.

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