HEALTH TECHNOLOGY ASSESSMENT

VOLUME 18 ISSUE 48 JULY 2014
ISSN 1366-5278

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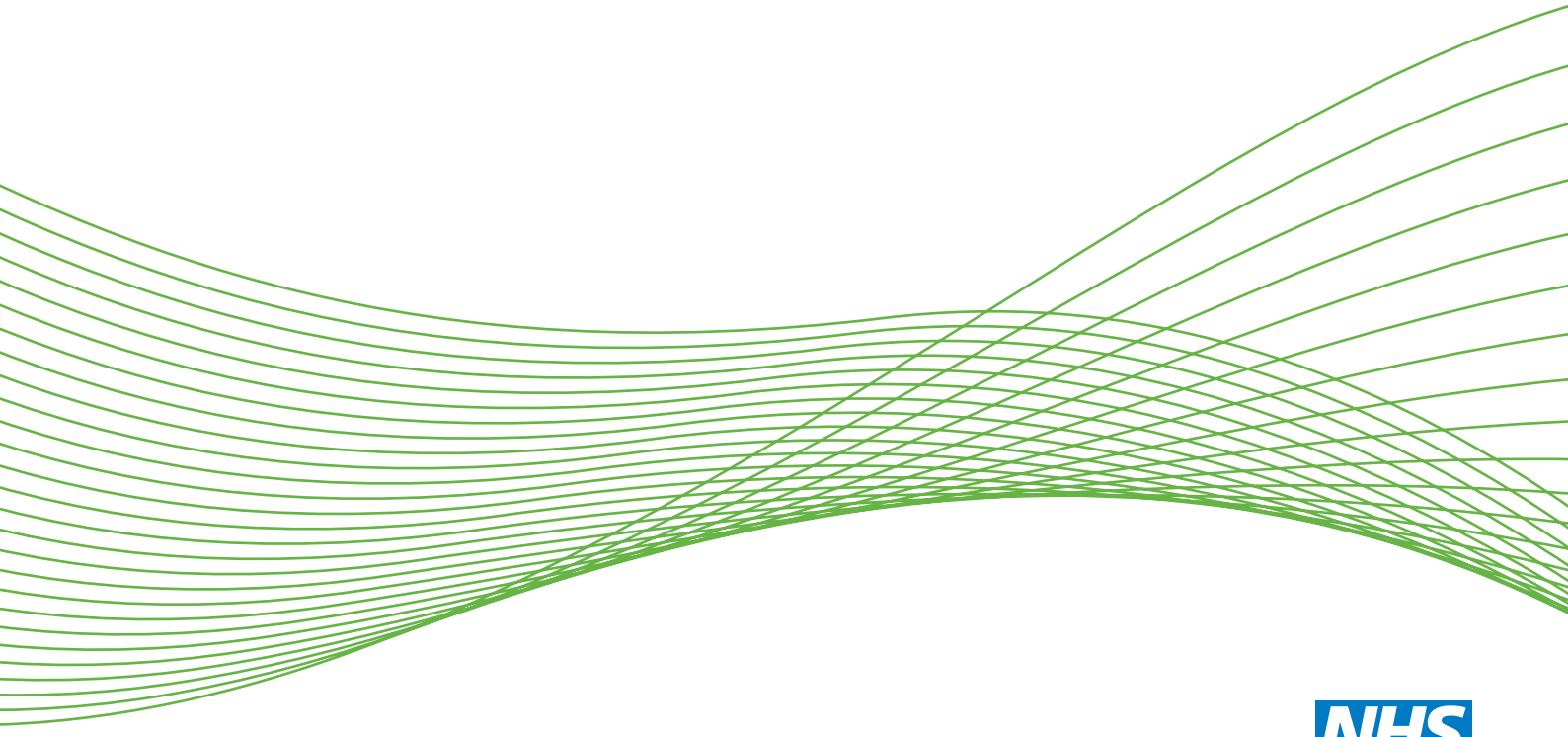

NHS
National Institute for Health Research

DOI 10.3310/hta18480

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Declared competing interests of authors: Andrew McCaddon is a scientific advisor and shareholder of COBALZ Ltd – a private limited company developing novel B vitamin and antioxidant supplements.

Published July 2014
DOI: 10.3310/hta18480

This report should be referenced as follows:

Bedson E, Bell D, Carr D, Carter B, Hughes D, Jorgensen A, *et al.* Folate Augmentation of Treatment – Evaluation for Depression (FolATED): randomised trial and economic evaluation. *Health Technol Assess* 2014;**18**(48).

Health Technology Assessment is indexed and abstracted in *Index Medicus*/MEDLINE, *Excerpta Medica*/EMBASE, *Science Citation Index Expanded* (SciSearch®) and *Current Contents®*/ Clinical Medicine.

Health Technology Assessment

HTA/HTA TAR

ISSN 1366-5278 (Print)

ISSN 2046-4924 (Online)

Impact factor: 5.116

Health Technology Assessment is indexed in MEDLINE, CINAHL, EMBASE, The Cochrane Library and the ISI Science Citation Index and is assessed for inclusion in the Database of Abstracts of Reviews of Effects.

This journal is a member of and subscribes to the principles of the Committee on Publication Ethics (COPE) (www.publicationethics.org/).

Editorial contact: nihredit@southampton.ac.uk

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This report

The research reported in this issue of the journal was funded by the HTA programme as project number 04/35/08. The contractual start date was in October 2006. The draft report began editorial review in May 2012 and was accepted for publication in August 2013. The authors have been wholly responsible for all data collection, analysis and interpretation, and for writing up their work. The HTA editors and publisher have tried to ensure the accuracy of the authors' report and would like to thank the reviewers for their constructive comments on the draft document. However, they do not accept liability for damages or losses arising from material published in this report.

This report presents independent research funded by the National Institute for Health Research (NIHR). The views and opinions expressed by authors in this publication are those of the authors and do not necessarily reflect those of the NHS, the NIHR, NETSCC, the HTA programme or the Department of Health. If there are verbatim quotations included in this publication the views and opinions expressed by the interviewees are those of the interviewees and do not necessarily reflect those of the authors, those of the NHS, the NIHR, NETSCC, the HTA programme or the Department of Health.

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Abstract

Folate Augmentation of Treatment – Evaluation for Depression (FolATED): randomised trial and economic evaluation

The FolATED trialists namely (in alphabetical order)
Emma Bedson,[1] Diana Bell,[2] Daniel Carr,[3] Ben Carter,[4]
Dyfrig Hughes,[5] Andrea Jorgensen,[6] Helen Lewis,[7]
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Background: Folate deficiency is associated with depression. Despite the biological plausibility of a causal link, the evidence that adding folate enhances antidepressant treatment is weak.

Objectives: (1) Estimate the clinical effectiveness and cost-effectiveness of folic acid as adjunct to antidepressant medication (ADM). (2) Explore whether baseline folate and homocysteine predict response to treatment. (3) Investigate whether response to treatment depends on genetic polymorphisms related to folate metabolism.

Design: FolATED (Folate Augmentation of Treatment – Evaluation for Depression) was a double-blind and placebo-controlled, but otherwise pragmatic, randomised trial including cost–utility analysis. To yield 80% power of detecting standardised difference on the Beck Depression Inventory version 2 (BDI-II) of 0.3 between groups (a 'small' effect), FolATED trialists sought to analyse 358 participants. To allow for an estimated loss of 21% of participants over three time points, we planned to randomise 453.

Settings: *Clinical* – Three centres in Wales – North East Wales, North West Wales and Swansea. *Trial management* – North Wales Organisation for Randomised Trials in Health in Bangor University. *Biochemical analysis* – University Hospital of Wales, Cardiff. *Genetic analysis* – University of Liverpool.

Participants: Four hundred and seventy-five adult patients presenting to primary or secondary care with confirmed moderate to severe depression for which they were taking or about to start ADM, and able to consent and complete assessments, but not (1) folate deficient, vitamin B_{12} deficient, or taking folic acid or anticonvulsants; (2) misusing drugs or alcohol, or suffering from psychosis, bipolar disorder, malignancy or other unstable or terminal illness; (3) (planning to become) pregnant; or (4) participating in other clinical research.

Interventions: Once a day for 12 weeks experimental participants added 5 mg of folic acid to their ADM, and control participants added an indistinguishable placebo. All participants followed pragmatic management plans initiated by a trial psychiatrist and maintained by their general medical practitioners.

Main outcome measures: Assessed at baseline, and 4, 12 and 25 weeks thereafter, and analysed by 'area under curve' (main); by analysis of covariance at each time point (secondary); and by multi-level repeated measures (sensitivity analysis): *Mental health* – BDI-II (primary), Clinical Global Impression (CGI), Montgomery–Åsberg Depression Rating Scale (MADRS), UKU side effects scale, and Mini International Neuropsychiatric Interview (MINI) suicidality subscale; *General health* – UK 12-item Short Form Health Survey (SF-12), European Quality of Life scale – 5 Dimensions (EQ-5D); *Biochemistry* – serum folate, B_{12}, homocysteine; *Adherence* – Morisky Questionnaire; *Economics* – resource use.

Results: Folic acid did not significantly improve any of these measures. For example it gained a mean of just 2.9 quality-adjusted life-days [95% confidence interval (CI) from –12.7 to 7.0 days] and saved a mean of just £48 (95% CI from –£292 to £389). In contrast it significantly reduced mental health scores on the SF-12 by 3.0% (95% CI from –5.2% to –0.8%).

Conclusions: The FolATED trial generated no evidence that folic acid was clinically effective or cost-effective in augmenting ADM. This negative finding is consistent with improving understanding of the one-carbon folate pathway suggesting that methylfolate is a better candidate for augmenting ADM. Hence the findings of FolATED undermine treatment guidelines that advocate folic acid for treating depression, and suggest future trials of methylfolate to augment ADM.

Trial registration: Current Controlled Trials ISRCTN37558856.

Funding: This project was funded by the NIHR Health Technology Assessment programme and will be published in full in *Health Technology Assessment*; Vol. 18, No. 48. See the HTA programme website for further project information.

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List of abbreviations

5-HT	5-hydroxytryptamine
5-MTHF	5-methyltetrahydrofolate
ADM	antidepressant medication
AE	adverse event
AHEAD	Assessing Health Economics of Anti-Depressants trial
AR	adverse reaction
AUC	area under the curve
BDI-II	Beck Depression Inventory (version 2)
BMI	body mass index
BNF	*British National Formulary*
bp	base pair
CGI	Clinical Global Impression
CI	confidence interval
CMHT	Community Mental Health Team
CNS	central nervous system
CRF	Case Report Form
CSF	cerebrospinal fluid
CTA	Clinical Trial Authorisation
CV	coefficient of variation
DMEC	Data Monitoring and Ethics Committee
EQ-5D	European Quality of life scale – 5 Dimensions
EQ-VAS	EQ-5D Visual Analogue Scale
FDR	false discovery rate
FolATED	Folate Augmentation of Treatment – Evaluation for Depression
GCP	Good Clinical Practice
GP	general medical practitioner
HADS	Hospital Anxiety and Depression Scale
HDRS	Hamilton Depression Rating Scale
HTA	Health Technology Assessment
ICD-10	*International Statistical Classification of Diseases & Related Health Problems* (10th Revision)
ICER	incremental cost-effectiveness ratio
IQR	interquartile range
MADRS	Montgomery–Åsberg Depression Rating Scale
MCAR	missing completely at random
MCS	Mental Component Score
MHRA	Medicines and Healthcare products Regulatory Agency
MHRN-Cymru	Mental Health Research Network for Wales
MINI	Mini International Neuropsychiatric Interview
MMA	methylmalonic acid
MRC	Medical Research Council
MREC	Multicentre Research Ethics Committee
MTHFR	methylenetetrahydrofolate reductase
MTR	methyltetrahydrofolate reductase
NICE	National Institute for Health and Care Excellence
NIHR	National Institute of Health Research
NISCHR	National Institute for Social Care and Health Research (Wales)
NISCHR CRC	NISCHR Clinical Research Centre
NWORTH	North Wales Organisation for Randomised Trials in Health
OLS	ordinary least squares
OR	odds ratio

PCS	physical component score
PHQ9	Patient Health Questionnaire – 9 items
PSS	Personal Social Services
QALY	quality-adjusted life-year
QOF	Quality & Outcomes Framework
RCF	red cell folate
RCT	randomised controlled trial
SAE	serious adverse event
SAR	serious adverse reaction
SD	standard deviation
SF-6D	UK Short Form Health Survey – 6 Dimensions
SF-12	UK 12-item Short Form Health Survey
SNP	single nucleotide polymorphism
SNRI	selective noradrenaline reuptake inhibitor
SOP	standard operating procedure
SSRI	selective serotonin reuptake inhibitor
SUSAR	suspected unexpected serious adverse reaction
TCA	tricyclic antidepressant
TSC	Trial Steering Committee
WHO	World Health Organization
WONCA	World Organization of National Colleges & Associations of Family Doctors

Plain English summary

Depression is common and serious. Only half of sufferers respond well to antidepressants. There is reason to hope that folic acid, which helps mothers and babies in pregnancy, will help. We conducted the clinical trial known as FolATED to test whether adding folic acid to antidepressants makes them work better and also gives good value for money. We also studied genetic and other scientific aspects of depression.

We aimed to recruit 450 adults from across Wales with confirmed moderate or severe depression for which they were taking or about to start antidepressants, but without other serious illness. Our target was for 360 (80%) of them to complete carefully designed questionnaires about their mental health on three occasions over 6 months. We actually recruited 475, and analysed 440 (93%) of them. Once a day for 12 weeks these participants added an extra pill to their antidepressants. For half of them, chosen at random, this pill contained 5 mg of folic acid. For the other half this pill looked the same but did not contain any folic acid. Only one person knew who had which pill.

Unfortunately the reported health of those who received active pills did not improve any more than the health of those who took inactive pills. So there is now no reason to believe that folic acid strengthens antidepressants. Fortunately recent research suggests that methylfolate may be better at this. So FolATED has undermined guidelines that advocate folic acid for depression, but suggested another way forward.

Scientific summary

Introduction

Depression is a prevalent and debilitating mental disorder. It often persists or recurs throughout life. Guidelines recommend antidepressants for moderate to severe depression, but only half of sufferers respond to initial treatment. Research is necessary to investigate ways of augmenting antidepressants to improve this.

Folic acid may enhance antidepressant treatment for three reasons:

1. Patients with depression often have folate deficiency.
2. Folate deficits correlate with severity of depression and poor response to treatment.
3. Folate is needed to synthesise neurotransmitters linked to depression.

Aim and objectives

The relevant Cochrane review recommended large randomised trials to investigate the therapeutic potential of folate augmentation of antidepressants. The National Institute of Health Research (NIHR) programme commissioned FolATED (Folate Augmentation of Treatment – Evaluation for Depression) to address this gap in knowledge. Our main objectives were to assess the clinical effectiveness and cost-effectiveness of adding folic acid to antidepressant treatment of moderate to severe depression. Our secondary objectives were to investigate whether baseline folate and homocysteine predict response to treatment, and whether response to treatment depends on genetic polymorphisms related to folate metabolism.

Design

FolATED was a double-blind, placebo-controlled, yet pragmatic, randomised trial. To yield 80% power at 5% significance level of detecting a 'small' effect size of 0.3 – equivalent to a difference between groups of three points on the Beck Depression Inventory version 2 (BDI-II) – we sought to analyse 358 participants. To allow for losses across three assessments, we aimed to randomise 453. We exceeded both targets. We undertook cost–utility analysis from the perspective of the health and personal social services. We also extracted genomic DNA from blood provided by each participant to test whether polymorphisms change the effectiveness of folic acid combined with antidepressants.

Settings

Clinical Three centres in Wales: North East Wales, North West Wales and Swansea, Swansea, UK.

Trial management, including telephone randomisation The Registered Clinical Trials Unit in Bangor University, Bangor, UK.

Biochemical analysis University Hospital of Wales, Cardiff, UK.

Genetic analysis University of Liverpool, Liverpool, UK.

Participants

Patients over 18 years old presenting to primary or secondary care with confirmed moderate to severe depression for which they were taking or about to start antidepressant medication (ADM), and able to consent and complete assessments, but not:

a. folate deficient, vitamin B_{12} deficient, or taking folic acid or anticonvulsants
b. misusing drugs or alcohol; or suffering from psychosis, bipolar disorder, malignancy or other unstable or terminal illness; or
c. pregnant or planning to become pregnant.

Interventions

All participants followed pragmatic management plans initiated by a trial psychiatrist and maintained by their general practitioners. Once a day for 12 weeks participants in the experimental group added 5 mg of folic acid to their antidepressants, and those in the control group added an indistinguishable placebo; neither group knew whether their adjunct was folate or placebo.

Main outcome measures

Assessed at baseline ('week 0'), and 4, 12 and 25 weeks thereafter:

Mental health BDI-II (primary), Clinical Global Impression (CGI), Montgomery–Åsberg Depression Rating Scale (MADRS), UKU side effects scale, and Mini International Neuropsychiatric Interview (MINI) suicidality subscale.

General health UK 12-item Short Form Health Survey (SF-12; both mental and physical components), European Quality of life scale – 5 Dimensions (EQ-5D).

Haematology Serum folate, B_{12}, homocysteine.

Compliance Morisky Questionnaire.

Resource use Client Service Receipt Inventory.

Results

We recruited participants in three centres – North East Wales, North West Wales and Swansea – between July 2007 and November 2010, and completed follow-up in May 2011. The trial received 1488 referrals; screened 863, of whom 635 consented to take part; and randomised 479, of whom 475 were valid. Of 156 consenters not later randomised, 68 dropped out between screening and randomisation, and 36 reported better BDI-II scores at randomisation interview. Of 237 randomised to folic acid, eight withdrew within 4 weeks and six never attended appointments; of 238 randomised to placebo, 10 withdrew and 11 never attended. We analysed the remaining 440 (93% of the 475 valid randomisations), if necessary by statistically imputing missing data.

Clinical effectiveness

The main analysis focused on the 'area under the curve' (AUC) of each of the 13 main outcomes – BDI-II (primary), MADRS, CGI (three scales), EQ-5D (two scores), SF-12 (two scales) and UKU (four scales) – adjusted for stratification variables and the baseline score of that variable. The only significant result favoured the

placebo in the SF-12 Mental Component Score (MCS). Five of the non-significant differences favoured placebo and seven favoured folate.

The 33 adverse events (AEs) reported in the folic acid arm did not differ significantly from the 45 reported in the placebo arm. We adjudged six of those in the folic acid arm to be serious, compared with 14 in the placebo arm – another difference not statistically significant. We classified four of the AEs reported in the intervention arm as adverse reactions because folic acid (if prescribed) was a possible cause, in comparison with three in the control arm – also not statistically significant. Fortunately none of these reactions was serious or unexpected.

We assessed adherence to trial medication at 12 weeks in four ways: we found no significant differences in scores on the Morisky Questionnaire or in the number of returned pills; we found that 83% of those taking folic acid achieved adherence defined as a serum folate greater than 15 mg/ml, and 60% achieved adherence defined as reduction of at least 15% in serum homocysteine between baseline and 12 weeks.

To test the sensitivity of our main analyses to the assumption that AUC is a valid summary of the various outcome measures over 6 months, we repeated them in the form of repeated measures analyses of variance. We also applied this technique to serum folate, red cell folate, homocysteine and serum B_{12}. These last four analyses summarise a wide range of biochemical predictors of folate metabolism. By analysing and reporting interactions between 'treatment allocated' and 'time', they show that added folic acid has statistically very significant effects on serum folate and homocysteine; a marginal but not significant effect on red cell folate; but no independent effect on B_{12}.

Cost-effectiveness

There were no differences in resource use or resulting costs between treatment groups. The largest component was for psychiatric services (£797 in the folic acid group and £886 in the placebo group). Costs differed more in the 3 months before baseline: £514 in the folic acid group compared with £746 in the placebo group. As this difference may reflect an imbalance in patient or disease characteristics, our primary cost analysis used regression to adjust for these baseline differences.

In responding to the EQ-5D at baseline, most patients described themselves as having difficulty with anxiety or depression (97% in both groups), pain or discomfort (about 60% in both), and usual activities (about 78%). Between baseline and 6 months those reporting anxiety or depression fell to 75% in both groups, and difficulty with usual activities to about 58%. The resulting utilities rose from 0.482 at baseline to 0.605 at 6 months in the folate group, and 0.514 to 0.607 in the placebo. After adjusting for differences at baseline, we found no significant differences between treatment groups in quality-adjusted life-years (QALYs) gained – as estimated by EQ-5D (primary analysis), EQ-VAS or SF-12 via Short Form Health Survey – 6 Dimensions (SF-6D); or in outcomes for the cost-effectiveness analyses – notably area under the BDI-II curve. Thus folic acid seems no more effective, but no more expensive, than placebo.

Biochemistry

Despite the lack of clinical response to folic acid, it was effective in increasing participants' folate and in reducing homocysteine. Nevertheless the few patients who had very low baseline red cell folate yielded weak but consistent evidence across multiple instruments that augmenting antidepressants with folic acid improved clinical outcome. However biochemical variables predicted only one of the eight clinical outcomes – the CGI improvement scale.

Genetics

We analysed associations between 104 relevant single nucleotide polymorphisms (SNPs) and each of seven outcome measures – BDI-II, MADRS, CGI severity, EQ-5D, EQ-VAS, SF-12 mental and SF-12 physical. We found two statistically significant main effects. The rs11627525 SNP in the methylenetetrahydrofolate dehydrogenase 1 (*MTHFD1*) gene was associated with MADRS scores [false discovery rate (FDR) = 4.67%; significance level $p = 0.04$] but none of the other six outcome measures analysed. The rs588458 SNP in the

folate hydrolase 1 (*FOLH1*) gene was associated with EQ-5D utilities (FDR = 3.37%; $p = 0.03$) but none of the other measures. We also found one statistically significant SNP-treatment interaction – that between the rs17102596 SNP in the methionine adenosyltransferase 1 alpha (*MAT1A*) gene and folic acid influenced SF-12 mental status (FDR = 2.55%; $p = 0.02$).

Discussion

Summary

Clinical effectiveness

FoLATED shows that routinely adding 5 mg of folic acid to ADM has no clinical benefit. This finding is very consistent across outcome measures and time points. The one exception is the MCS of the SF-12, which shows a statistically significant difference favouring placebo, especially at 12 weeks. However there were no significant differences in reported side effects or (serious) AEs between groups.

Cost-effectiveness

No economic criterion was significant: folic acid saved only £48 per patient; folic acid gained only three EQ-5D-adjusted days per patient, while EQ-VAS showed a very small loss. We conclude that folic acid is not cost-effective.

Biochemistry

Folic acid was effective in increasing participants' folate. However biochemical variables predicted only one of the eight chosen clinical outcomes.

Genetics

FolATED identified only two polymorphisms within genes of the one-carbon folate pathway associated with clinical outcome regardless of treatment, and one such polymorphism associated with the outcome of folic acid. However these polymorphisms could not replicate these associations with any other outcome measure, including the primary outcome. Furthermore one polymorphism associated with depression in previous studies did not modify the effect of either antidepressant therapy or folic acid supplementation. We judge this consistent with the trial finding that folic acid does not influence the treatment of depression. In future a whole-genome approach to the FolATED data could identify markers of efficacy beyond those already analysed.

Strengths and limitations of FolATED

FolATED is by far the largest trial to evaluate folic acid in augmenting ADM. We powered it to detect a clinically small difference between treatment groups, and followed rigorous procedures for randomisation and blinding. We recruited a wide range of patients treated for moderate or severe depression in primary or secondary care. There were few exclusion criteria, and our sample included comorbidities like substance misuse, often excluded from less pragmatic trials.

Of our sample 36% achieved 'response to treatment', namely 50% reduction in BDI-II baseline score; 27% achieved 'remission' at 6 months, namely BDI-II score less than 13; and ADM reduced depressive symptoms markedly over the first few weeks, then more slowly until 6 months. All these findings were expected from a study mixing new and continuing treatment episodes. Hence the lack of any effect of folic acid is not attributable to unusual treatment resistance to antidepressants in our sample. In short FolATED was both robust and representative.

Interpretation

Clinical effectiveness

Our negative outcomes contrast with positive findings in smaller trials. For example Coppen and Bailey reported a significantly greater reduction in Hamilton Depression Rating Scale (HDRS) scores with fluoxetine and 400 mg of folic acid than with fluoxetine and placebo, but only in females (Coppen A, Bailey J. Enhancement of the antidepressant action of fluoxetine by folic acid: randomised, placebo controlled trial. *J Affect Disord* 2000;**60**:121–30.). However their sample was much less representative than that recruited by FolATED. Furthermore, while FolATED took care at all stages to avoid unblinding researchers, Coppen and Bailey seemed less rigorous, especially in handling blood results.

Biochemical interpretation

Folate is a naturally occurring B vitamin, needed in the brain to synthesise serotonin, noradrenaline and dopamine. In humans the biologically active form is methylfolate – derived from ingested folates, taken up by cells and transported to the cerebrospinal fluid (CSF) via folate receptors. Folic acid is an inactive form of folate not naturally found in the human body, which needs transformation to methylfolate. There is evidence that commercial preparations of folic acid can compete with methylfolate for folate receptors, thus exacerbating folate deficiency in the central nervous system (CNS). Hence our finding that folic acid had a statistically significant negative effect on the widely used SF-12 MCS may reflect folate deficiency rather than type 1 error.

Thus better understanding of the one-carbon folate pathway has raised questions about the most appropriate formulation of folate to use for folate deficiency. Stahl argues that methylfolate is therapeutically better than folic acid as it does not need transformation, which may be difficult for some patients (Stahl SM. Novel therapeutics for depression: L-methylfolate as a trimonamine modulator and antidepressant-augmenting agent. *CNS Spectr* 2007;**12**:739–44). Furthermore high doses of inactive folic acid may compete with methylfolate for transport across the blood–brain barrier.

Against this biomedical background our rigorous and powerful trial has established that folic acid has no general role as adjunct in antidepressant therapy. However studies in patients with cardiovascular disease have shown that higher doses of folic acid produce greater concentrations of methylfolate in plasma. Moreover the reductions in homocysteine in participants on folic acid relative to those on placebo suggests that they were successfully metabolising folic acid to methylfolate. Nevertheless we suspect that the beneficial increase in methylfolate was masked by excess folic acid that competed for the folate receptors and led to negative results. Before we dismiss all folates, however, we recommend an updated systematic review and meta-analysis, ideally at patient level, of the many trials of folate augmentation.

At the time of our initial proposal little information was available on the use of methylfolate in patients with depression. Now there is evidence that methylfolate given as adjunct or monotherapy reduces depressive symptoms in patients with low folate levels or alcoholism, and improves cognitive function and depressive symptoms in elderly patients with dementia and folate deficiency. Furthermore there are long-standing concerns that folate may increase cancer risk, mask B_{12} deficiency and exacerbate depressive symptoms. As methylfolate may reduce some of these risks, it may now be a candidate for a large multi-centre trial. In that context we offer the design of FolATED as a proven model. In hindsight, however, we judge that a trial recruiting for 1 year in 10 centres would yield better value for money than one recruiting for 3 years in three centres.

Conclusions

This rigorous and powerful trial has established that folic acid is not an effective adjunct to antidepressant therapy.

The NIHR commissioned FolATED at a time when there was considerable scientific interest in the role of folate in causing and treating depression. Since then this interest has grown, with increasing international pressure to use folate as an adjunct to antidepressants and in algorithms for treating depression. The unequivocally negative findings of FolATED demand reappraisal of this consensus and associated treatment guidelines.

There is a strong case for appraising whether future trials of methylfolate would yield value for money.

Trial registration

This trial is registered as ISRCTN37558856.

Funding

Funding for this study was provided by the Health Technology Assessment programme of the National Institute for Health Research.

Chapter 1 Introduction

Background

Prevalence of depression

Depression is one of the main mental health disorders presenting in primary care.[1] The prevalence of major depression in the general population ranges from 3% to 10% with more than 150 million people at a time suffering from depression across the world.[2] In the UK prevalence of depression in 2009–10 was 11% in England, 11.5% in Northern Ireland, 8.6% in Scotland, and 7.9% in Wales.[3] Unipolar depression leads to 12.15% of years lived with disability, and is ranked as the third leading contributor to the global burden of diseases.[2] Indeed depression is currently the second cause of disability worldwide for males and females between the ages of 15 and 44 years and is predicted to reach second place for all ages by 2020.[4] Depression has become the leading cause of disability in Europe, leading to a loss of one in every 10 healthy years of life, and the leading cause of early retirement.[5] Depression and stress are now the commonest reported causes for sickness absence from work in the UK[6] with over 100 million working days lost across Europe at a cost of 1% Gross Domestic Product (GDP).[7] A higher prevalence of depression is observed in women than men across the 18- to 64-year age range with women up to 2.5 times more likely to develop depression.[8]

Characteristics of depression

The core symptoms of depression are low mood and loss of interest or enjoyment in usually pleasurable activities. Associated symptoms include disturbances to sleep and appetite, reduced energy and concentration, negative thoughts of guilt or worthlessness and suicidal ideation. The *International Statistical Classification of Diseases & Related Health Problems* (ICD-10) states that for a diagnosis of depression at least five symptoms need to be present, including at least one of the core symptoms, at an intensity that causes functional impairment and for a minimum duration of 2 weeks. Depression is classified as mild, moderate or severe according to the number of symptoms present and degree of functional impairment, and the grading of severity is of direct relevance to the treatment approaches recommended.[9] Depression is associated with increased mortality linked to suicide, alcohol and drug misuse, and increased rates of cardiovascular disease.[10] Depression thus burdens individuals, families, the NHS, and the national economy.[11] One UK study estimated the total cost of depression to the UK in 2000 at £9 billion; at that time, before the introduction of Improving Access to Psychological Therapies (also known as IAPT) in NHS England, the direct cost of treatment, mainly antidepressant medication (ADM), was £370 million; and indirect costs of 110 million working days lost to depression accounted for the vast majority of the total cost.[12] The sub-optimal treatment of depressive disorders is therefore of great public health concern.

Treatments for depression

In accordance with the joint report of the World Health Organization (WHO) and World Organization of National Colleges & Associations of Family Doctors (WONCA)[1] and the National Institute for Health and Care Excellence (NICE) guidance[9] the majority of people with depression are identified, treated and managed within primary care. Treatment aims to relieve symptoms, restore functioning and, in the long term, prevent relapse. The goal of treatment is complete remission which is associated with better functioning and reduced risk of relapse.[13] While there is some evidence that people with depressive symptoms improve over time without treatment,[14,15] a significant proportion follow a chronic course with significant levels of depressive symptoms and functional continuing for several years.[16,17]

Antidepressants are recommended as a treatment option for moderate to severe depression either in combination with psychotherapy[9] or as monotherapy.[18] Owing to their greater tolerability selective serotonin reuptake inhibitors (SSRIs) are recommended as first-line treatment in primary care.[9] Patients treated with an SSRI are seven times more likely to complete a therapeutic course than those treated with

tricyclic antidepressants (TCAs).[19] In clinical trials of antidepressants typically 50% of patients with depression respond to active treatment, while one-third respond to placebo[20] with the placebo response appearing to increase over time in clinical trials.[21] With first-line treatments about one-third of patients achieve remission from depression, increasing to two-thirds with refinement of treatment.[22] A study of mental disorders in 14 centres worldwide found that 50% of patients continued to have a diagnosis of depression after 1 year[23] with at least 10% having persistent or chronic depression.[24] Furthermore, at least 50% of people will go on to have at least one further episode of depression following their first episode of major depression.[25] The risk of further recurrences after second and third episodes rises to 70% and 90% respectively.[25] Cumulative rates of recurrence remain linear over long periods of follow-up (30–40 years), indicating a constant risk of recurrence over the lifespan.[26] Therefore recurrence rates increase with length of follow-up, and for the majority of patients depression is a recurrent condition.

Depression is a prevalent global health problem resulting in high levels of disability. While effective treatments are available outcomes remain sub-optimal with a significant proportion of patients failing to achieve remission and experiencing chronic illness, early relapse and multiple recurrences across the lifespan. There remains a pressing need for research to optimise outcomes from antidepressant treatment.

Review of the literature

Depression and folate

Over recent years there has been a growing interest and an increasing body of evidence exploring the relationship between B vitamins, in particular folate, and depression.[27–30] Folate is a naturally occurring B vitamin and can be found in leafy green vegetables, fruits, dried beans and peas.[31] Folic acid is the synthetic form of folate, which is inexpensive and found in supplements and fortified food.[31,32]

There is evidence to suggest that low folate intake is associated with symptoms of depression.[33–36] Studies report that up to one-third of patients with depressive illness have decreased serum and red cell folate levels.[37] Many people with depression have lower concentrations of folate than people with other psychiatric disorders or no psychiatric disorder.[34,38,39] Associations between folate deficiency and depressive symptoms, symptom severity and treatment outcomes in adults and the older adult population have been reported.[38,40–44] Low folate intake may also increase the risk of recurrent depression[33] and depression in later life.[43] Gilbody and colleagues conducted a systematic review of observational epidemiological studies investigating the relationship between low folate status and depression.[45] They concluded that low folate status was associated with depression but could not conclude that that was a causal relationship. Though low folate may result from poor nutrition or socio-economic disadvantage, confounders are common in chronic mental illness. Other recent evidence suggests that low folate may be a consequence rather than a cause of depressive symptoms.[46] While there is weak evidence that increased folate intake may prevent depressive symptoms,[35,47] this is not a consistent finding.[48] In one randomised controlled trial (RCT), for example, giving folate (2 mg) with vitamins B_{12} and B_6 did not reduce severity of depressive symptoms.[49]

The role of homocysteine

Folate is absorbed and transported in the blood in the form of 5-methyltetrahydrofolate (5-MTHF)[50] and is measured in the blood as either serum folate or red cell folate.[50]

Homocysteine is a highly sensitive marker of folate status[51] and functional folate deficiency is indicated by elevated homocysteine. Tiemeier *et al.* found a significant relationship between depression and hyperhomocysteinemia, folate and B_{12} deficiency.[52] Observational studies indicate that patients with depression have increased plasma total homocysteine concentrations.[53,54] A recent meta-analysis showed that older adults with a high total homocysteine concentration have an increased risk of depression [odds ratio (OR) = 1.70; 95% confidence interval (CI) from 1.38 to 2.08].[55]

There are important relationships between folic acid and vitamin B_{12} that have implications for how folate can be used therapeutically. For people who are deficient in vitamin B_{12}, exposure to high levels of folate can result in subacute combined degeneration of the spinal cord, which may be linked to impaired methionine biosynthesis.[56] As methylmalonyl CoA mutase, a vitamin B_{12}-dependent enzyme, converts methylmalonyl-CoA to succinyl-CoA, blood methylmalonic acid (MMA) levels increase with suboptimal B_{12} status. The cross-sectional study of > 10,000 participants in the US National Health and Nutrition Examination Survey (NHANES) observed a novel relationship between MMA and folate:[57] in patients with low B_{12} (< 148 pmol/l ≈ 200 ng/l) MMA increased significantly with increasing serum folate. This finding is due, either to adverse oxidative effects of unmetabolised folic acid on B_{12} homeostasis, or inability of patients with low B_{12} to retain intracellular folate.[58]

Antidepressant treatment and folate

Folate is an essential cofactor for the biosynthesis of both serotonin (5-hydroxytryptamine or 5-HT) and noradrenaline. Thus folate deficiency leads to impaired serotonin synthesis in the human brain.[59] This may provide a theoretical model for claims that folate can play a role in the treatment and prevention of depression.[60] Virtually all antidepressants are thought to act by prolonging the activity of serotonin or noradrenaline in neurotransmission or by modulating monoamine receptor sensitivity.[61] Lower folate levels have been associated with poorer antidepressant response.[62] Further evidence suggests that baseline levels of folate within the normal range predict antidepressant response.[63]

This raises the possibility of folate being used to augment antidepressants. Initial small feasibility studies seemed to confirm the potential of this augmentation strategy.[32] A Cochrane review and meta-analysis[50,64] explored the role of folate augmentation in depression. Only two RCTs were identified (combined $n = 151$), both of which suggest possible beneficial effects of folate augmentation.[65,66] Two further small trials have given contradictory results. One study, [67] with a clinical sample of 42 patients with major depressive disorder, reported that 10 mg of escitalopram (Cipralex®, Lundbeck) alone produced greater improvement than the combination of escitalopram and folic acid (2.5 mg/day). The other study, [68] with a clinical sample of 27, reported a greater reduction in depressive symptoms when 20 mg of fluoxetine (non-proprietary) was augmented with folic acid (10 mg/day) than when augmented with placebo. However, emerging evidence within an older depressed population suggests there may be no benefit from folate augmentation of antidepressants.[69] Despite this negative finding in the elderly there is still interest in a potential augmentation role for the B vitamins in general, with the currently recruiting B-VITAGE trial exploring B vitamin supplementation in later life.[70] These data convey mixed messages about the use of folic acid to improve ADM. To advance this debate about the clinical effectiveness of folic acid, we plan to update the Cochrane systematic review to include this and other recently reported studies of augmentation by folic acid.[50]

Folate, depression and genetics

There has recently been much research aimed at identifying genetic aspects of depression and antidepressant therapy. Indeed, a number of large genome-wide association studies, and some subsequent meta-analyses, have identified genetic polymorphisms associated with both risk of depression[71–73] and response to antidepressant therapy.[74,75] However, these studies have been unable to demonstrate consistent and reproducible genetic associations.

Only a few studies have described an association between risk of depression and genetic variation of genes of the one-carbon folate and methionine biosynthesis pathways. Most have focused on, and identified, an association with depression and the frequently characterised c.677C > T polymorphism (rs1801133) of the methyltetrahydrofolate reductase (MTR) gene.[76,77] This variant encodes a valine to alanine amino acid substitution at residue 222. The variant protein has reduced catalytic activity and thermolability and is associated with elevated homocysteine levels under conditions of impaired folate status. Others, in addition to MTR c.677C > T, have also described the p.D919G variant (rs1805087) of the MTR gene as a statistically significant risk factor for moderate and severe depression in postmenopausal women.[78] The MTR gene encodes the protein methyltetrahydrofolate reductase, which is a key enzyme in the biosynthesis of homocysteine to methionine.

Furthermore, it has recently been reported that the MTR c.677C > T polymorphism modifies the protective effect of folic acid against depression after pregnancy.[79] Others have demonstrated an association between c.677C > T and folate and homocysteine concentrations.[80] These observations further support the hypothesis that genetic variation in the one-carbon folate pathway may affect folic acid efficacy as an adjuvant to antidepressant therapy by altering folate bioavailability and increasing homocysteine levels.

Summary

Depression is a prevalent and debilitating mental health disorder. It often follows a chronic or recurrent course across the lifespan. Antidepressants are the recommended treatment for moderate to severe depression. Only half of people will respond to first-line treatment, and only one-third will achieve remission. Further research is needed to investigate ways of augmenting antidepressants to boost treatment response and rates of remission.

Evidence suggests that folic acid may be a useful adjunct to antidepressant treatment for four reasons:

1. Patients with depression often have a functional folate deficiency.
2. The severity of deficiency, indicated by elevated homocysteine, correlates with depression severity.
3. Low folate is associated with poor antidepressant response.
4. Folate is required for the synthesis of neurotransmitters in the pathogenesis and treatment of depression.

Objectives

The Cochrane review by Taylor and colleagues concluded that adequately powered randomised trials were needed to investigate the therapeutic potential of folate augmentation of antidepressants.[50] The National Institute of Health Research Health Technology Assessment (NIHR HTA) programme commissioned FolATED to address this gap in the literature.

The main objectives of FolATED were to assess the clinical effectiveness and cost-effectiveness of adding folic acid to the antidepressant treatment of moderate to severe depression. Our secondary objectives were to explore whether baseline folate and homocysteine predict response to treatment, and investigate whether response to treatment depends on genetic polymorphisms related to folate metabolism.

Chapter 2 Methods

Trial design

FolATED was a three-centre, double-blind and placebo-controlled, but otherwise pragmatic, randomised trial of folic acid augmentation of antidepressant treatment for people with moderate to severe depression. Participants were allocated to folic acid or matching placebo in equal proportions. Assessment took place at weeks –2 (to screen for eligibility and initiate ADM if needed); –1 (to check by telephone for tolerability of antidepressants); 0 (baseline – to randomise to folate or placebo); and 4, 12 and 25 weeks (to assess outcomes). *Figure 1* shows the flow diagram for the trial.[81,82]

Participants

Settings

We recruited participants from primary and secondary care at three centres across Wales between July 2007 and November 2010. The sites were North East Wales, North West Wales and Swansea; and covered a population of about 1.35 million people in 2009.[83] We screened potential participants referred by their primary or secondary care clinicians or themselves for eligibility – in a variety of settings including general practice, secondary mental health services, research clinics, and patients' homes.

Informed consent

All potential participants received a copy of the information sheet and consent form (see *Appendix 1*) from their referring clinician or the research team at least 24 hours before screening to ensure they had time to consider the study. Trial psychiatrists or screening nurses checked that eligible patients fully understood the study and gave them the opportunity to ask questions. To all potential participants we stressed that taking part in the study was voluntary and that their clinical care would not change if they did not want to take part in the trial.

Inclusion and exclusion criteria

Trial psychiatrists could assess eligibility of any potential participant. For self-referred participants registered mental health nurses liaising with a trial psychiatrist could also assess eligibility.

Potential participants were eligible to take part in the trial if they met all these criteria:

1. presenting with moderate to severe depressive symptoms confirmed by a trial psychiatrist during the screening interview and reporting a score of at least 19 on the Beck Depression Inventory version 2 (BDI-II) at screening, and at least 17 at baseline[84]
2. being treated with ADM, or about to commence ADM treatment
3. aged at least 18 years
4. able to give informed consent, and
5. able to complete the research assessments.

We excluded potential participants from the trial if they:

a. were folate deficient
b. were B_{12} deficient
c. had taken supplements containing folic acid within 2 months
d. suffered from psychosis
e. suffered from bipolar disorder
f. were participating in other clinical research

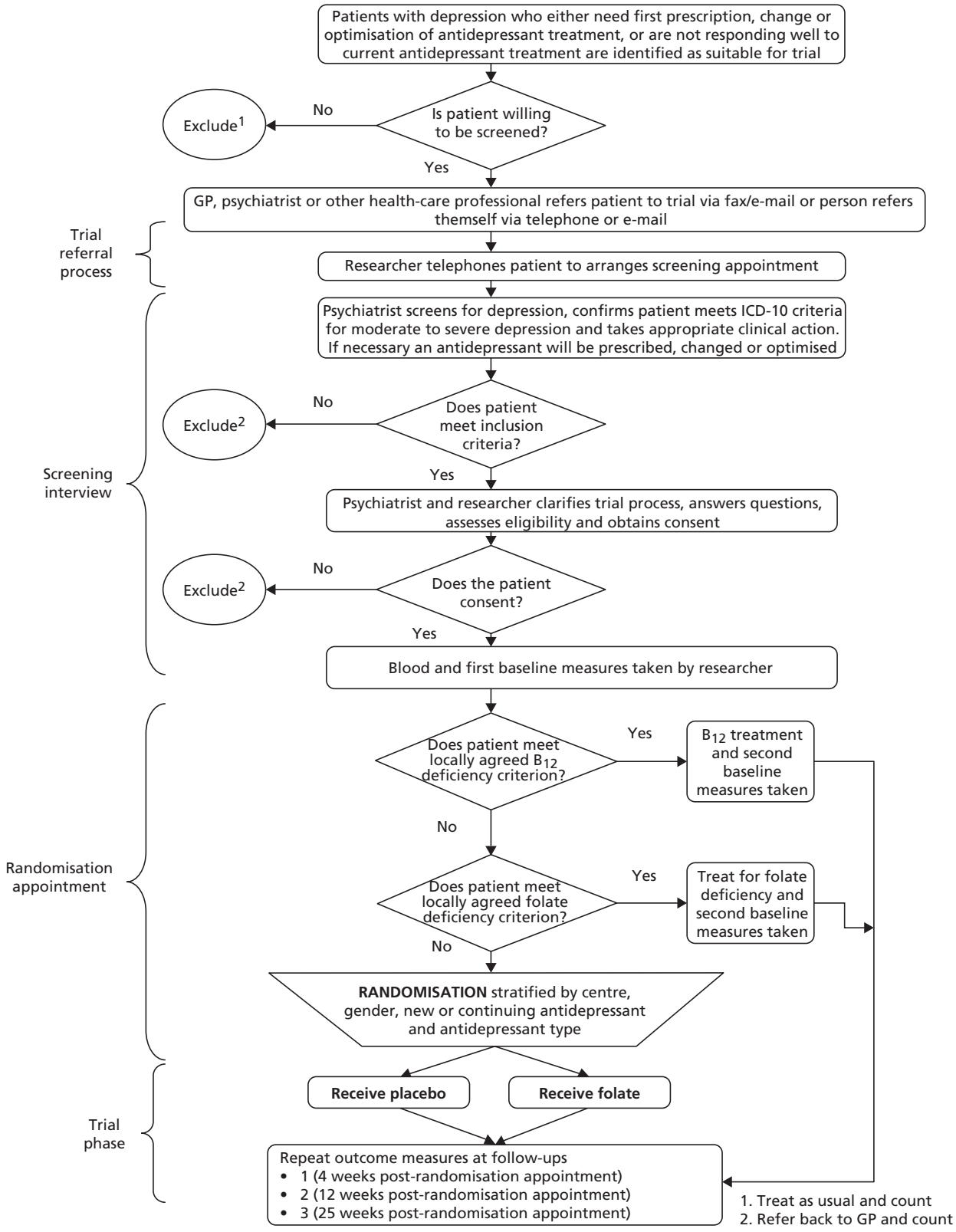

FIGURE 1 Flow diagram of the trial.

g. were pregnant or planning to become pregnant
h. were taking anticonvulsants
i. had a serious, advanced or terminal illness with a life expectancy of less than 1 year
j. had recently started treatment for a medical condition that had not yet been stabilised, or
k. had a diagnosis of, or treatment for, any malignant disease or related condition like intestinal polyposis.

Sample size

We originally powered FolATED to detect a difference between the two treatment groups of three points on the BDI-II at 25 weeks, judging that a clinically important difference. As we estimated the standard deviation (SD) of BDI-II scores in the trial population at 10.7, our protocol proposed a completed sample size of 400 at 25 weeks to yield 80% power to detect this difference using a significance level of 5%. As interim analysis of baseline BDI-II scores showed that their SD was about 10, we revised the target completed sample size to 358 at 25 weeks. The original protocol allowed 10% loss at each of the three follow-up assessments, thus requiring to randomise 549 to achieve 400 completers at 25 weeks. As interim analysis also showed that retention at 25 weeks was 79% rather than the 73% expected, the new target of 358 completers needed a randomised sample of 453.

Randomisation

At screening the screener took a blood sample to assess B_{12} and folate status, and arranged a further appointment within 14 days to confirm the B_{12} and folate results. We excluded participants who were B_{12} or folate deficient from the main trial but offered them the opportunity to continue in the 'comprehensive cohort' of recruited patients.

Eligible participants completed the baseline assessments and the recruiting centre telephoned the randomisation centre at NWORTH, Bangor University. NWORTH used dynamic allocation to protect against subversion while ensuring that each arm of the trial was balanced for the stratification variables. For each participant the adaptive algorithm recalculated the likelihood of their allocation between treatment groups from the distribution of stratification variables among participants already recruited and allocated. This process keeps the balance between strata within acceptable limits of the target allocation ratio of 1 : 1 while maintaining unpredictability.[85] The selected stratification variables were:

1. centre (North East Wales, North West Wales or Swansea)
2. sex (male or female)
3. timing of ADM (new or continuing)
4. type of ADM (SSRI or other)
5. whether participant had received counselling for depression (ever or never).

Intervention

By the time participants entered the trial, we ensured they were receiving antidepressant treatment optimised to therapeutic dosages – equivalent to SSRI of at least 20 mg per day or TCA of at least 150 mg per day. Most had received an antidepressant prescription from their general practitioner (GP) before referral to the trial. For patients not on ADM, trial psychiatrists initiated ADM to meet clinical need and patient preference in accordance with routine practice. For patients on sub-therapeutic ADM, trial psychiatrists optimised the treatment regime according to the *British National Formulary* (BNF),[86] namely citalopram dose of at least 20 mg per day or equivalent. For participants who had been receiving a therapeutic dose of ADM, we encouraged trial psychiatrists to optimise dose according to the BNF, for example by increasing citalopram dosage to 40 mg per day, or change the antidepressant, again depending on clinical need and patient preference.

Participants received a 12-week supply of either 5 mg folic acid or placebo in addition to their ADM. We selected the 5-mg dose of folic acid because that is effective for other indications,[87] carries a low risk of adverse events (AEs), and is routinely used to treat folate deficiency.[31]

Bilcare (formerly DHP Ltd) supplied the trial drugs and achieved the blinding needed by the trial by the process of over-encapsulation. They placed each tablet inside a size '1' opaque hard gelatin capsule and added lactose BP, an ingredient of the tablet, to fill the capsule. To produce placebo for the trial they filled the same capsules with lactose. They packed capsules into high-density polyethylene bottles with tamper-evident child-resistant screw caps. They tested to confirm that the over-encapsulated tablet complied with the British Pharmacopoeia[88] standard for disintegration in vitro. They also checked each batch for the presence or absence of 5 mg folic acid.

Blinding

The North Wales Organisation for Randomised Trials in Health coded the identically packaged folic acid and placebo randomly for each stratification group. Each patient's prescription indicated his or her trial number and package serial number generated by NWORTH, thus determining the appropriate trial package. NWORTH and the local pharmacies held the key to the randomisation codes. The telephone numbers of those pharmacies were available to break codes in emergency.

This ensured that throughout recruitment treatment allocations were unknown to participants, healthcare professionals, investigators, and researchers. We broke randomisation codes for two participants. One was diagnosed with lower oesophageal cancer, and the other collapsed with agitation, breathing difficulty, raised pulse, and reduced consciousness. On both occasions the local pharmacist revealed the code from a scratch card. This ensured that we revealed only the individual allocation, only to those who needed to know.

An independent GP monitored blood results, notably to check for B_{12} and folate deficiency at follow-up. To avoid accidental unblinding researchers engaged in clinical data collection or analysis did not have access to these blood results. Furthermore we separated pharmacogenetic and biochemistry analysis from clinical effectiveness analysis, and combined these results only when analyses were complete. Formal unblinding of the randomisation codes took place at the joint final meeting of the Trial Steering Committee (TSC) and the Data Monitoring and Ethics Committee (DMEC) on 10 October 2011.

Data collection

We collected data at screening, baseline and randomisation, and 4, 12 and 25 weeks after randomisation, using the trial case report forms (CRFs). Though we aimed to collect the data on the day due, this was not always possible. Hence there was a window for each data collection (*Table 1*).

We designed some questionnaires for completion by researchers or clinicians, and others by participants. The preferred mode was face to face. When that was not possible, we permitted completion over the telephone and mailed the questionnaire to the participant in advance.

TABLE 1 Permissible windows for data collection

Data collection	Due date (days since randomisation)	Window
Screening for eligibility	–14 days	± 10 days
Randomisation	0	Origin
4-week follow-up	28 days	+ 14 days
12-week follow-up	84 days	± 14 days
25-week follow-up	175 days	± 28 days

Primary outcome measure

The main outcome measure was self-rated symptom severity as measured by the Beck Depression Inventory version 2 (BDI-II).[84] The BDI-II consists of 21 items, each rated on a four-point scale ranging from 0 to 3; a total score of 1–13 indicates no depression, 14–18 mild depression, 19–28 moderate depression, and 29–63 severe depression. As BDI-II scores at 25 weeks are useful in assessing participants' medium-term recovery, that was the basis of our original power calculation (see *Sample size*, above). When updating that calculation, we also designated the primary outcome as the area under the curve ('AUC' for short) of mean BDI-II scores between randomisation and the 25-week follow-up, because this summarises participants' recovery across the whole of that period.[84] Though there was less prior information on AUCs, notably on clinically important differences, we judged that the combination of well-behaved BDI-II scores and the mathematically robust trapezium method of estimating AUCs[89] would make 358 an appropriate target sample size (see *Sample size*, above). In the event we were able to analyse many more than 358 trial participants. Because the actual follow-up time could vary by up to 4 weeks from the target of 25 weeks (see *Table 1*), we converted the area under the curve to a more meaningful 'AUC average', which represents a participant's BDI-II (or other outcome) score averaged over that participant's follow-up period.

Secondary outcome measures

Symptom severity

Clinicians rated symptom severity using the Montgomery–Åsberg Depression Rating Scale (MADRS) and the Clinical Global Impression (CGI) of change at baseline and 4, 12 and 25 weeks. The MADRS[90] consists of 10 items each rated on a seven-point scale (0–6), which yield a total score between 0 and 60. The CGI[91] comprises three separate clinician-rated items: an ordinal scale of current severity of illness between 1 and 7; an ordinal scale of global improvement since recruitment between 1 and 7; and an efficacy index ranging from 0.25 to 4.0 derived from a 4×4 matrix plotting therapeutic effect against side effects.

Researchers at all centres received training in completing the rated scales (MADRS and CGI) from standard training videos. To estimate inter-rater reliability, we collated researchers' ratings of these videos on several occasions.

Health status

Participants reported their mental and physical health by version 2 of the UK 12-item Short Form Health Survey (SF-12) and their quality of life by the EQ-5D, both at baseline, 4, 12 and 25 weeks. The SF-12 is a functional measure of quality of life comprising 10 five-point items and two three-point items.[92] Using scoring algorithms designed to achieve standardisation to a mean of 50 and a SD of 10 in the 1998 general US population, these items yield separate physical and mental component scores (PCS-12 and MCS-12). Though we used EQ-5D as a secondary measure of clinical effectiveness, its main purpose was to measure health utility for economic analysis.[93]

Proportion of participants with moderate depression

Though we adopted the standard definition of moderate depression as a BDI-II score of 19 or more, we estimated the proportion of participants with moderate depression by statistical inference from the observed distribution of BDI-II scores. This technique is more robust to random variation than mere counting.

Adverse events and side effects

Though we asked centres to report all AEs, we focused on serious adverse events (SAEs) including inpatient admissions, attempted or completed suicide, and other mortality. We asked centre principal investigators to assess whether folic acid could possibly have caused each SAE, and whether it was an expected consequence. The chief investigators reviewed these data blind to the random allocations.

We assessed side effects through the UKU side effects scale,[94] which sums scores on 48 distinct side effects – 10 'psychic', 8 'neurologic', 11 'autonomic' and 19 other – and adds two global items.

It rates each item on a four-point scale ranging from 0 to 3, yielding system-specific scores out of 30, 24, 33 and 57, two global scores out of three, and a total score out of 150.

Adherence to the trial drug

We assessed adherence to the trial medication from dispensing records; returned tablet count at 12 weeks; folate and homocysteine levels at 12 weeks; and the Morisky Questionnaire[95] administered only at 12 weeks. This instrument asks four binary questions about adherence, and reports the number of positive responses as a score between 0 and 4.

Folate status and B_{12} status

We measured red cell folate at baseline; and serum folate, homocysteine and B_{12} from blood samples collected at baseline, 12 and 25 weeks. We sent all of those samples to local NHS laboratories on the day of collection. For homocysteine analysis we centrifuged venous blood within 30 minutes of collection and stored the plasma at –20 °C until analysis. We assayed all samples from individual participants in the same batch to minimise the effect of inter-batch variation. We measured plasma total homocysteine using a one-step immunoassay following reduction with dithiothreitol, commercially available from the Abbott Diagnostics ARCHITECT system. The average intra-batch coefficient of variation (CV) for homocysteine was less than 3%.

Suicidality

We rated suicidality by Section C of the Mini International Neuropsychiatric Interview (MINI)[96] at baseline and 4, 12 and 25 weeks. Summing the scores allocated to 'yes' responses yields a total score between 0 and 33, which the MINI criteria categorise into low risk (0–5), moderate risk (6–9) or high risk (≥ 10).

Other data

We collected basic demographic information for each participant including sex, age, ethnicity, employment status, marital status and number of dependent children. We also recorded smoking and alcohol consumption, which are known to affect homocysteine levels.

Follow-up

Thus we thoroughly assessed participants at 4, 12 and 25 weeks after randomisation. Antidepressants show a delayed and variable onset of clinical improvements in depression.[97–99] Previous trials suggest that 50% of those who eventually respond to ADM start to respond within 2 weeks, 75% within 4 weeks, and almost all within 6 weeks.[100] Hence we scheduled the first assessment at week 4, 6 weeks after the start or optimisation of antidepressant treatment. Non-response at 4 weeks may lead to changes in the ADM in accordance with the BNF and NICE guidelines. Hence the second assessment at 12 weeks could measure both continuing and late responses to ADM and folate augmentation. The third assessment at 25 weeks addressed any changes in effectiveness after the end of folic acid therapy, but during ADM, since that is the minimum duration of maintenance antidepressant treatment.[18]

Quality assurance

The conduct of this trial followed the principles of good clinical practice (GCP) outlined by the ICH-GCP and complied with EU directive 2001/20/EC.[101] The research also adhered to the Medical Research Council (MRC) guidelines for clinical trials[102–104] and the Research Governance Frameworks for England and Wales.[105–107] In particular we anonymised all research data and stored them securely. All research team members received general training in GCP and trial-specific training in the protocol, recruiting participants, taking blood, completing CRFs, conducting assessments, and reporting AEs. We also developed a fieldworker's manual to maintain consistency between sites.

Independent Trial monitoring

We established a TSC and a DMEC to oversee FolATED through biannual meetings or telephone conferences. The TSC comprised an independent chair, three independent members, and five members of the FolATED trial management team. The DMEC comprised an independent chair and two independent members, with the trial statistician in attendance (see *Appendix 2*).

Approvals

The Multicentre Research Ethics Committee (MREC) for Wales gave initial ethical approval on 6 November 2006, and the Medicines and Healthcare products Regulatory Agency (MHRA) issued the Clinical Trial Authorisation (CTA) on 21 December 2006. *Appendix 3* lists the dates of approvals for individual centres.

Summary of changes to the project protocol

Appendix 4 lists all substantial changes to the protocol approved by TSC, DMEC, MHRA, MREC, and primary and secondary care R&D Departments.

Statistical methods

Statistical analysis plan

Before starting analysis we developed our analysis plan for approval by the DMEC (see *Appendix 5*).

Trial populations

'Analysed' population

Randomisation allocated all participants to one of the two treatments. The CONSORT guidelines require that the main analysis be 'by treatment allocated'. Ideally, therefore, this population should comprise all randomised participants. In practice only participants who contributed at least one BDI-II measured after baseline can usefully contribute. To get the most from their data, we used established methods to impute their missing data.

Complete case population

This population comprises only those participants whose outcome data are complete. It provides a sensitivity analysis of two issues: whether primary and secondary findings are sensitive to the absence of missing data, and the methods we used to impute those missing data.

'Randomised' population

At first sight it is difficult to draw inferences about this population because some contributed no data after baseline, even on the BDI-II. Because we know the baseline characteristics of all these participants, however, it is possible to reweight the 'analysed population' so that they match the characteristics of the randomised population, notably allocated treatment, stratifying variables and baseline BDI-II.

Imputation of missing data for 'analysis by treatment allocated'

We excluded participants without follow-up data from the primary analysis 'by treatment allocated'.
For each variable we summarised missing data by reason (mainly participant withdrew; questionnaire not returned; page missing; item missing). Where < 10% of data were missing, we treated them as if they were missing completely at random (MCAR).[108] If > 10% of data were missing, we explored the missing data and tabulated them by the stratification variables both as reported at randomisation and as validated after quality assurance; by participant demographics; and by other important covariates. Rather than exclude participants missing some data, we chose to impute these data (see *Appendix 5*).

Missing items within a subscale

For missing items within a subscale we took account of methodological publications about the instrument. To impute missing items we used the principle that, if < 25% of the items within a subscale were missing for a participant at a time point, one should impute them by the weighted mean of the completed items, but if > 50% of the items within a subscale were missing for a participant at a time point, one should treat that subscale as wholly missing and impute it accordingly.

Missing subscales

Where between 25% and 50% of the items within a subscale were missing, we proceeded thus: if < 40% of the subscales for a participant at a time point were missing, we imputed all missing subscales by a single application of the general regression model for missing data imputation used in SPSS (Statistical Product and Service Solutions, SPSS Inc., Chicago, IL, USA),[109] taking account of all validated stratification variables. If > 40% of the subscales for a participant at a time point were missing, but < 20% of participants experienced that problem, we imputed all missing subscales by a single multivariate imputation across all time points that also took account of all validated stratification variables. Fortunately these rules covered the whole of FolATED.

Missing time points

If one of the four time points for a participant was missing, we imputed all subscales within that time point by five iterations of the repeated-measures model for missing data imputation used in SPSS using all other subscales at all time points together with age, gender, centre and group.[107]

Data description and transformation

Initially we summarised data by allocated treatment and centre. Rather than test for statistical differences between allocated groups at baseline, we adjusted for any imbalance by analysis of covariance. Our analysis plan assumed that residual variation from our statistical models follows Normal distributions. This is a robust assumption in the sense that only a substantial deviation would invalidate each analysis. So we plotted and reviewed residual distributions. As none of these was substantial, we did not need to transform data to improve consistency with the assumption of Normality. Hence we present all data as collected.

Methods for analysing outcomes

All of our statistical tests were two-sided with a significance level of 5%.

Continuous outcomes with baseline and more than one follow-up

We used the AUC average, not only to summarise treatment outcome across the whole of the 25 weeks of data collection, but also to take account of the correlation between successive measurements for the same participant. We calculated the AUC average by using the trapezium rule[89,110] to weight the outcome scores at baseline and the three actual follow-up points. From the imputed data set we estimated the average score for each participant over his or her total follow-up period as the area under the EQ-5D utility curve divided by the duration of follow-up, using the trapezoidal rule specified by the formula:

$$U_{\text{av}} = \sum_{j=0}^{2} \left[\left(\frac{(U_{\text{j}} + U_{\text{j}+1})}{2} \right) \times \left(\frac{(t_{\text{j}+1} - t_{\text{j}})}{\text{T}} \right) \right] \quad (1)$$

where U_{j} is the utility attributed to the jth measurement, T is the duration in days of the participant's study period, t_{j} is the time in days at which the jth measurement takes place for that participant,[111] and values j = 0, 1, 2 and 3 correspond to the baseline and three subsequent follow-ups respectively. We used similar formulae to calculate AUC averages for BDI-II, MADRS and SF-12 physical and mental component scores.

As covariates in these analyses we used the validated stratification variables – centre, sex, new or continuing case, type of antidepressant and previous counselling. For the individual time points, which contribute to and illustrate the AUC, we used analysis of covariance to adjust for the corresponding baseline score.

Continuous outcome with no baseline and only one follow-up (Morisky scale)

We used analysis of covariance with baseline depression scores and validated stratification variables as covariates, to test whether medication adherence, measured on the Morisky scale, differs significantly

between the two treatment arms. If so, we would have added the Morisky score and ADMs recorded by GPs to the usual covariates.

Dichotomous outcomes (serious adverse events and adverse events)

We used logistic regression of (S)AE compared with no (S)AE over each participant's time in the trial to test whether the proportion of (S)AEs differs between treatment arms, using baseline scores and validated stratification variables as covariates. We transformed all estimated fixed effects back from their logistic form and summarised them by OR, standard error, 95% CI, and significance level.

Covariates to be adjusted within the statistical model

We kept baseline depression scores and validated stratification variables as covariates throughout. We also explored covariates of potential scientific relevance, including demographic (notably age, ethnicity, marital status, number of dependants and employment status, coded in accordance with usual demographic practice) and clinical (e.g. referral source, smoking, alcohol consumption and medication adherence, measured by both Morisky scale and recorded prescriptions). We fitted and retained these if they showed evidence of an effect at a significance level of 10%.

Interactions to be tested

Within each analysis we tested for interaction between treatment and centre, not least because of substantial differences in psychiatric practice and recruitment policy. On finding no evidence of interaction we estimated the treatment effect for each centre. We also tested for interactions between treatment and significant covariates.

Deviations from protocol

During the trial there were two protocol deviations that resulted in systematic missing data – one within a centre at one time point, the other within a single instrument early in the trial. First, early in the trial 13 participants in one centre did not receive appointments for visits at 4 weeks as the centre was under pressure from a large number of referrals; fortunately preventive action prevented any recurrence. Second, early in the trial 83 participants completed an incorrect version of the MADRS instrument: 40 at screening; 29 at randomisation; eight at 4 weeks; and six at 12 weeks. As both were administrative errors balanced between treatment groups, however, sensitivity analysis suggested that neither resulted in systematic bias. We therefore invoked our standard missing data procedures (see *Imputation of missing data for 'analysis by treatment allocated'*, above).

Sensitivity analyses

We applied three main sensitivity analyses – to the BDI-II as primary outcome in the first instance, with the intention of applying them to other outcome measures if the BDI-II proved sensitive to alternative assumptions. First we used 'complete case' analysis to test the sensitivity of findings to the absence of missing data; and the methods we used to impute those missing data. Secondly we used multi-level modelling with the same covariates, also known as repeated measures analysis of variance, to test the sensitivity of findings to our choice of AUC as main method of analysis; we estimated parameters for three fixed factors – the three time points (4, 12 and 25 weeks), centre and treatment group. Finally we reweighted the 'analysed population' to match the characteristics of the 'randomised population', and test the sensitivity of findings to non-response. To do so, we matched the participants completely lost to follow-up to participants from the analysed population. First we linked them by allocated treatment, centre and gender. Then we used a hierarchical cluster analysis to identify the best set of variables to match the non-responders to members of the responding trial population – age, marital status, reported alcohol intake, and BDI-II at screening and at baseline. We conducted this procedure both on raw data and on imputed data.

Biochemical analyses

The first of our secondary objectives was to explore whether baseline folate and homocysteine predict response to treatment – the difference between baseline and follow-up. We followed participants at

12 weeks, as they completed the trial medication, and at 25 weeks, the usual endpoint of antidepressant trials. Though many of our analyses of effectiveness use simple linear regression, this is less well suited to analyse topics where multicollinearity, that is multiple correlation, plays a major role. Instead we use repeated measures analysis of variance, which examines all four time points (i.e. baseline and 4, 12 and 25 weeks) simultaneously by fitting all four measures and adjusting for stratification variables, baseline measurements, biochemistry, demography and other covariates.

Health economics methods

Introduction

There are no economic evaluations of folic acid in managing depression. If shown to be effective, however, folic acid could represent a highly cost-effective treatment option. As it costs only 3 pence a day, the main cost drivers are likely to be hospital admissions, use of health and personal social services, ADM, and other aspects of care which might change following therapeutic benefit.

The aim of the economic analysis was therefore to assess whether the addition of 5 mg folic acid, once daily for 12 weeks, in new or existing users of antidepressants, represents a cost-effective use of healthcare resources. We limited this analysis to trial-generated estimates of costs and benefits, without modelling wider effects.

Perspective

In line with the NICE reference case,[112] we adopted the costing perspective of the National Health Service (NHS) and Personal Social Services (PSS). We estimated all costs in 2009–10 prices.

Data sources

Resource use

We derived participants' use of services from:

1. self-completed questionnaires
2. GPs' records of prescribed medications, and
3. our register of serious adverse events (SAEs), specifically for hospital admissions.

We based our resource use questionnaire on that used in the Assessing Health Economics of Anti-Depressants (AHEAD) trial of the cost-effectiveness of tricyclic antidepressants, selective serotonin re-uptake inhibitors and lofepramine.[113] It comprises four sections, relating to patients' use of general practice and generic community nursing services, social services, psychiatric hospital and community services, and other health services, notably hospital admissions and attendances, including at Emergency Departments (*Table 2*). Research professionals completed the questionnaire by asking participants to recall their use of these services for the 3 months before the baseline visit, and 12 and 25 weeks thereafter.

We sought details of participants' prescribed medicines, over the 25 weeks they were in the trial, from their GPs. Two pharmacy technicians compiled a database of prescription data, normally supplied as printouts or screen dumps, and a pharmacist checked it for accuracy. We also checked data on hospital admissions, obtained directly from participants, against our SAE register.

Unit costs

The costs of the intervention were: folic acid 5 mg – 84 tablets costing £2.67;[114] dispensing fee equal to NHS average of £3.03;[115] and serum vitamin B_{12} testing from the NHS reference costs for biochemistry.[116]

We derived the unit costs of other resources from standard sources (see *Table 2*). We took drug costs from the *Prescription Cost Analysis*,[117] which derives products' net ingredient costs (i.e. excluding discounts and

TABLE 2 Average cost of units of healthcare resources

Item	Unit	Cost	Comments and assumptions	Reference
General practice and community pharmacy and nursing services				
GP consultation	Visit	£36	11.7 minutes/consultation, including direct care staff costs and qualification costs	119
GP home visit	Visit	£120	23.4 minutes/visit, including travel, direct care staff costs and qualification costs	119
GP telephone contact	Call	£22	7.1 minutes/call, including direct care staff costs and qualification costs	119
Practice nurse at surgery	Visit	£12	15.5 minutes/consultation, including qualification costs	119
District nurse at home	Visit	£27	20 minutes/visit, including qualification costs	119
Counsellor at surgery	Visit	£71	96.6 minutes/consultation	119
Health visitor	Visit	£37	20 minutes/visit	119
Vitamin B_{12} test	Test	£1.29	NHS reference cost code DAP841	116
Pharmacy dispensing fee	Prescription item	£3.03	Average NHS cost/item dispensed, assuming all prescribed items dispensed	115
Social services				
Social worker (home or office)	Visit	£213	1 hour face-to-face contact	119
Home help	Contact	£75	3 hours/week of local authority home care	119
Care assistant	Contact	£214	10 hours/week of local authority community care	119
Day centre	Day	£36	Based on community care package	119
Psychiatric hospital and community services				
Consultant psychiatrist at hospital	Visit	£205	NHS reference cost code PS25B	116
Consultant psychiatrist at home	Visit	£328	Cost/hour of patient contact, including qualification costs	119
Clinical psychologist	Visit	£81	Cost/hour of client contact	119
Community psychiatric nurse	Visit	£56	Cost/per hour of client contact	119
Other services				
Day hospital	Day	£99	NHS reference cost code DCF41	116
Emergency Department	Visit	£116	NHS reference cost code 180	116
Hospital clinic	Visit	£199	NHS reference cost code 430	116
Mental health inpatient stay	Night	£302	NHS reference cost code MHIPA2	116
Occupational health services	Visit	£46	Hospital occupational therapist	119
NHS Direct	Contact	£21.37	Cost/nurse adviser contact	120
Ambulance or paramedic	Contact	£246	NHS reference cost code PS25A	116

dispensing costs) from actual NHS expenditure.[118] We took the cost of pharmacy dispensing from a report commissioned by the Department of Health to estimate the cost of providing community pharmacies.[115] We retrieved the costs of healthcare professionals' time from the *Unit Costs of Health and Social Care 2010*.[119] These include salaries and expenses, costs of training and qualifications, and capital and overhead costs. We took hospital costs from the NHS reference costs,[117] which underpin the calculation of the tariff for 'payment by results' in England.

Health outcomes

The primary measure of health outcome for economic analysis was the quality-adjusted life-year (QALY), estimated from the EQ-5D questionnaire administered at baseline and 4, 12 and 25 weeks. This assesses health-related quality of life on five dimensions – mobility, self-care, usual activities, pain-discomfort and anxiety-depression. The three possible responses on each dimension are 'no problems', 'moderate problems' and 'extreme problems'. We converted participants' responses into a single, preference-based utility using on the UK tariff.[121]

Secondary measures of health outcome for economic analysis included the EQ-VAS, the UK Short Form Health Survey – 6 Dimensions (SF-6D) and BDI-II, all completed at the same times as the EQ-5D. The EQ-VAS is a vertical 20-cm visual analogue scale for recording participants' rating of their current health-related quality of life. The SF-6D derives an alternative preference-based utility from SF-12 responses using weights estimated from a sample of the general population by the standard gamble technique.[122]

For the cost-effectiveness analysis, we calculated the number of weeks free from moderate or severe depression (defined as a BDI-II score < 13)[123] by statistical inference from the observed distribution of BDI-II scores, assuming linear interpolation between time points (baseline, 4, 12 and 25 weeks).

Data analysis

We combined data on costs and outcomes in the 'treatment allocated' population (see *Statistical methods, Imputation of missing date for analysis by treatment allocated, Missing items within a subscale*, above) over 25 weeks to estimate an incremental cost-effectiveness ratio (ICER) for comparison against accepted thresholds. Primary analysis was on the data set in which we had imputed missing data in the manner described above (see *Statistical methods, Trial populations*).

Analysis of costs

We estimated costs over 25 weeks for each participant by aggregating across resource categories. To draw inferences from this skewed distribution, we used 'bootstrapping', that is resampling with replacement and re-estimating sample means for each replicate. We used 10,000 replicates, corrected for bias and skewness by the technique known as 'bias correction and acceleration', and generated 95% CIs. We inferred whether differences in mean costs between treatment and control groups were statistically significant from those bootstrapped CIs.[124] To adjust for differences at baseline and in duration of follow-up, we used the regression model:[125]

$$\mathrm{Ln}(Cost_i) = \beta_0 + \beta_1 g_i + \beta_2 \ln(C_i) + \beta_3 T_i \qquad (2)$$

where patient i in treatment group g_i has a pre-baseline cost of C_i and a time between randomisation and final follow up of T_i and β_1 represents the difference in costs after adjusting for imbalance in mean costs before baseline. We used the logarithmic transformation to address the natural skewness of costs, and transformed the results of the regression back to recover the differential cost. As there were essentially no differences in demographic variables between groups, we did not need to adjust for any other variable.

Analysis of health outcomes

While the AUC average U_{av} is the measure analysed in effectiveness tables (see *Methods for analysing outcomes: Continuous outcomes with baseline and more than one follow-up* above), economic analysis uses QALY: the area under the utility curve over the participant's follow-up period in years. Hence:

$$\text{QALY} = U_{av} \times \left(\frac{T}{365}\right) \tag{3}$$

where U_{av} is the participant's AUC average utility and T is the duration in days of the participant's study period. However, as this period may vary from 21 to 29 weeks, we adjusted QALYs, like costs, for duration as well as baseline.

To adjust QALYs for differences in baseline utility and duration of follow-up, we used the regression model:[126]

$$\text{QALY}_i = \beta_0 + \beta_1 g_i + \beta_2 B_i + \beta_3 T_i \tag{4}$$

where patient i in treatment group g_i has baseline utility of B_i [equal to U_0 in equation (1) above] and time between randomisation and final follow-up of T_i; and β_1 represents the difference in QALYs after adjusting for imbalance in mean utility at baseline. As again there were essentially no differences in demographic variables between the two groups, we did not need to adjust for any other variable. We applied the same procedure to other measures of health outcome.

For all economic measures of health outcome we used 10,000 replicates to generate non-parametric bootstrapped 95% CIs, again corrected for bias and skewness, for the differences in means between treatment and control groups.

Cost–utility analysis

Comparing two treatments results in one of four scenarios. The intervention 'dominates' if it saves costs and improves health outcomes. The intervention 'is dominated' if it increases costs and outcomes deteriorate. More commonly the intervention improves outcomes at greater cost, or saves costs at the expense of outcomes. Then one must estimate an incremental cost-effectiveness ratio (ICER) by dividing the difference in adjusted mean costs (ΔC) by the difference in adjusted mean benefits (ΔB). NICE is more likely to recommend an intervention for use by the NHS if the ICER falls below the threshold for cost-effectiveness, which ranges from £20,000 to £30,000 per QALY.[112]

We used non-parametric bootstrapping to map the joint distribution of costs and outcomes on the cost-effectiveness plane and generate cost-effectiveness acceptability curves to show the probability that the intervention was cost-effective across a range of thresholds of cost-effectiveness.

Sensitivity analysis

To examine the extent to which the ICERs are sensitive to basic assumptions, we used two alternative approaches to measuring utility – the EQ-VAS and the SF-6D, and restricted analysis to all participants who gave complete EQ-5D responses. We used R software[111] for all analyses.

Genetic methods

Our aim was to test whether genetic polymorphisms affect the efficacy of folic acid in combination with ADM, with a view to using them as predictive markers of adjuvant folic acid efficacy. There is strong evidence to suggests that folic acid can play a role in the treatment and prevention of depression.[60] It is the effect of genetic variability on this role that we aim to investigate. This study focuses on variability in genes encoding proteins and enzymes implicated in the carbon folate and methionine synthesis pathways,

rather on genome-wide analysis.[127] We justify this approach by the folic acid intervention in this study and the weight of evidence to suggest decreased folate is associated with depressive illness.[41,77,128]

Given the level of complexity of the one-carbon folate pathway,[127] the genetic characteristics of FolATED trial participants span a comprehensive set of folate pathway genes beyond the commonly analysed methylenetetrahydrofolate reductase (MTHFR) polymorphisms. Similar pathway-wide candidate gene approaches to genotyping have previously successfully identified genetic risk factors for several clinical phenotypes including colorectal,[129,130] breast,[131] and bladder cancers,[132] and cleft lip or palate.[133]

DNA isolation

We extracted genomic DNA from 5 ml of whole blood using the Chemagic Magnetic Module (MSM) 1 system according to the manufacturer's protocol (Chemagen Biopolymer-Technologie AG, Baesweiler, Germany). We eluted samples in 500 µl of the manufacturer's elution buffer.

Single nucleotide polymorphism selection

We compiled a list of 25 candidate genes[127] associated with either the one-carbon folate or methionine synthesis pathways. We identified 48 non-synonymous single nucleotide polymorphisms (SNPs) within these genes from the Single Nucleotide Polymorpism Database [dbSNP;[134] www.ncbi.nlm.nih.gov/SNP (accessed May 2008)] and selected for analysis those with minor allele frequency greater than 5%. We included a further 100 SNPs from the HapMap (http://hapmap.ncbi.nlm.nih.gov) population of Utah residents with ancestry from northern and western Europe which, when analysed by Haploview software version 402 (www.broadinstitute.org/haploview/haploview), tagged at least one other SNP. We added a 19 base pair (bp) deletion polymorphism of intron 1 of the dihydrofolate reductase gene (*DHFR*) and a 28 base pair double or triple tandem repeat polymorphism of the thymidylate synthase (*TYMS*) gene because both have been extensively characterised in clinical studies.[127]

Genotyping

We designed multiplex assays for the MALDI-TOF-based Sequenom iPLEX system (Sequenom Inc., San Diego, CA, USA) using the software at https://mysequenom.com/default.aspx. We included 140 SNPs in five-assay plexes ranging from 11 to 35 SNPs in size. We excluded five SNPs which we could not incorporate in assays because of proximal nucleotide sequence constraints and another three SNPs which we could not include at a minimum plexing level of > 10 SNPs per assay (see *Appendix 6*, *Table 35*).

We genotyped patients for these 140 SNPs according to the manufacturer's protocol using 40 ng/reaction genomic DNA. We obtained sequence-specific polymerase chain reaction (PCR) and extension reaction oligonucleotides from Metabion GmbH (Martinsried, Germany). *Table 36* of *Appendix 6* defines the corresponding primer and probe sequences.

We typed the 19 bp deletion polymorphism from the dihydrofolate reductase gene (*DHFR*) and the 28 bp tandem repeat polymorphism from the thymidylate synthase (*TYMS*) gene using previously published protocols and PCR primer sequences[21,22] with minor modification. Briefly the 25-µl PCR reaction consisted of 20 ng genomic DNA, 5 pmol each of primer and 18 µl 1.1× ReddyMix™ PCR mastermix (Abgene Ltd, Epsom, UK).

We resolved all PCR products with ethidium bromide staining on a 3% agarose gel. For the DHFR 19 bp deletion, a 92 bp product identified the deletion allele and a 113 bp product identified the insertion allele. For the TYMS tandem repeat a 144 bp product distinguished the triple repeat allele from the double (116 bp).

We undertook all genotyping with 10% of DNA samples duplicated as well as positive and negative controls to confirm genotype calling accuracy and concordance.

Genetics outcomes

For this genetic sub-study, the primary outcome was self-rated symptom severity on the Beck Depression Inventory (BDI-II) at baseline, and 4, 12 and 25 weeks, consistent with the trial's primary outcome. Secondary outcomes were:

1. symptom severity rated by clinicians on the MADRS and the CGI of change (also at baseline and 4, 12 and 25 weeks)
2. mental and physical aspects of self-reported health status on the SF-12 (ditto)
3. side effects assessed by the UKU side effects scale and reported AEs (ditto), and
4. proportion of patients with self-rated moderate or severe depression (i.e. BDI-II score $\geq$ 19) at 25 weeks.

Genetics statistical methods

Before the analyses of association, we tested each SNP for Hardy–Weinberg Equilibrium, and excluded those found to deviate at a significance level of 0.1%. We also excluded SNPs which did not meet all our genotype quality criteria:

a. minor allele frequency greater than 1%
b. genotyping rate greater than 95% per SNP, and
c. samples more than 90% of SNPs called.

We fitted three mixed models to all five outcomes for each included SNP. The first ('baseline model') included the baseline value of the outcome, covariates representing the three time points (4, 12 and 25 weeks), three validated stratifying variables – centre, type of antidepressant, new or continuing patient – and treatment received, that is whether participants supplemented their medication with folic acid or not. We also tested non-genetic factors known to be generally associated with outcome (age, gender, marital status, employment status, number of dependents, smoking and alcohol consumption, previous counselling and treatment adherence as assessed by the Morisky scale) for univariate association with each outcome and included them in the model if the significance level was less than 10%.

The second ('SNP') model was identical to the first with the addition of the SNP as covariate. The third ('interaction') model was identical to the second with the addition of interaction between SNP and treatment received. To test for statistical significance of SNP main effects, we used likelihood ratio tests to compare the specific SNP model with the baseline model. To test for statistical significance of the SNP-folated interaction, we again used the likelihood ratio test to compare the specific interaction model with the specific SNP model. Each test tried two models – one making no assumption about the underlying mode of inheritance, the other assuming an additive mode of inheritance – and used the lower significance level for each SNP.

To take account of the multiple comparisons due to four tests on each of more than 100 SNPs, we estimated the false discovery rate ('FDR') for each comparison, and treated FDRs less than 5% as statistically significant associations. We used the statistical software packages: R;[111] PLINK version 1.07 from http://pngu.mgh.harvard.edu/~purcell/plink/; and PASW version 18[135,136] for these analyses.

Systematic review of the effectiveness of folate in augmenting antidepressant medication

Introduction

At the start of the FolATED trial understanding of the benefits of folate augmentation of ADM stemmed from a recent Cochrane systematic review.[50,64] The authors concluded that there was limited evidence that adding folate to ADM was helpful, and recommended larger trials to test this hypothesis thoroughly. That recommendation led directly to the funding of FolATED.

Method

Data sources and study selection

We updated the current Cochrane systematic review[50] following analysis of the FolATED trial. The authors of that review searched the Cochrane Central Register of Controlled Trials and MEDLINE from 1966 until May 2005. In August 2012 we reran their search for randomised trials evaluating folate in any form to augment ADM in treating depression. We followed the Cochrane systematic review search strategy in PubMed until December 2011 but without language restrictions. Consistent with the design of FolATED we selected randomised trials evaluating folate to augment antidepressants in treating depressive disorder rather than folate as sole therapy.

Data extraction and synthesis

Two of us (BRC and ITR) independently assessed potential trials for eligibility and quality, and extracted data. The Cochrane systematic review had used the Hamilton Depression Rating Scale (HDRS) as primary outcome.[50] In contrast the FolATED trial used the Beck Depression Index (BDI-II). To compare these instruments we converted both to standard Normal distributions with a SD of 1 and mean equal to the trial effect size, namely the mean difference between trial groups divided by trial SD. We gave each trial a weight inversely proportional to the variance with which it estimated that difference. We used a random-effects model to estimate the standardised mean difference and associated 95% CI. We assessed the heterogeneity of findings by the I^2-statistic.[137]

Chapter 3 Results

Recruitment: identification and eligibility

We recruited participants in three centres: North East Wales, North West Wales and Swansea. The first randomisations took place in North West Wales in July 2007, in Swansea in August 2007, and in North East Wales in October 2007. We randomised the final participant in November 2010 and completed follow-up in May 2011.

Figure 2 shows that FolATED received 1488 referrals; screened 863, of whom 635 consented to take part; randomised 479, of whom four were in error and removed from analysis; and analysed 440 (92% of the 475 valid randomisations). Though the four randomised in error had BDI-II scores of at least 19 at –2 weeks, these had fallen below 17 at randomisation, so they should have been excluded. The reasons why 625 referred patients did not reach screening were: 44% did not wish to take part; 26% did not respond to the research team; 16.5% were ineligible; and 13.5% did not attend the screening appointment.

At screening to assess eligibility for the trial, the primary reason for exclusion was that people did not meet the trial specified criteria for moderate to severe depression (54%). The other criteria that excluded more than 5% of those screened were: presence of malignancy or similar disorder (10%); not currently taking antidepressants (9%); and taking anticonvulsants (7%).

Randomisation interviews took place 2 weeks after screening when blood test results were available to verify eligibility to enter the trial. Of the 635 people who had consented to take part, we could not randomise 156: 68 people dropped out between screening and randomisation and a further 42 at the randomisation interview, of whom 36 scored too low on the BDI-II. Forty-six people entered the comprehensive cohort and 20 who were eligible to do so declined. *Table 3* cross-tabulates reasons for losses by stage of recruitment and *Appendix 7* does so by centre.

Centre differences in recruitment patterns

North West Wales received 47% of the referrals to the trial and randomised 50% of the final sample. North East Wales and Swansea received and randomised very similar proportions of the total – 27% and 26% respectively of referrals received and 25% of the randomised sample each). *Table 4* summarises these flows by centre.

Loss to follow-up

We randomised 475 participants (excluding four randomised in error): 237 to receive folic acid and 238 to receive placebo. In the folic acid group 15 people withdrew and 26 were lost to follow-up by 25 weeks. In the placebo group 18 people withdrew and 32 were lost to follow-up by 25 weeks. *Table 5* shows the reasons for loss at each stage.

Participant drop-out and missing data

There were no follow-up data for 35 randomised participants; 18 withdrew before the 4-week follow-up (8 folic acid group, 10 placebo group) and 17 did not attend any appointments (6 folic acid group, 11 placebo group). Thus 14 dropped out of the folic acid group and 21 out of the placebo group. We removed these from further analysis. Therefore 440 entered the main analysis, 223 from the folic acid group and 217 from the placebo group. If these evaluable participants missed follow-up appointments, we imputed their data in accordance with *Chapter 2, Statistical methods, Trial populations*, above (*Table 6*). However we imputed no baseline measures.

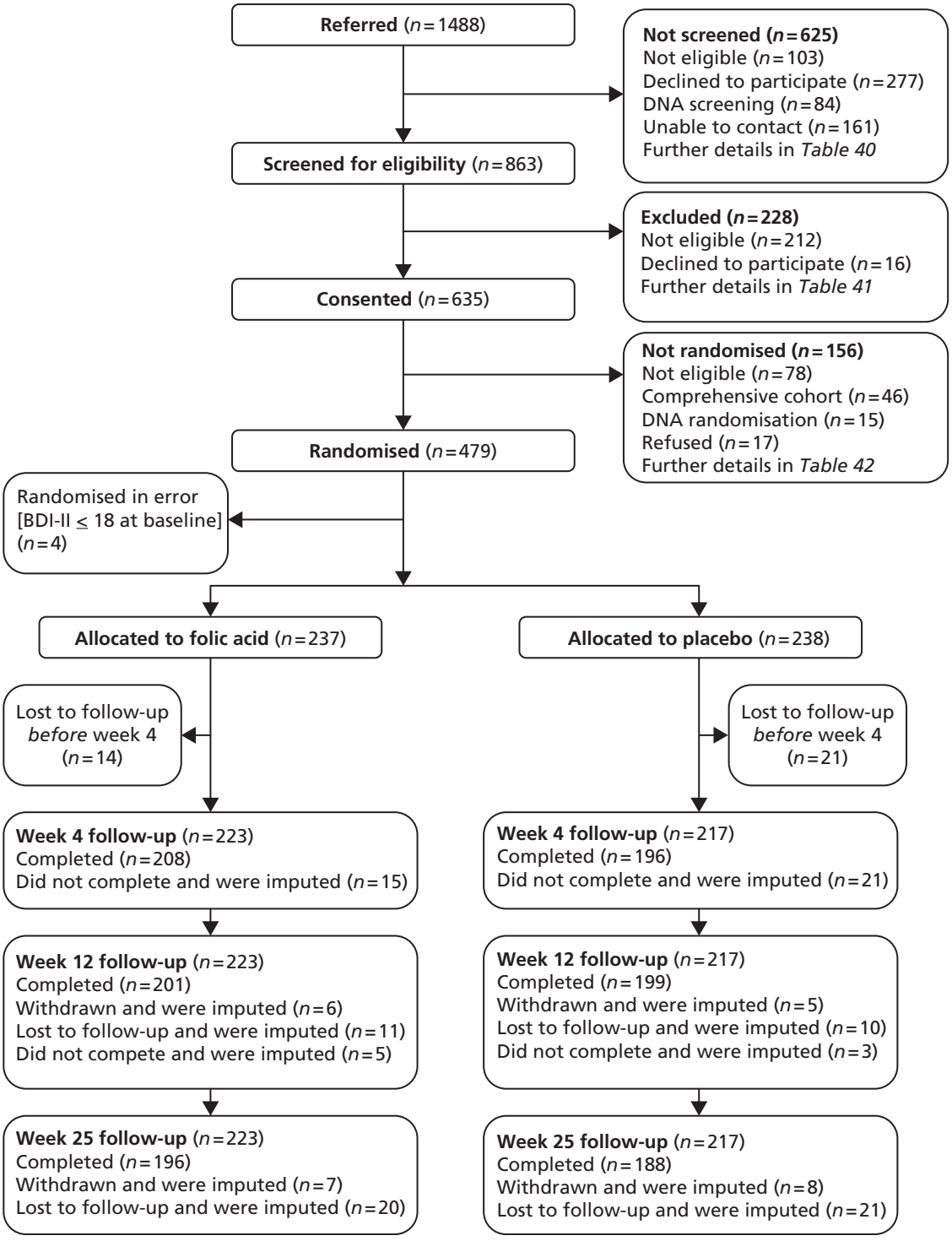

FIGURE 2 'CONSORT diagram' of flow of participants through trial. Note: Hence we included 223 + 217 = 440 participants in primary analysis.

TABLE 3 Reasons for losses between referral and randomisation by stage

Reason for not randomising	Between referral and screening	Screening	Randomisation	Total
Pre-specified exclusion criteria				
Are under 18 years	4	0	0	**4**
Not depressed by ICD – 10 criteria	0	122	36	**158**
Folate deficient	0	1	15	**16**
B_{12} deficient	2	0	8	**10**
Have taken folate supplementation	14	8	2	**24**
Suffered from psychosis	3	2	0	**5**
Bipolar disorder	2	4	0	**6**
Are already in another research trial	2	0	0	**2**
Are pregnant or planning to be so	9	0	0	**9**
Taking anticonvulsants	5	16	1	**22**
Serious, advanced or terminal illness	0	0	0	**0**
Treatment for a medical condition not yet stabilised	1	2	0	**3**
Taking lithium	1	0	0	**1**
Have had diagnosis of malignant disease	19	22	2	**43**
Subtotal	**62**	**177**	**64**	**303**
Other exclusions				
Not on antidepressants	36	21	9	**66**
Other	5	14	5	**24**
Subtotal	**41**	**35**	**14**	**90**
Refusal	277	16	17	**310**
Did not attend appointment	84	0	15	**99**
Could not contact	161	0	0	**161**
Subtotal	**522**	**16**	**32**	**570**
Comprehensive cohort	0	0	46	**46**
Total	**625**	**228**	**156**	**1009**

TABLE 4 Participant flow from referral to randomisation by centre

Reason for not randomising	North East Wales	North West Wales	Swansea	Total
Number referred	**400**	**698**	**390**	**1488**
Trial exclusion criteria	15	31	16	62
Other exclusions	15	17	9	41
Refusal	91	128	58	227
Did not attend	21	29	34	84
Could not contact	26	75	60	161
Number screened	**232**	**418**	**213**	**863**
Trial exclusion criteria	57	75	45	177
Other exclusions	7	25	3	35
Refusal	4	12	0	16
Other loss	0	0	0	0
Number consented	**164**	**306**	**165**	**635**
Trial exclusion criteria	6	42	16	64
Other exclusions	1	9	4	14
Refusal	9	4	4	17
Did not attend	3	6	6	15
To comprehensive cohort	24	7	15	46
Number randomised	**121**	**238**	**120**	**479**

Of the 440 evaluable participants 36 (8%) missed follow-up at 4 weeks, 40 (9%) at 12 weeks, and 56 (13%) at 25 weeks. Thus 10% of follow-up assessments were missing. Sixty-two participants missed one assessment: 33 at 4 weeks, 6 at 12 weeks and 23 at 25 weeks. Thirty-five participants missed two assessments: 2 at 4 and 12 weeks; 1 at 4 and 25 weeks; and 32 at 12 and 25 weeks. Thus 343 (78%) participants undertook all three assessments.

For the eight main outcome measures (BDI-II, MADRS, CGI, SF-12, EQ-5D, EQ-VAS, MINI and Morisky) a full data set over the four times would have comprised 102,520 data items. Only 2572 (2.5%) were missing, of which 2476 (2.4%) items were in missing subscales or times while 96 (0.1%) items were isolated missing values within otherwise complete subscales. Reassuringly there was no hint of significant differences between trial groups in either respect.

The missing item rate for seven of these measures is fairly consistent: BDI-II, EQ-5D, EQ-VAS, and MINI all 2.3%; MADRS 2.4%; SF-12 3.2% and CGI 3.3%. In contrast the Morisky data had a missing item rate of 10%, not explained by being collected only at 12 weeks. Predictably the pattern of missing data differed very significantly between centres: North West Wales, the best recruiting centre, missed more 4-week data than other centres, but fewer at 25 weeks. This pattern was due to heavy workload early in the trial when several 4-week appointments were missed; fortunately routine monitoring recognised and rectified the problem. Reassuringly there was no significant difference between centres in the proportion followed up.

TABLE 5 Participants not attending at each time point with reasons for withdrawal

		Folate				Placebo			
Type of drop-out	Reason for drop-out	4 weeks	12 weeks	25 weeks	Total	4 weeks	12 weeks	25 weeks	Total
Withdrawals	Too many personal commitments	2	0	0	**2**	2	0	0	**2**
	Felt better	1	0	0	**1**	1	0	0	**1**
	Stopped taking antidepressants	0	0	0	**0**	1	1	0	**2**
	Pregnant	0	0	0	**0**	1	0	0	**1**
	No reason given or did not want to take part	3	4	0	**7**	3	0	0	**3**
	Withdrew owing to adverse side effects	1	0	0	**1**	0	0	0	**0**
	Had an abnormal electrocardiogram	0	0	0	**0**	1	0	0	**1**
	Participant elderly and too ill to take part	0	0	0	**0**	1	0	0	**1**
	Unable to contact because moved out of area	1	0	0	**1**	0	4	3	**7**
	Newly diagnosed with bipolar disorder	0	1	0	**1**	**0**	0	0	**0**
	Started folic acid supplements – asked to withdraw	0	1	0	**1**	**0**	0	0	**0**
	New diagnosis of multiple sclerosis	0	0	1	**1**	**0**	0	0	**0**
	Subtotal	**8**	**6**	**1**	**15**	**10**	**5**	**3**	**18**
Lost to follow-up	Did not attend any further follow-up appointments	6	11	9	**26**	11	10	11	**32**
	DNAd but attended at least one further follow-up	15	5	0	**20**	21	3	0	**24**
	Total	**29**	**22**	**10**	**61**	**42**	**18**	**14**	**74**

ECG, electrocardiogram; MS, multiple sclerosis.

TABLE 6 Number of participants with complete and imputed data by time point

	Folate (*n* = 223)		Placebo (*n* = 217)	
Follow-up	Completed	Imputed	Completed	Imputed
4 weeks	208	15	196	21
12 weeks	201	22	199	18
25 weeks	196	27	188	29

Validation of stratification variables

The trial used five variables to balance allocation between treatment groups during dynamic randomisation: centre; sex; antidepressant type; whether the participant was new or continuing on treatment; and whether the participant had ever received counselling for depression. Not surprisingly given the speed of the recruitment process, data validation identified a few inconsistencies in these data. However we found no misclassification of centre, sex or counselling, and minimal misclassification of antidepressant type or new patient (*Table 7*).

Baseline characteristics of participants

Table 8 compares the baseline characteristics of those included in the trial analysis with those excluded for lack of follow-up data. Those who dropped out were significantly younger, less likely to have a current partner and more likely to exceed safe limits of alcohol consumption, and had a higher mean BDI-II score. All of these are consistent with these participants having worse depression. However the systematic exclusion of these 35 (7.4%) consented participants from 475 created little risk of bias since they were equally distributed across the arms of the trial. Nevertheless this may have removed those who could have benefited most from the intervention.

TABLE 7 Stratification variables by group – at randomisation and after validation

	Recorded at randomisation, no. (%)		Amended following validation, no. (%)	
Stratification variable or category	Folate	Placebo	Folate	Placebo
Type of antidepressant				
SSRI	157 (70)	145 (67)	155 (70)	143 (66)
Other	66 (30)	72 (33)	68 (30)	74 (34)
Previous treatment?				
No – new ADM	56 (25)	52 (24)	45 (20)	42 (19)
Yes – continuing ADM	167 (75)	165 (76)	178 (80)	175 (81)
Previous counselling?				
Yes	101 (45)	97 (45)	No change	
No	122 (55)	120 (55)		
Gender				
Male	79 (35)	81 (37)	No change	
Female	144 (65)	136 (63)		
Centre				
North East Wales	57 (26)	53 (24)		
North West Wales	110 (49)	113 (52)	No change	
Swansea	56 (25)	51 (24)		

TABLE 8 Baseline characteristics of participants by whether included in final analysis

Characteristic	Included ($n = 440$)	Excluded ($n = 35$)	Significance test
Age			
Range	19–81	20–66	
Mean (SD)	45 (13)	39 (14)	
Median (IQR)	46 (36 to 54)	40 (26 to 47)	$U = 6200$, $p = 0.005$
Gender, no. (%)			
Male	160 (36)	11 (31)	$\chi^2 = 0.34$, df = 1, $p = 0.56$
Female	280 (64)	24 (69)	
Ethnicity, no. (%)			
White	427 (97)	34 (97)	Fisher's: $p = 0.46$
Other	5 (1)	1 (3)	
Not stated	8 (2)	0 (0)	
Marital status, no. (%)			
Single	109 (25)	13 (37)	$\chi^2 = 7.0$, df = 2, $p = 0.031$
Had a partner	91 (21)	11 (31.5)	
Have a partner	240 (54)	11 (31.5)	
Number of dependent children, no. (%)			
0	269 (61)	22 (63)	Fisher's: $p = 1$
1	70 (16)	5 (14)	
2	61 (14)	5 (14)	
3 or more	40 (9)	3 (9)	
Employment, no. (%)			
Full time employed	121 (28)	11 (31)	$\chi^2 = 1.1$, df = 2, $p = 0.58$
Part time or in education	124 (28)	7 (20)	
Smoking status, no. (%)			
Inactive	195 (44)	17 (49)	$\chi^2 = 0.093$, df = 2, $p = 0.96$
Smoker	162 (37)	14 (40)	
Non smoker	194 (44)	15 (43)	
Ex-smoker	77 (17)	6 (17)	
Not stated	7 (2)		
Alcohol consumption per week, no. (%)			
None	177 (40)	10 (29)	$\chi^2 = 7.1$, df = 2, $p = 0.029$
Within safe limit[a]	215 (49)	16 (45)	
Above safe limit[a]	48 (11)	9 (26)	
Centre, no. (%)			
Bangor	223 (51)	15 (43)	$\chi^2 = 1.1$, df = 2, $p = 0.59$
Wrexham	110 (25)	9 (26)	
Swansea	107 (24)	11 (31)	

continued

TABLE 8 Baseline characteristics of participants by whether included in final analysis (*continued*)

Characteristic	Included ($n = 440$)	Excluded ($n = 35$)	Significance test
Baseline BDI-II score			
Range	17–61	18–57	
Mean (SD)	34 (10)	39 (11)	$t = -2.793$, $df = 461$, $p = 0.005$
Median (IQR)	33 (26 to 41)	39 (31 to 48)	
Missing	10	2	
Group allocated			
Folate	223	14	$\chi^2 = 1.5$, $df = 1$, $p = 0.22$
Placebo	217	21	

IQR, interquartile range.
a Safe limits: females = 14 units per week; males = 21 units per week.

Differences between centres at baseline

The management of the FolATED trial included a monthly telephone conference between the three clinical centres and NWORTH, the coordinating Clinical Trials Unit. These conferences soon identified substantial differences between centres, notably in psychiatric practice and recruitment policy. Judging that these differences would enhance the generalisability of the trial provided the conduct of the research was consistent across centres, we pursued such consistency, notably by maintaining a rigorous fieldwork handbook and arranging regular inter-centre training.

Table 9 shows that participants differed significantly between centres, notably in:

(a) *Mean age* Those in North West Wales were on average more than 3 years younger than those in the other centres.
(b) *Numbers of dependent children* Half of those in North West Wales had children, but only 30% of those elsewhere.
(c) *Employment status* Swansea had many more students, while North East Wales had more unemployed.
(d) *Smoking rates* Nearly half of those in Swansea smoked, but only 30% of those elsewhere.

However there was no significant difference in mean BDI-II scores across centres; or in alcohol consumption.

Another difference between centres identified by our monthly management conferences is that one centre did more to optimise medication than the other two, notably by using reboxetine (Edronax®, Pfizer) to augment basic ADM. Though we plan to analyse the process and outcome of optimisation in detail, we have confirmed that this reboxetine augmentation did not differ between allocated groups; hence there was no danger of bias from differential optimisation.

Baseline demographic profile

Predictably our randomisation algorithm generated similar treatment groups (*Tables 10* and *11*).

TABLE 9 Baseline demographic characteristics of trial participants by centre

Characteristic	North East Wales (*n* = 110)	North West Wales (*n* = 223)	Swansea (*n* = 107)	Total (*n* = 440)
Age				
Range	19–75	19–81	19–75	19–81
Mean (SD)	46 (11)	44 (12)	47 (14)	45 (13)
Gender, no. (%)				
Male	30 (27)	90 (40)	40 (37)	160 (36)
Female	80 (73)	133 (60)	67 (63)	280 (64)
Ethnicity, no. (%)				
White	107 (97)	220 (99)	100 (94)	427 (97)
Other	1 (1)	2 (1)	2 (2)	5 (1)
Not stated/missing	2 (2)	1 (0)	5 (4)	8 (2)
Marital status, no. (%)				
Single	18 (16)	62 (28)	29 (27)	109 (25)
Had a partner	27 (25)	40 (18)	24 (22)	91 (21)
Have a partner	65 (59)	121 (54)	54 (51)	240 (54)
Number of dependent children, no. (%)				
0	72 (65)	117 (52)	80 (75)	269 (61)
1	15 (14)	40 (18)	15 (14)	70 (16)
2	18 (16)	37 (17)	6 (5.5)	61 (14)
3 or more	5 (5)	29 (13)	6 (5.5)	40 (9)
Employment status,[a] no. (%)				
Full time employed	38 (35)	59 (26)	24 (22)	121 (28)
Part time or in education	31 (28)	48 (22)	45 (42)	124 (28)
Inactive	41 (37)	116 (52)	38 (36)	195 (44)
Smoking status, no. (%)				
Smoker	27 (24)	97 (44)	38 (35)	162 (37)
Non smoker	57 (52)	94 (42)	50 (47)	201 (46)
Ex-smoker	26 (24)	32 (14)	19 (18)	77 (17)
BDI-II				
Mean (SD)	33.7 (9.3)	34.3 (9.2)	33.1 (10.8)	33.7 (9.6)
Range	18–61	19–58	17–60	17–61

a *Appendix 9* elaborates on this table and describes how we recorded these variables.

TABLE 10 Baseline demographic characteristics of trial participants by treatment allocated

Characteristic	Folate (*n* = 223)	Placebo (*n* = 217)
Age		
Range	19–81	20–75
Mean (SD)	45 (14)	45 (12)
Median (IQR)	47 (35 to 55)	45 (36 to 53)
Gender, no. (%)		
Male	79 (35)	81 (37)
Female	144 (65)	136 (63)
Ethnicity, no. (%)		
White	215 (97)	212 (98)
Other	5 (2)	0 (0)
Not stated	3 (1)	5 (2)
Marital status, no. (%)		
Single	60 (27)	49 (23)
Had a partner	124 (56)	116 (53)
Have a partner	39 (17)	52 (24)
Number of dependent children, no. (%)		
0	146 (66)	123 (56)
1	29 (13)	41 (19)
2	27 (12)	34 (16)
3 or more	21 (9)	19 (9)
Employment status, no. (%)		
Full time employment	49 (22)	72 (33)
Part time employment or education	69 (31)	55 (25)
Inactive	105 (47)	90 (42)
Smoking status, no. (%)		
Smoker	82 (37)	80 (37)
Non smoker	98 (44)	103 (47)
Ex-smoker	43 (19)	34 (16)
Smoking consumption, no. (%)		
Non-smoker	141 (63)	137 (63)
Low (≤ 10)	30 (14)	34 (16)
Medium (between 10 and 20)	43 (19)	32 (15)
High (≥ 20)	9 (4)	14 (6)
Alcohol consumption per week, no. (%)		
None	86 (39)	82 (38)
Below safe limit	106 (47)	101 (46)
Above safe limit	31 (14)	34 (16)

IQR, interquartile range.

Baseline clinical profile

TABLE 11 Baseline clinical measures and blood results by treatment allocated

Measure or scale		Folate (*n* = 223)	Placebo (*n* = 217)
Symptom severity instruments			
BDI-II	Range	17 to 60	17 to 61
	Mean (SD)	33 (9)	34 (10)
MADRS	Range	1 to 50	13 to 53
	Mean (SD)	28 (7)	29 (7)
CGI: Severity of illness, no. (%)	Normal to mild	3 (1)	0 (0)
	Mild to moderate	148 (67)	147 (68)
	Moderate to severe	72 (32)	70 (32)
Health status and utility			
EQ-5D	Range	–0.2 to 1.0	–0.3 to 1.0
	Mean (SD)	0.48 (0.30)	0.51 (0.30)
EQ-VAS	Range	0 to 100	0 to 95
	Mean (SD)	45 (20)	44 (20)
SF-12: Physical component scale	Range	17 to 69	17 to 71
	Mean (SD)	44 (12)	44 (13)
SF-12: Mental component scale	Range	4 to 50	–1 to 57
	Mean (SD)	26 (9)	26 (10)
Biochemistry			
Serum folate level	Range	2 to 20	2 to 20
	Mean (SD)	7.1 (4.2)	7.2 (4.2)
B_{12} level	Range	142 to 1019	150 to 928
	Median (IQR)	300 (228 to 391)	306 (248 to 402)

Clinical effectiveness results

Is folic acid clinically effective?

Primary clinical effectiveness outcomes

The primary clinical effectiveness outcome measure was self-rated symptom severity as measured by BDI-II scores over 25 weeks, as summarised by the AUC of mean BDI-II scores from randomisation till 25 weeks. This provided no evidence that folic acid was effective (*Figure 3*). The adjusted difference in AUC between folic acid and placebo was 1.09 (95% CI from –0.48 to 2.66; $p = 0.17$). The adjusted difference between folic acid and placebo at 25 weeks was 1.27 (95% CI from –0.99 to 3.54; $p = 0.27$). Similarly there was no significant difference in the proportion of patients who were depressed at 25 weeks (at least moderately, i.e. BDI-II $\geq$ 19): there were 126/223 (57%) depressed participants in the folate group and 118/217 (54%) in the placebo group. The adjusted OR of being depressed in the folate group compared with placebo group was 1.09 (95% CI from 0.75 to 1.59; $p = 0.65$).

Secondary clinical effectiveness outcomes

Table 12 shows the unadjusted AUC results for all the main outcome measures, together with two-sample *t*-tests. The only significant result favoured the placebo in the SF-12 mental component. By convention generic outcome measures like EQ-5D and SF-12 show good health by high scores, while condition-specific outcome measures like BDI-II, CGI and MADRS show good health by scores that are low, if not zero. Thus five of the non-significant differences favoured placebo and seven favoured folate. *Table 42* of *Appendix 10* elaborates on *Table 12* by showing the results of unadjusted two-sample *t*-tests for each variable at each time point separately.

Area under the curve analysis adjusted for stratification variables and baseline score of the variable in question gave a very similar picture. *Table 13* shows these results and reports all significant stratification and baseline variables. For most variables the baseline score and antidepressant type were significant covariates, but allocated treatment was not. As for the unadjusted AUCs the only outcome on which the allocated treatment had a statistically significant effect was the SF-12 mental component, again favouring the placebo.

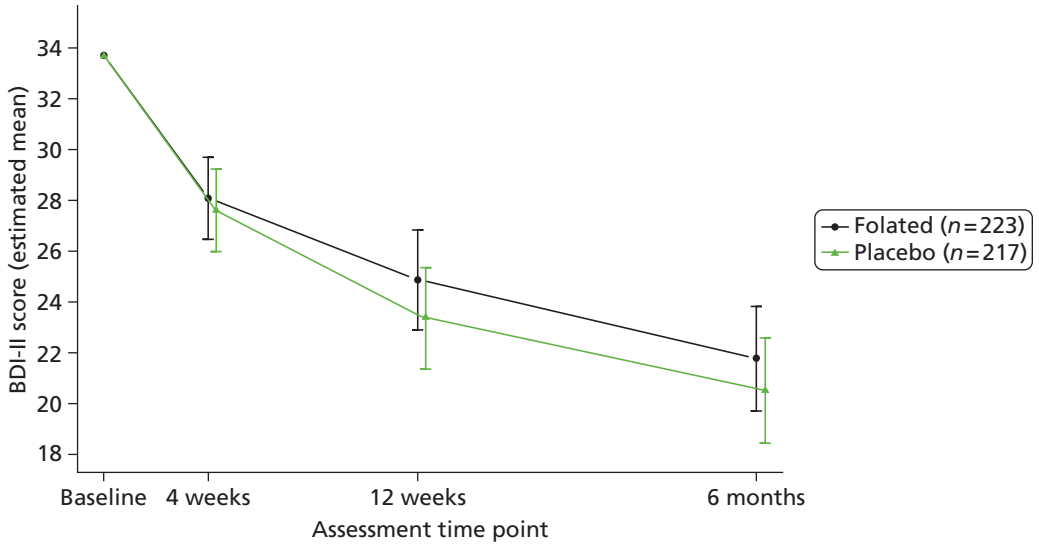

FIGURE 3 Estimated mean BDI-II scores over time adjusted for baseline score and stratification variables – by treatment allocated. Baseline score = observed population mean BDI-II score.

TABLE 12 Unadjusted AUC average (using values at baseline and 4, 12 and 25 weeks) of main outcomes by treatment allocated

	Folate	Placebo	Difference (folate minus placebo)		
Outcome variable	Mean (SD)	Mean (SD)	Mean (SE)	95% CI	Significance
BDI-II	25.70 (10.83)	25.64 (11.43)	0.06 (1.06)	−2.02 to 2.15	0.953
MADRS	22.17 (7.59)	22.39 (8.34)	−0.22 (0.76)	−1.71 to 1.27	0.771
Euroqol					
EQ-5D	0.573 (0.259)	0.591 (0.262)	−0.018 (0.025)	−0.067 to 0.031	0.476
EQ-VAS	54.58 (18.38)	54.34 (17.85)	0.25 (1.73)	−3.15 to 3.64	0.887
SF-12					
SF-12 PCS	44.89 (11.13)	44.28 (11.48)	0.61 (1.08)	−1.51 to 2.73	0.571
SF-12 MCS*	31.99 (9.34)	34.09 (9.01)	**−2.09 (0.88)**	**−3.81 to −0.37**	**0.017**
CGI					
CGI: Severity	3.60 (0.87)	3.61 (0.94)	−0.00 (0.09)	−0.17 to 0.17	0.980
CGI: Improvement	3.11 (0.86)	3.09 (0.95)	0.02 (0.09)	−0.15 to 0.19	0.794
CGI: Efficacy[a]	0.29 (0.49)	0.30 (0.50)	−0.01 (0.05)	−0.10 to 0.08	0.826
Estimated CGI	1.34[b] (–)	1.35[b] (–)	0.99[c] (–)	0.90 to 1.08[c]	
UKU					
UKU: Psychic	8.21 (3.86)	8.16 (4.01)	0.05 (0.38)	−0.68 to 0.79	0.894
UKU: Neurologic[d]	0.73 (0.67)	0.79 (0.71)	−0.06 (0.07)	−0.19 to 0.07	0.371
Estimated UKU	0.53[e] (–)	0.62[e] (–)	(–) (–)	(–)	
UKU: Autonomic	2.93 (2.22)	2.85 (2.51)	0.08 (0.23)	−0.37 to 0.52	0.723
UKU: Other	3.62 (2.68)	4.06 (3.11)	−0.44 (0.28)	−0.98 to 0.11	0.114

a After logarithmic transformation.
b Estimated CGI scale scores in each group from the transformed model.
c Ratio of estimated CGI scores and CI for the ratio.
d After square-root transformation.
e Estimated UKU scale scores in each group from the transformed model.
* Difference significant at 5% level with effect size = 0.23.

TABLE 13 Area under curve average of main outcomes adjusted for stratification variables and their own baselines – by treatment allocated

	Difference (folate minus placebo)				
Outcome variable	Mean (SE)	95% CI	Significance	Significant covariates	Significance
BDI-II	1.09 (0.86)	–0.48 to 2.66	0.173	Baseline scores	< 0.001
				Type of antidepressant	0.028
				Centre	0.025
MADRS	0.71 (0.62)	–0.51 to 1.93	0.252	Baseline scores	< 0.001
				Type of antidepressant	0.002
				Centre	0.026
EQ-5D	0.00 (0.017)	–0.034 to 0.033	0.982	Baseline scores	< 0.001
EQ-VAS	–0.48 (1.36)	–3.16 to 2.20	0.726	Baseline scores	< 0.001
				Type of antidepressant	0.001
SF-12 PCS	0.40 (0.60)	–0.78 to 1.59	0.501	Baseline scores	< 0.001
				Previous treatment	0.002
SF-12 MCS*	–1.97 (0.78)	–3.49 to –0.44	**0.012**	Baseline scores	< 0.001
				Type of antidepressant	0.001
CGI: Severity	0.05 (0.08)	–0.11 to 0.2	0.553	Baseline scores	< 0.001
				Type of antidepressant	0.001
				Centre	< 0.001
CGI: Improvement	0.04 (0.08)	–0.13 to 0.21	0.649	Type of antidepressant	0.002
				Centre	0.009
CGI: Efficacy[a]	0.98[b] (0.04)	–0.89 to 1.07	0.604	Type of antidepressant	< 0.001
				Centre	< 0.001
UKU: Psychic	0.31 (0.31)	–0.3 to 0.92	0.319	Baseline scores	< 0.001
				Type of antidepressant	0.001
				Centre	0.029
UKU: Neurologic	–0.01 (0.04)	–0.10 to 0.07	0.738	Baseline scores	< 0.001
				Centre	0.008
UKU: Autonomic	–0.11 (0.15)	–0.42 to 0.19	0.458	Baseline scores	< 0.001
UKU: Other	–0.38 (0.20)	–0.78 to 0.02	0.065	Baseline scores	< 0.001
				Centre	0.002

a Ratio (folate/placebo) and its CI.
* Difference significant at 5% level with effect size = 0.24.

Figures 4–11 display the remaining adjusted analyses, except those relating to the UKU, by individual time points. Though receiving folic acid or placebo does not affect the outcome of treatment in the trial, the pattern of results over time is consistent across measures: ADM achieves major benefit over the first 4 weeks, and continuing though reducing improvement over 25 weeks. In particular *Figure 11* shows how the adjusted SF-12 MCSs differ between arms, with the difference favouring placebo: the mean scores diverge by 4 weeks, achieve a substantial gap by 12 weeks, and converge a little by 25 weeks. *Table 14* records the statistical analyses underpinning these nine figures, together with the corresponding analyses from the UKU side effects scale.

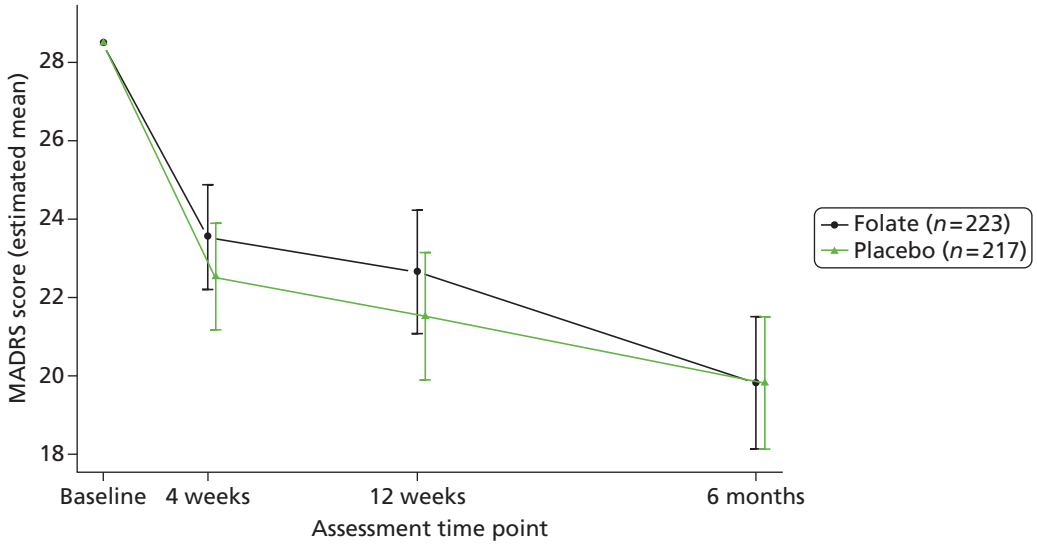

FIGURE 4 Estimated mean MADRS scores over time adjusted for baseline score and stratification variables – by treatment allocated. Baseline score = observed population mean MADRS score.

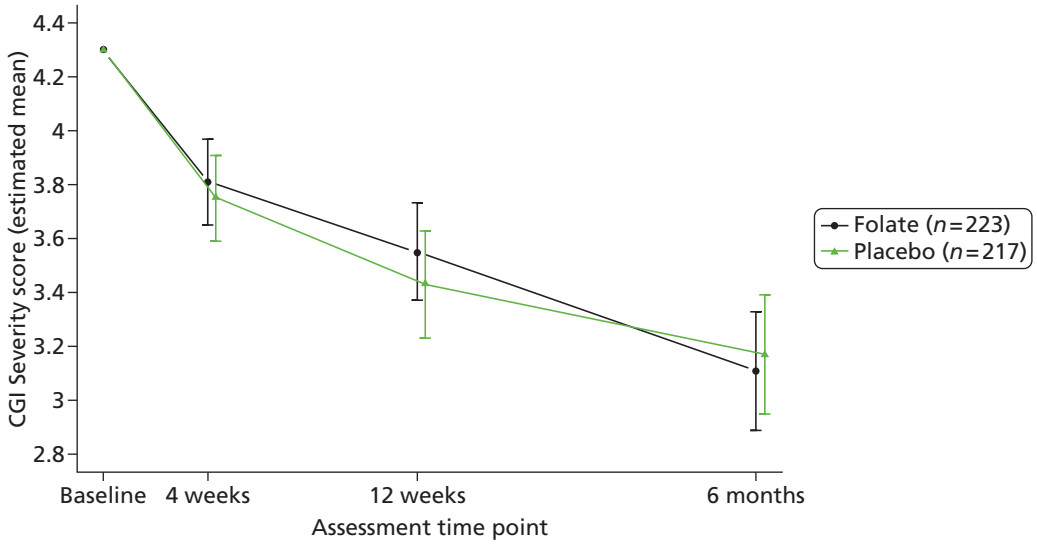

FIGURE 5 Estimated mean CGI severity scores over time adjusted for baseline score and stratification variables – by treatment allocated. Baseline score = observed population mean CGI severity score.

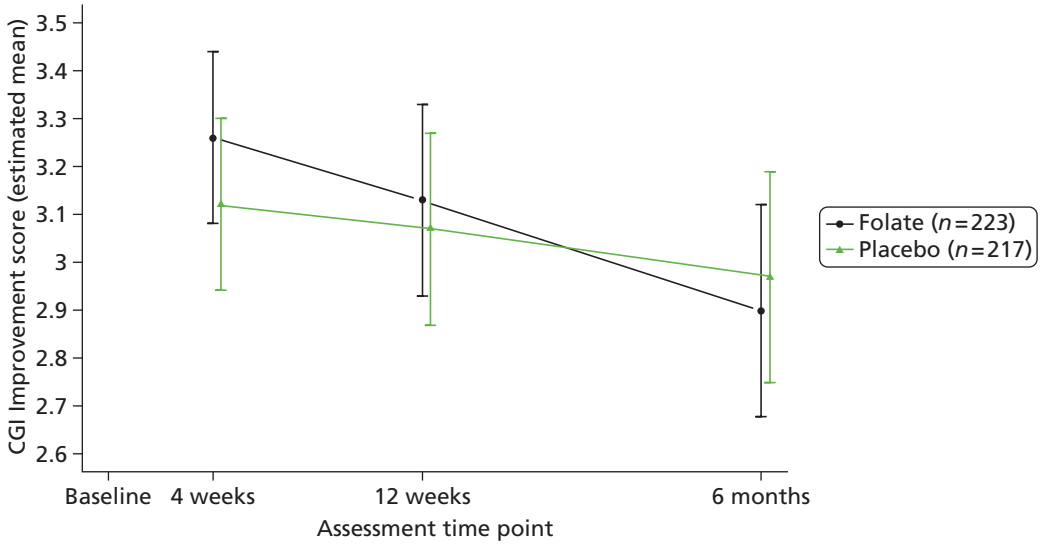

FIGURE 6 Estimated mean CGI improvement scores at follow up adjusted for stratification variables – by treatment allocated.

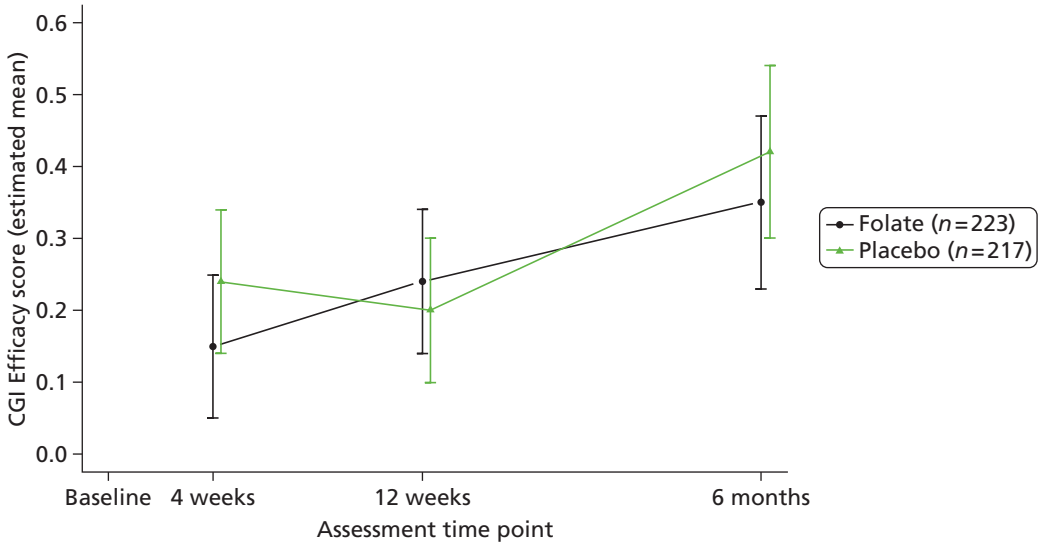

FIGURE 7 Estimated mean CGI efficacy scores at follow up adjusted for stratification variables – by treatment allocated.

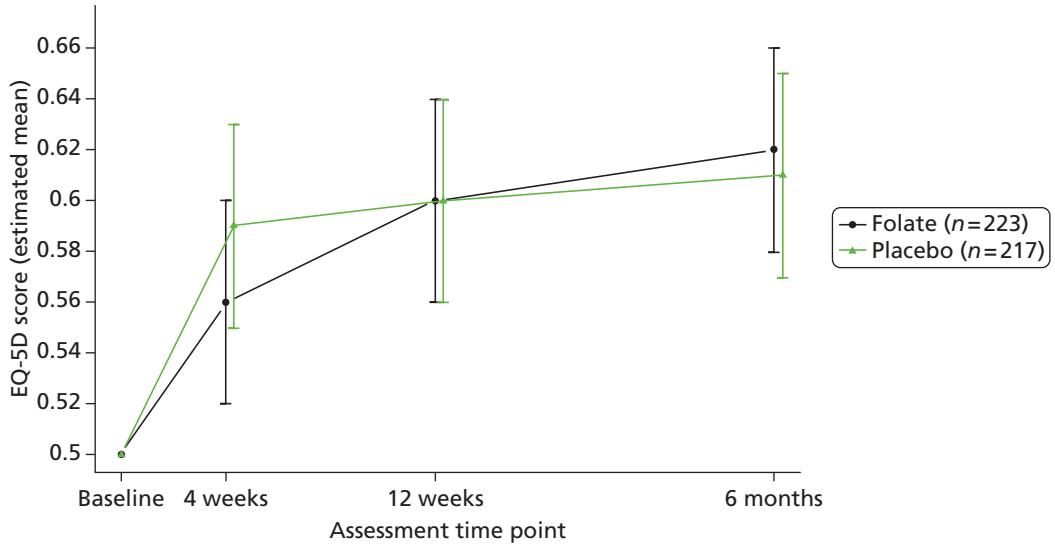

FIGURE 8 Estimated mean EQ-5D scores over time adjusted for baseline score and stratification variables – by treatment allocated. Baseline score = observed population mean EQ-5D score.

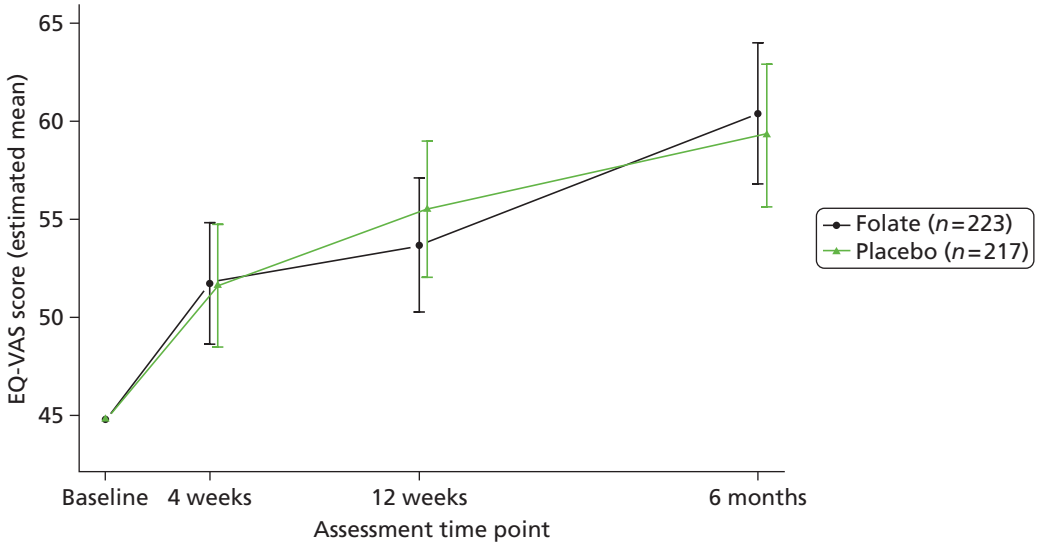

FIGURE 9 Estimated mean EQ-VAS scores over time adjusted for baseline score and stratification variables – by treatment allocated. Baseline score = observed population mean EQ-VAS score.

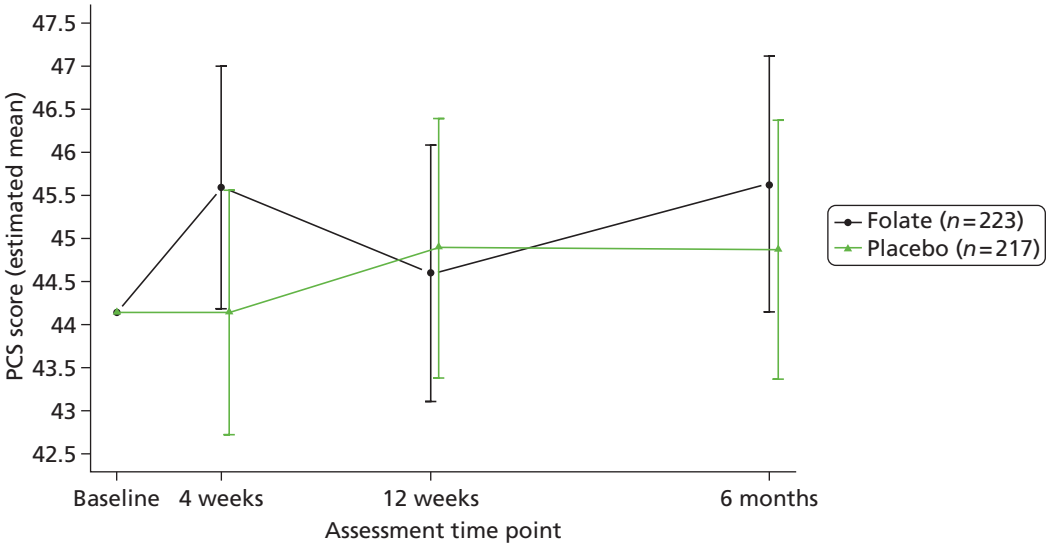

FIGURE 10 Estimated mean SF-12 Physical Component Scale scores over time adjusted by baseline score and stratification variables – by treatment allocated. Baseline score = observed population mean SF-12 Physical Component Scale score.

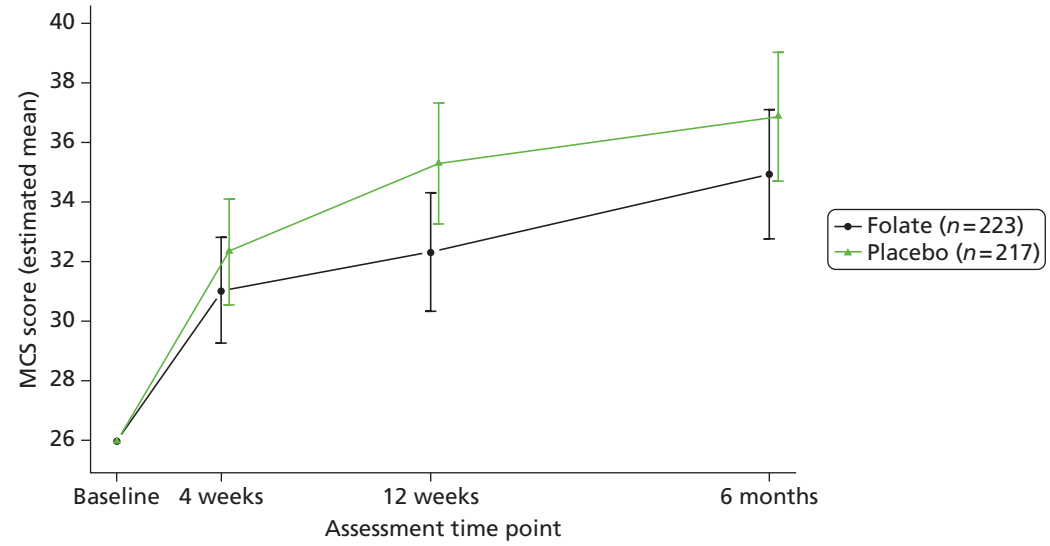

FIGURE 11 Estimated mean SF-12 Mental Component Scale scores over time adjusted for baseline score and stratification variables – by treatment allocated. Baseline score = observed population mean SF-12 Mental Component Scale score.

The absence of the binary variable 'Previous treatment?' from the significant covariates in all but the SF-12 Physical Component Score of the 13 rows of *Table 13*, and from all but four non-psychiatric rows of the 39 rows of *Table 14*, confirms that this does not seem to predict the clinical outcome of ADM or of adjunctive folate.

Tables 43–46 of *Appendix 10* complement *Table 14*, specifically the BDI-II row, by reporting the analogous logistic regression analyses for two levels of response to treatment – 50% or full improvement – both at 12 and at 25 weeks, thus illustrating the primary analysis in clinical terms.

Side effects, adverse events and suicidality

Side effects

There were 33 reported AEs in the folic acid arm of the trial and 45 in the placebo arm – difference not statistically significant ($\chi^2 = 2.66$; df = 1; $p = 0.10$; OR = 0.66; 95% CI from 0.40 to 1.09). We adjudged six of

TABLE 14 Outcomes over time adjusted for significant stratification variables and their own baselines by treatment allocated

Outcome variable and time point	Difference (folate minus placebo)			Significant covariates	Significance
	Mean (SE)	95% CI	Significance		
BDI-II					
BDI-II (4 weeks)	0.45 (0.91)	–1.34 to 2.23	0.623	Baseline scores	< 0.001
BDI-II (12 weeks)	1.52 (1.10)	–0.64 to 3.69	0.168	Baseline scores	< 0.001
				Centre	0.013
BDI-II (25 weeks)	1.27 (1.15)	–0.99 to 3.54	0.270	Baseline scores	< 0.001
				Type of antidepressant	0.004
				Centre	0.011
MADRS					
MADRS (4 weeks)	1.02 (0.75)	–0.45 to 2.49	0.174	Baseline scores	< 0.001
MADRS (12 weeks)	1.14 (0.89)	–0.62 to 2.89	0.204	Baseline scores	< 0.001
				Type of antidepressant	0.012
MADRS (25 weeks)	0.02 (0.95)	–1.84 to 1.88	0.981	Baseline scores	< 0.001
				Type of antidepressant	0.003
EQ-5D					
EQ-5D (4 weeks)	–0.028 (0.024)	–0.074 to 0.017	0.247	Baseline scores	< 0.001
				Previous treatment	0.005
EQ-5D (12 weeks)	0.010 (0.024)	–0.037 to 0.057	0.681	Baseline scores	< 0.001
EQ-5D (25 weeks)	0.019 (0.024)	–0.027 to 0.065	0.450	Baseline scores	< 0.001
EQ-VAS					
EQ-VAS (4 weeks)	0.15 (1.74)	–3.28 to 3.58	0.932	Baseline scores	< 0.001
				Type of antidepressant	0.004
EQ-VAS (12 weeks)	–1.83 (1.90)	–5.57 to 1.91	0.337	Baseline scores	< 0.001
				Type of antidepressant	0.026
EQ-VAS (25 weeks)	1.11 (2.01)	–2.85 to 5.07	0.582	Baseline scores	< 0.001
				Type of antidepressant	0.002
				Previous treatment	0.018
SF12 PCS					
SF-12 PCS (4 weeks)	1.45 (0.78)	–0.08 to 2.98	0.064	Baseline scores	< 0.001
				Previous treatment	0.008
SF-12 PCS (12 weeks)	–0.28 (0.83)	–1.91 to 1.35	0.733	Baseline scores	< 0.001
				Previous treatment	0.019
				Centre	0.063
SF-12 PCS (25 weeks)	0.76 (0.83)	–0.87 to 2.38	0.360	Baseline scores	< 0.001
				Previous counselling	0.080
				Previous treatment	0.009

continued

TABLE 14 Outcomes over time adjusted for significant stratification variables and their own baselines by treatment allocated (*continued*)

Outcome variable and time point	Difference (folate minus placebo)			Significant covariates	Significance
	Mean (SE)	95% CI	Significance		
SF-12 MCS					
SF-12 MCS (4 weeks)	–1.28 (0.99)	–3.22 to 0.67	0.198	Baseline scores	< 0.001
SF-12 MCS (12 weeks)*	**–2.95** (1.12)	–5.16 to –0.75	**0.009**	**Baseline scores**	< 0.001
				Type of antidepressant	0.002
SF-12 MCS (25 weeks)	–1.93 (1.21)	–4.30 to 0.45	0.112	Baseline scores	< 0.001
				Type of antidepressant	0.005
				Previous counselling	0.02
CGI: Severity					
CGI: Severity (4 weeks)	0.06 (0.09)	–0.12 to 0.23	0.517	Baseline scores	< 0.001
				Type of antidepressant	0.008
CGI: Severity (12 weeks)	0.12 (0.11)	–0.09 to 0.32	0.276	Baseline scores	< 0.001
				Type of antidepressant	0.035
				Centre	< 0.001
CGI: Severity (25 weeks)	–0.06 (0.12)	–0.30 to 0.18	0.616	Baseline scores	< 0.001
				Type of antidepressant	< 0.001
				Centre	< 0.001
CGI: Improvement					
CGI: Improvement (4 weeks)	0.14 (0.10)	–0.05 to 0.33	0.139	Type of antidepressant	0.030
CGI: Improvement (12 weeks)	0.06 (0.11)	–0.16 to 0.28	0.594	Type of antidepressant	0.052
CGI: Improvement (25 weeks)	–0.07 (0.13)	–0.32 to 0.18	0.596	Type of antidepressant	0.005
				Centre	0.012
CGI: Efficacy					
CGI: Efficacy (4 weeks)[a]	–0.10 (0.05)	–0.20 to 0.01	0.079	Type of antidepressant	0.003
				Centre	< 0.001
CGI: Efficacy (12 weeks)[a]	0.04 (0.06)	–0.08 to 0.15	0.560	Type of antidepressant	0.007
				Centre	0.005
CGI: Efficacy (25 weeks)[a]	–0.07 (0.06)	–0.19 to 0.05	0.272	Type of antidepressant	0.004
				Centre	< 0.001

TABLE 14 Outcomes over time adjusted for significant stratification variables and their own baselines by treatment allocated (*continued*)

Outcome variable and time point	Difference (folate minus placebo)			Significant covariates	Significance
	Mean (SE)	95% CI	Significance		
UKU: psychic					
UKU: psychic (4 weeks)	0.81 (0.47)	–0.11 to 1.73	0.084	Baseline scores	< 0.001
UKU: psychic (12 weeks)	0.08 (0.47)	–0.84 to 1.00	0.869	Baseline scores	< 0.001
				Type of antidepressant	0.005
				Centre	0.003
UKU: psychic (25 weeks)	0.37 (0.46)	–0.54 to 1.28	0.426	Baseline scores	< 0.001
				Type of antidepressant	0.002
				Centre	0.013
UKU: neurologic					
UKU: neurologic (4 weeks)[b]	–0.00 (0.06)	–0.12 to 0.12	0.989	Baseline scores	< 0.001
				Centre	< 0.001
UKU: neurologic (12 weeks)[b]	–0.03 (0.06)	–0.16 to 0.10	0.642	Baseline scores	< 0.001
				Centre	0.039
UKU: neurologic (25 weeks)[b]	–0.02 (0.07)	–0.15 to 0.12	0.821	Baseline scores	< 0.001
UKU: autonomic					
UKU: autonomic (4 weeks)	–0.13 (0.22)	–0.55 to 0.30	0.565	Baseline scores	< 0.001
				Centre	0.004
UKU: autonomic (12 weeks)	–0.17 (0.23)	–0.63 to 0.28	0.460	Baseline scores	< 0.001
UKU: autonomic (25 weeks)	0.03 (0.24)	–0.43 to 0.49	0.907	Baseline scores	< 0.001
UKU: other					
UKU: other (4 weeks)	–0.11 (0.28)	–0.65 to 0.44	0.694	Baseline scores	< 0.001
				Centre	< 0.001
UKU: other (12 weeks)**	**–0.63** (0.30)	–1.22 to –0.04	**0.037**	**Baseline scores**	< 0.001
UKU: other (25 weeks)	–0.39 (0.30)	–0.98 to 0.20	0.195	Baseline scores	< 0.001
				Previous treatment	0.047
				Gender	0.018

a After logarithmic transformation.
b After square-root transformation.
* Difference significant at 5% level with effect size = 0.25 in favour of placebo.
** Difference significant at 5% level with effect size = 0.44 in favour of folic acid.

the AEs in the intervention arm to be serious, compared with 14 in the control arm – difference also not statistically significant ($\chi^2 = 3.59$; df = 1; $p = 0.058$; OR = 0.40; 95% CI from 0.15 to 1.06). We classified seven of the 78 AEs as adverse reactions because folic acid (if prescribed) was a possible cause; unblinding revealed that four had received folic acid and three not – difference again not statistically significant ($\chi^2 = 0.12$; df = 1; $p = 0.73$; OR = 1.30; 95% CI from 0.29 to 5.89). None of these was serious or unexpected.

Suicidality using the MINI and UKU

We measured side effects by the UKU side effects scale. Comparison of the areas under subscale curves over 25 weeks showed no significant differences between treatment groups (*Table 15*). When we examined differences at each of the three follow-up times for each of the four subscales, we found a marginally significant difference at 12 weeks in the 'other' subscale, which includes mostly sexual side effects ($p = 0.037$). As this is one of many comparisons for the UKU, we treat this finding with caution.

Table 16 tabulates the numbers and percentages of patients classified in each of three suicidal risk categories from the MINI suicidality scale by follow-up time and treatment arm. All four differences are small and non-significant.

Adherence to trial medication

We assessed adherence to the trial medication at 12 weeks in four ways: scores on the Morisky Questionnaire; counting returned trial medication; serum folate levels; and homocysteine levels. We followed the published instructions for calculating respondents' scores on the Morisky Questionnaire. We defined the biochemical criteria for adherence to folic acid treatment as: serum folate at 12 weeks greater than 15 µg/ml, and reduction of at least 15% in serum homocysteine between baseline and 12 weeks. *Table 17* shows no significant difference between randomised groups in Morisky score or tablet count. Of the biochemical criteria, serum folate shows better adherence than homocysteine.

TABLE 15 Area under UKU curve adjusted by stratification variables and their own baselines – by treatment allocated

	Difference (folate minus placebo)			
UKU subscale	Mean	(SE)	95% CI	Significance
Psychic	0.31	(0.31)	–0.30 to 0.92	0.319
Neurologic[a]	–0.01	(0.04)	–0.10 to 0.07	0.738
Autonomic	–0.11	(0.15)	–0.42 to 0.19	0.458
Other (mostly sexual)	–0.38	(0.20)	–0.78 to 0.02	0.065

a After square-root transformation.

TABLE 16 Suicide risk on MINI suicidality scale by treatment allocated

	Folate ($n = 223$)				Placebo ($n = 217$)			
	Suicide risk: number (%)				Suicide risk: number (%)			
Week	None	Low	Medium	High	None	Low	Medium	High
0	0 (0)	138 (62)	36 (16)	49 (22)	0 (0)	143 (66)	34 (16)	40 (18)
4	0 (0)	169 (76)	21 (9)	33 (15)	0 (0)	155 (71)	32 (15)	30 (14)
12	0 (0)	152 (68)	35 (16)	36 (16)	0 (0)	168 (77)	21 (10)	28 (13)
25	0 (0)	163 (73)	35 (16)	25 (11)	0 (0)	156 (72)	33 (15)	28 (13)

TABLE 17 Measures of adherence at 12 weeks by treatment allocated

Adherence at 12 weeks	Folate ($n = 223$)	Placebo ($n = 217$)
Morisky score – no. (%)		
0 (best)	4 (2)	6 (3)
1	18 (8)	17 (8)
2	43 (19)	36 (17)
> 2 (worst)	158 (71)	158 (73)
Missing	Nil	Nil
Tablet count – median (IQR)	14 (7 to 20)	14 (7 to 20)
Missing	43	45
Serum folate > 15 µg/ml – no. (%)	163 (73)	Not applicable
Missing	29 (13)	
Homocysteine reduction of 15% – no. (%)	103 (46)	Not applicable
Missing	51 (23)	

IQR, interquartile range.

Sensitivity analyses of clinical effectiveness outcomes

Complete case analysis

Table 18 comparing the main and complete case analyses of BDI-II found no essential differences between the alternative estimates of the AUC of folic acid minus placebo: in particular the complete case analysis's estimate of the adjusted AUC was 1.26 (95% CI from –0.56 to 3.08; $p = 0.18$), very close to that of the main analysis.

Multi-level repeated-measures analysis

We fitted a mixed-effects multi-level model with the same covariates as the AUC analysis. *Table 19* shows that both analyses reported the same general findings; in particular the repeated measures analysis's estimate of the AUC was 0.67 (95% CI from –1.00 to 2.34; $p = 0.429$), close to that of the main analysis.

TABLE 18 Estimated effectiveness on BDI-II of folic acid vs. placebo – sensitivity to missing data

	Difference (folate minus placebo)					
	Main analysis (imputing missing) ($n = 440$)			Complete case analysis ($n = 323$)		
BDI-II scores	Mean	95% CI	Significance	Mean	95% CI	Significance
AUC average: unadjusted	–0.06	–2.02 to 2.15	0.953	0.11	–2.30 to 2.52	0.930
AUC average: adjusted	1.09	–0.48 to 2.66	0.173	1.26	–0.56 to 3.08	0.175
ANCOVA: adjusted						
4 weeks	0.45	–1.34 to 2.23	0.623	0.91	–1.14 to 2.96	0.384
12 weeks	1.52	–0.64 to 3.69	0.168	1.75	–0.68 to 4.19	0.157
25 weeks	1.27	–0.99 to 3.54	0.270	1.24	–1.44 to 3.91	0.364

TABLE 19 Estimated effectiveness of folic acid vs. placebo – sensitivity to analysis by AUC

	Difference (folate minus placebo)					
	Main analysis (AUC)			Repeated measures analysis		
Outcome variable	Mean	95% CI	Significance	Mean	95% CI	Significance
BDI-II	1.09	–0.48 to 2.66	0.173	0.67	–1.00 to 2.34	0.429
MADRS	0.71	–0.51 to 1.93	0.252	0.49	–0.80 to 1.78	0.452
EQ-5D	0.001	–0.033 to 0.031	0.982	0.013	–0.062 to 0.087	0.760
EQ-VAS	–0.48	–3.16 to 2.20	0.726	0.03	–0.02 to 0.08	0.230
SF-12 PCS	0.40	–0.78 to 1.59	0.501	0.64	–0.61 to 1.90	0.313
SF-12 MCS*	**–1.97**	**–3.49** to **–0.44**	**0.012**	**–1.83**	**–3.48** to **–0.19**	**0.029**
CGI: Severity	0.05	–0.11 to 0.20	0.553	0.00	–0.16 to 0.16	0.995
CGI: Improvement	0.04	–0.13 to 0.21	0.649	0.01	–0.15 to 0.16	0.942
CGI: Efficacy[a]	–0.02	–0.11 to 0.07	0.604	–0.05	–0.17 to 0.08	0.482
Estimated CGI	0.98[b]	0.90[b]	1.07[b]	0.95[b]	0.84 to 1.08[b]	

a After logarithmic transformation.
b Ratio of estimated CGI scores and CI for the ratio.
* Differences significant at 5% level.

Analysis reweighted to adjust for participants who did not respond after baseline

Table 20 comparing the main and reweighted analyses of BDI-II found no essential differences between the alternative estimates of the AUC of folic acid minus placebo: in particular the reweighted analysis's estimate of the adjusted AUC was 1.17 (95% CI from –0.36 to 2.69; $p = 0.135$), very close to that of the main analysis. *Table 47* in *Appendix 10* shows that the 35 participants completely lost to follow-up were very similar to their 'nearest neighbours' in the analysed population. For 30 participants the matches were unambiguous; we resolved the ambiguity for the remaining five participants by matching them with the candidate with the closest BDI-II at baseline.

TABLE 20 Estimated effectiveness of folic acid compared with placebo – sensitivity to non-response

	Folate minus placebo					
	Main analysis (responders) ($n = 440$)			Reweighted analysis ($n = 475$)		
BDI-II	Mean	95% CI	Significance	Mean	95% CI	Significance
AUC: unadjusted	–0.06	–2.02 to 2.15	0.953	1.02	–1.83 to 2.18	0.864
AUC: adjusted	1.09	–0.48 to 2.66	0.173	1.17	–0.36 to 2.69	0.135
ANCOVA: adjusted	0.45	–1.34 to 2.23	0.623	0.52	–1.19 to 2.22	0.554
4 weeks						
12 weeks	1.52	–0.64 to 3.69	0.168	1.51	–0.59 to 3.62	0.158
25 weeks	1.27	–0.99 to 3.54	0.270	1.36	–0.83 to 3.55	0.222

Biochemistry analysis

Adjusting clinical effectiveness outcomes for biochemistry

Table 21 shows that baseline BDI-II was a powerful predictor of subsequent BDI-II scores, to the exclusion of all biochemical covariates in stepwise linear regression.

The alternative repeated measures analysis adjusted for baseline biochemistry but still found no consistent evidence of differences between folate and placebo groups in any outcome (*Table 22*). For example after adjustment for serum folate the estimated difference between the folate and placebo groups in the BDI-II after treatment was 0.68 (95% CI from –0.96 to 2.32; $p = 0.413$). Again only the SF-12 mental health component score (MCS) showed statistical significance with an estimated difference adjusted for baseline serum folate of –1.85 (95% CI from –3.48 to –0.21; $p = 0.027$).

The estimated change in BDI-II after a unit increase in baseline serum folate was –0.07 (95% CI from –0.24 to 0.10; $p = 0.431$). Only in the CGI Improvement scale did any baseline biochemical variables predict clinical outcome. There was some evidence that red cell folate predicts outcome and strong evidence for homocysteine: a single unit of baseline homocysteine increased CGI improvement by 0.05 (95% CI from 0.03 to 0.08; $p < 0.001$).

The repeated measures analysis adjusted for biochemical measures while on treatment in week 12 found no evidence of difference between folate and placebo groups in any instrument (*Table 23*). However there was clear evidence across most instruments except SF-12 showing that homocysteine measured in week 12 while on treatment predicted clinical outcomes like MADRS, CGI (severity and improvement) and EQ-5D. In particular a unit increase in homocysteine at week 12 increased BDI-II by 0.34 (95% CI from 0.15 to 0.52; $p = 0.001$). *Tables 48 and 49* of *Appendix 10* elaborate on *Tables 22* and *23* by reporting more extensive models in more detail.

Biochemistry outcomes

Despite the lack of clinical response to folic acid, it was effective in increasing participants' folate. *Table 24* shows that the folate group had higher serum folate by 15.1 (95% CI from 12.4 to 17.8) at 12 weeks, and by 15.6 (95% CI from 13.3 to 17.8) at 25 weeks. The difference in red cell folate was 272 (95% CI from 210 to 334) at 12 weeks, but only 82 (95% CI from 26 to 139) at 25 weeks. Baseline scores enhanced the prediction of all four biochemical outcomes. Each unit of baseline serum folate increased serum folate on treatment by 0.49 (95% CI from 0.30 to 0.68); each unit of baseline red cell folate increased red cell folate on treatment by 0.34 (95% CI from 0.22 to 0.45). Age was another good predictor for red cell folate: each

TABLE 21 Beck Depression Inventory at 12 and 25 weeks by treatment allocated

	Difference (folate minus placebo)			
	Mean (SE)	95% CI	Significant covariates	Significance
Unadjusted				
BDI-II (12 weeks)	1.21 (1.14)	–1.02 to 3.44	Baseline BDI-II score	< 0.001
BDI-II (25 weeks)	0.71 (1.20)	–1.65 to 3.07	Baseline BDI-II score	< 0.001
Adjusted by stratification variables and allocated treatment				
BDI-II (12 weeks)	1.43 (1.12)	–0.79 to 3.65	Baseline BDI-II score	< 0.001
			Centre	0.019
BDI-II (25 weeks)	0.90 (1.18)	–1.42 to 3.22	Baseline BDI-II score	< 0.001
			Type of ADM	0.007
			Centre	0.012

TABLE 22 Clinical effectiveness by repeated measures analysis adjusting for baseline biochemistry

		Difference (folate minus placebo)		
Outcome variable	Covariate	Mean (SE)	95% CI	Significance
BDI-II	Serum folate	0.682 (0.833)	–0.96 to 2.32	0.413
	Red cell folate	–0.045 (0.990)	–1.99 to 1.90	0.964
MADRS	Serum folate	0.530 (0.649)	–0.75 to 1.81	0.415
	Red cell folate	0.351 (0.744)	–1.11 to 1.82	0.638
EQ-5D[a]	Serum folate	0.012 (0.038)	–0.064 to 0.068	0.763
	Red cell folate	0.028 (0.022)	–0.015 to 0.070	0.202
EQ-VAS[a]	Serum folate	1.019 (0.850)	–0.65 to 2.69	0.231
	Red cell folate	–0.026 (1.016)	–2.03 to 1.97	0.980
MCS	Serum folate	–1.848 (0.832)	–3.48 to –0.21	0.027
	Red cell folate	–0.610 (0.991)	–2.56 to 1.34	0.538
PCS	Serum folate	0.657 (0.635)	–0.59 to 1.91	0.302
	Red cell folate	0.996 (0.743)	–0.47 to 2.45	0.181
CGI: Severity	Serum folate	0.003 (0.082)	–0.16 to 0.16	0.971
	Red cell folate	–0.085 (0.095)	–0.27 to 0.10	0.368
CGI: Improvement	Serum folate	0.059 (0.109)	–0.15 to 0.27	0.586
	Red cell folate	–0.022 (0.124)	–0.27 to 0.22	0.859

a Raw scores rather than QALYs.

TABLE 23 Clinical effectiveness by repeated measures analysis adjusting for biochemistry 'on treatment'

		Difference (folate minus placebo)		
Outcome variable	Covariate	Mean (SE)	95% CI	Significance
BDI-II	Serum folate	0.107 (1.093)	–2.04 to 2.26	0.922
	Red cell folate	–1.794 (1.490)	–4.73 to 1.14	0.230
MADRS	Serum folate	0.260 (0.894)	–1.50 to 2.02	0.772
	Red cell folate	–0.623 (1.188)	–2.97 to 1.72	0.601
EQ-5D	Serum folate	0.021 (0.022)	–0.011 to 0.074	0.363
	Red cell folate	0.059 (0.031)	–0.003 to 0.121	0.060
EQ-VAS	Serum folate	0.107 (1.093)	–2.04 to 2.26	0.922
	Red cell folate	–1.794 (1.490)	–4.73 to 1.14	0.230
MCS	Serum folate	–1.172 (1.146)	–3.42 to 1.08	0.307
	Red cell folate	0.096 (1.565)	–2.99 to 3.18	0.951
PCS	Serum folate	1.136 (0.825)	–0.49 to 2.76	0.170
	Red cell folate	1.502 (1.114)	–0.69 to 3.70	0.179
CGI: Severity	Serum folate	–0.042 (0.112)	–0.26 to 0.18	0.706
	Red cell folate	–0.233 (0.144)	–0.52 to 0.05	0.107
CGI: Improvement	Serum folate	–0.063 (0.119)	–0.30 to 0.17	0.597
	Red cell folate	–0.116 (0.165)	–0.44 to 0.21	0.480

TABLE 24 Biochemistry outcomes at 12 weeks – by treatment allocated

	Difference (folate minus placebo)			Statistical significance of other variables	
Outcome variable	Mean (SE)	95% CI	Significance	Covariate	Significance
Serum folate	15.1 (1.4)	12.4 to 17.8	< 0.001	Centre	0.034
				Time	< 0.001
				Baseline serum folate	< 0.001
				Type of antidepressant	0.209
				Previous treatment	0.856
				Red cell folate at 12 weeks	< 0.001
				Allocated treatment by time	< 0.001
Red cell folate	272.4 (31.4)	210.5 to 334.3	< 0.001	Centre	0.504
				Time	0.004
				Baseline red cell folate	< 0.001
				Type of antidepressant	0.914
				Previous treatment	0.118
				Age	0.008
				Serum folate at 12 weeks	< 0.001
				Allocated treatment by time	0.066
Homocysteine	0.93 (0.37)	0.19 to 1.66	0.014	Centre	0.726
				Time	0.703
				Baseline homocysteine	< 0.001
				Type of antidepressant	0.285
				Previous treatment	0.773
				Age	0.002
				Gender	< 0.001
				Serum folate at 12 weeks	< 0.001
Vitamin B_{12}	6.20 (8.28)	–10.1 to 22.5	0.454	Centre	0.005
				Time	0.036
				Baseline B_{12}	< 0.001
				Type of antidepressant	0.017
				Previous treatment	0.998
				Homocysteine at 12 weeks	0.005

Note: Threshold significance level for main effects to be included in the model is $p < 0.05$, and for interactions is $p < 0.10$.

year increased mean red cell folate by 2.17 (95% CI from 0.58 to 3.75). The reduction in homocysteine caused by folic acid was –0.93 (95% CI from –1.66 to –0.19). Prediction of homocysteine improved after adjustment for baseline homocysteine and serum folate at 12 weeks. Age and gender were also good predictors for homocysteine: for every year of life homocysteine reduced by 0.048 (95% CI from 0.021 to 0.076); and females had higher homocysteine by 1.64 (95% CI from 0.91 to 2.34).

Many expected that patients deficient in folate and randomised to the folated group would achieve improvements in biochemical and clinical outcomes. So we analysed the subset of patients who at baseline had a serum folate concentration of less than 3 µg/l, a red cell folate less than 200 µg/l, or homocysteine greater than 15.3 µmol/l. *Table 25* found no clinical or statistical difference between folate and placebo

TABLE 25 Participants with baseline deficiency in biochemistry – by treatment allocated

			Difference (folate minus placebo)		
Outcome variable	Deficiency	*n*	Mean (SE)	95% CI	Significance
BDI-II	Serum folate < 3 µg/l	27	–1.67 (3.99)	–9.85 to 6.51	0.679
	Red cell folate < 200 µg/l	9	–18.4 (8.2)	–36.8 to 0.2	0.052
	Homocysteine > 15.3 µmol/l	53	3.51 (2.63)	–1.77 to 8.78	0.188
MADRS	Serum folate < 3 µg/l	27	2.51 (2.40)	–2.42 to 7.43	0.305
	Red cell folate < 200 µg/l	9	–7.49 (3.66)	–15.8 to 0.79	0.071
	Homocysteine > 15.3 µmol/l	53	1.04 (1.94)	–2.85 to 4.94	0.593
EQ-5D	Serum folate < 3 µg/l	27	–0.080 (0.074)	–0.226 to 0.072	0.281
	Red cell folate < 200 µg/l	9	0.189 (0.131)	–0.109 to 0.488	0.179
	Homocysteine > 15.3 µmol/l	53	0.03 (0.07)	–0.11 to 0.17	0.661
EQ-VAS	Serum folate < 3 µg/l	27	0.5 (6.0)	–11.8 to 12.8	0.937
	Red cell folate < 200 µg/l	9	34.5 (15.5)	–0.6 to 69.6	0.054
	Homocysteine > 15.3 µmol/l	53	–1.9 (5.9)	–13.6 to 9.9	0.753
MCS	Serum folate < 3 µg/l	27	0.83 (3.69)	–6.75 to 8.40	0.824
	Red cell folate < 200 µg/l	9	15.9 (6.2)	2.0 to 29.9	0.029
	Homocysteine > 15.3 µmol/l	53	–0.38 (2.47)	–5.33 to 4.56	0.877
PCS	Serum folate < 3 µg/l	27	2.26 (2.65)	–3.18 to 7.69	0.402
	Red cell folate < 200 µg/l	9	–1.88 (3.94)	–10.8 to 7.02	0.644
	Homocysteine > 15.3 µmol/l	53	–3.13 (2.04)	–7.21 to 0.94	0.129
CGI: Severity	Serum folate < 3 µg/l	27	–0.19 (0.34)	–0.88 to 0.50	0.581
	Red cell folate < 200 µg/l	9	–1.40 (0.52)	–2.57 to –0.23	0.024
	Homocysteine > 15.3 µmol/l	53	–0.14 (0.23)	–0.61 to 0.33	0.549
CGI: Improvement	Serum folate < 3 µg/l	27	0.09 (0.32)	–0.56 to 0.73	0.787
	Red cell folate < 200 µg/l	9	–1.35 (0.63)	–2.75 to 0.05	0.058
	Homocysteine > 15.3 µmol/l	53	–0.06 (0.23)	–0.52 to 0.40	0.802

groups among those with deficiency in serum folate or homocysteine. For example the estimated difference in BDI-II in patients who had less than 3 µg/l serum folate was –1.67 (95% CI from –9.85 to 6.51; $p = 0.68$). In contrast the few patients who were deficient in baseline red cell folate (RCF) yielded necessarily weak but consistent evidence across multiple instruments that augmenting ADM with folic acid improved clinical outcome. Since high scores in EQ-5D and SF-12, and low scores in depression scales are all good, folic acid achieved (near) significant improvements in six of the eight criteria, specifically in the RCF-deficient group.

Cost-effectiveness

Resource use

Table 26 presents participants' use of NHS and PSS resources over their 25 weeks in the trial. The majority of participants, 78% and 81% in folic acid and placebo groups respectively, visited their GP on at least one occasion. The mean number of visits was 3.3 and 3.9. Many participants reported telephone contact with their GPs – 21% of those in the folic acid group and 27% in the placebo group; and visits to practice nurses – 35% and 39% respectively. Despite the NICE recommendation that people with moderate to severe depression should receive psychological therapy, only 7% of intervention participants and 6% of controls did so (0.4 times on average). However 22% of those randomised to folic acid consulted psychiatrists at hospital clinics (0.6 times on average), compared with 29% of control participants (0.8 times on average). Other hospital visits were reported by similar numbers of participants – 22% and 25% respectively; and only 6% of patients in each group were admitted to hospital. All patients received prescribed medication during the trial, with a mean of 6.5 and 7.2 antidepressant items in folic acid and placebo groups respectively. Total prescribing was comparable at 21.0 and 21.9 items respectively. Participants' use of social services was low. Thus there were no significant differences in resource use between the two groups.

The mean percentage of responses missing from the resource use questionnaire, across all questions and time points, was 10.1% in the folic acid group and 11.0% in the control group. The level of missing data was not related to the treatment allocation at any time point (Student's *t*-test: $p > 0.1$ for each point). The lowest rate of missing data was in response to consultations at general practices (8.1% across both treatment groups) while the highest rate of missing data related to contact with health visitors (23.5%). The high response rate is in marked contrast to the AHEAD study, which used the same questionnaire, but required patients to return their completed forms by mail, leaving 73.8% of questionnaires incomplete.[138]

Costs

Table 27 presents unadjusted costs incurred during the 25 weeks of follow-up. There were no statistically significant differences in categorised or total cost between treatment groups, although there was a tendency towards lower costs in the control group for all categories. Psychiatric services in hospital or community services took more than half the total cost – £797 in the folic acid group, and £886 in the placebo control group. Participants' attendances at hospital clinics for consultations with psychiatrists was the main driver of this. The costs of prescribed medicines were the second highest cost category at £240 and £257 in intervention and control groups respectively. Antidepressants accounted for around 30% of total medication costs.

There were differences in baseline costs, evident from the resource use questionnaire relating to the 3 months before baseline visits. Baseline costs in the folic acid group were £514 compared with £746 in the placebo group (a difference of £232, 95% CI from –£9 to £487). Although baseline costs do not contribute to the total costs, imbalances at baseline may reflect an imbalance in patient or disease

TABLE 26 Resource use over 25 weeks – by treatment allocated

	Folate ($n = 223$)			Placebo ($n = 217$)		
		Non-zero responses			Non-zero responses	
Type of resource	Mean (SD)	no. (%)	[Median][a]	Mean (SD)	no. (%)	[Median][a]
General practice, community pharmacy, and nursing services						
GP surgery visits	3.3 (3.3)	173 (78)	[3]	3.9 (4.1)	176 (81)	[3]
GP home visits	0.1 (0.6)	11 (5)	[2]	0.0 (0.2)	3 (1)	[1]
GP telephone contacts	0.4 (1.3)	46 (21)	[1]	0.6 (1.4)	59 (27)	[1]
Visits to practice nurse	0.7 (1.2)	79 (35)	[1]	1.3 (3.9)	84 (39)	[2]
District nurse home visits	0.0 (0.1)	1 (0)	[2]	0.0 (0.2)	2 (1)	[2]
Counsellor at surgery	0.6 (1.8)	31 (14)	[3]	0.4 (1.5)	27 (12)	[2]
Vitamin B_{12} testing	1.0 (0)	223 (100)	[1]	0.0 (0)	0 (0)	[0]
Folic acid dispensing fee	1.0 (0)	223 (100)	[1]	0.0 (0)	0 (0)	[0]
Antidepressant items dispensed	6.5 (3.9)	223 (100)	[6]	7.2 (5.7)	217 (100)	[6]
All prescription items dispensed	21.0 (17.8)	223 (100)	[17]	21.9 (27.3)	217 (100)	[14]
Other	0.2 (0.9)	19 (9)	[3]	0.1 (0.6)	11 (5)	[2]
Health visitor	0.2 (2.6)	4 (2)	[4]	0.1 (0.5)	7 (3)	[1]
Social services						
Social worker	0.3 (1.4)	15 (7)	[2]	0.3 (1.4)	14 (6)	[3]
Home help	0.2 (3.2)	1 (0)	[48]	1.8 (19.1)	3 (1)	[162]
Care assistant	0.3 (3.3)	3 (1)	[8]	0.9 (10.0)	5 (2)	[6]
Day centre visits	0.1 (0.8)	2 (1)	[6.5]	0.2 (1.8)	5 (2)	[12]
Other (social services)	0.0 (0.4)	5 (2)	[2]	0.1 (1.3)	6 (3)	[2.5]
Psychiatric hospital and community services						
Psychiatrist at hospital clinic	0.6 (1.7)	50 (22)	[2]	0.8 (1.9)	63 (29)	[2]
Psychiatrist at home	0.0 (0.2)	3 (1)	[2]	0.0 (0.3)	2 (1)	[2.5]
Psychologist	0.4 (2.2)	15 (7)	[4]	0.4 (2.3)	14 (6)	[2]
Community psychiatric nurse	0.5 (2.0)	25 (11)	[3]	1.4 (4.0)	35 (16)	[5]
Other (psychiatric services)	0.4 (1.6)	25 (11)	[2]	0.3 (2.2)	16 (7)	[2.5]
Other services						
Day hospital	0.2 (0.9)	18 (8)	[1.5]	0.3 (1.3)	17 (8)	[2]
Accident and Emergency	0.2 (0.6)	31 (14)	[1]	0.3 (1.1)	38 (18)	[1]
Hospital clinic	0.5 (1.5)	48 (22)	[1]	0.6 (1.5)	55 (25)	[2]
Nights spent on hospital ward	0.8 (5.2)	13 (6)	[6]	0.4 (2.5)	13 (6)	[3]
Occupational or employment health services	0.1 (0.5)	12 (5)	[1.5]	0.3 (1.0)	21 (10)	[2]
Other (hospital)	0.2 (0.9)	14 (6)	[1]	0.1 (0.7)	9 (4)	[1]
NHS Direct	0.2 (1.7)	19 (9)	[1]	0.1 (1.0)	12 (6)	[1.5]
Ambulance or paramedic	0.1 (0.4)	14 (6)	[1]	0.1 (0.5)	11 (5)	[1]

a Median of non-zero responses.

TABLE 27 Unadjusted costs through 25 weeks – by treatment allocated

Type of cost	Folate Mean (95% CI)	Placebo Mean (95% CI)	Difference (folate minus placebo) Mean (95% CI)
GP costs	164.20 (144.72 to 185.02)	186.46 (163.60 to 210.61)	–22.26 (–53.61 to 8.64)
Social care costs	148.46 (84.63 to 233.98)	324.86 (144.51 to 569.11)	–176.40 (–428.14 to 19.63)
Psychiatric hospital and community services costs	797.37 (562.94 to 1090.52)	886.40 (712.65 to 1089.37)	–89.03 (–404.23 to 249.42)
Antidepressant drug costs	72.93 (62.39 to 84.47)	75.30 (63.84 to 88.44)	–2.37 (–19.37 to 13.96)
All medication costs	239.95 (206.94 to 275.14)	256.79 (201.15 to 324.42)	–16.84 (–91.66 to 49.17)
Other costs	60.72 (44.60 to 78.85)	66.44 (48.50 to 86.49)	–5.72 (–31.42 to 19.50)
Total cost	1410.21 (1147.28 to 1729.31)	1719.12 (1398.10 to 2088.25)	–308.94 (–764.14 to 155.18)

characteristics. Hence they may bias cost estimates, particularly if previous use of health and social care predicts future use. The primary cost analysis therefore corrected for baseline differences by regression.[125]

Adjusting for these differences at baseline reduced the mean difference in total costs from £309 (95% CI from –£155 to £764) to £48 (95% CI from –£292 to £389), still not significant.

Health outcomes

Figure 12 shows the distribution of EQ-5D scores by time, treatment group and dimension. At baseline the majority of patients described themselves as having either moderate or severe problems in relation to anxiety or depression (97% in both groups), pain or discomfort (61% and 59% for folic acid and placebo groups respectively), and usual activities (77% and 80% respectively). Improvements in the states of anxiety or depression (to 75% in both groups), and ability to perform usual activities (to 62% and 55% respectively) were evident between baseline and 25 weeks. The corresponding changes in unadjusted mean utilities from baseline to 25 weeks were 0.481 to 0.605 for folic acid and 0.514 to 0.607 for placebo.

Table 28 presents the numbers of gross and net QALYs gained, as measured by the EQ-5D (primary analysis), EQ-VAS and SF-6D. There were no statistically significant differences between treatment groups. Similarly there were no differences in either outcome measure in the cost-effectiveness analyses – the AUC for BDI-II scores and the number of 'depression-free weeks' (when participants' BDI-II scores were less than 13). The number of participants reporting time free from depression was low – 18 (11%) in the folate group and 23 (15%) in the placebo group at 4 weeks, rising to 56 (33%) and 63 (41%), respectively, at 25 weeks.

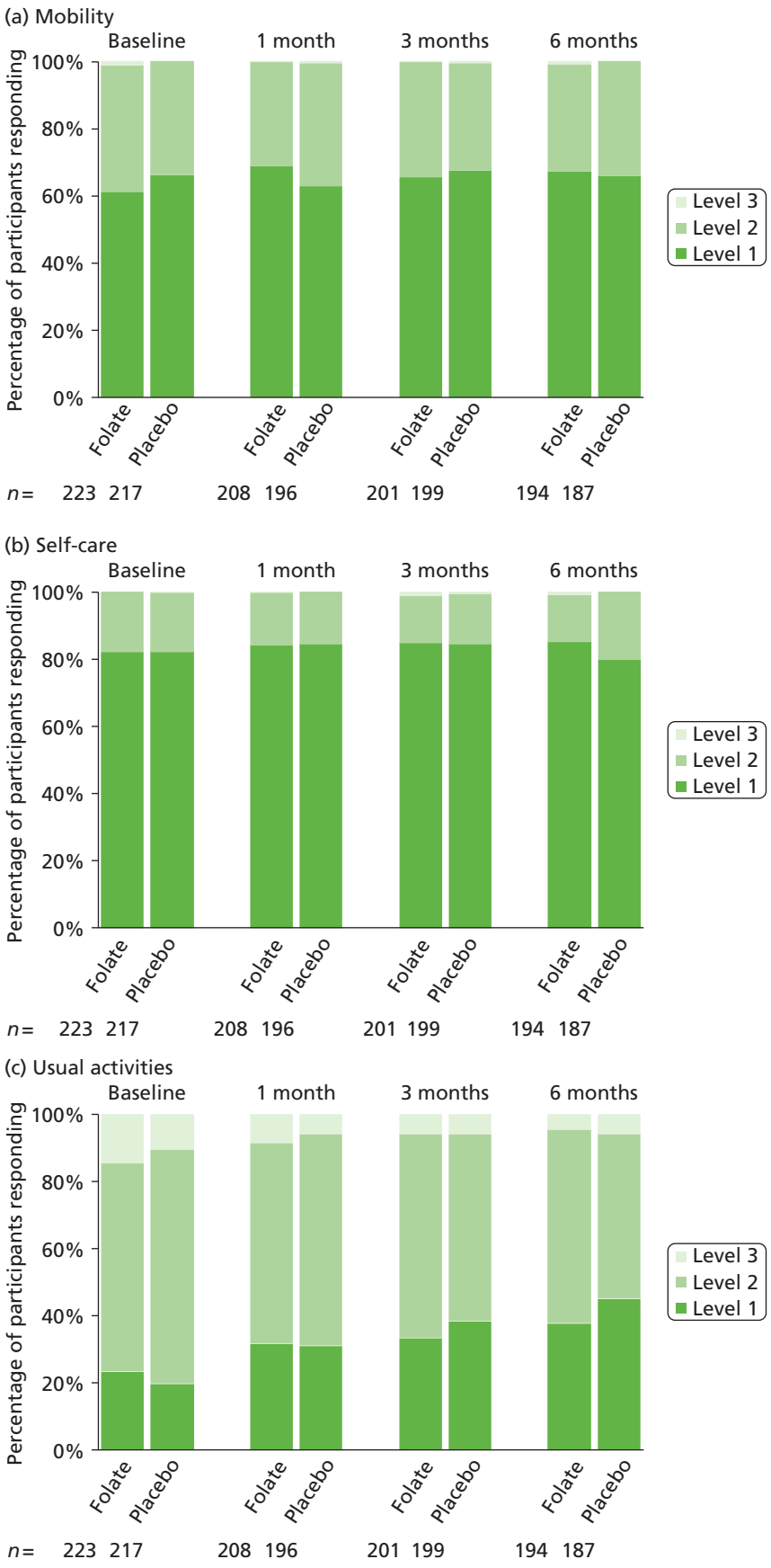

FIGURE 12 Participants responding to each dimension of the EQ-5D – by time and treatment allocated. *n* shows the number of completed responses within each treatment group. Level 3 represents the most severe problems. (*continued*)

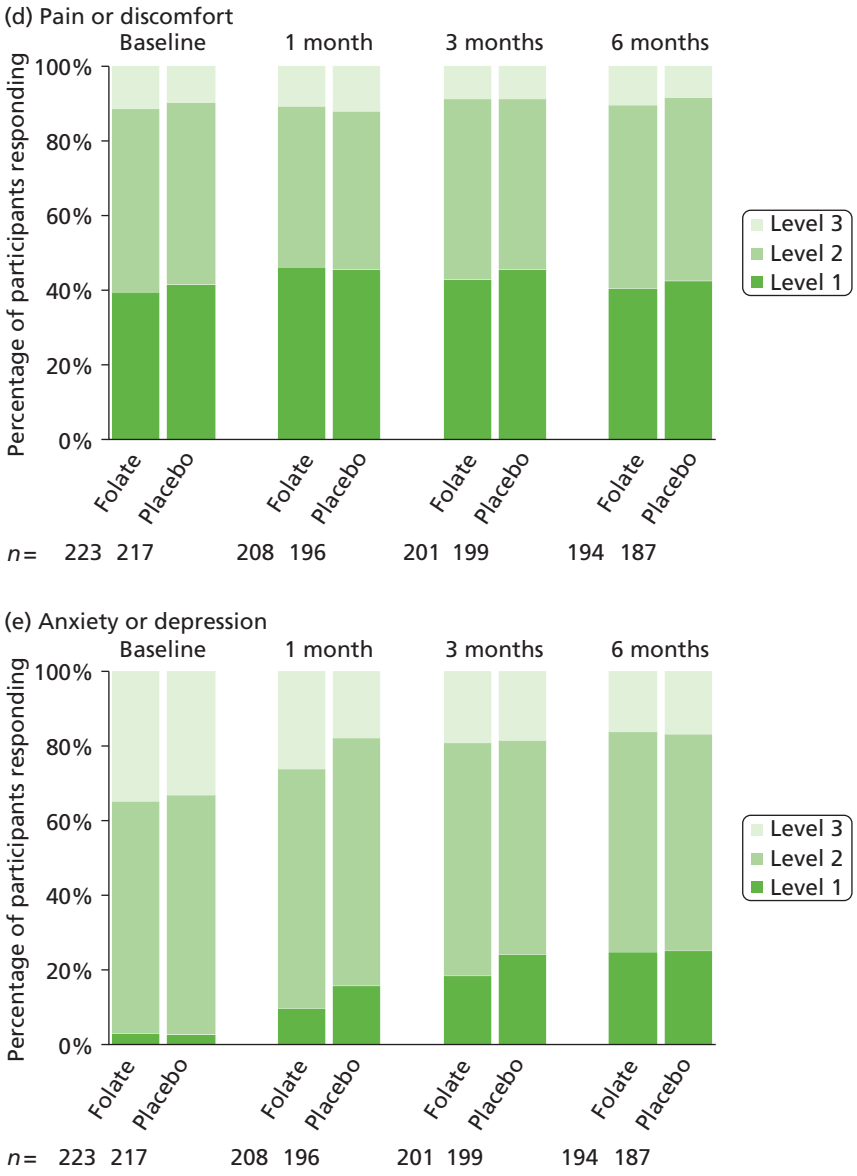

FIGURE 12 Participants responding to each dimension of the EQ-5D – by time and treatment allocated. *n* shows the number of completed responses within each treatment group. Level 3 represents the most severe problems.

TABLE 28 Unadjusted health outcomes over 25 weeks – by treatment allocated

Outcome measure	Full responses no. (%)		Mean (95% CI) including inputed response		
	Folate	Placebo	Folate ($n = 223$)	Placebo ($n = 217$)	Difference
QALYs (EQ-5D)	172 (77.1)	165 (76.0)	0.290 (0.270 to 0.308)	0.298 (0.279 to 0.316)	–0.0079 (–0.0346 to 0.0191)
QALYs (EQ-5D – complete cases)			0.294[a] (0.274 to 0.314)	0.290[b] (0.269 to 0.309)	0.0046 (–0.0254 to 0.0328)
QALYs (EQ-VAS)	173 (77.6)	162 (74.7)	0.276 (0.262 to 0.290)	0.275 (0.262 to 0.288)	0.0008 (–0.0187 to 0.0197)
QALYs (SF-6D)	157 (70.4)	141 (65.0)	0.292 (0.273 to 0.311)	0.303 (0.284 to 0.322)	–0.0113 (–0.0378 to 0.0156)
AUC (BDI-II)[c]	169 (75.8)	154 (71.0)	12.91 (12.19 to 13.66)	12.95 (12.16 to 13.76)	–0.030 (–1.12 to 1.06)

a $n = 172$.
b $n = 165$.
c True area under curve, not 'AUC average'.

Cost-effectiveness and uncertainty

In the primary analysis after baseline adjustment following Manca *et al.*,[126] folic acid is on average £48 less expensive than the placebo group, and more effective by 0.0012 QALYs. As those findings put folic acid in the south-east quadrant of the cost-effectiveness plane (*Figure 13*) it is therefore the dominant strategy. However there is considerable uncertainty surrounding these estimates, shown by the distribution of costs and QALYs over all four quadrants of the cost-effectiveness plane. The estimated probability of folic acid saving costs is 64%, and that of it gaining QALYs is 55% (*Table 29*). The resulting cost-effectiveness

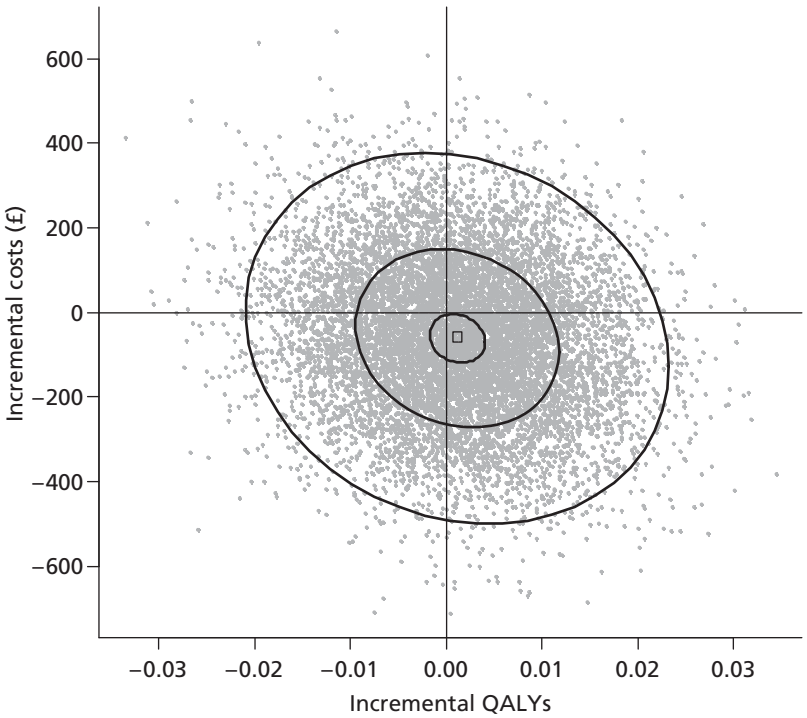

FIGURE 13 Cost-effectiveness plane showing joint distribution of costs and QALYs. Note: Confidence ellipses represent the 5%, 50% and 95% levels of confidence.

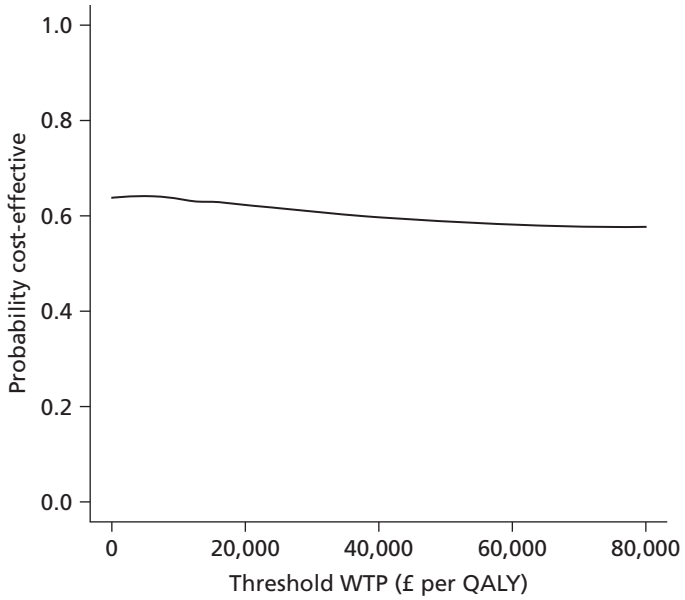

FIGURE 14 Cost-effectiveness acceptability curve showing probability of folate being cost-effective – by cost-effectiveness threshold (£ per QALY). WTP, willingness to pay.

acceptability curve (*Figure 14*) shows that the probability of folic acid being cost-effective is 0.62 at the threshold of £20,000 per QALY and 0.61 at the £30,000 threshold (see *Table 29*).

As this ICER is close to the origin of the cost-effectiveness plane, comparison with other QALY outcomes is labile. For example QALYs derived from both the EQ-VAS and SF-6D located the ICER in the south-west quadrant, where folic acid is both less effective and less expensive than placebo.

The interpretation of cost-effectiveness results suffers from lack of a benchmark for economic efficiency. The unstable direction of differences in mean effect exacerbates this. The primary clinical outcome of area under the BDI-II curve shows folic acid dominating placebo, being more effective and less costly. However the cost per depression-free week averted suggests that folic acid is less effective by an average of 6 depression-free days over 25 weeks, though less expensive. In short the proximity of all possible criteria to the origin of the cost-effectiveness plane, and associated uncertainty suggests that folic acid is no more effective, but no more expensive than placebo.

Genetics

Introduction

Of the 440 patients included in the main analysis by treatment allocated, we excluded from genetic analysis: five with low genotype call rates; 14 non-Caucasians; and 38 who did not consent to the genetic study. Thus we included 383 patients in genetic analysis.

Of the 142 variants genotyped, 38 were omitted as they failed to meet minimum inclusion criteria. These included 19 SNPs owing to quality controls issues with the call rate of the specific SNP assay; 13 SNPs due to a minor allele frequency < 0.01, and six with a Hardy–Weinberg p-value of < 0.0001. In total 104 genetic variants were carried forward for analysis.

TABLE 29 Deterministic and stochastic costs and effects, adjusted for baseline and time in trial

Outcome	Difference	95% CI	ICER	Pr (cost saving)	Pr (clinically superior)	Pr (cost-effective at £20,000/QALY)	Pr (cost-effective at £30,000/QALY)
Costs	–£48.40	–£389 to £292	–	–	–	–	–
QALYs (EQ-5D)	0.0012	–0.0132 to 0.0186	Folate is dominant	0.6373	0.5480	0.6240	0.6093
QALYs (EQ-VAS)	–0.0017	–0.0155 to 0.0121	£28,110 per QALY[a]	0.6316	0.4024	0.5420	0.5135
QALYs (SF-6D)	0.0008	–0.0167 to 0.0183	Folate is dominant	0.6251	0.5314	0.6064	0.5926
QALYs (EQ-5D complete cases)[b]	0.0129	–0.0062 to 0.0319	Folate is dominant	0.7847	0.9011	0.9176	0.9226
BDI-II (AUC)	0.4702	–0.3240 to 1.2643	Folate is dominant	0.6276	0.8789		
Depression-free weeks (BDI-II < 13)	–0.8673	–2.1471 to 0.4124	£56 per depression-free week[a]	0.6278	0.0857		

Pr, probability.
a ICERs arise from the south-west quadrant of the cost-effectiveness plane, where folate is less effective, but incurs less cost, than placebo.
b For complete cases, the baseline-adjusted incremental cost was –£158 (unadjusted –£150).

Results of the analysis of association between each SNP and each of the seven outcome measures (BDI-II, MADRS, CGI1 severity of illness, EQ-5D, EQ-5D visual analogue score (VAS), SF-12 mental, and SF-12 physical) are given in *Table 30*. Two associations gave a FDR < 0.05. Results of assessing for the statistical significance of a SNP-treatment group interaction term for each SNP and each outcome are given in *Table 31* – for this only one SNP gave a FDR < 0.05.

Statistically significant main single nucleotide polymorphism effects

The rs11627525 SNP in the methylenetetrahydrofolate dehydrogenase (NADP$^+$ dependent) 1 (*MTHFD1*) gene was associated with MADRS ($p = 0.0004$, FDR = 0.0467). This association was not replicated with any of the other six outcome measures analysed ($p > 0.05$; FDR > 0.05).

A plot of mean MADRS at each study time point compared with rs11627525 genotype, stratifying by genotype [CC wild-type group and a combined CT and TT group since the homozygote variant allele frequency was so low ($n = 4$)], is given in *Figure 15*. At baseline, there was no difference observed [28.3 ± 0.4 (CC) compared with 29.0 ± 0.7 (CT/TT)]. The data suggest a more dramatic reduction in MADRS between baseline and 12 weeks between CC individuals (mean difference between baseline and 12 weeks: 6.11) and the combined CT/TT group (mean difference between baseline and 12 weeks: 9.82).

The rs588458 SNP in the folate hydrolase 1 (*FOLH1*) gene was associated with EQ-5D ($p = 0.0003$, FDR = 0.0337). This association was not replicated with any of the other six outcomes (FDR > 0.05), although both EQ-VAS ($p = 0.0414$) and SF-12 mental ($p = 0.0140$).

A plot of mean EQ-5D scores at baseline, 4 12 and 25 weeks, stratified into the three genotype groups, is given in *Figure 16a*. At baseline, TT carriers had a mean EQ-5D of 0.534 (± 0.024) – the mean scores for heterozygotes (TC carriers) were 0.049 lower, while they were 0.102 points lower for CC carriers. Similar differences were observed at 4 weeks with TC carriers being 0.078 lower than TT (0.625 ± 0.023) with CC carriers being 0.140 lower than TT. A significant difference in score was apparent at 12 weeks where the mean score (± SE) for TT carriers was 0.645 (± 0.023) compared with 0 for TC genotype (0.058 lower) and (0.140 lower than baseline) for individuals with the CC genotype. Similarly, at 25 weeks, mean EQ-5D for TT individuals (0.673 ± 0.023) was significantly higher (0.09 points) than TC which, in turn, was significantly higher (0.192 points) than for CC genotype). The overall trajectory of improvement (by EQ-5D) appears to stabilise first in the CC group (at around 4 weeks); then the TC group at around 12 weeks; whilst the wild-type TT group appear to continue to improve.

Plots for EQ-VAS and SF-12 Mental Component Scale (MCS) against rs588458 genotype are also provided (see *Figure 16b* and *c*, respectively) since the associations with rs588458 in the main SNP effect model gave $p < 0.05$. For mean EQ-VAS, there was a significant divergence between genotypes at 12 weeks with TT genotype (62.2 ± 1.9) significantly higher (difference of means = 4.5) than CT (57.7 ± 1.8) and CC (52.6 ± 2.8) (difference in means = 9.6). No difference was observed at any earlier time points. Mean SF-12 did not show an obvious divergence between genotypes at any of the time points though a moderate difference was seen at 12 weeks between TT (37.1 ± 1.0) and TC (34.3 ± 1.0).

TABLE 30 Significant associations between folate pathway gene polymorphisms and seven outcome measures

SNP	Gene symbol	Associated outcome measure	Significance
rs10380	*MTRR*	PCS	0.0491
rs1051266	*SLC19A1*	EQ-VAS	0.0103
rs10640	*AMT1*	MCS	0.0023
rs1127717	*ALDH1L1*	EQ-5D	0.0064
rs11545078	*GGH*	EQ-VAS	0.0162
rs11627525	*MTHFD1*	MADRS	0.0004*
rs11995525	*GGH*	BDI-II	0.0382
		MADRS	0.0337
		EQ-VAS	0.0383
rs12347	*MTRR*	CGI1	0.0329
rs1532268	*MTRR*	PCS	0.0489
rs16837178	*ALDH1L1*	BDI-II	0.0493
		MADRS	0.0460
		CGI1	0.0356
rs1801133	*MTHFR*	PCS	0.0297
		MCS	0.0481
rs2236225	*MTHFD1*	MADRS	0.0354
		MCS	0.0322
rs2273026	*SHMT1*	MADRS	0.0147
rs2273028	*SHMT1*	BDI-II	0.0431
		MADRS	0.0195
rs2330183	*SLC19A1*	EQ-VAS	0.0060
rs2461838	*SHMT1*	BDI-II	0.0159
		MADRS	0.0338
rs2853532	*TYMS*	PCS	0.0494
rs3772426	*ALDH1L1*	MADRS	0.0407
		MCS	0.0180
rs383028	*FOLH1*	MADRS	0.0303
		EQ-VAS	0.0143
rs4817577	*GART*	MCS	0.0181
rs4920037	*CBS*	MADRS	0.0361
		EQ-5D	0.0195
rs4933327	*MAT1A*	MADRS	0.0071
rs588458	*FOLH1*	EQ-5D	0.0003*
		EQ-VAS	0.0414
		MCS	0.0140
rs6435899	*ATIC*	BDI-II	0.0202
		MADRS	0.0046

continued

TABLE 30 Significant associations between folate pathway gene polymorphisms and seven outcome measures (*continued*)

SNP	Gene symbol	Associated outcome measure	Significance
		CGI1	0.0275
		EQ-5D	0.0212
		EQ-VAS	0.0023
		MCS	0.0488
rs6494509	*MTFMT*	PCS	0.0077
rs6668344	*MTR*	BDI-II	0.0489
rs6800400	*ALDH1L1*	PCS	0.0035
		EQ-5D	0.0090
rs7010484	*GGH*	BDI-II	0.0423
		CGI1	0.0087
		MCS	0.0342
rs7553194	*MTHFR*	PCS	0.0138
rs8012229	*MTHFD1*	MADRS	0.0023
TSER	*TYMS*	PCS	0.0088
		MCS	0.0369

* False discovery rate of < 0.05.

TABLE 31 Significant interactions between folate pathway gene polymorphisms, treatment and seven outcomes

SNP	Gene	Interacting outcome measure	Significance
rs1004474	*TYMS*	BDI-II	0.0456
		EQ-VAS	0.0369
rs1127717	*ALDH1L1*	EQ-5D	0.0368
rs13268472	*GGH*	PCS	0.0100
rs16837171	*ALDH1L1*	MADRS	0.0402
		MCS	0.0038
rs17102596	*MAT1A*	BDI-II	0.0135
		MADRS	0.0151
		CGI1	0.0086
		MCS	0.0002*
rs1801394	*MTRR*	MCS	0.0336
rs1950902	*MTHFD1*	CGI1	0.0097
rs2236225	*MTHFD1*	PCS	0.0023
		EQ-5D	0.0164
		EQ-VAS	0.0224
rs2276726	*ALDH1L1*	EQ-5D	0.0175
rs2372536	*ATIC*	EQ-5D	0.0069
		MCS	0.0016
rs2586154	*MTHFS*	BDI-II	0.0371
rs2586183	*MTHFS*	BDI-II	0.0073
		CGI1	0.0111
		EQ-VAS	0.0179
		MCS	0.0026
rs2733107	*MTHFS*	CGI1	0.0347
rs2853532	*TYMS*	MCS	0.0491
rs3862534	*MAT1A*	MADRS	0.0443
		MCS	0.0022
rs4779165	*MTHFS*	PCS	0.0057
rs4920037	*CBS*	BDI-II	0.0246
		CGI1	0.0333
		MCS	0.0315
rs535112	*CTH*	EQ-VAS	0.0350
rs7010484	*GGH*	MADRS	0.0025
rs8042012	*MTHFS*	CGI1	0.0262
		MCS	0.0061
TSER	*TYMS*	MCS	0.0182

* False discovery rate of < 0.05.

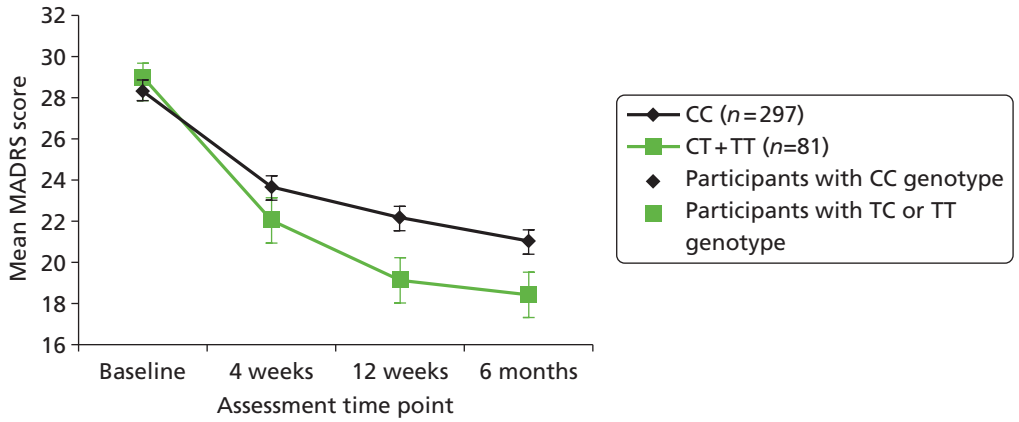

FIGURE 15 Montgomery–Åsberg Depression Rating Scale depression scores by *MTHFD1* rs11627525 genotype. Note: Graphs plot mean MADRS scores at each point with vertical bars showing standard errors of means.

Statistically significant single nucleotide polymorphism–treatment interaction

For the model incorporating the treatment interaction term, the association of the rs17102596 SNP in the methionine adenosyltransferase I alpha (*MAT1A*) gene and SF-12 mental status was statistically significant ($p = 0.0002$, FDR = 0.0255).

A plot of mean SF-12 Mental Component Score at each study time point stratified by genotype and treatment group (folic acid or placebo) is given in *Figure 17*. At baseline, no difference in SF-12 is observed between TT (25.0 ± 0.8) and TC + CC (25.2 ± 1.2) groups in the folate arm of the trial. In the placebo arm, there was a modest difference at baseline between TT (25.8 ± 0.9) and TC + CC (28.2 ± 1.3). In both arms of the trial, wild-type (TT) individuals showed a similar trajectory for increase in SF-12 with similar mean SF-12 at 25 weeks (folic acid = 25.0 ± 0.8; placebo = 25.8 ± 0.9). In individuals with TC or CC genotypes in the placebo group, there was a marginally steeper trajectory of the ΔSF-12 resulting in a significantly higher mean value at 12 weeks (39.6 ± 1.7) compared with TT individuals though much of this difference could be explained by the variability in baseline measure. This is considerably higher than those individuals with TC or CC genotype receiving folic acid (31.2 ± 1.7) (difference in mean = 8.4) whose Δ mean SF-12 trajectory stabilises after 4 weeks. The SNP effect model was run on the two arms of the trial separately (data not shown) with resulting statistically significant difference in both the folate group ($p = 0.02$) and the placebo group ($p = 0.00006$).

As three other outcomes measure (BDI-II, MADRS, and CGI severity of illness) gave $p < 0.05$ for tests of association with rs17102596-treatment interaction (see *Table 31*), these too were plotted (not shown). In all three instances the pattern demonstrated with SF-12 was not replicated and no clear distinction in the trajectory of the improvement determined by each outcome was seen.

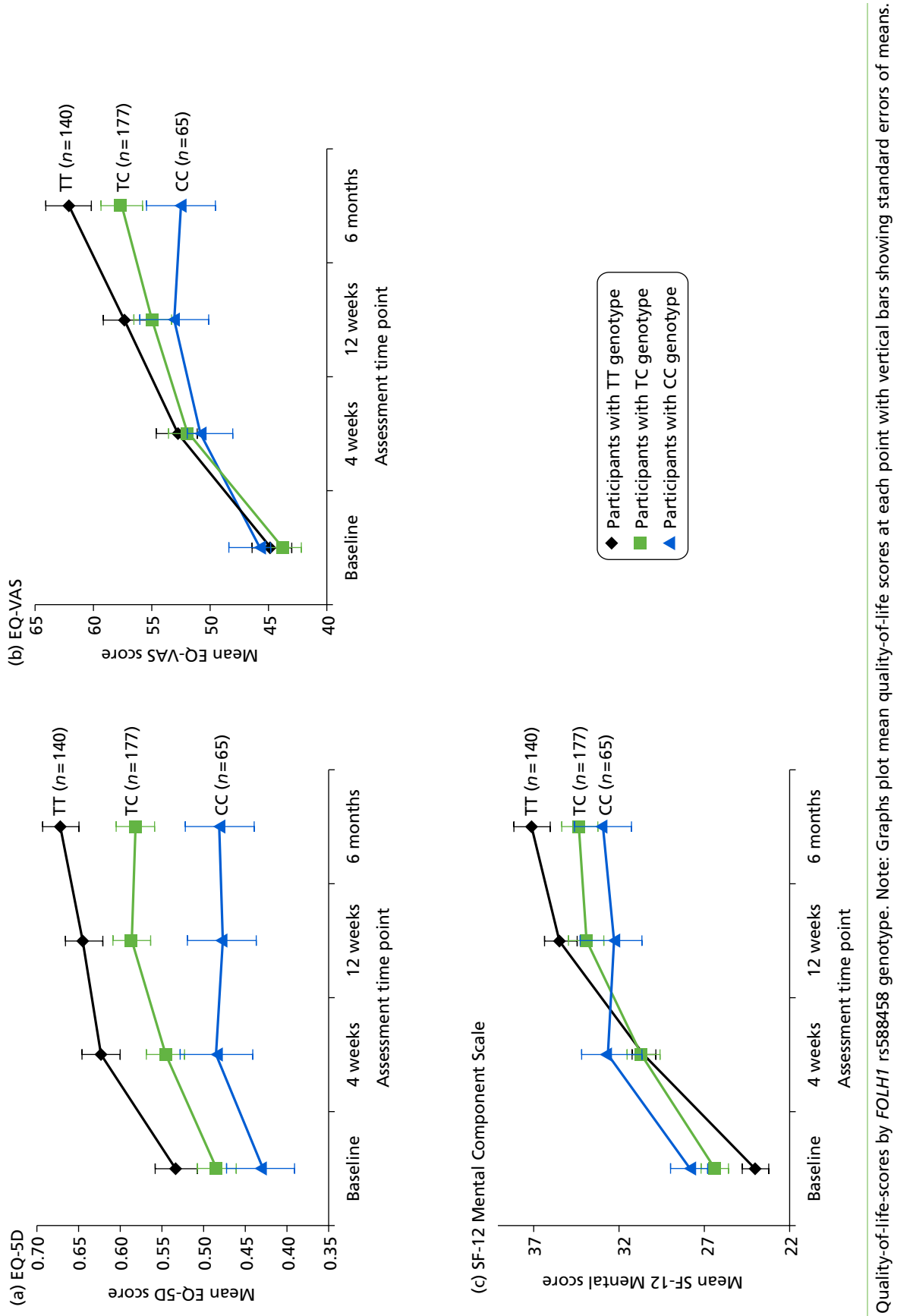

FIGURE 16 Quality-of-life-scores by *FOLH1* rs588458 genotype. Note: Graphs plot mean quality-of-life scores at each point with vertical bars showing standard errors of means.

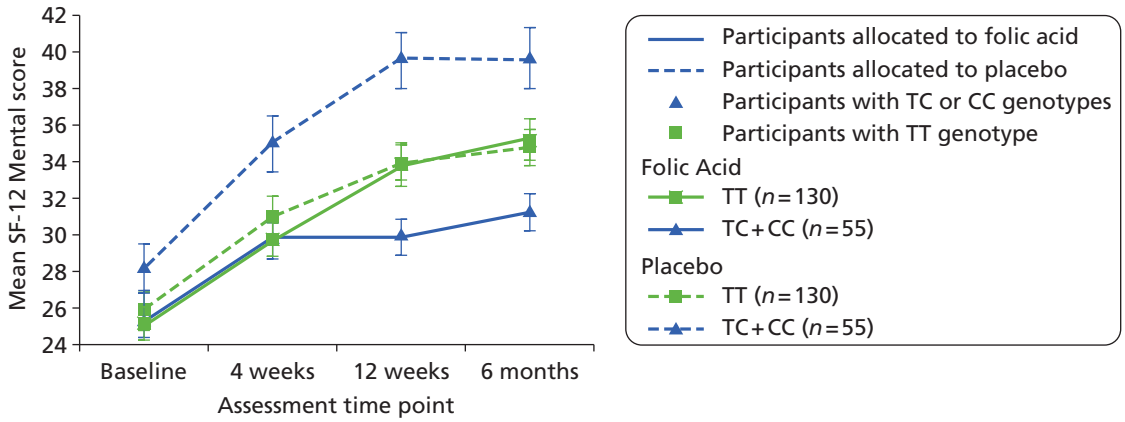

FIGURE 17 SF-12 Mental Component Scores by *MAT1A* rs17102596 genotype – by treatment allocated.
Note: Graphs plot mean SF-12 MCS scores at each point with vertical bars denoting standard error of the mean.

Comprehensive cohort

We recruited participants to this cohort as part of the basic trial process until screening results were known. During the screening appointment the screener took a blood sample to assess B_{12} and serum folate status, and arranged a further research appointment within 14 days to confirm these results. As we then needed to treat patients who were B_{12} or folate deficient, we had to exclude them from the main trial. Nevertheless we invited them to continue in the 'comprehensive cohort' of recruited patients. Though they could not contribute to our evaluation of folic acid as adjunct to ADM, they help us to describe the experiences of the entire cohort of patients invited to join FolATED.

Recruitment and baseline characteristics of the comprehensive cohort

Of the 635 people who originally consented to take part, we could not randomise 156 (see *Figure 2*). Of these 68 dropped out between screening and randomisation, and another 42 at the randomisation interview, 36 because their BDI-II scores were too low for the trial. The remaining 46 people continued as 'residual' members of the comprehensive cohort alongside the 475 properly randomised into the trial. *Table 3* tabulates the reasons for losses by stage of recruitment; and *Appendix 7* does so by centre. *Table 32* compares the baseline characteristics of the 'residual' cohort with those of trial completers: though residual members included more economically inactive females, the most notable difference is that, while Bangor recruited most to the trial, Wrexham recruited most to the residual cohort.

Methods of analysis and results from the residual cohort

We followed the residual cohort like trial participants, but limited data collection to BDI-II at baseline, 4, 12 and 25 weeks. We also used similar methods of analysis. As the findings of fully imputed and complete case analyses were very similar, *Table 33* displays the former. Though both residual and control groups show steady improvement in BDI-II scores from baseline over 25 weeks, the residual members start slower but finish with greater net improvement. We attribute this to the delayed effects of therapy to correct known deficiencies in B_{12} or folate.

TABLE 32 Baseline characteristics – residual cohort vs. trial completers

Participant characteristic	Residual cohort (*n* = 46)	Trial completers (*n* = 440)
Age: mean (SD) in years	47 (12)	45 (13)
Gender: female	35 (76%)	280 (64%)
Race: white	44 (96%)	427 (97%)
Marital status		
Single	9 (20%)	109 (25%)
Had a partner	18 (39%)	91 (21%)
Have a partner	19 (41%)	240 (54%)
No dependent children	30 (65%)	269 (61%)
Employment		
Full time employed	7 (15%)	121 (28%)
Part time or in education	10 (22%)	124 (28%)
Inactive	29 (63%)	195 (44%)
Smoker? Never	17 (37%)	194 (44%)
Alcohol? None	14 (30%)	177 (40%)
Centre		
Bangor	7 (15%)	223 (51%)
Wrexham	24 (52%)	110 (25%)
Swansea	15 (33%)	107 (24%)
Baseline BDI-II score		
Mean (SD)	34 (13)	34 (10)

TABLE 33 Changes in BDI-II – residual cohort vs. control group

			Change from baseline			
Patient sample	Time of analysis	*n*	Mean	(SD)	95% CI	Significance
Residual cohort (imputed)	4 weeks	45	4.33	(10.82)	1.00 to 7.65	0.012
	12 weeks	45	7.22	(9.41)	4.08 to 10.40	< 0.001
	25 weeks	45	14.53	(13.50)	9.67 to 19.40	< 0.001
Control participants (imputed)	4 weeks	217	6.06	(9.96)	4.72 to 7.39	< 0.001
	12 weeks	217	9.54	(11.81)	7.96 to 11.12	< 0.001
	25 weeks	217	11.33	(12.56)	9.65 to 13.01	< 0.001
			Change in residual cohort minus change in control			
Patient sample	Time of analysis		Mean	(SE)	95% CI	Significance
Residual cohort (imputed) minus control participants (imputed)	4 weeks		−1.73	(1.75)	−5.18 to 1.72	0.323
	12 weeks		−2.32	(1.62)	−5.50 to 0.86	0.151
	25 weeks		3.20	(2.19)	−1.11 to 7.51	0.143

Systematic review of the effectiveness of folate in augmenting antidepressant medication

Introduction

There is general consensus in current clinical guidelines for depression that the augmentation of ADM with folate improves patient symptoms. However the supporting evidence is little more than biochemical theory and two small trials.[65,66] Indeed the authors of the current Cochrane systematic review concluded that there was limited evidence that adding folate to ADM was helpful, and recommended a trial like FolATED to test this hypothesis thoroughly.[50]

Effects of interventions

Our updated search found no additional trials that met the criteria of the Cochrane review.[50] Hence our updated review analysed three trials – FolATED with 440 analysable participants, Coppen and Bailey with 100,[65] and Godfrey *et al.* with 24.[66] As the primary analysis of the much larger FolATED trial favours the placebo (*Figure 18*), our updated review reverses the findings of the Cochrane review. As higher scores on both alternative outcomes – HDRS and BDI-II – show more depression, the resulting standardised mean difference of 0.05 (95% CI from –0.11 to 0.22; $p = 0.52$) also favours placebo. Furthermore there was no heterogeneity between studies. Hence there is no evidence to support the use of folic acid as an adjunct to ADM.

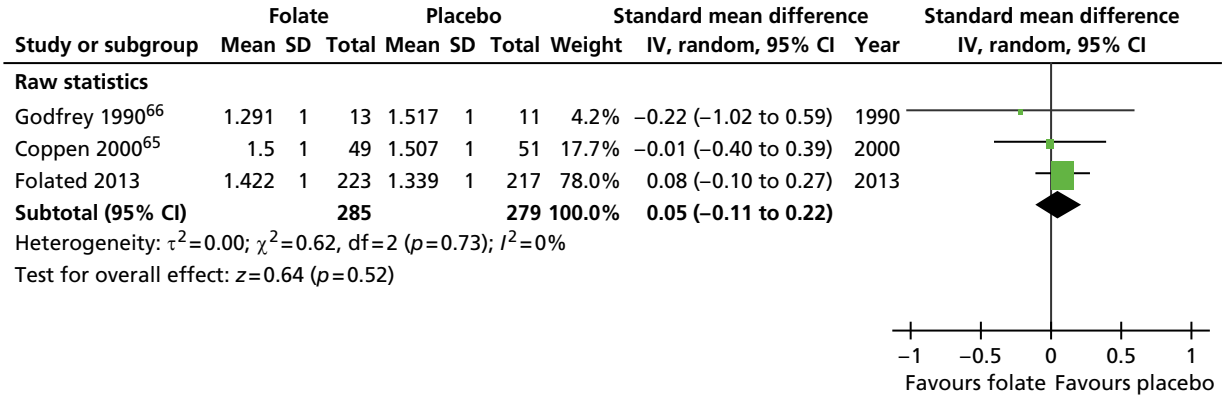

FIGURE 18 Updated systematic review of the effectiveness of folic acid augmentation of antidepressants.

Chapter 4 Discussion

Summary of findings

Clinical effectiveness

FolATED shows that on average routinely adding 5 mg of folic acid to antidepressants prescribed at therapeutic dosages has no clinical benefits. The lack of any mean treatment effect is highly consistent across all outcome measures and time points. The trajectory of response across time is also consistent between measures. The one exception to this is the MCS of the SF-12, which shows a significant difference favouring placebo, especially at 12 weeks. It is not immediately clear whether this single statistically significant adverse result in a secondary outcome measure has any clinical significance. As we used many secondary measures in the trial, this may be a statistical artefact of multiple testing. Given high levels of correlation between the MCS and measures of depression;[139,140] however, it seems unlikely that the MCS detected a real treatment effect not detected by the other outcome measures.

To put this disappointing finding into context, we record that the biochemical outcomes confirmed that folic acid was delivered successfully, as in other studies.[141] Fortunately the few patients who were deficient in baseline red cell folate yielded consistent evidence that augmenting ADM with folic acid improved their clinical outcomes. Furthermore folic acid appeared well tolerated, resulting in no more reports of side effects, AEs or SAEs than placebo.

Cost-effectiveness

The FolATED trial showed no significant clinical effect of 5 mg folic acid once daily for 12 weeks in new or existing users of antidepressants. The economic analysis suggested that folic acid might save costs of about £48 per patient (equivalent to about two-thirds of the cost of antidepressants). According to economic theory, one might therefore conclude that folic acid has positive net benefits, and should be recommended for use. However it seems unlikely that the prescribing of an additional, ineffective medicine would result in reduced costs, since the probability of folic acid being cost saving was not high. A more appropriate conclusion, therefore, is that the economic evaluation was unable to demonstrate cost-effectiveness. We interpret results suggestive of dominance in the primary analysis with caution, as the mean difference in effect was less than half a quality-adjusted day (or six depression-free days) over the 25 weeks of the trial. Furthermore complete case analysis and alternative methods of calculating QALYs did not contradict the principal findings given the small differences in costs and effects, and their associated uncertainty.

Although it is not generally possible for an intervention to be cost-effective when clinical effectiveness has not been established, the application of a cost minimisation analysis would be inappropriate for several reasons.[142,143] First, lack of significant effects does not confirm equivalence.[144] Second, equivalence in one clinical end point does not necessarily mean equivalence in others. Third, preference-based measures of outcome may reveal differences unrelated to the effect of the intervention on depression. Finally it is arguable that hypothesis testing is arbitrary and irrelevant to decision making because the intervention with the highest net benefit should be adopted whether or not difference in benefit reach conventional levels of statistical significance.[145]

We considered it inappropriate to extend the economic analysis to evaluate the cost-effectiveness of pharmacogenetic testing, as the evidence had not supported the clinical utility of testing in relation to the predicting patients' responsiveness to folic acid.

Genetics

Our study has identified two polymorphisms within genes of the one-carbon folate and methionine metabolism pathways associated with outcome regardless of treatment arm (a main SNP effect), and one polymorphism–treatment interaction which is associated with outcome. However the well characterised c.677C > T (rs1801133) polymorphism of the MTHFR associated in previous studies with depression risk or outcome[76,77,79,128] did not modify the effect of either the antidepressant therapy or the folic acid supplementation. This seems to correlate with the study finding that folic acid and modification of homocysteine levels does not influence antidepressant outcome in treatment of moderate to severe depression.

MTHFD1 *genetic variation association with outcome measures*

Our data suggest an association between the rs11627525 SNP of the *MTHFD1* gene locus and outcome. Though statistically significant only for the MADRS outcome, the trend was also present in the BDI-II and CGI measures of severity of illness. The main difference in MADRS between genotypes appears to occur between 4 and 12 weeks with the trajectory of improvement similar after the end of folic acid supplementation at 12 weeks. Given that the effect is seen in the study cohort as a whole regardless of arm, however, any relationship to folic acid supplementation is questionable. Further support comes from the lack of an association of the SNP with any outcome when analysed using the model incorporating the treatment interaction term.

The *MTHFD1* gene encodes an enzyme which catalyses three sequential reactions in the inter-conversion of derivatives of one-carbon tetrahydrofolate, key substrates for methionine, thymidylate, and purine synthesis de novo. Given its function within the one-carbon folate metabolism, *MTHFD1* is a very good candidate. As only one outcome measure reached statistical significance, however, this finding does not provide compelling evidence that the SNP in question might be a reliable predictive marker of antidepressant therapy outcome. Furthermore a difference of three points on MADRS scale after 12 weeks between the two genetics groups, as well as a decrease of 10.5 (variant carriers) and 7.3 (wild-type) is not clinically significant; so prognostic utility is debatable. However previous studies have estimated the minimal clinically relevant change in the MADRS as 1.6 to 1.9.[146]

FOLH1 *genetic variation association with outcome measures*

In addition to the *MTHFD1* SNP, our data suggest that the rs588458 SNP in the *FOLH1* gene locus is significantly associated with outcome, particularly the SF-12 mental component score (MCS) well-being tool. This is an intronic variant with no obvious consequence for the functionality of the FOLH1 protein. Within the HapMap (http://hapmap.ncbi.nlm.nih.gov) CEPH population of Utah residents with ancestry from northern and western Europe, however, rs588458 is in high linkage disequilibrium ($D'' = 1$) with rs202676, a c.484T>C polymorphism, encoding a tyrosine to histidine substitution at amino acid residue 60 (p.Y60H). This variant has recently been identified as a potential risk factor for anencephaly, a neural tube defect, in a Chinese population.[147]

FOLH1 acts as a glutamate carboxypeptidase which performs the initial hydrolysis of glutamate residues of the main dietary form of folates, folylpoly-γ-glutamates. Thus *FOLH1* is a key regulator of intestinal absorption of dietary folate. Studies have demonstrated that the presence of a p.H475Y amino acid substitution polymorphism in *FOLH1* is associated with impaired absorption of dietary folate, with associated low blood levels and hyperhomocystinuria.[148] Indeed studies have identified an association between the c.1561C>T (rs61886492) polymorphism and depressive symptoms on the Center for Epidemiologic Studies Depression scale (CES-D),[149] and cognitive function.[150]

The association with rs588458 is seen in our study group as a whole regardless of treatment arm. Folic acid is likely to neutralise any effect of *FOLH1* genetic variation on folate levels, and subsequent improvement in outcome measures. As folic acid supplementation will swamp dietary folate levels, any effect of *FOLH1* variation is likely to occur before or after administration of folic acid or placebo. From our data, baseline EQ-5D, as well as the trajectory of increase after baseline is greatest in wild-type (TT) and is

still increasing between 12 and 25 weeks when both heterozygote (TC) and homozygote variant (CC) appear to plateau. It is plausible that the difference between genotypes is due to variability in the availability of dietary folates. Observations for two other outcome measures (EQ-VAS and SF-12 mental) appear to support the differences in outcome trajectory, particularly between 12 and 25 weeks.

However these observations need caution. Analysis of correlation between serum and red cell folate levels and genotype suggested that there was no effect on folate levels driven by *FOLH1* genetic variation. It is possible that the association with outcome is independent of folate status. *FOLH1*, or *GCPII* as it is also known, is expressed in brain tissue and hydrolyses extracellular, *N*-acetylaspartylglutamate, *N*-acetylaspartate and glutamate thus affecting glutamatergic transmission. Protein expression and functionality of *FOLH1* may therefore play an important role in the pathophysiology of psychiatric disorders.[151] It is possible that worse outcomes in patients with variant *FOLH1* may be due to a *GCPII* functionality in the brain and its effect on the depressive symptoms rather than the *FOLH1* effect on folate levels. So this is an encouraging observation that requires considerable further investigation.

MAT1A *genetic variation and treatment group interaction with outcome measures*

A single interaction between SNP rs17102596 and treatment group was associated with the SF-12 Mental Scale. This SNP lies within an intronic region of the *MAT1A* gene. *MAT1A* encodes the enzyme, methionine adenosyltransferase I alpha, which catalyses the transfer of adenosyl moiety of ATP to methionine, forming *S*-adenosylmethionine, the source of methyl groups for most biological methylations. Mutations of the *MAT1A* have been identified in patients with methionine adenosyltransferase deficiency, also known as hypermethionaemia.[152,153] So it is unlikely that variability in this gene would have any direct effect on either folate or homocysteine levels. This requires further investigation.

Curiously this association suggests that participants with the variant for the *MAT1A* polymorphism have better outcomes on the SF-12 Mental Component Scale when receiving placebo, and worse outcomes when receiving folate. However no other outcome analysed was able to demonstrate a similar effect. Thus we suspect this is a spurious observation. As such it should be viewed with caution until such time as it is independently validated.

Strengths and limitations of FolATED

FolATED is by far the largest trial to evaluate the clinical effectiveness and cost-effectiveness of folic acid to augment ADM for depression. We powered it to detect a clinically small difference between treatment groups, and followed rigorous procedures for randomisation and blinding. We recruited a wide range of patients being treated for moderate or severe depression in primary or secondary care. There were few exclusion criteria, and our sample included common comorbidities like substance misuse, often excluded from less pragmatic trials.

The reported response to ADM was within the range expected from a mixed study of new and continuing treatment episodes. After 25 weeks 36% of our sample had achieved 'response to treatment' defined as a 50% reduction in BDI-II score from baseline; this is consistent with clinical response rates of about 50% reported in the literature for new patients.[18] Also after 25 weeks 27% had achieved 'remission', defined as a BDI-II score less than or equal to 12;[123] this is consistent with remission rates between 35% and 50% reported in the literature for new patients.[18] Again after 25 weeks 46% had improved to the point where they reported BDI-II scores less than 19 (i.e. depression so mild that they would not have been eligible for FolATED), also consistent with reported recovery rates.

This suggests that the lack of treatment effect for folic acid is not attributable to unusual treatment resistance to antidepressants in our sample. Indeed the trajectory of response to ADM in our sample, with a clear reduction in depressive symptoms over the first few weeks followed by slower improvement up to

25 weeks, is entirely consistent with the model reported in the large GENDEP sample of 807 patients with major depressive disorder treated with escitalopram or nortriptyline.[154]

Also in line with the published literature, baseline severity of depressive symptoms predicted greater response to ADM.[155] Treatment with SSRIs also led to better outcomes over 25 weeks than treatment with other antidepressants. This supports previous reviews suggesting better practical outcomes for SSRIs are due to much better completion of therapeutic courses for SSRIs compared with other antidepressants like TCAs.[156] Other findings also accord with observations from antidepressant trials, with both continuing, rather than new, antidepressant treatment, and economic inactivity like unemployment or long-term sickness, leading to poorer response. In short FolATED was both robust and representative.

Interpretation

Clinical effectiveness

Our negative outcomes contrast with positive findings in two small, selective trials. Coppen and Bailey reported a significantly greater reduction in HDRS scores in females treated with fluoxetine and 400 µg of folic acid, compared with fluoxetine and placebo.[65] They suggested that males required higher doses of folic acid. In response we selected 5 mg of folic acid for FolATED to cover the entire dose–response curve They achieved very high clinical response rates – 82% in the folic acid arm and 62% in the placebo arm – surpassing those in the general literature, because their sample was much less representative than that recruited by FolATED. For example they excluded patients with continuing episodes of depression, previous poor response to fluoxetine, or comorbid substance use. One must also question the robustness of their blinding, especially in handling blood results, whereas FolATED took great care at all stages to avoid unblinding clinicians, patients and researchers. While females treated with folic acid by Coppen showed a 21% reduction in homocysteine levels, similar to the FolATED sample, the males did not. In FolATED, however, there was no gender difference in homocysteine levels following treatment with folic acid, suggesting that we covered the dose–response curve, yet without treatment effect.

In contrast Godfrey and colleagues recruited only from secondary care, specifically patients with folate deficiency (viz. red cell folate less than 200 µg) but suffering from a wide range of psychiatric disorders including depression and schizophrenia; hence they had to adapt clinical outcomes to diagnosis.[66] This makes comparison difficult as FolATED examined the routine use of folic acid augmentation in depressed patients recruited predominantly from primary care. Though Godfrey used 15 mg of the biologically active MTHF, the gross differences between studies generate no evidence for the superiority of MTHF over folic acid.

Hence, after updating the systematic review[50] by including FOLATED, we found no evidence to support the previous suggestion that folic acid could improve the clinical benefits of ADM.

Cost-effectiveness

Our economic evaluation showed that folic acid, clinically ineffective, could not be a cost-effective use of resources. With no other economic evaluations of folic acid in managing depression, we assessed external validity in comparison with other studies of depression. For example the AHEAD trial pragmatically compared TCAs, SSRIs and lofepramine in UK primary care, and provided the prototype for our resource use questionnaire. Though AHEAD followed patients for 12 months, their rates of contact with healthcare professionals was comparable pro rata with FolATED findings. AHEAD trial participants visited GPs on 9.1 occasions over 12 months, compared with 3.6 over 25 weeks in FolATED; the mean length of inpatient stay was 1.05 days in AHEAD, compared with 0.58 in FolATED. With one exception we consider our participants' use of NHS and PSS resources to be generally representative of UK practice, and typical of locations other than the recruiting sites. The exception lies in divergence from the NICE recommendation that people with moderate to severe depression receive psychological therapy, as only 6.5% of participants did so. Fortunately there is no evidence that greater use of psychotherapy, engendered for example by the

English 'Improving Access to Psychological Therapy' programme would have changed any of our insignificant findings. As our genetic analysis found no support for pharmacogenetic testing in this field, that too could not be a cost-effective use of resources.

Genetic implications

Our study has identified three polymorphisms associated with modification of depression outcomes. For *MTHFD1* rs11627525 and *FOLH1* rs588458 this association was independent of treatment allocated; in particular the new link between EQ-5D and the rs588458 genotype needs further study. The *MAT1A* rs17102596 SNP modified the SF-12 Mental Component Scale through interaction between SNP and treatment.

In general, however, there is little evidence that any of the variants analysed can predict patients' response to ADM or the efficacy of folic acid. Even the three SNPs showing association could not replicate this with any other outcome measure. Above all none of the polymorphisms analysed could achieve statistically significant modification of BDI-II, the primary outcome measure.

However we limited the variants analysed to genes within the one-carbon folate or methionine synthesis pathways. It is possible that other factors, genetic or otherwise, may influence patient response to ADM or folic acid. Thus it is conceivable that in future a whole-genome approach to the FolATED data set could identify markers of efficacy beyond those already analysed.

Biochemical interpretation

Folate is a naturally occurring B vitamin, needed in the brain to synthesise serotonin, noradrenaline and dopamine. In humans the biologically active form is 5-methyltetrahydrofolate (5-MTHF), which is derived from ingested folates and is the form taken up by cells and transported to the cerebrospinal fluid (CSF) via folate receptors. Folic acid is an inactive, stable, synthesised, oxidised form of folate, not naturally found in the human body; it has to undergo transformation to 5-MTHF – an inefficient process as unmetabolised folic acid remains in the blood even after an intake of only 400 µg, the recommended daily intake of folate. Folinic acid (calcium folinate) is a stable reduced form of 5-MTHF that needs no transformation before entering the CSF.[157] There is evidence that commercial preparations of folic acid can compete with 5-MTHF for the folate receptor and thus exacerbate a folate deficiency in the central nervous system (CNS).[158] Hence we wonder whether our finding that folic acid had a statistically significant negative effect on the widely used SF-12 MCS was a manifestation of such a folate deficiency rather than the type 1 error that we first supposed.

Thus better understanding of the one-carbon folate and methionine biosynthesis pathways have raised questions about the most appropriate formulation of folate to use clinically for functional folate deficiency. Stahl argues that 5-MTHF is therapeutically better than folic acid as it bypasses conversion of folic acid to the biologically active 5-MTHF, which may be difficult for some patients.[159] Furthermore high doses of inactive folic acid may compete with 5-MTHF for transport across the blood–brain barrier, potentially reducing active 5-MTHF in the brain. Other reasons why 5-MTHF synthesis may be difficult include inhibition of the enzyme by anticonvulsants. Fortunately we avoided this potential confounding factor by excluding patients on anticonvulsant medication.

Against this biomedical background our rigorous and powerful trial has established that folic acid has no general role as adjunct in antidepressant therapy. However studies in patients with cardiovascular disease have shown that higher doses of folic acid produce greater concentrations of 5-MTHF in plasma.[160] Moreover the reductions in homocysteine in FolATED participants on folic acid relative to those on placebo suggests that they were successfully metabolising folic acid to 5-MTHF. Nevertheless we suspect that the beneficial increase in 5-MTHF was masked by the excess folic acid that competed with 5-MTHF for the folate receptors and led to the negative results.

Recommendations for research

Folate and depression

Our inclusive multi-disciplinary trial has shown beyond doubt that folic acid is an ineffective adjunct in antidepressant therapy. Furthermore better understanding of the one-carbon pathway offers a plausible explanation why.[158] Before we can write off folic acid entirely, however, we recommend updating the Cochrane systematic review and meta-analysis,[64] and summarising the systematic review data more thoroughly than was possible in *Chapter 3* (see *Systematic review of the effectiveness of folate in augmenting antidepressant medication*).

At the time of our initial grant proposal little information was available on the use of 5-MTHF or folinic acid in patients with depression. Now there is evidence that 5-MTHF given as adjunct or monotherapy reduces depressive symptoms in patients with low folate levels or alcoholism, and improves cognitive function and reduces depressive symptoms in elderly patients with dementia and folate deficiency.[161] Furthermore there are long-standing concerns that folate may increase cancer risk, mask B_{12} deficiency and exacerbate depressive symptoms. As 5-MTHF may reduce some of these risks, we judge that that is now a candidate for a large multi-centre trial. There is also evidence that adjunctive 5-MTHF reduces depressive symptoms in patients who were partially responsive or non-responsive to a selective SSRI.[161]

In that context we offer the design of FolATED as a proven model. With the benefit of hindsight, however, we judge that a trial recruiting for 1 year in 10 centres would yield better value for money than one recruiting for 3 years in three centres.

Recruitment

Like many trials FolATED recruited more slowly than expected. We based the original target of randomising 550 participants in three centres over 2 years – slightly less than 24 per month across all three centres – on experience with a previously successful trial in one of the three centres. Initially, however, the complex design of FolATED, designed to exclude patients suffering from folate or B_{12} deficiency while allowing psychiatric teams to optimise antidepressant treatment in normal clinical practice (*Figure 1*), restricted randomisation across all three centres to eight a month. We attribute our success in eventually increasing these initial rates by 50% to 12 a month across all three centres to a lot of hard work, a combination of creative protocol changes summarised in *Appendix 4*, and the mutual support engendered by joint training and joint monthly telephone conferences about management and recruitment.

Late in the conduct of FolATED we used a qualitative reflective data collection tool to undertake a retrospective study of recruitment and retention issues (see *Appendix 11*). We sought, not to evaluate which methods were the most effective in increasing recruitment and retention, but rather to gain insights into problems we faced and methods we used to overcome recruitment and retention problems within FolATED. We found little research into participants' preferences between different approaches. This leads us to recommend that future large trials include smaller trials or qualitative studies or both to assess the effectiveness of materials used in recruitment such as posters, leaflets, patient information sheets and newsletters to participating practitioners.

Chapter 5 Conclusions

This rigorous and adequately powered trial has established that folic acid is not an effective adjunct to antidepressant therapy. The NIHR Health Technology Assessment programme commissioned FolATED at a time when there was considerable scientific interest in the role of folate in the aetiology of depression and in treating depression. During the lifetime of FolATED this interest has grown, with increasing international pressure to use folate as an adjunct to antidepressants and in algorithms for treating depression.

The unequivocally negative findings of FolATED demand reappraisal of this consensus and associated treatment guidelines. Thus there is a strong case for research to investigate whether future trials of 5-MTHF would yield value for money.

Acknowledgements

Contributions of authors

All 19 authors contributed to design and data collection or analysis and interpretation, commented on successive drafts, and approved the version to be published. More specifically:

Emma Bedson (Research Officer, Clinical Trials) was co-applicant and trial manager; she contributed to developing and implementing the design, management and quality assurance of the trial; and coordinated this report.

Diana Bell (Research Nurse, Clinical Trials) was local trial coordinator for North West Wales; she coordinated recruitment, data collection, local reporting and administration.

Daniel Carr (Tenure Track Fellow Pharmacogenetics) contributed to developing and implementing the genetic component of the study and interpreting data; he led the writing of the pharmacogenetics sections of this report.

Ben Carter (Lecturer, Statistics) was senior trial analyst; he undertook validatory statistical analysis and contributed to interpreting data and drafting this report.

Dyfrig Hughes (Professor of Pharmacoeconomics) was applicant and principal investigator for health economics.

Andrea Jorgensen (Lecturer, Medical Statistics and Pharmacogenetics) undertook the pharmacogenetic analysis, and contributed to interpreting data and drafting this report.

Helen Lewis (Research Officer, Clinical Trials) was local trial coordinator for North East Wales; she contributed to developing and coordinating recruitment, data collection, local reporting and administration, and led the writing of *Appendix 11* describing recruitment into FolATED.

Keith Lloyd (Professor of Psychological Medicine) was co-applicant, clinical chief investigator and principal investigator for Swansea.

Andrew McCaddon (Principal in General Practice) was principal investigator for the sub-study of MMA.

Stuart Moat (Director, Medical Biochemistry) was co-applicant and principal investigator for medical biochemistry.

Joshua Pink (Doctoral Student, Health Economics) contributed to analysing and interpreting the economic data, and drafting this report.

Munir Pirmohamed (NHS Professor of Pharmacogenetics) was co-applicant and principal investigator for pharmacogenetics.

Seren Roberts (Research Fellow, Psychology) was co-applicant and principal investigator for North East Wales; she coordinated the development and implementation of the study design, led the writing of the published protocol, and contributed to interpreting data and drafting this report.

Ian Russell (Professor of Clinical Trials) was co-applicant, methodological chief investigator and chair of the trial management group; he edited this report.

Yvonne Sylvestre (Research Officer, Statistics) was assistant trial statistician; she undertook primary statistical analysis and contributed to interpreting data and drafting this report.

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Clare Wilkinson (Professor of General Practice) was co-applicant and principal investigator for general practice.

Nefyn Williams (Senior Lecturer in General Practice) contributed to developing and implementing recruitment from primary care and prescribing data for economic evaluation; assessing laboratory test results and communicating abnormal results to clinicians; and interpreting data.

Profound thanks to:

The National Institute for Health Research Health Technology Assessment Programme for funding FolATED; and their staff for generous and patient support.

The trial participants, especially for completing a high proportion of responses.

General practitioners, psychiatrists and other healthcare practitioners for generously referring participants to the study.

Psychiatrists who generously screened participants and consented them into the study – Yinka Abiodun, Ahmed Alastal, Angela Ambrose, Aneeba Anwar, Mohammed Butt, Padmaja Chalsani, Jude Chukwuma, Alexis Economou, Fiona Farquahar, Qamar Jabeen, Archana Jauhari, Kristy Khonji, Ravi Krishnaiah, Stefanie Linden, Danielle Loveday, Richard Mellor, Jorge Diaz-Monoz, Emma Morgan, Jisha Mukundan, Askey Nair, Subhash Pinnaka, Mohtasim Qamruddan, Yusuf Ragoonwala, Javier Rodriguez-Mendieta, Divya Sakuja, Saurabh Saxena, Adeel Siddiqui, Nick Smith, Mujahid Ali Syed, Faye Tarrant, Ranjit Tatineni, Johannes Thome and Joe John Vattakatuchery.

Researchers who assiduously recruited participants and completed trial documentation – Kim Davies (North West Wales), Azzam Farroha and Faye Griffith-Noble (Swansea), and Diane Pasterfield (North East Wales).

Research professionals from the National Institute for Social Care and Health Research – Clinical Research Centre (NISCHR CRC) who energetically supporting the trial in many ways – Nathan Bray, Theresa Gill, Michelle Grey, Jenny Griffiths, Martin Jones, Adwoa Hughes-Morley, Vaughn Price, Leanne Quinn, Julia Roberts, Farah Shiraz, Sue Thomson, Melissa Van Der Bijl and Sally Williams.

Staff from the biochemistry, haematology and pharmacy departments of Singleton Hospital in Swansea, Wrexham Maelor Hospital and Ysbyty Gwynedd in Bangor– Keith Dadds, Gareth Davies, Robert Jones, Christine Lloyd, Sue Lord, Dewi Morris, Adele Sparks, Rachel Still, Lisa Wakeman, Robert Walters, David Watson and Chris Wight.

Angela Hollett and Stephanie Rodger (pharmacy technicians) who helped to analyse prescription data.

Vittorio Simeon of the Wolfson Centre for Personalised Medicine at the University of Liverpool, who helped to analyse genetic data.

Daphne Russell of Swansea University College of Medicine for scientific proofreading.

And not least the staff of the North Wales Organisation for Randomised Trials in Health (NWORTH) – Darren Baker, Zoe Hoare and Rashesh Mehta for data management, Debbie Skelhorn for quality assurance, and Victoria Buckley for administering FolATED.

Publications

Roberts SH, Bedson E, Hughs D, Lloyd K, Moat S, Pirmohamed M, *et al.* Folate Augmentation of Treatment – Evaluation for Depression (FolATED: protocol of a randomised controlled trial). *BMC Psychiatry* 2007;**15**:65.

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Appendix 1 Participant Information Leaflet and Informed Consent Form

Augmentation of Treatment – Evaluation for Depression: a randomised controlled trial (FolATED)

Study Booklet

All you need to do for now is read the information leaflet and consent form in this booklet

STUDY INFORMATION LEAFLET

Does taking the vitamin folate help antidepressants work better?

You are being invited to take part in a large research study. Before you decide to take part in the study, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?

One in five people experience depression during their lives and some, but not all, will need to be prescribed antidepressants tablets to help them overcome their problems. Only half of those treated with drugs will get better, many others experience symptoms for a very long time. It has been suggested that taking folic acid (i.e. tablets containing the vitamin folate) every day may make the antidepressants work better and we are hoping to find out whether this is true.

Why have I been chosen?

You have been chosen because your doctor thinks you need to start a course of antidepressants. If you are already taking antidepressants, you have been chosen because you have not made a full recovery. You have been given this information leaflet because your doctor thinks you may be willing to take part in the study.

If you contacted the team directly and have spoken to a researcher, the researcher has sent you this information leaflet because you have asked for further details and might be eligible to take part.

What is the drug or procedure that is being tested?

This study is investigating folate. Folate is a water-soluble vitamin that is present in many food stuffs such as fruit, vegetables, grains, legumes, nuts and seeds. We need a sufficient amount of folate in our bodies to help build red blood cells and to help form the genetic material in every cell in the body. This vitamin is available 'over the counter' in low doses known as "folic acid 400 micrograms". In this study we shall use a higher dose of 5 milligrams, which is designed to treat folate deficiency and only is available on prescription. There is no reason for you to keep taking the folic acid 5mg per day beyond the three months of the study. If you feel better and want to take the vitamins longer you can buy supplements over the counter at a dosage of 400micrograms

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you are free to withdraw at any time. If you decide not to take part or withdraw this will not affect your treatment or the care you receive in anyway.

What will happen to me if I take part?

If you choose to take part in the study, a researcher will contact you to make arrangements for you to see one of our doctors.

First appointment: The researcher and doctor or nurse will tell you all about the study and answer any questions you have about it. You will be asked to complete the consent form and will be given a copy of your consent form and this information leaflet to keep. The doctor or nurse will assess you and you will be asked to complete 3 short questionnaires. A blood sample will also be taken to measure your levels of vitamins (folate and B12) and an amino acid (homocysteine) to see whether it is appropriate for you to take folic acid.

If you need antidepressant medication, it will be prescribed. If you are already taking an antidepressant, we will make sure that you are on the right dose.

Telephone contact: You will be telephoned by the researcher a week after your first appointment to check how you are doing with the antidepressants.

Second appointment: Two weeks after you first appointment you will be given the results of your blood test, your symptoms of depression will be assessed and you will be asked to complete a set of short questionnaires again. If your levels of the folate and vitamin B12 are within the normal range you will be randomly placed in one of two groups for comparison.

One group will be given folic acid 5mg and the other group will be given a dummy or placebo tablet to take every day. The folic acid and placebo will look the same so nobody, not even the researchers will know who is taking folic acid until the results are compared at the end of the study. You have a 50/50 chance of being placed in the group that takes folic acid.

If your blood results show that you have low levels of folate or vitamin B12 in your blood, then we will make sure that you get the treatment you need. You can still take part in the study by attending the remaining appointments and completing the questionnaires.

Third, fourth and fifth appointments: You will be asked to attend three more appointments. One will be after 4 weeks, one after 12 weeks and one after 6 months. You will only be taking folic acid or placebo for 12 weeks but will continue to take the antidepressants unless the doctor asks you to stop taking them. At each appointment, your symptoms of depression will be assessed and you will be asked to complete a set of questionnaires again. In addition to the blood sample taken in the first appointment, two more blood samples will be taken, one after 12 weeks and another after 6 months, to monitor any changes in your folate and homocysteine levels. You will only need to take part in the study for 6 months.

Each appointment should take no longer than one and a half hours.

What do I have to do?

All you need to do is take the antidepressant and the extra pill given to you for the study regularly as prescribed and attend the study appointments to complete the questionnaires.

What are the possible benefits and risks of taking part?

If the results of the study confirm that adding folate to antidepressant treatment is helpful this will benefit future patients with depression. This may help doctors make decisions about how best to treat depression.

Folic acid is normally well tolerated but occasionally nausea, allergic reactions, anorexia and abdominal distension can occur. There is a risk associated with taking folate for people who have low levels of vitamin B12 so anyone with low levels of vitamin B12 will be treated and not given folate. There is good evidence that high folate intake lowers the risk of developing cancer. However, if you have already had a malignant disease there is

some evidence that high folate intake may increase cancer growth. That is why we are not including in our trial anybody who has had a diagnosis or treatment for any malignant disease.

Pregnant women or women planning to become pregnant are not eligible for the trial. Women who become pregnant during the trial should stop taking the trial tablets. If you do become pregnant you need to speak to your GP and inform your local researcher. Depending on the stage of your pregnancy your GP may advise you to take folic acid tablets. The dosage of folic acid being used in this study is higher than the dosage usually given to pregnant women, but there is no evidence from previous studies that it is harmful to the unborn baby. Researchers for the study would collect information from your medical records about the progress of your pregnancy, the birth and the health of your child. They may talk directly with clinicians involved in your care, and the care of your baby. All this information will be kept with other information from the study and will be treated as strictly confidential.

Will my taking part in this study be kept confidential?

All information collected in this study will be kept strictly confidential. Your study information will be kept separate from your personal details so that you cannot be recognised from it. We will only share information with your doctor if you wish it. The results from this study may be published so that any potential benefits to treatment can be shared with other health professionals. You will not be identified in any report or publication.

OPTIONAL Genetics Study

We know from experience that different people respond differently to drugs and vitamins, and there is increasing evidence to show that genetic factors are involved. Part of this study involves looking at some of the genes known to be related to:

(a) folate metabolism – these are the genes involved in taking up folate into cells, and the conversion of folate in the body to breakdown products before being removed from the body through the kidneys. At least 27 genes are involved in this, and we will look at all of these in due course;

(b) how antidepressants work – there are many genes involved in this, including those that breakdown the antidepressants, and the receptors and enzymes on which the drugs work.

If you agree to have the genetic tests on your blood sample, you will first of all be asked to give additional consent to look at the above genes. Your blood samples will be kept confidential for 5 years after the study, at which point they will be destroyed. If you wish to withdraw your consent at any time your blood samples will be destroyed.

You can also consent to us storing your blood samples anonymously after the 5 years for future research. So as well as looking at the specific genes mentioned above, this also allows us to look at other genes as our knowledge of genes advances.

To do this, we will irreversibly anonymise your clinical details and blood samples, so it will not be possible to trace the blood sample back to you once the study has been completed, and therefore will have no direct bearing on your clinical care. After anonymisation however, it will not be possible for you to withdraw the blood sample as we will not be able to identify which blood sample is yours.

If you consent to the genetic test, a sample of your blood will be sent to the University of Liverpool where DNA will be extracted. It will be stored here until it is used up. It is important to note that all blood samples going to the University of Liverpool will be identified by a code number only. The Chief Investigator will keep any personal and clinical information confidential.

Only a small amount of blood is need for this part of the study. If you agree, this will be taken from the blood sample you give in your first appointment. This sample will be considered to be a gift to the University of Liverpool, which will act as custodians of all the samples obtained as part of this study. In some cases, a small amount of your sample will be provided to other researchers either in the UK or other parts of the world. However, it is important to remember that this will only be identified by a code.

In the short-term, it is unlikely that the samples will be of any commercial value to the Universities, hospitals and GP practices involved in the study. However, it is possible that there may be some commercial value in the future, although it is important to note that any commercial value is likely to be due to findings in a group of patients rather than from samples from a single patient.

Participation in this part of the study is optional. You can opt out of this part but continue with the main study if you wish.

Who is organising and funding the research?

This study is funded by the NHS Health Technology Assessment Programme. It will take place in three areas of Wales, North East Wales, North West Wales and Swansea. The study will be managed by Bangor University.

Healthcare professionals will be reimbursed £50 for each patient included in the study to cover administration costs.

Who has reviewed the study?

This study has been reviewed by the Multicentre Research and Ethics Committee for Wales and by three Local Research and Ethics Committees (North East Wales, North West Wales Research, and Swansea Research)

Contact for further information or if there are any problems

If you have any problems with your tablets such as side effects you will need to inform [name of local researcher and tel no.] so that appropriate actions can be taken.

If you have a complaint about the conduct of the trial you can write to the Chief Investigator (contact details below) or you can follow the usual National Health Service complaints procedure.

Professor I. T. Russell, Chief Investigator

Institute of Medical and Social Care Research (IMSCaR), Bangor University, Y Wern, , George Site, Holyhead Road Bangor, LL57 2PZ.

Thank you for reading this information leaflet

Folate Augmentation of Treatment - Evaluation for Depression: a randomised, controlled trial (FolATED)

CONSENT FORM

Centre Number: _____

Study Number: _____

Participant ID Number: _____

Does taking the vitamin folate help antidepressants work better?

Name of Local Investigator: _____

Please initial box

I confirm that I have read and understood the information sheet dated_____ version_____ for the above study, including the section which explains the OPTIONAL genetics study. ☐

I have had the opportunity to discuss and ask questions about the study and the OPTIONAL genetics study. ☐

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. ☐

I understand that sections of my medical notes may be looked at by responsible individuals from the research team, who are either healthcare professionals within the Trust or have honorary contract with the Trust, or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐

I agree for any relevant information about my participation in this trial to be passed on to my GP. ☐

I agree to have my blood taken for folate, B12 and Homocysteine tests ☐

I agree to take part in the above study. ☐

OPTIONAL Genetics Study

1. I understand that if I agree to have my blood samples used for genetic tests, the results will not be added to my medical records ☐

I agree to have a blood sample taken for genetic tests on the genes involved in folate metabolism and antidepressant response (according to our current knowledge) and understand that this sample will be kept for only 5 years after the study has finished. ☐

I understand that if I agree to have my blood samples kept for future ethically approved genetic studies after the five years, my sample will be anonymised so that I cannot be identified as the blood donor. I understand that this means I cannot withdraw my blood sample once it has been anonymised on completion of the study (i.e. after 5 years). I agree to have my blood sample anonymised and kept after the study has finished for future genetic testing. ☐

_____	_____	_____
Name of participant	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature
_____	_____	_____
Researcher	Date	Signature

Appendix 2 Independent members of Trial Steering Committee and Data Monitoring and Ethics Committee

Trial Steering Committee independent members

Allan House (Chair) – Professor of Liaison Psychiatry, University of Leeds

Ian Anderson – Professor of Psychiatry, University of Manchester

Glyn Lewis – Professor of Psychiatric Epidemiology, University of Bristol

Lynne Pike – patient representative

Data Monitoring and Ethics Committee independent members

Francis Creed (Chair 2006–8) – Professor of Psychological Medicine, Manchester University

David Baldwin (Chair 2008–11) – Professor of Psychiatry, University of Southampton

Chris Dowrick – Professor of Primary Medical Care, University of Liverpool

Gareth Griffiths – Scientific Director of Wales Cancer Trials Unit

Appendix 3 Dates of initial approvals

Regulatory body		Date of approval
Multi-centre Research Ethics Committee for Wales		6 November 2006
Medicines & Healthcare products Regulatory Authority		21 December 2006
Secondary Care	Swansea NHS Trust	21 September 2006
	North East Wales NHS Trust	16 October 2006
	North West Wales NHS Trust	17 November 2006
Primary Care	Swansea Local Health Board	13 November 2006
	North Wales Local Health Boards	12 June 2007

Appendix 4 Summary of protocol changes approved by DMEC, TSC, MHRA, MREC and NCCHTA

Referrals

Initially we accepted referrals from GPs and psychiatrists only. To be more inclusive, and to improve recruitment into the trial, on 15 May 2009 we started to accept referrals from other healthcare professionals including primary care liaison workers, social workers and mental health nurses.

To improve recruitment in the Swansea centre, we amended our protocol to accept self referrals from people already taking antidepressants. From 26 June 2010 we recruited participants through advertisements in the community, including posters, local newspapers, pharmacies, clinics and general practices. To help with the expected increase in referrals we gained permission for registered mental health nurses to screen people who refer themselves to the study.

Our original protocol stated that we would reimburse general practices £50 to cover administration costs for every patient recruited. In November 2010 we gained permission to extend reimbursement to secondary care teams.

Recruitment

We initially planned to complete all randomisations by August 2008. However unforeseen delays in research governance delayed the start of recruitment by 6 months for reasons including: change of sponsor; delays in appointing researchers; delays in obtaining regulatory approval; and delays in obtaining honorary contracts. We also encountered obstacles to recruitment including: shortage of psychiatrists to screen patients; fewer referrals from secondary care than expected; fewer referrals of newly diagnosed patients than expected; limited space in general practices for screening; and difficulty in contacting referred patients. The NIHR HTA programme therefore granted us a 24-month costed extension to extend recruitment by 18 months.

On 25 June 2009 we gained permission to extend the Wrexham centre to include Conwy and Denbighshire.

Exclusion criteria

In July 2007 we removed substance misuse from the list of exclusion criteria for three reasons:

1. to make the trial more pragmatic and inclusive
2. to increase recruitment, and
3. because we could detect folate deficiency from alcohol use through blood tests at screening.

We added malignancy and related conditions like intestinal polyposis as exclusion criteria, following advice from the TSC in January 2007 based on evidence from rat studies that high folate intake may increase growth of existing tumours.

In response to our original application for a CTA the MHRA asked us to add taking lithium as an exclusion criterion, which we did. However we found no evidence of adverse interactions between lithium and folate. Furthermore we identified that this exclusion criterion was impeding recruitment into the trial,

particularly from secondary care. In April 2008, we therefore amended the exclusion criterion for taking lithium to exclude patients with bipolar disorder whether taking lithium or not. This ensured that only patients with unipolar depression entered the trial.

Data collection

It had been our intention for the FolATED trial to be paperless. However IT constraints led us had to substitute a rigorous paper-based system to collect data and we amended the protocol accordingly.

We identified a need for researchers to make home visits to conduct the research assessments. Depression can often make it difficult for patients to attend hospital and many potential participants had requested that researchers go to their homes for appointments. In May 2009 we amended the protocol to allow researchers to visit homes when needed.

Assessment and follow-up

Following the initial application for approval in October 2006, we received further requests from the MREC, local R&D Departments and Trial Management Group to amend the protocol, including:

1. taking extra samples of blood for homocysteine analysis, to guard against losing samples in the post
2. collecting red cell folate at screening to ensure that we did not randomise patients with folate deficiency
3. collecting serum folate at weeks 12 and 25 to check for compliance
4. measuring plasma vitamin B_{12} concentrations at minus 2, 12 and 25 weeks, to guard against participants becoming B_{12} deficient during treatment with folic acid and resulting in a neuropathy, and
5. asking patients at each follow-up whether they are taking additional supplements.

We found that the initial screening appointment with patients took longer than expected owing to the number of assessments. In April 2008 we gained permission to remove the majority of the assessments including the MADRS, UKU, SF12, EQ5D and CGI from this appointment. The full battery of assessments at randomisation, which provided the true baseline, continued as planned. This increased the number of patients be screened by psychiatrists and reduced the burden on participants.

After the MHRA recommended following up any pregnancy that occurs during a trial, we gained permission in April 2008 to alter the information sheet to allow us to track any such pregnancy.

On 17 September 2008, the TSC identified the need to ask participants explicitly about attempts at self-harm. Though we define self-harm as a SAE, this relies on participants reporting it without prompts. We therefore added the question: 'Since we last saw you, have you tried to harm yourself? For example, have you taken any extra tablets or cut yourself or done anything else to injure yourself?'

Additional analyses

In December 2009 we gained permission to investigate the interaction between folic acid and MMA, a metabolic marker of the enzymatic function of vitamin B_{12} and a more sensitive marker of B_{12} status. High-dose folate is generally considered safe, provided that B_{12} deficiency is excluded.[162] However, there is evidence that high folate levels combined with low B_{12} levels are associated with significant cognitive impairment in the elderly.[163] Furthermore the US National Health and Nutrition Examination Survey observed a relationship between folic acid and MMA (57). The FolATED trial offers a rare opportunity to observe the effects of folic acid supplementation on MMA concentration (not reported in this monograph).

To ensure the safety of participants in the trial fieldworkers informed their Principal Investigator (PI) of any participant who attempted suicide or was at high risk. For this purpose they completed the MINI suicidality scale at the end of the MADRS. If there was a marked increase in suicidal risk, they reported this as a SAE. Though we had approval to monitor suicidality through our safety reporting system, we did not name the MINI suicidality explicitly in our protocol. The MREC therefore asked us not to analyse these data. However our TSC were concerned that not to report fully on the safety of our intervention would be unscientific. So they recommended that we seek permission to publish this information in the public domain. We therefore gained permission in May 2011 to publish analyses comparing differences between the two treatment groups in MINI suicidality scores at follow-up.

Clarification of protocol

Following meetings of TSC and Data Monitoring Committee early in 2007 we replaced the lay summary with a technical abstract at the beginning of the protocol.[81] We elaborated on screening, informed consent, randomisation, withdrawal and safety reporting. We improved clarity, not least by integrating the flow diagram and outcome measures table into the main text of the protocol.[81,82]

Review of power calculation (approved by DMEC, TSC and NCCHTA)

We originally powered FolATED to detect a difference between the two treatment groups of three points on the Beck Depression Inventory (BDI-II) at 25 weeks, judging that a clinically important difference. As we estimated the SD of BDI-II scores in the trial population at 10.7, our protocol proposed a completed sample size of 400 at 25 weeks to yield 80% power to detect this difference using a significance level of 5%. As interim analysis of baseline BDI-II scores showed that their SD was about 10, we revised the target completed sample size to 358 at 25 weeks. The original protocol allowed 10% loss at each of the three follow-up assessments, thus requiring a randomised sample of 549 to achieve 400 completers at 25 weeks. As interim analysis also showed that retention at 25 weeks was 79% rather than the 73% expected, the new target of 358 completers needed a randomised sample of 453 participants.

Appendix 5 Analysis plan

Introduction

Trial design

FolATED is a three-centred, double-blind, placebo-controlled, pragmatic randomised trial of folic acid augmentation of moderate-to-severe depression. It investigates the effect of augmentation on new and continuing ADM. Assessments take place 2 weeks before randomisation ('week –2') to screen for eligibility and initiate antidepressant if required; 1 week before randomisation ('week –1') by telephone to check for tolerability of antidepressant; baseline ('week 0') to randomise to folate or placebo; and at weeks 4, 12 and 25 to assess outcomes. To estimate the effectiveness of folic acid in augmenting ADM, the trial uses standardised instruments to measure changes in depressive symptoms from two perspectives – clinical and participants'.

Primary outcome measures

The primary clinical effectiveness outcome measure is self-rated symptom severity as measured by the Beck Depression Inventory (BDI-II). Though BDI-II scores at 25 weeks are useful in assessing participants' medium-term recovery, the primary outcome is the 'AUC' of mean BDI-II scores between randomisation and the 25-week follow-up, to aggregate participants' paths to recovery over that timeframe. The primary economic measure is ICER, namely cost per QALY gained.

Secondary outcome measures

a. Symptom severity as measured by clinicians using the MADRS and the CGI of change.
b. Health status (mental and physical components) as measured by SF-12.
c. Health utility as measured by the EuroQoL (EQ-5D).
d. Proportion of patients with moderate depression (defined as BDI-II score $\geq$ 19) at week 25 – estimated by statistical inference from observed distribution of BDI-II scores rather than statistically weaker technique of counting cases.
e. Side effects as measured by the UKU side effects scale and reported AEs – serious examples include psychiatric inpatient admission, attempted or completed suicide, and other mortality.
f. Compliance and adherence of patients to take the medication as prescribed.
g. Suicidality as measured by the MINI suicidality scale.
h. Folate status as measured by homocysteine derived from blood samples taken at baseline, 12, and 25 weeks; and B_{12} status as measured by MMA at 12 weeks only.
i. Resource use as measured by the self-completed health and social care resource use questionnaire, and
j. Genetic analysis of SNPs between the two arms.

Scope of statistical analysis plan

The statistical analysis plan focuses on clinical effectiveness as measured by its primary outcome and secondary outcomes a to g. *Annexes 2* and *3* summarise the assessment timetable and outcome measures. *Annex 4* outlines the methods for economic analyses; *Annex 5* those for genetic analysis; and *Annex 6* those for biochemical analysis.

Management of analysis of trial

The trial data manager will coordinate the preparation and provision of suitable data sets for the various analysts, and the transfer of data between them. He will manage all data in accordance with the NWORTH data management standard operating procedure (SOP) – 6.01.[164] Statistical and health economic analysis

will follow the principles set out in the corresponding NWORTH SOPs – 5.02[165] and 7.01.[166] The genetic analyses will follow the relevant SOPs in use in Liverpool University.

Data collection

Visit windows

Though we aim to complete questionnaires 2 weeks before randomisation and 12 and 25 weeks thereafter, we have defined an advisory window for each. We monitor reasons for collecting the data outside these windows.

Mode of data collection

Some of the trial instruments are administered by researcher or clinician, and others by participant. There is evidence from the MODE-ARTS systematic review that pragmatic changes of administrator 'at random' are unlikely to bias outcomes within placebo-controlled trials.[167]

Allocation concealment and unblinding the data

In principle throughout recruitment to the trial we conceal treatment allocation from participants, healthcare professionals, investigators, and study team. We shall continue this during data analysis. The trial report will specify occasions when blinding was broken for specific participants.

We shall unblind the data at the joint final meeting of the TSC and the DMEC meeting on 10 October 2011. The chair of this meeting will have a sealed envelope to unblind the data when the TSC and DMEC members are content. After correcting errors detected up to, and as a consequence of, the TSC-DMEC meeting, the trial statistician (RhW) will freeze the database. No longer than a week before that meeting an independent senior statistician (CJW) will check the allocations and thus become unblinded to the treatment allocation. However the trial statisticians (RhW, YS as the assistant trial statistician, and BRC as senior trial analyst responsible for the second, validating analysis) will continue to analyse clinical effectiveness blind to allocated treatment.

Delivery of data sets to biochemistry, genetics and health economics teams

These teams will receive cleaned data from NWORTH. *Annexes 4–6* describe the analysis of these data.

TABLE 34 Data collection end point windows

Data collection	Day due (days since randomisation)	Window
Screening for eligibility (week –2)	–14 days	± 10 days
Telephone monitoring	–7 days	± 3 days
Randomisation (baseline)	0	Origin
4-week follow-up	28 days	+ 14 days
12-week follow-up	84 days	± 14 days
25-week follow-up	175 days	± 28 days

Statistical methodology

Populations

'Analysed' population

This population – the subject of the principal analyses – comprises all randomised participants with at least one post-baseline BDI-II outcome. If they fail to return any questionnaire or to complete all items for each instrument, we shall impute these data using the principles and methods described under Missing Data above.

'Complete case' population

This population comprises only those participants whose outcome data are complete. It will provide a useful sensitivity analysis of primary and secondary findings, especially whether they are sensitive to the presence of missing data and the methods of imputation we use to augment them.

'Randomised' population

At first sight it is difficult to draw inferences about this population because some contributed no data after baseline, even on the BDI-II. Because we know the baseline characteristics of all these participants, however, it is possible to reweight the analysed population so they match the characteristics of the randomised population, notably allocated treatment, stratifying variables and baseline BDI-II.

Instruments

BDI-II

We shall compare the effects of adding folic acid or placebo on self-rated symptom severity by comparing the total scores of the BDI-II.[84]

Scoring: This instrument collected data at: screening; baseline; 4; 12; and 25 weeks. We shall score it according to the validated manual, namely by summing the ratings of the 21 items. Each item is rated on a four-point scale ranging from 0 to 3, yielding a total possible score of 63. If a participant ticks more than one category, we shall score the highest.

MADRS

We shall compare the effects of adding folic acid or placebo on researcher-rated depression severity by comparing total MADRS scores.[90]

Scoring: This instrument collected data at: baseline; 4; 12; and 25 weeks. We shall score it according to the validated manual by adding the ratings of the 10 items. Each item is rated on a seven-point scale ranging from 0 to 6, yielding a possible score of 60. If a rater selects more than one category, we shall score the highest.

CGI

We shall compare the effects of adding folic acid or placebo on the researcher-rated CGI.[91]

Scoring: This instrument collected data at: baseline; 4; 12; and 25 weeks. It comprises three separate clinician-rated items: an ordinal scale of current severity of illness using a range of responses from 1 to 7; an ordinal scale of global improvement since recruitment ranging from 1 to 7; and an efficacy index ranging from 0.25 to 4.0 derived from a 4 × 4 matrix plotting therapeutic effect against side effects.

SF-12 version 2

We shall compare the effects of adding folic acid or placebo on self-rated quality of life in the form of SF-12 scores for mental and physical components.[91]

Scoring: We collected SF-12 data at baseline 4, 12, and 25 weeks. The SF-12 comprises ten × 5-point items and three 3-point items. We shall score it according to the validated method, namely by applying Norm-Based Scoring (NBS) algorithms using the recommended standardisation (mean = 50, SD = 10 in the 1998 general US population) to calculate the physical and mental component scores PCS-12 and MCS-12.

Missing items: We shall use the SF-12 missing data software which uses Missing Data Estimation.

EQ-5D

We shall compare the effects of adding folic acid or placebo on self-rated health utility in the form of the EQ-5D (also known as EuroQol). This consists of a self-reported matrix and a self-rated visual analogue scale (EQ-VAS).[93] The self-reported matrix comprises five dimensions: mobility, self-care, usual activities, pain-discomfort and anxiety-depression.

Scoring: This instrument collected data at: 4, 12, and 25 weeks. The five dimensions use three-point scales ranging from 0 to 2. If a participant ticks more than one category, we shall score the highest. In the clinical effectiveness section we shall analyse this as an outcome in its own right, rather than convert it to QALYs. In contrast the health economics section will convert these data to QALYs.

UKU side-effects scale

We shall compare the effects of adding folic acid or placebo on four UKU researcher-rated system-specific side-effect scores and their total.[94]

Scoring: Participants completed the instrument at telephone monitoring 1 week before baseline; baseline; 4; 12; and weeks. The instrument sums scores on 48 distinct side effects – 10 'psychic', 8 'neurologic', 11 'autonomic' and 19 other, of which 11 are gender-free, four are male-specific and four are female-specific. It finishes with three generic items, of which we used two – global assessments by 'patient' and researcher – for imputation. It rates each item on a four-point scale ranging from 0 to 3, yielding possible system-specific scores of 30, 24, 33 and 45 (different questions for each sex, but same total).

Missing items: If respondents fail to answer system-specific items in the instrument, we shall consider them informative missing items and impute by assuming they did not experience those items If raters score above the range of the instrument, we shall censor at the upper limit.

Analysis: As the authors developed the UKU scale specifically for psychotropic drugs, they planned mainly to analyse specific side effects, but acknowledged the case for planned analyses of system-specific scores.[93] Believing that folate does not have independent psychotropic properties, however, we shall test whether it is safe by analysing the four system-specific scores.

Morisky compliance scale

We shall use this four-item instrument to compare the effects of adding folic acid or placebo on self-rated compliance with medication.[95]

Scoring: Participants completed the instrument at 12 weeks. We shall score it as the number of 'yes' responses, yielding a score between 0 and 4.

MINI suicidality scale

We shall use this six-item instrument to compare the effects of folic acid and placebo on self-reported suicidality.[96]

Scoring: Participants completed the instrument at all six data collection points: screening; monitoring; baseline; 4; 12 and 25 weeks. Though we selected it primarily to fulfil our duty of care to participants, the TSC and DMEC asked us to analyse it as an outcome in its own right, and the Wales MREC agreed.

We shall score it by summing the scores allocated to 'yes' responses, which range between 0 and 10, yielding a total score between 0 and 33.

Missing data

We shall adopt a consistent approach to missing data relating to both effectiveness and cost-effectiveness except where individual outcome measures require some variation in that approach; for example the SF12 has its own missing data software. In particular we shall exclude participants without follow-up data. Then for each variable we shall summarise the frequency of missing data by type (e.g. participant withdrew; questionnaire not returned; page missing; item missing). Where < 10% of data are missing, we shall assume they are missing completely at random in the sense that there is no systematic reason for absences (MCAR).[108] Where > 10% of data are missing, we shall explore the missing data and tabulate them by stratification variables [namely centre (Swansea, North East Wales or North West Wales); sex (male or female); new or continuing prescription (where participants in the second category have taken the same daily antidepressant for at least 2 months with a stable dose in the therapeutic range reported in the BNF for at least 1 month); type of antidepressant prescribed (SSRI or other) and whether or not they have ever received counselling for depression], both as reported at randomisation and as validated after quality assurance; patient demographics; and other important scientific covariates. If there is no reason to suspect that the data are not MCAR, we shall impute values to be used in the main analyses by the following methods. If there is reason to suspect that the missing data are not MCAR, the trial statistician and CI will discuss the findings.

Missing items within a subscale

If a subscale comprises three or fewer items, we shall treat each as a separate subscale. In addressing missing items within a subscale thus defined, we shall take account of methodological publications about the validated instrument. In principle we seek to impute missing items to complete instruments thus:[108]

a. If < 25% of the items within a subscale are missing for a participant at a time point, we shall impute them by the weighted mean of the completed items.
b. If > 50% of the items within a subscale are missing for a participant at a time point, we shall treat that subscale as missing and impute it.

Missing subscales

Where between 25% and 50% of the items within a subscale are missing, we shall proceed thus:

1. If < 40% of the subscales for a participant at a time point are missing, we shall impute all missing subscales by a single application at that point of the SPSS multivariate imputation algorithm that also takes account of all validated stratification variables.
2. If > 40% of the subscales for a participant at a time point are missing, but < 20% of participants experience that problem, we shall impute all missing subscales by a single multivariate imputation that also takes account of all validated stratification variables across all time points.
3. If > 40% of the subscales for a participant at a time point had been missing, and > 20% of participants had experienced that problem, we would have used multiple multivariate imputations; in the event, however, this never happened.

Missing time points

1. If < 15% of all time points are missing, we shall impute all subscales within each time point by one iteration of the SPSS multiple imputation algorithm using all other subscales at all time points, together with age, gender, centre and group.
2. If > 15% but < 30% of all time points are missing, we shall impute all subscales within each time point by five iterations of the SPSS multiple imputation algorithm using all other subscales at all time points, together with age, gender, centre and group.

Preliminary data description

We shall summarise baseline and demographic data by both treatment allocation and centre. Conscious that our three centres differed in many respects notably psychiatric practice and recruitment policy, we shall present outcomes believed to follow a Normal distribution in the form: number of responses; mean and SD. If Normal plots show evidence of non-normality however, we shall them present them in the form: number of responses; median and first and third quartiles.

Data transformations

Our analysis plan assumes that residual variation from our statistical models will follow approximately Normal distributions. This is a robust assumption in the sense that only a substantial deviation would invalidate each analysis. Hence the trial statistician will plot all residual distributions and discuss any evidence against normality, as shown for example by Normal plots, with the CI. If necessary we shall seek an optimal transformation to improve approximation to Normality.

Analytical methods

All tests will be two-sided with a significance level of 5% but no correction for multiple testing.

Continuous outcomes with baseline and more than one follow-up (for example BDI-II)

We shall use 'AUC' to combine outcomes over all time points to create the primary outcome. As covariates in the AUC analysis we shall use validated stratification variables – centre, gender, new or old patient, type of antidepressant and previous counselling. For individual time points we shall use analysis of covariance to adjust for the corresponding baseline score.

As a sensitivity analysis we shall use multi-level modelling with the same covariates, also known as repeated measures analysis of variance. We shall estimate parameters for three fixed factors – the three time points (4, 12 and 25 weeks), centre and treatment group. We shall also include interactions between treatment and both time point and centre. We shall summarise all effects by parameter estimate, standard error, significance level, and 95% confidence level.

Continuous outcome with no baseline and only one follow-up (Morisky scale)

We shall use analysis of covariance, with baseline depression scores and validated stratification variables as covariates, to test whether medication adherence, measured on the Morisky scale, is significantly different between the treatment groups. If so, or if there is other evidence that the Morisky score influences the main psychological outcomes, we shall test in secondary analysis whether adding it to the usual covariates improves the fit of each model and refine those models accordingly. In these circumstances we shall test whether also to add prescribed medications recorded by GPs to the usual covariates.

Dichotomous outcomes (serious adverse effects and adverse events)

We shall use logistic regression of the binary response of (S)AE or no (S)AE over each participant's time in the trial to test whether the proportion of participants who experienced (S)AEs differs between treatment arms. Covariates will include baseline scores and validated stratification variables. We shall transform all estimated fixed effects back from their logistic form and summarise them by OR; standard error; significance level and 95% CI.

Covariates for adjustment within statistical model

We shall keep baseline depression scores and validated stratification variables as covariates throughout. We shall explore covariates of potential scientific relevance, including: demographic (e.g. age, ethnicity, marital status, number of dependants and employment status, coded in accordance with usual demographic practice); and clinical (e.g. referral source, smoking, alcohol consumption and medication adherence, measured by both Morisky scale and recorded prescriptions). We shall retain these if they achieve significance levels of 10%.

Interactions to be tested

Within each analysis we shall test for interaction between treatment and centre, not least because our three centres differed in many respects, notably psychiatric practice and recruitment policy. Any evidence of interaction between treatment and centres will lead to exploratory analysis to explain the effects, initially by covariates within the 'treatment allocated' population. Failing that, we shall estimate the treatment effect for each centre separately. We shall also test interactions with significant covariates and include these interactions within the model if significant at the 10% level.

Sensitivity analyses

In addition to the planned sensitivity analyses described in *Chapter 2* (see *Statistical methods, Methods for analysing outcomes, Sensitivity analyses*), we shall use sensitivity analysis ad hoc to test whether the validity of the trial is at risk, for example to protocol deviations that result in systematic missing data or potentially differential reasons for withdrawal from the trial and loss to follow-up.

Annex 2: Assessment timetable

			Screening		Baseline	Follow-ups		
Assessment type	Outcome measure	Respondent	Week 2 (eligibility)	Week 1 (telephone contact)	Week 0	Week 4	Week 12	Week 25
Screening tool for ICD-10	Symptoms: BDI-II	Patient	✓					
Screening tool	MINI depression	Clinician	✓					
Blood testing	FBC	Researcher	✓					
	Serum folate	Researcher	✓				✓	✓
	Red cell folate	Researcher	✓					
	B_{12}	Researcher	✓				✓	✓
	Homocysteine	Researcher	✓				✓	✓
	MMA	Researcher	✓				✓	
	Genetics	Researcher	✓ (needs extra consent)					
Depression status	BDI-II	Patient	✓		✓	✓	✓	✓
	MADRS	Researcher			✓	✓	✓	✓
Health status and quality of life	CGI	Researcher			✓	✓	✓	✓
	SF-12	Patient			✓	✓	✓	✓
	EQ-5D	Patient			✓	✓	✓	✓
Health economics	Resource usage	Patient			✓		✓	✓
Suicidality	MINI suicidality	Researcher	✓	✓	✓	✓	✓	✓
Compliance and side effects	Morisky Questionnaire	Patient					✓	
	UKU side effects scale	Researcher		✓	✓	✓	✓	✓

Annex 3: Outcome measures

Depression outcomes

Tool	Purpose	Time points (weeks)
BDI-II	**Primary outcome for clinical effectiveness:** Self-rated measure of symptom severity	–2
		0
		4
		12
		25
		0
MADRS	**Secondary outcome:** Researcher-rated measure of symptom severity	0
		4
		12
		25
CGI	**Secondary outcome:** Researcher-rated measure of three dimensions – illness severity, global improvement and response to treatment	0
		4
		12
		25

General quality-of-life outcomes

Tool	Purpose	Time points (weeks)
SF-12	**Secondary outcome:** Self-rated measure of health status in two dimensions – mental and physical	0
		4
		12
		25
EQ-5D	**Primary outcome for cost-effectiveness:** Self-rated measure of health-related quality of life	0
		4
		12
		25

Biochemistry outcomes

Tool	Purpose	Time points (weeks)
Serum folate	**Clinical outcome:** Blood concentration level	–2 12 25
Vitamin B_{12}	**Clinical outcome:** Blood concentration level	–2 12 25
Homocysteine	**Clinical outcome**: Blood concentration level	–2 12 25
MMA	**Clinical outcome:** Blood concentration level (not reported in this monograph)	–2 12

Side effects and compliance

Tool	Purpose	Time points (weeks)
UKU side effects scale	**Secondary outcome:** Self-rated measure of side effects on four main dimensions – psychic, neurologic, autonomic and other	–1 0 4 12 25
Morisky Questionnaire	Self-rated measure of drug compliance	12

Annex 4: Economic analysis plan

Aim

To assess whether the use of folic acid supplementation is cost-effective by estimating the incremental cost–utility and cost-effectiveness ratios of ADM plus folic acid relative to ADM alone.

Data

Healthcare resources: We measure participants' use of health and social care services by:

a. self-completed questionnaires (collected by research professionals at baseline, and weeks 12 and 25), which ask patients to recall their use of general practice, community services and social services over previous 3 months
b. general practice records of prescribed medications over 25 weeks of follow-up, and
c. data on hospitalisations, triggered by notified SAEs.

Unit costs: We shall derive the cost of most resources used from national sources.[114,116,119] We shall estimate specialised costs like pharmacogenetic testing from appropriate local sources. The cost year will be 2010 and the perspective will be that of the NHS and PSS.

Health outcomes: Participants completed the EQ5D instrument at: baseline, 4, 12, and 25 weeks. We shall convert their responses into a single, preference-based utility value based on the UK tariff.[168] They completed the SF12 at: baseline, 4, 12, and 25 weeks. To generate the SF6D utility score from their responses we shall apply a valuation algorithm using preference weights obtained from a sample of the general population using the standard gamble technique.[122] For the cost-effectiveness analysis, we shall estimate the number of weeks free from moderate or severe depression from participants' responses to the BDI-II.

Analysis

Cost analysis: In principle we shall impute missing data on resource use according to the section of the statistical analysis plan on 'Missing items within an instrument'. We shall estimate the mean cost per patient over 25 weeks across both arms of the trial together with their respective bootstrapped 95% CIs using 10,000 replicates. We shall analyse cost data by assuming that the large samples generate nearly Normal distributions of sample means, thus justifying Student's *t*-test and ordinary least squares (OLS) linear regression. If quantile–quantile plots,[169] and Shapiro-Wilk[170] and Shapiro-Francia[171] tests for normality show problems like skewness and excess zeros (at least 20% of samples),[124] we shall develop an appropriate generalised linear model. To gain precision in estimating mean costs, we shall include covariates in the cost regression models. Selection of covariates will accord with the section of the statistical analysis plan on 'Covariates for adjustment within statistical model'.

Analysis of health outcomes: We shall impute data missing from EQ5D responses according to the section of the statistical analysis plan on 'Missing items within an instrument'. We shall present descriptive statistics of fully imputed responses to individual items within the EQ5D (mobility, self-care, usual activity, pain-discomfort and anxiety-depression) and the derived utility scores and Visual Analogue Scale scores for each time point across treatment groups. We shall estimate the number of QALYs experienced by each patient over 25 weeks as the area under the EQ5D utility curve, using the trapezoidal rule and adjusting for baseline utility score.[170] We shall derive non-parametric 95% CIs from 10,000 bootstrapped replicates. We shall analyse these QALY data by Student's *t*-test and OLS linear regression, after testing by quantile-quantile plots[169] and the Shapiro-Wilk[170] and Shapiro-Francia[171] tests that the large samples generate Normal distributions. To gain precision in estimating mean QALYs, we shall include covariates in the QALY regression models. Selection of covariates will include baseline utility score[126] and accord with the section of the statistical analysis plan on 'Covariates for adjustment within statistical model').

Cost-effectiveness analysis: We shall estimate the number of weeks free from moderate or severe depression (when BDI-II scores are less than 13) by statistical inference from the observed distribution of BDI-II scores and assuming linear interpolation between time points – baseline and at 4, 12 and 25 weeks.

Incremental and uncertainty analysis: We shall derive ICERs by dividing differences in adjusted mean costs by differences in adjusted mean effects. We shall explore uncertainty around ICERs using non-parametric bootstrapping. We shall display results on cost-effectiveness planes and as cost-effectiveness acceptability curves showing when the results fall below given cost-effectiveness thresholds. We shall conduct sensitivity analyses to test the robustness of our findings and examine the extent to which ICERs are sensitive to key assumptions in the analysis, notably on unit costs and SF6D utilities and through complete case analysis.

Secondary analysis: Depending on the analysis of clinical data, secondary analyses will assess relationships between cost-effectiveness and potential predictors of response, notably adverse reactions and high-cost episodes by including them in generalised linear regression models.[172] These are likely to include baseline disease severity and class of prescribed ADM, along with gender, age and genetic factors. If the results of the genetic analysis suggest clinical value, we shall undertake exploratory analysis of the cost-effectiveness of pharmacogenetic testing.

Annex 5: Genetic analysis plan

We shall analyse baseline samples for 140 single nucleotide polymorphisms and two tandem repeat polymorphisms within the genomic loci of 25 genes related to either one-carbon folate metabolism or methionine biosynthesis pathways. Initially we shall examine each single nucleotide polymorphism ('SNP') in turn for association with participants' response to antidepressant medication (ADM).

We shall fit two mixed models. The first will include baseline values, the three time points (4, 12 and 25 weeks), centre, genetic factors potentially associated with outcome a priori [age, gender, body mass index (BMI), co-medications, type of antidepressant and new or continuing patient] as well as an indicator variable to indicate whether a patient supplemented their treatment with folic acid or not. This indicator variable will specify treatment received rather than treatment allocated.

The second model will be the same as the first except for a covariate representing the SNP. We shall compare both models by the likelihood ratio test, and record the significance level. Initially we shall assume an additive mode of inheritance for each SNP, with patients having the wild-type genotype coded '0', those having the heterozygous genotype coded '1', and those having the homozygous variant genotype coded '2'. Later sensitivity analysis will test whether a dominant allele model provides a better fit to the data.

Before the analyses of association, we shall test each SNP for Hardy–Weinberg Equilibrium. We shall flag those found to deviate at the 1% significance level but include them in analysis. We shall include in the association analyses only SNPs passing the following genotype quality criteria:

a. minor allele frequency > 0.01
b. genotypes call > 95% of SNPs
c. samples call > 90% of SNPs.

In addition we shall exclude any patient samples with > 5% missing genotypes from analysis. Also before the analyses of association, we shall reduce the number of SNPs investigated by assessing the extent of linkage disequilibrium ('LD') between SNPs. Where the LD is significant ($r^2 > 0.81$) for a group of two or more SNPs, we shall include only the SNP with the least missing data in the analyses.

If the study includes only a small proportion of self-reported non-Caucasian patients (5%), we shall exclude them from the genetic association analyses. If the proportion exceeds 5%, we shall adjust for ethnic origin in analysis by including additional covariates in the regression models.

Once all genetic analyses of association with participants' response to ADMs are complete, we shall estimate the FDR for each association. We shall treat FDRs less than 0.05 as statistically significant associations. For marginally significant SNPs (i.e. FDR < 0.10), we shall fit a further regression model including a folic acid × SNP interaction term to identify genetic predictors of the efficacy of folic acid adjuvant to ADM. We shall compare this model with the model without interaction, and re-estimate the FDR with 5% again the criterion of significance.

We shall also undertake exploratory analyses for association between each SNP and changes from baseline in the biochemical markers specified in *Annex 6* below, following the same format as for the primary analyses. We shall use the statistical packages R, SPSS and PLINK.[111,135,136]

Hence the genetic component of the study will need the clinical and biochemical data specified in *Annex 2* including the following stratifying variables: centre, age, gender, BMI (i.e. weight/height^2), co-medications, type of antidepressant, and whether new or continuing patient.

Annex 6: Biochemistry analysis plan

Homocysteine

Folate and vitamin B_{12} concentrations are major determinants of one-carbon metabolism, in which the essential methyl donor S-adenosylmethionine (SAM) is formed. SAM is essential for neurologic function. Low plasma folate concentrations are associated with poor response to ADM, and treatment with folic acid can improve that response. Plasma total homocysteine (tHcy) is a sensitive marker of folate (and B_{12}) status. We measure this at baseline and following treatment with folic acid or placebo primarily to assess the response to folate therapy. It will be informative to characterise how response to folate augmentation depends upon baseline homocysteine levels. We will assay homocysteine by an automated analyser (Abbott Architect) using one-step immunoassay with chemiluminescence detection (Axis-Shield). This assay is standardised using the National Institute of Standards and Technology (NIST) Standard Reference Material. It is used to assess patient samples routinely, and is subject to rigorous internal and external quality assurance assessments.

Biochemistry analysis

As patients with depression have increased homocysteine concentrations, the primary analysis of homocysteine will follow essentially the same analysis plan as for genetics in *Annex 5*. We shall compare the effects of folic acid and placebo on tHcy using the mixed model approach to repeated-measures analysis of variance. As potential covariates we shall use baseline Hcy, age, gender, BMI (weight/height^2), co-medications, type of ADM, and new or continuing patient. We shall estimate parameters for three fixed factors – the three time points (4, 12 and 25 weeks), centre (especially important as tHcy sample handling differs between centres) and treatment group. We shall also include the interaction between treatment group and time point to test whether differences between treatments vary over time.

We shall add reported compliance with folate therapy or placebo to this model and test whether this leads to a fall in tHcy. We shall investigate how type of ADM, lifestyle factors (e.g. smoking, BMI and alcohol) and genetic polymorphisms in the folate pathway affect patients' baseline homocysteine concentrations; and how folic acid affects BDI-II scores, the primary outcome measure. We shall also test the interaction between biochemical variables and the final set of genetic polymorphisms analysed by the genetics analysis plan (see *Annex 5*). We shall explore the extent to which tHcy and MMA, both baseline and subsequent, predict BDI-II, MADRS and CGI scores.

Methylmalonic acid

Methylmalonic acid is a sensitive marker of B_{12} status. Hence the trial affords a unique opportunity to estimate the effects of folic acid supplementation on MMA concentration specifically in individuals with low-to-normal B_{12} levels, and thus assist in elucidating the nature of the relationship, if any, between folic acid and MMA. If folic acid is clearly shown to influence MMA concentrations, this will be of considerable importance for public health decisions relating to the issue of food folate fortification in the UK and elsewhere. There is evidence from cross-sectional studies that the combination of high plasma folate and low B_{12} status is associated with cognitive decline. Hence we shall later explore the extent to which MMA acts as a prognostic variable for other outcomes.

Thus the non-genetic data required for the biochemical component of the study are the same as those listed in *Annex 2* for the genetic component of the study.

Imputing serum folate measurements censored at 20

We shall impute separately for groups A and B without using demographic variables like age or sex.

1. Step 1:

    a. Regress log (serum folate) on log (red cell folate – RCF) using raw data where both values are present; and derive slope and intercept.
    b. Replace serum folates recorded as > 20 by 20.
    c. Regress log (serum) on log(RCF) after including the extra data points added in step 1b, expecting the intercept to increase and the slope to decrease.

2. Step 2:

    a. Use slope and intercept from step 1c to impute serum folates recorded as > 20.
    b. Retain all imputations that yield serum folates > 20; and replace by 20 all imputations that yield serum folates < 20 in 2.1.
    c. Regress log(serum) on log(RCF) after including the extra data points imputed in step 2b, expecting the intercept to increase and the slope to decrease.

3. Final steps:

    a. Repeat steps 2a to 2c till slope changes by < 0.1 × SD of slope or no imputed folates < 20.
    b. Impute all serum folates recorded as > 20 using slope and intercept from final iteration of step 2c; add normally distributed residual with mean zero and SD = residual SD of final model.
    c. Repeat entire process after replacing RCF by B_{12} to impute missing values when RCF is missing but B_{12} is present; however imputation is not possible when folate, RCF and B_{12} are all missing.
    d. Assess imputation process by scatter plots of serum folate against RCF and B_{12}.

Appendix 6 Supplementary tables for single nucleotide polymorphisms

TABLE 35 Single nucleotide polymorphisms included in Sequenom genotyping assays or excluded before analysis for assay design factors

Gene (HUGO)	Accession number	Chromosome position
AHCY	rs866027	chr20:32874310
	rs13043752	chr20:32883308
ALDH1L1	rs4646760	chr3:125822871
	rs4646756	chr3:125824256
	rs1127717	chr3:125826059
	rs2276726	chr3:125826287
	rs3772426	chr3:125829830
	rs1868130	chr3:125837819
	rs12106789	chr3:125838351
	rs4646739	chr3:125844079
	rs6763254	chr3:125848067
	rs6799991	chr3:125856916
	rs6800400	chr3:125857342
	rs11923466	chr3:125859367
	rs10934751	chr3:125868513
	rs16837171	chr3:125871773
	rs16837178	chr3:125872293
	rs3796191	chr3:125872384
	rs6774807	chr3:125883082
	rs4679102	chr3:125885196
	rs9842910	chr3:125896572
AMD1	rs7768897	chr6:111212283
AMT1	rs10640	chr3:49454277
	rs1464568	chr3:49458266
ATIC	rs7585489	chr2:216181868
	rs2372536	chr2:216190020
	rs10932606	chr2:216198374
	rs6737407	chr2:216207121
	rs6435899	chr2:216207271
	rs4672768	chr2:216214124
BHMT	rs6875201	chr5:78410564
	rs506500	chr5:78414337

continued

TABLE 35 Single nucleotide polymorphisms included in Sequenom genotyping assays or excluded before analysis for assay design factors (*continued*)

Gene (HUGO)	Accession number	Chromosome position
	rs3733890	chr5:78421959
CBS CTH	rs12613	chr21:44473691
	rs4920037	chr21:44481891
	rs234706	chr21:44485350
	rs535112	chr1:70889476
	rs525276	chr1:70893391
	rs17131305	chr1:70896165
	rs473334	chr1:70896967
	rs1021737	chr1:70904800
DHFR	rs1677692	chr5:79937014
	rs10072026	chr5:79945140
DNMT1	rs10407514	chr19:10255112
	rs8111085 (merged with rs2228612)	chr19:10273372
	rs6511677	chr19:10277799
FOLH1	rs16906158	chr11:49170774
	rs383028	chr11:49174141
	rs202687	chr11:49180269
	rs7126892	chr11:49198799
	rs202712	chr11:49198924
	rs588458	chr11:49214048
FPGS	rs2230270	chr9:130570894
	rs34354111	chr9:130575702
FTCD	rs17004505	chr21:47571209
	rs28941768	chr21:47571859
GART	rs8971	chr21:34883618
	rs6517178	chr21:34888621
	rs2834234	chr21:34894623
	rs4817577	chr21:34894797
GGH	rs11995525	chr8:63934988
	rs7010484	chr8:63937675
	rs11545078	chr8:63938764
	rs3780130	chr8:63940776
	rs13268472	chr8:63942016
	rs10957267	chr8:63944251
	rs17194931	chr8:63944344
	rs13270305 (merged with rs11545077)	chr8:63951237
	rs1800909	chr8:63951312

TABLE 35 Single nucleotide polymorphisms included in Sequenom genotyping assays or excluded before analysis for assay design factors (*continued*)

Gene (HUGO)	Accession number	Chromosome position
MAT1A	rs4933327	chr10:82033683
	rs17102596	chr10:82035173
	rs2236568	chr10:82035923
	rs873395	chr10:82037215
	rs1143693	chr10:82040484
	rs3862534	chr10:82048978
MTFMT	rs34507711	chr15:65295441
	rs6494509	chr15:65307463
	rs35302908	chr15:65308791
	rs11638255	chr15:65321037
MTHFD1	rs8006686	chr14:64868671
	rs1950902	chr14:64882380
	rs10133855	chr14:64890227
	rs34181110	chr14:64892470
	rs11627525	chr14:64894362
	rs10498514	chr14:64899055
	rs2236225	chr14:64908845
	rs8012229	chr14:64911562
	rs2281603	chr14:64926097
MTHFR	rs2184226	chr1:11847436
	rs35737219	chr1:11850750
	rs2274974	chr1:11851319
	rs13306556	chr1:11852110
	rs1801131	chr1:11854476
	rs1801133	chr1:11856378
	rs4846052	chr1:11857951
	rs17367504	chr1:11862778
	rs2066472	chr1:11862971
	rs7553194	chr1:11864149
	rs2244976	chr8:19122545
MTHFS	rs8923	chr15:80137560
	rs4779165	chr15:80151013
	rs2586154	chr15:80165368
	rs12899781	chr15:80168282
	rs7166189	chr15:80172385
	rs2733107	chr15:80178612

continued

TABLE 35 Single nucleotide polymorphisms included in Sequenom genotyping assays or excluded before analysis for assay design factors (*continued*)

Gene (HUGO)	Accession number	Chromosome position
	rs8042012	chr15:80179847
	rs2586183	chr15:80180106
	rs12898642	chr15:80182050
	rs8040104	chr15:80183342
MTR	rs12749581	chr1:236966848
	rs6668344	chr1:237001326
	rs4659727	chr1:237006914
	rs1805087	chr1:237048500
	rs10158222	chr1:237050682
	rs1252252	chr1:237056002
	rs11799647	chr1:237060921
MTRR	rs1801394	chr5:7870973
	rs326124	chr5:7877178
	rs161869	chr5:7877831
	rs1532268	chr5:7878179
	rs10380	chr5:7897191
	rs12347	chr5:7897283
	rs716537	chr5:7899419
	rs8659	chr5:7900833
SHMT1	rs1979277	chr17:18232096
	rs2273028	chr17:18239012
	rs9910090	chr17:18250399
	rs2273026	chr17:18256979
	rs2461838	chr17:18265264
SLC19A1	rs1051296	chr21:46934861
	rs12659	chr21:46951556
	rs2330183	chr21:46953292
	rs1051266	chr21:46957794
	rs3177999 (merged with rs1131596)	chr21:46957916
	rs35789560	chr9:130575515
TYMS	rs1004474	chr18:660383
	rs11540152	chr18:662215
	rs596909	chr18:669087
	rs11540153	chr18:669117
	rs2853532	chr18:670414

TABLE 35 Single nucleotide polymorphisms included in Sequenom genotyping assays or excluded before analysis for assay design factors (*continued*)

Gene (HUGO)	Accession number	Chromosome position
Unable to plex owing to sequence constraints		
Gene (HUGO)	**rs number**	**Chromosome position**
ALDH1L1	rs4646750	chr3:125826003
CBS	rs1801181	chr21:44480616
DNMT1	rs8112801	chr19:10253099
MTHFR	rs3927589	chr1:11854493
TYMS	rs2853542	chr18:657685
SNPs excluded as incompatible with assay containing ≤ 10 SNP plex		
MTHFS	rs16971449	chr15:80152997
SLC19A1	rs35786590	chr21:46935675
SLC19A1	rs7278825	chr21:46935942

TABLE 36 Sequenom Assay Oligonucleotide sequences for PCR primers and extension probes

rs number	Gene (HUGO)	Forward PCR primer (5′ to 3′)	Reverse PCR primer (5′ to 3′)	Extension probe sequence (5′ to 3′)
Assay 1 (35-plex)				
rs10072026	*DHFR*	ACGTTGGATGGCAGCTTCATCAATAGCTCC	ACGTTGGATGTGTCTCATAGTGGAGATCAG	gAGTGGAGATCAGTATATGATAA
rs1127717	*ALDH1L1*	ACGTTGGATGCGGGCTTTTATCTCTCTTGC	ACGTTGGATGCCTTGGCTATGAACATGTGG	ACATGTGGTCTTCCACG
rs1143693	*MAT1A*	ACGTTGGATGCATCCTCCTCATTTCTGTCC	ACGTTGGATGTCAAGACTTGCAACGTGCTG	acTCCCCAGATATTGCCCA
rs11540152	*TYMS*	ACGTTGGATGAGTCCCCTTCTTCTCTGGTG	ACGTTGGATGGGGAGTGAAAATCTGGGATG	ggtgaCAATGGATCCCGAGAC
rs11540153	*TYMS*	ACGTTGGATGCCAAGCGCACATGATGATTC	ACGTTGGATGAGTTGACCAACTGCAAAGAG	gccaAACTGCAAAGAGTGATTGACA
rs11638255	*MTFMT*	ACGTTGGATGAAAAGTGGAAGGAGGACTGC	ACGTTGGATGCGTATATACTTCTCTGCCTC	CTGCCTCTTTTTCCCAATT
rs11799647	*MTR*	ACGTTGGATGTGGCTGAGGTTGAGAAATGG	ACGTTGGATGCCTTGAGGATCATCAAGAAT	TTAGTCTGTATCATATCCCAAAA
rs11923466	*ALDH1L1*	ACGTTGGATGTTATGCACTTGCCTATTGTC	ACGTTGGATGAAGGCCACTACATAGGTAAG	gATGAAAGTCCATATTATTGTATTTTA
rs12106789	*ALDH1L1*	ACGTTGGATGTGACACCTCCCTTTTCCATC	ACGTTGGATGGAGTGGTGGAAGATGGAGG	cGATGGAGGGACTGGA
rs12347	*MTRR*	ACGTTGGATGTGGCCATGAAGCTGGATGTT	ACGTTGGATGTCCTTCTCAAGAGATGCTCC	tggtgGCCCCAGCAAAGTATGT
rs1252252	*MTR*	ACGTTGGATGGGGCTGGAACTTAACATTAG	ACGTTGGATGAAATCCTCACTGTATGCGCC	atGCTCGGACGCCACAGAAT
rs12659	*SLC19A1*	ACGTTGGATGAGCACTGAGTCCCCACAGG	ACGTTGGATGTGCGAAACCTCGGCTTCGGA	gtCTGGAGCGCATGAATCC
rs12749581	*MTR*	ACGTTGGATGGCGGGAGAAGCTAAACGAAG	ACGTTGGATGGTCATTGTTGCCTTTCAGCG	ccttGCATGATCTTTAAATTCCTGACCT
rs16906158	*FOLH1*	ACGTTGGATGATCTGGATCATTATACCGAG	ACGTTGGATGAAATGACGAGTCCTCTGTGG	tcACAAACTACCAAACAAATTAAA
rs17102596	*MAT1A*	ACGTTGGATGACAAAGGCATTAGGGAGTGG	ACGTTGGATGTTGAAAACAGGTAACCTGCC	CCTGCCAAAGCTTCA
rs1801131	*MTHFR*	ACGTTGGATGGAGCTGCTGAAGATGTGGG	ACGTTGGATGACCATTCCGGTTTGGTTCTC	ttggCAAAGACTTCAAAGACACTT
rs1801394	*MTRR*	ACGTTGGATGGTGAAGATCTGCAGAAAATCC	ACGTTGGATGATATGCTACACAGCAGGGAC	agtCAAAGGCCATCGCAGAAGAAAT
rs202687	*FOLH1*	ACGTTGGATGATAGGAATAGCACTGAATC	ACGTTGGATGCCATGCTCATGGATAGGAAG	cccccAACGGCCATATTGCCC
rs2066472	*MTHFR*	ACGTTGGATGTGGAATCTGGTGACAAGTGG	ACGTTGGATGGAGATGAGATTGACAGCTCC	ttgcCAGCTCCCTCAGCAGTT
rs2236225	*MTHFD1*	ACGTTGGATGCACTAACCTACAAACCCTTC	ACGTTGGATGATCGCACATGGCAATTCCTC	gttaCTCCATCATTGCAGACC
rs2236568	*MAT1A*	ACGTTGGATGAGTGGAGGAATCCTAGGGAC	ACGTTGGATGCATTTAGTGCACTCTCTGGG	aggaGTCTGACAGTGTGCAAGAAAT
rs2330183	*SLC19A1*	ACGTTGGATGGCCTCTTCCTCTGGACTGT	ACGTTGGATGACTACAGGCAGGCACCAAAC	GCACCAAACGTCCTCAG
rs234706	*CBS*	ACGTTGGATGTCACACTGCTGCAGGATCTC	ACGTTGGATGTTCTATGCAGTACCGCAACG	ctcccCGCCAGCAACCCCCTGGCTCACTA

rs number	Gene (HUGO)	Forward PCR primer (5′ to 3′)	Reverse PCR primer (5′ to 3′)	Extension probe sequence (5′ to 3′)
rs2834234	*GART*	ACGTTGGATGTCACTTGGGATATCTTCTGC	ACGTTGGATGTTAAAAACAAGTCATCACC	cttTTAAAAACAAGTCATCACCTAAACG
rs2853532	*TYMS*	ACGTTGGATGTCAGAGCTGAAGGGATCTGG	ACGTTGGATGTACCAGAAGCACCAGTTTCC	cccTTATTCCTGCTGTATTTGTAAT
rs34354111	*FPGS*	ACGTTGGATGGACTAGGTGGCTGGAAGATG	ACGTTGGATGCTCGTCTTCAGCTGCATTTC	ggggaATGCCTTGCAATGGATCA
rs35737219	*MTHFR*	ACGTTGGATGTCAGGACGCAGGGTCATGGA	ACGTTGGATGTGGTGGAAGACACATTGGAG	CCCAGAATGCGAGAGAAA
rs3733890	*BHMT*	ACGTTGGATGTTGTTGCAGTCAGGAGTGTG	ACGTTGGATGAGTGAAGCTCATGAAGGAGG	GCTTGGAGGCTGCCC
rs3862534	*MAT1A*	ACGTTGGATGCCAAGCCTATACTGAGGGA	ACGTTGGATGCTCATGGATCAAAACAACAC	tcataGATCAAAACAACACACGGTAC
rs4646739	*ALDH1L1*	ACGTTGGATGCTGCTTCTCTCCAGGACTTC	ACGTTGGATGACAGCCTGAGTAGTCTATCC	tttttATGTCCCTTATGTGGCTTCC
rs4817577	*GART*	ACGTTGGATGAATAGCGGTTCTCAGGGATG	ACGTTGGATGTTTAACTGGTCTAGGGCAGG	ggcgGGGAATTGAAATGAGTTTGAAC
rs506500	*BHMT*	ACGTTGGATGTGACAGAATGAGACTCCATC	ACGTTGGATGAGATTTCCAAGCACAGTTCC	aaaaCAAGCACAGTTCCATGGATGAAT
rs6494509	*MTFMT*	ACGTTGGATGGTTCCTTGGAATATCAGGGC	ACGTTGGATGGAGTACCTATTCTGTGCCAG	ACTGTACAAGATGCTGTAAATA
rs6763254	*ALDH1L1*	ACGTTGGATGTCTCTAGGGCTCTAAGGGTC	ACGTTGGATGGCAGATGAACAACCAACTCC	CCAACTCCTCAAGCA
rs8111085	*DNMT1*	ACGTTGGATGCCTTTACCTTTTCATCCTCG	ACGTTGGATGGAGGCCCGAAGAAAAAGAAC	ggagGAACCTGAAAAAGTAAATCCACAG
Assay 2 (35-plex)				
rs1004474	*TYMS*	ACGTTGGATGTAAAACTGTGACTCTCCCCC	ACGTTGGATGGGGAAAGGCTGACATACATC	GATGGTGATGTTCGTCTA
rs1021737	*CTH*	ACGTTGGATGGCAGCTTCTAATAGCAGCTC	ACGTTGGATGGCACTGTTATTATAGCACCC	ACCCTCCAAGTGGAA
rs1051266	*SLC19A1*	ACGTTGGATGCGTAGAAGCAAAGGTAGCAC	ACGTTGGATGAGAAGCAGGTGCCCGTGGAA	cccaaCCCGAGCTCCGGTCCTGGCGGC
rs13268472	*GGH*	ACGTTGGATGTTCCCACCTTGCATGAAGAC	ACGTTGGATGTCTCACCTGATGGATTACGG	ctccGGATTACGGGGTGACT
rs13306556	*MTHFR*	ACGTTGGATGTCAAGTTCAGGAGAGGATGC	ACGTTGGATGACAGATGACCCTCTGAGAAG	GGTGAGATATCCCTCCT
rs1464568	*AMT1*	ACGTTGGATGTCCCCACTGGATGGACAAAA	ACGTTGGATGAGACCTGCTGAATCAGAACC	aAGAACCTTCATTTTAACAAGATC
rs161869	*MTRR*	ACGTTGGATGACCGTAGCTGAAAACATGAG	ACGTTGGATGGGAGGAATTCTATTCCTGTC	tgCCTATCCCTCTTCATTTCTT
rs16837171	*ALDH1L1*	ACGTTGGATGAACTCACGGTACCAGCAAAC	ACGTTGGATGCTCATAGTGTTGTTAGGCAG	ATTGTTCTGGTATAAAAGGTG
rs1805087	*MTR*	ACGTTGGATGCTTTGAGGAAATCATGGAAG	ACGTTGGATGTCTACCACTTACCTTGAGAG	CCTTGAGAGACTCATAATGG
rs2184226	*MTHFR*	ACGTTGGATGACCAGCTCTGTGGCCTGTGT	ACGTTGGATGCCAGGAAGTCCAAGCCCAT	cGCTCCTCTCTGGCTA
rs2281603	*MTHFD1*	ACGTTGGATGGACCCTTTGATATCCTTGTC	ACGTTGGATGATCCTGTGTGGAACAGGTTG	ggggCTGAGAAAAACTAGAAAAAGC
rs2372536	*ATIC*	ACGTTGGATGGTTGCCTGCAATCTCTATCC	ACGTTGGATGTCAATTTGCTCCACAGCCTC	ACAGCCTCCTCAACA

continued

TABLE 36 Sequenom Assay Oligonucleotide sequences for PCR primers and extension probes (*continued*)

rs number	Gene (HUGO)	Forward PCR primer (5′ to 3′)	Reverse PCR primer (5′ to 3′)	Extension probe sequence (5′ to 3′)
rs2461838	*SHMT1*	ACGTTGGATGAGGTGACTTCACTTACTAGG	ACGTTGGATGTTTCTGTAGAGGCACTGTTG	GTGGCATGATCCTCAAC
rs2586154	*MTHFS*	ACGTTGGATGCTAGCTGACCATTAGGGAGA	ACGTTGGATGGCTGTTCTCACTCTGTTTCC	ttatGTTTTACTGAGTGCCCTG
rs2586183	*MTHFS*	ACGTTGGATGTCTACCTCCTTCATGTCCTG	ACGTTGGATGCTTGCAATCTTTCCCCTTGA	ATCTTTCCCCTTGAAAATATG
rs2733107	*MTHFS*	ACGTTGGATGCATCACACTCTGTACTGTGG	ACGTTGGATGTGTCCCAAACACTGTAAAGC	gacaAAACACTGTAAAGCTAGATG
rs3177999	*SLC19A1*	ACGTTGGATGATCTTCCAAGGTGCCCTGAC	ACGTTGGATGACCATCCTGCTCAGGCCAC	ctgcGGGGACGAAGGTGAC
rs34181110	*MTHFD1*	ACGTTGGATGGAATCATCCACTTTCCTGGC	ACGTTGGATGGCTTAGAGCACAGTAGAGAG	ctccCAGTAGAGAGTGCCAAGC
rs3772426	*ALDH1L1*	ACGTTGGATGTGCACACAAGGTCCTGTCTG	ACGTTGGATGGAATTATCCCTGGGACTCTG	cccgCTGTGGAAGTCTCATGACTCC
rs4646756	*ALDH1L1*	ACGTTGGATGTGCTACCCAGACCTGCATAG	ACGTTGGATGCTGAACCCTCAGCCAGAAAC	ccttGCCCCTGGGTCTTCA
rs525276	*CTH*	ACGTTGGATGACAGAGCAAGACTCCATTTC	ACGTTGGATGTGTAAAGGGAAAGATGTTG	AGGGAAAGATGTTGATAATGTAT
rs6435899	*ATIC*	ACGTTGGATGGGTGGAAGCCATATCAAGTG	ACGTTGGATGTGAAGACACAGGGCATTTCC	tccaaTGCAGTGATCCCTCATGTTCTT
rs6511677	*DNMT1*	ACGTTGGATGAGTCTTCACCTCCCACTCTG	ACGTTGGATGTTGAGACTGAGCCTGAATCC	AGGTGGGCAGAATACC
rs6517178	*GART*	ACGTTGGATGGAACAGTCCAAAGTAGTGGG	ACGTTGGATGAATCCCATGTTGGTTTGATG	tTTGGTTTGATGATAATACTTTTACA
rs6668344	*MTR*	ACGTTGGATGAGACATCCCTGATCTGACTC	ACGTTGGATGCTAAAGGGAAGGTTGAATTTG	TGGCTAGAGGGCTGT
rs7010484	*GGH*	ACGTTGGATGTCGGAGATCAAGTAACCCAC	ACGTTGGATGCCAGGACAAAGACCAGATAG	gaAGGACAAAGACCAGATAGATTCTTA
rs7126892	*FOLH1*	ACGTTGGATGTTCCCTTTTTCACTAAGGAG	ACGTTGGATGGCTTTATACGTGGCATTTT	agTACATTACTTGAAATTCTGTTTAAT
rs716537	*MTRR*	ACGTTGGATGTCCGACGTTAGAAACGTCTG	ACGTTGGATGGTGAGTTACACTTCCTACCC	gCCTACCCTCAAACAACTTA
rs7166189	*MTHFS*	ACGTTGGATGCGAGACTCCGTCTTAAAAAG	ACGTTGGATGCCATCTCATGATTTGCTGCC	TGCTGCCTACTTCATCC
rs7585489	*ATIC*	ACGTTGGATGAAGGGTCAAGTGAAGGACAG	ACGTTGGATGAAAGTCTGCCAGAGTGTTCG	gaggCTGCCAGAGTGTTCGTGGTTA
rs8012229	*MTHFD1*	ACGTTGGATGGGCCCATGTGGAAATGAATG	ACGTTGGATGAGCACACTGCAGGCCTTTTG	ctCCTTTTGGCACCCTGC
rs8040104	*MTHFS*	ACGTTGGATGATGGGATTTGAGGAAGGAAG	ACGTTGGATGGATCGAGAGTTGAATAAGCAG	gggaAGTTAACACTCAATGTGAGACTGT
rs8042012	*MTHFS*	ACGTTGGATGCATCTCTATAATGTGCCTGC	ACGTTGGATGGCTGCCTCTAGAGGAATTAG	ACTGGAGGAAACCAAGTAAAATT
rs8923	*MTHFS*	ACGTTGGATGGGTCCCAGTGAATGAAAACG	ACGTTGGATGCACTGATTATTTGGCTGTAG	gggaCTGTAGTAATCCAGATTTAAGCTG
rs9842910	*ALDH1L1*	ACGTTGGATGAAGGTACAACACCAAGAGGG	ACGTTGGATGGCCTGGCCAATTTTGTTATAC	aACTTGGTGAGCCAATAT

rs number	Gene (HUGO)	Forward PCR primer (5′ to 3′)	Reverse PCR primer (5′ to 3′)	Extension probe sequence (5′ to 3′)
Assay 3 (34-plex)				
rs10133855	*MTHFD1*	ACGTTGGATGAAATCAGCTGGGCTTGGTAG	ACGTTGGATGCCTCCCGGGTTCAAGTGAGTC	cgtgCAAGTGAGTCTCCTGC
rs1051296	*SLC19A1*	ACGTTGGATGATACCAAGGCCAGCACGTC	ACGTTGGATGAGTGTGTCCATCCTGACCTG	ggggCTCAGCTGCTCCCACACT
rs10957267	*GGH*	ACGTTGGATGCCTTCCCCTGTGACAATTAG	ACGTTGGATGTAAATGGCTCCCACACTGTC	ccgcCTATAGTTTTGGTCCCCATTTATC
rs11545078	*GGH*	ACGTTGGATGAGTGAAGTTCAGCGGCATTG	ACGTTGGATGGAGCTTTCACTGCTGATTAG	gaATTAGTGGAGAGTGCTTATTAA
rs11995525	*GGH*	ACGTTGGATGGGAAACATTCAGAATCCAAC	ACGTTGGATGCCTCCAGATTCCAACCTTTC	gaccCATTTGGCATTTACATTTACAT
rs12613	*CBS*	ACGTTGGATGTTGAGAGAGAAGTCGGCCAG	ACGTTGGATGGGACTCTTCTCTCTTTTGCC	TGCCTTTAATCCACTCTG
rs12899781	*MTHFS*	ACGTTGGATGGAAACAATCATTGGCTCACC	ACGTTGGATGTATTTTTTGGCCTGGCTTCC	ctttCTGCTCCTATGTCCATAT
rs13043752	*AHCY*	ACGTTGGATGTGGACATTGCTGAGAACGAG	ACGTTGGATGGCCCTTCAGTGGCTTGGAG	cctaTTGGAGGCCGAGTACC
rs13270305	*GGH*	ACGTTGGATGAGCCTCGAGCTGTCTAGACC	ACGTTGGATGCAGCCTCCTTACCGATGATG	TGATGGGCTTCTTGG
rs1532268	*MTRR*	ACGTTGGATGAGCAGCTCTGACTTCACAAG	ACGTTGGATGGGACAAGAGGAGATAAGTGG	ttgtCATCACCTGCATCCT
rs17004505	*FTCD*	ACGTTGGATGGGGATTTTCCAGGGAACGAG	ACGTTGGATGAAGTCGCTGTTTCCCAGCAG	GGAAATGCTGCCACA
rs17367504	*MTHFR*	ACGTTGGATGAGGAAGGAGAAGGCAGAGTG	ACGTTGGATGCACTGTGGAGGGACTTTTAC	tttttGAGGGACTTTTACAGGCAAC
rs1801133	*MTHFR*	ACGTTGGATGCTTGAAGGAGAAGGTGTCTG	ACGTTGGATGCTTCACAAAGCGGAAGAATG	cccaTGCGTGATGATGAAATCG
rs2244976	*MTHFR*	ACGTTGGATGGTGGACCACTCCCCTTAAAA	ACGTTGGATGTGAGAAATCTCTGATTTCC	TCCAAATCAATTCTGAAACCTTC
rs2273026	*SHMT1*	ACGTTGGATGGCCCTAGGCTCTTGCTTAAA	ACGTTGGATGCCTATGACTGTGTCAGAACC	gggCAGAACCAGGCCTTTGAAGATATT
rs2273028	*SHMT1*	ACGTTGGATGCTTTTCACTCCTGGAGGAAG	ACGTTGGATGCTGGGTTTGAGCCTAAAAAG	GCCCCTATGATCCCA
rs2274974	*MTHFR*	ACGTTGGATGAGCCGAATGCTGTCACTTGG	ACGTTGGATGTCCAGAACATGAAGCTGACG	ggggtCTGGATGATCTCTCGC
rs35789560	*SLC19A1*	ACGTTGGATGTTCCAGTGCTGCTGGTGTTC	ACGTTGGATGCCACAGACCAACAGAACTTC	ctGACCAGGTCCTGCTC
rs3780130	*GGH*	ACGTTGGATGTCTGCTTGGATTTGTGGTAG	ACGTTGGATGGGAAGATCACAGTCTGGTAG	GGTAGAAAACATATAAGCATCTG
rs3796191	*ALDH1L1*	ACGTTGGATGTCCCCTTGACAGAAACTGAC	ACGTTGGATGATGGGCAAAGCGTCTCCCTC	ccTCTCCCTCGGGCACC
rs383028	*FOLH1*	ACGTTGGATGGCCATGTTTCTTTGCAGGTG	ACGTTGGATGGAACCAGCTGAAAATATCGG	GGTAAGAAACAAGACAAATAATTTTA
rs4659727	*MTR*	ACGTTGGATGACCTCTCTGGTTCTTTGTGG	ACGTTGGATGCCTAACCATCACCATATCTC	CATCACCATATCTCATCTGAA
rs473334	*CTH*	ACGTTGGATGGAAACTAAAAATTCACTAGG	ACGTTGGATGGGCTTTATGATATGATATAAG	GTCCATCTTAATTATAATCATAATTTG

continued

TABLE 36 Sequenom Assay Oligonucleotide sequences for PCR primers and extension probes (*continued*)

rs number	Gene (HUGO)	Forward PCR primer (5′ to 3′)	Reverse PCR primer (5′ to 3′)	Extension probe sequence (5′ to 3′)
rs4933327	*MAT1A*	ACGTTGGATGTGTTACAGTTCGTTGCTCCC	ACGTTGGATGAGGAAGGGCATTGGAAGATG	tGCATTGGAAGATGGAACAG
rs535112	*CTH*	ACGTTGGATGCGTTTTCTGTTGTGGGTGTC	ACGTTGGATGATGCCAGAAACTCTAGAAAC	TCTAGAAACTATAGAATAAGTATCTAAG
rs588458	*FOLH1*	ACGTTGGATGCTCTGGGGTATGTAGAGTTG	ACGTTGGATGTGGGTGATCCAAATCCCATC	tcccCCCATCAATGTACAGCATAT
rs6774807	*ALDH1L1*	ACGTTGGATGAGGGTGAGGCAGAAAAGAAG	ACGTTGGATGAGAGAAAGCTACACCAAAAC	acatAGAAAGCTACACCAAAACCTGAAT
rs6799991	*ALDH1L1*	ACGTTGGATGATCTGAGTCCACAGCCTGAG	ACGTTGGATGAAACACCCCAGAGAGGCTCC	ccccGAGAGGCTCCATCCCAGCACCG
rs6875201	*BHMT*	ACGTTGGATGAAGAGTCAGGAAGCCCTATG	ACGTTGGATGGAAATTAAGACCCAGAGTCC	ggatAGTCCCTGAGTAATTTATACA
rs7553194	*MTHFR*	ACGTTGGATGGGCTGCTCTTCTTACACATC	ACGTTGGATGTCAGGCAATCCTTCTGCCTC	CTTCTGCCTCAGCCTT
rs7768897	*AMD1*	ACGTTGGATGCTTCATCTGGCCATTCACTC	ACGTTGGATGCCTGGGAGACAAAGTGAAAC	GAACCACTGAGTGAGACA
rs8006686	*MTHFD1*	ACGTTGGATGCTACACATCTGTATGAAGCC	ACGTTGGATGATCTGCCTCTGCATTCAGTC	GCTGTGTGCTGGTTTGA
rs8659	*MTRR*	ACGTTGGATGCACTCTGGCATATGATTTATC	ACGTTGGATGGTACGTACTGGTACCTGTAA	CCCAAAATTCTGAAATTGTGACTT
rs8971	*GART*	ACGTTGGATGCCTTGTGGTATCAAAGGAGC	ACGTTGGATGCCAATCACCCAGGCTTCTTC	tgCCTTGTGCTGCTGGATA
Assay 4 (25-plex)				
rs10158222	*MTR*	ACGTTGGATGGTAACTCCTTTTTTTACGTGG	ACGTTGGATGACTCTGACTCACGGAGTGAC	TTGAGCAGCTTGCTTCCA
rs10380	*MTRR*	ACGTTGGATGTGACAACCTTTTAGTGATCC	ACGTTGGATGGATGAGTTAAGATCCCATGC	gggtATCCCATGCTTAAGGAAAT
rs10407514	*DNMT1*	ACGTTGGATGTGACAGAGCAAGACTCCATC	ACGTTGGATGTGCGTGCTTCAAACTGTGAG	CTTCAAACTGTGAGACTAAATCT
rs10498514	*MTHFD1*	ACGTTGGATGTCAGGTGGTATGGTGTATGC	ACGTTGGATGGGGTCTGTGTTAAACAGTTA	gTTTATTTGAATTTCTGTAACAAATAC
rs10640	*AMT1*	ACGTTGGATGTCCTCCCAGACTTGCCTTAC	ACGTTGGATGTTTCAGGATTCAGTGGAGTC	ttgcGTCCATTAATGCATACCAGG
rs10934751	*ALDH1L1*	ACGTTGGATGATGCCTCATGTCCATCCTTG	ACGTTGGATGAGAGTCAGGTGGGCACAGC	GGGGACCTTGGGTGT
rs12898642	*MTHFS*	ACGTTGGATGGTAGTCTTCGGTCATTTCAC	ACGTTGGATGAAGGAGTGGAGAAGCTTGTG	AGGTAAAATAAAGCATACTCTAGG
rs17131305	*CTH*	ACGTTGGATGCCTCTATTTTTCATTTCTTC	ACGTTGGATGCCTATGACACTCTCAGTGAC	gcgCTAAGTGATGTGAAGTGATG
rs17194931	*GGH*	ACGTTGGATGCTCATACCTTGTTGAAAGGG	ACGTTGGATGCCCTGGCAGAGAATACAGC	gtgtCAGAGAATACAGCTGTTAGTT
rs1800909	*GGH*	ACGTTGGATGGCAGAGCTTTTGAAAGGCGG	ACGTTGGATGCAGAGTAGCAGGCCCAGCA	tccccGCACGCACAGCAGGC
rs1868130	*ALDH1L1*	ACGTTGGATGTCAACGAGACTTCAGAGTGG	ACGTTGGATGCAAGATAATGGCTTAAGTGGG	aAGTCTGGGAGGAGGCAG
rs1950902	*MTHFD1*	ACGTTGGATGAGATTGACTAGCATCAATG	ACGTTGGATGCCTTAGGCGTACAAGGAATG	GGTCACCTCTAGCAAGT

rs number	Gene (HUGO)	Forward PCR primer (5′ to 3′)	Reverse PCR primer (5′ to 3′)	Extension probe sequence (5′ to 3′)
rs202712	*FOLH1*	ACGTTGGATGGCCTTTTGCAAATTTTCTG	ACGTTGGATGATCAAGCAACATGAAGAGGC	TGGTAGAAAGCACAATACATAG
rs2230270	*FPGS*	ACGTTGGATGTATACCTGTGTCCGATGCTG	ACGTTGGATGAAGGCCAAGGCGGCGTTGGA	ctccaCCTCCAGGCCCAGGG
rs326124	*MTRR*	ACGTTGGATGTAGTGTACCACATGAGCACC	ACGTTGGATGAAGAGGAGGCAGATTTCAGG	TGTGTCATTATGACTCAGG
rs35302908	*MTFMT*	ACGTTGGATGAGAACTGCAACCTGACTTCC	ACGTTGGATGGGAGGAACAAACTTCAGAAC	CTTCAGAACAAATATTCAGACTTTAC
rs4646760	*ALDH1L1*	ACGTTGGATGAAATGGCTGCCTCAGATTGG	ACGTTGGATGCGAGGTCATGAATCTTACCC	CCTGGCAGTGGATAG
rs4672768	*ATIC*	ACGTTGGATGGAGAACCATTTGACTTCTCC	ACGTTGGATGGATACACTCAGTCAAAAAGGC	TAACAACAGGATTTGGGTT
rs4679102	*ALDH1L1*	ACGTTGGATGCCCTCTCATTATCACAGGGA	ACGTTGGATGAAGGTAAGGGTGGATTTTGG	CAAGTTAAATGAAAGGATTGAGTTAT
rs4779165	*MTHFS*	ACGTTGGATGATCTGTCTCTGTGGACTAAC	ACGTTGGATGGGCTATTTATGAGATGGATTG	gggagTTCCAGCAGATGTCAG
rs4846052	*MTHFR*	ACGTTGGATGCCAGCACTCCATGTAGTTTC	ACGTTGGATGGACTAAACTTACTAGCCGCC	gggctGTCAGGCAAGCAGGA
rs4920037	*CBS*	ACGTTGGATGATGCCACTGACTAGCCACAC	ACGTTGGATGTTAGAAGCTGGTGTGTGCTC	ccccCCCCAGAGGTCTAGATCAACT
rs6737407	*ATIC*	ACGTTGGATGGAGATAAAAGTTGTAGATTC	ACGTTGGATGTAGGAGAAATAGGGTAACGG	GTTACATGGGAACTCTTACAA
rs6800400	*ALDH1L1*	ACGTTGGATGAGATTGAACAGATGCCTCTC	ACGTTGGATGCCCAGTGCCTAATGATGTTG	cTATTCTTATTTGCCGTCCATA
rs9910090	*SHMT1*	ACGTTGGATGTGGTTTTGGTGCTGGAGCTG	ACGTTGGATGTGGTGACATCTCTGCTCGG	aAGCAGCAGCACAAGGA
Assay 5 (11-plex)				
rs10932606	*ATIC*	ACGTTGGATGAAGGACTTCTATCTCTATG	ACGTTGGATGAAATAGGAAATCCTATAATG	AGGAAATCCTATAATGAAACAAA
rs11627525	*MTHFD1*	ACGTTGGATGATCACCCATCCCAGAGAATC	ACGTTGGATGCCTCTGTAGTCATGCTTTTG	ATGAAGGCTGTCATTGGAT
rs1677692	*DHFR*	ACGTTGGATGAAGGCTCAGTATGAAGGGTC	ACGTTGGATGCCCTGAAATGGAATAGTGTC	GGAATAGTGTCTTAACCAGAA
rs16837178	*ALDH1L1*	ACGTTGGATGTCACCAAAGCAGGACTCATC	ACGTTGGATGTTTGAGCAGGCAAGTGACCC	CGTGCCCTTACCATTTT
rs1979277	*SHMT1*	ACGTTGGATGGGAGGAGGTTGAGAGCTTC	ACGTTGGATGCTCCTTTAGAAGTCAGGCAG	aaCAGGCAGAGGGAAGA
rs2276726	*ALDH1L1*	ACGTTGGATGCATTTCCCTCAACAGCACTC	ACGTTGGATGCCCACCTGGCTTGTTCTTTG	CTTTGATGTGGGTCTCA
rs28941768	*FTCD*	ACGTTGGATGTTCTTAGGGAGGGCCTCGTA	ACGTTGGATGGTGCCAGTTTACCTGTACGG	GCCAGGATGGACAGT
rs34507711	*MTFMT*	ACGTTGGATGAAGCTCAACCAAGCCAATGC	ACGTTGGATGGCATTGTTGCATAGCAACAG	GCAACAGTTTTTTTCTGCT
rs596909	*TYMS*	ACGTTGGATGGGGTTGGTTTTGATGGTGTC	ACGTTGGATGGTACCTGTCCTCTCTTTTTG	gggACAGATTATTCAGGACAGG
rs866027	*AHCY*	ACGTTGGATGAGCTTCTGAGGTGATTCCAA	ACGTTGGATGAGGACAGATCCCATGTTCTC	ttAGCTGAATGCCGTGGC

Appendix 7 Loss between referral and randomisation

TABLE 37 Withdrawals after referral and before screening – by centre

Exclusion criteria	North East Wales	North West Wales	Swansea	Total
Aged under 18 years	0	4	0	**4**
B_{12} deficient	0	0	2	**2**
Have taken folate supplementation	4	6	4	**14**
Suffered from psychosis	1	1	1	**3**
Bipolar disorder	1	0	1	**2**
Already in another research trial	1	1	0	**2**
Pregnant or planning to be	3	4	2	**9**
Taking anticonvulsants	1	2	2	**5**
Treatment for medical condition not yet stabilised	0	1	0	**1**
Taking lithium	0	0	1	**1**
Diagnosed with malignant disease	4	12	3	**19**
Subtotal	**15**	**31**	**16**	**62**
Not on antidepressants	13	16	7	**36**
Other	2	2	1	**5**
Subtotal	**15**	**17**	**9**	**41**
Self exclusion: refused	91	128	58	**277**
Did not attend screening	21	29	34	**84**
Could not be contacted	26	75	60	**161**
Subtotal	**138**	**235**	**152**	**522**
Total	**168**	**280**	**177**	**625**

Note: When recruiters reported more than one exclusion criterion, we tabulated the first criterion in this list.

TABLE 38 Withdrawals and exclusions at screening – by centre

Exclusion criteria	North East Wales	North West Wales	Swansea	Total
Aged under 18 years	0	0	0	**0**
Not depressed by ICD-10 criteria or low BDI-II	42	45	35	**122**
Folate deficient	0	1	0	**1**
Have taken folate supplementation	4	3	1	**8**
Suffered from psychosis	1	0	1	**2**
Bipolar disorder	1	3	0	**4**
Taking anticonvulsants	6	6	4	**16**
Treatment for medical condition not yet stabilised	1	0	1	**2**
Diagnosis with malignant disease	2	17	3	**22**
Subtotal	**57**	**75**	**45**	**177**
Not on antidepressants	1	20	0	**21**
Other	6	5	3	**14**
Subtotal	**7**	**25**	**3**	**35**
Self exclusion: refused	4	12	0	**16**
Did not attend or could not be contacted	0	0	0	**0**
Subtotal	**4**	**12**	**0**	**16**
Total	**68**	**112**	**48**	**228**

Note: When recruiters reported more than one exclusion criterion, we tabulated the first criterion in this list.

TABLE 39 Withdrawals and exclusions at randomisation – by centre

Exclusion criteria	North East Wales	North West Wales	Swansea	Total
Not depressed by ICD-10 criteria or low BDI-II	6	23	7	**36**
Folate deficient	0	8	7	**15**
B_{12} deficient	0	7	1	**8**
Have taken folate supplementation	0	1	1	**2**
Taking anti convulsants	0	1	0	**1**
Diagnosed with malignant disease	0	2	0	**2**
Subtotal	**6**	**42**	**16**	**64**
Not on antidepressants	0	7	2	**9**
Other	1	2	2	**5**
Subtotal	**1**	**9**	**4**	**14**
Self exclusion: refused	9	4	4	**17**
Did not attend or could not be contacted	3	6	6	**15**
Subtotal	**12**	**10**	**10**	**32**
Randomised in error	2	0	2	**4**
Recruited to comprehensive cohort	24	7	15	**46**
Subtotal	**26**	**7**	**17**	**50**
Total	**45**	**68**	**47**	**160**

Note: When recruiters reported more than one exclusion criterion, we tabulated the first criterion in this list.

Appendix 8 Follow-ups completed and imputed

TABLE 40 Participants lost to follow up or imputed – by centre and randomisation group

		North East Wales		North West Wales		Swansea		Total	
	Group	no. (%)[a]		no. (%)[a]		no. (%)[a]		no. (%)[a]	
Total randomised		***119***		***238***		***118***		***475***	
No follow-up ($n = 35$)	Folate	3	2.5	8	3.4	3	2.5	14	2.5
	Placebo	6	6.8	7	2.9	8	6.8	21	5.0
Analysed ($n = 440$)	**Folate**	**57**	**47.5**	**110**	**46.2**	**56**	**47.5**	**223**	**47.9**
	Placebo	**53**	**43.2**	**113**	**47.5**	**51**	**43.2**	**217**	**44.5**
4 weeks imputed	Folate	2	3.5	10	9.1	3	5.4	15	6.7
	Placebo	1	1.9	16	14.2	4	7.8	21	9.7
12 weeks imputed	Folate	4	7.0	5	4.5	13	23.2	22	9.9
	Placebo	7	13.2	6	5.3	5	9.8	18	8.3
25 weeks imputed	Folate	8	14.0	6	5.5	13	23.2	27	12.1
	Placebo	12	22.6	12	10.6	5	9.8	29	13.4
Two time points imputed	Folate	4	8.5	4	1.7	10	8.5	18	3.4
	Placebo	8	2.5	6	2.5	3	2.5	17	6.7

a Denominator for analysed and no follow up = total randomised. Denominator for imputed = number analysed for folate or placebo.

Appendix 9 Elaborated demographic data

TABLE 41 Full baseline demographic characteristics of trial participants by centre

Participant characteristic		North East Wales (*n* = 110) number (%)	North West Wales (*n* = 223) number (%)	Swansea (*n* = 107) number (%)
Ethnicity[a]	White	8 (7)	88 (40)	14 (14)
	White British	89 (81)	123 (55)	79 (74)
	White Irish	1 (1)	5 (2)	
	Other white background	9 (8)	4 (2)	7 (6)
	White and black Caribbean		1 (0)	
	Black (British)	1 (1)		1 (1)
	Caribbean			1 (1)
	Other Asian background		1 (0.5)	
	Not stated	2 (2)	1 (0.5)	5 (5)
Marital status[b]	Single (never married)	17 (16)	60 (27)	24 (24)
	Married (first marriage)	43 (39)	73 (33)	42 (41)
	Divorced	19 (17)	27 (12)	16 (16)
	Separated	4 (4)	6 (3)	4 (4)
	Widowed	4 (4)	7 (3)	4 (4)
	Cohabiting	12 (11)	37 (17)	5 (5)
	Remarried	10 (9)	11 (5)	7 (7)
Number of dependent children[c]	0	51 (57)	87 (45)	31 (53)
	1	15 (17)	40 (21)	15 (26)
	2	18 (20)	37 (19)	6 (10)
	3	3 (3)	13 (7)	4 (7)
	4	1 (1)	7 (4)	2 (3)
	5	1 (1)	2 (1)	
	6		3 (2)	
	7		3 (2)	
	8		1 (1)	
Employment status[d]	Full time employed	35 (32)	52 (23)	22 (22)
	Part time employed	14 (13)	22 (10)	7 (7)
	Self-employed full time	3 (3)	7 (3)	2 (2)
	Self-employed part time	4 (4)	4 (2)	1 (1)
	Unemployed	8 (7)	47 (21)	14 (14)
	Retired	10 (9)	16 (7)	22 (22)
	Student	2 (2)	5 (2)	10 (10)

continued

TABLE 41 Full baseline demographic characteristics of trial participants by centre (*continued*)

Participant characteristic		North East Wales (*n* = 110) number (%)	North West Wales (*n* = 223) number (%)	Swansea (*n* = 107) number (%)
	Looking after family/home	4 (4)	10 (5)	4 (4)
	Permanently sick/disabled	18 (17)	32 (14)	11 (11)
	Temporarily sick/disabled	11 (10)	27 (12)	9 (9)
Smoking status[e]	Smoker	27 (25)	97 (44)	38 (37)
	Non-smoker	56 (51)	93 (42)	45 (44)
	Ex-smoker	26 (24)	32 (14)	19 (19)
Drinking (units/week)[f]	None	52 (48)	78 (35)	38 (38)
	1–7	30 (28)	79 (36)	27 (27)
	8–14	16 (15)	20 (9)	15 (15)
	15–21	6 (6)	12 (5)	10 (10)
	22–35	3 (3)	12 (5)	7 (7)
	36–50	2 (2)	6 (3)	1 (1)
	51 or more		14 (6)	3 (3)

a We did not use ethnicity in the analysis because it provides no useful discrimination and threatens anonymity.
b For analysis, recoded as single or missing (109) vs. have a partner (240) vs. had a partner (91).
c For analysis, recoded as zero (269) vs. one (70) vs. two (61) vs. three or more (40).
d For analysis, recoded as full time (121) vs. part time or in education (124) vs. none (195).
e For analysis, recoded as smoking status as non-smoker (201) vs. smoker (162) vs. ex-smoker (77).
f For analysis,recoded as none (168) vs. safe (201) vs. less safe or missing (71).

Appendix 10 Elaborated clinical effectiveness results

TABLE 42 Unadjusted results by time point

	Folate	Placebo	Difference (folate minus placebo)			
Outcome variable	Mean (SD)	Mean (SD)	Mean (SD)	95% CI	Favours	Significance
BDI-II (4 week)	27.30 (11.56)	27.84 (12.83)	–0.54 (12.20)	–2.83 to 1.75	FOLATE	0.643
BDI-II (12 week)	25.13 (13.56)	24.66 (13.6)	0.47 (13.58)	–2.07 to 3.02	PLACEBO	0.714
BDI-II (25 week)	22.61 (13.53)	22.33 (14.09)	0.28 (13.81)	–2.31 to 2.87	PLACEBO	0.832
MADRS (4 week)	23.29 (8.92)	23.24 (9.49)	0.05 (9.21)	–1.67 to 1.78	PLACEBO	0.953
MADRS (12 week)	21.83 (10.06)	21.62 (10.73)	0.21 (10.39)	–1.74 to 2.16	PLACEBO	0.832
MADRS (25 week)	19.77 (9.99)	20.54 (11.24)	–0.77 (10.62)	–2.76 to 1.22	FOLATE	0.449
EQ-5D (4 week)	0.54 (0.31)	0.59 (0.31)	–0.04 (0.31)	–0.10 to 0.01	PLACEBO	0.133
EQ-5D (12 week)	0.58 (0.3)	0.59 (0.3)	–0.01 (0.30)	–0.06 to 0.05	PLACEBO	0.807
EQ-5D (25 week)	0.60 (0.3)	0.6 (0.31)	0.00 (0.30)	–0.05 to 0.06	EQUAL	0.949
EQ-VAS (4 week)	52.50 (21.69)	51.66 (20.41)	0.84 (21.07)	–3.11 to 4.79	FOLATE	0.677
EQ-VAS (12 week)	54.61 (23)	55.76 (22.2)	–1.15 (22.61)	–5.39 to 3.09	PLACEBO	0.594
EQ-VAS (25 week)	59.98 (22.71)	58.13 (23.57)	1.85 (23.14)	–2.48 to 6.19	FOLATE	0.401
SF-12 PCS (4 week)	45.39 (12.56)	43.71 (12.49)	1.68 (12.52)	–0.66 to 4.03	FOLATE	0.159
SF-12 PCS (12 week)	44.67 (12.03)	44.78 (13.05)	–0.11 (12.54)	–2.46 to 2.24	PLACEBO	0.925
SF-12 PCS (25 week)	45.20 (12.05)	44.21 (12.34)	0.98 (12.20)	–1.30 to 3.27	FOLATE	0.398
SF-12 MCS (4 week)	30.61 (11.35)	32.13 (11.61)	–1.52 (11.48)	–3.67 to 0.63	PLACEBO	0.165
SF-12 MCS (12 week)*	**32.66 (12.34)**	**35.64 (12.19)**	**–2.97 (12.27)**	**–5.27 to –0.68**	**PLACEBO**	**0.011**
SF-12 MCS (25 week)	34.56 (13.18)	36.47 (12.87)	–1.90 (13.03)	–4.34 to 0.54	PLACEBO	0.126
CGI: Severity (4 week)	3.79 (0.98)	3.78 (1.01)	0.01 (0.99)	–0.17 to 0.20	PLACEBO	0.875
CGI: Severity (12 week)	3.59 (1.13)	3.52 (1.24)	0.07 (1.18)	–0.16 to 0.29	PLACEBO	0.555
CGI: Severity (25 week)	3.21 (1.34)	3.33 (1.35)	–0.12 (1.34)	–0.37 to 0.14	FOLATE	0.363
CGI: Improvement (4 week)	3.30 (1.02)	3.17 (1.01)	0.13 (1.01)	–0.06 to 0.32	PLACEBO	0.179
CGI: Improvement (12 week)	3.14 (1.16)	3.09 (1.21)	0.05 (1.18)	–0.17 to 0.27	PLACEBO	0.678

Outcome variable	Folate Mean (SD)	Placebo Mean (SD)	Difference (folate minus placebo) Mean (SD)	95% CI	Favours	Significance
CGI: Improvement (25 week)	2.95 (1.31)	3.04 (1.4)	–0.09 (1.35)	–0.34 to 0.16	FOLATE	0.482
CGI: Efficacy (4 week)[a]	0.09 (0.59)	0.17 (0.59)	–0.08 (0.59)	–0.19 to 0.03	FOLATE	0.159
CGI: Efficacy (12 week)[a]	0.22 (0.64)	0.17 (0.64)	0.05 (0.64)	–0.07 to 0.17	PLACEBO	0.447
CGI: Efficacy (25 week)[a]	0.27 (0.66)	0.32 (0.7)	–0.05 (0.68)	–0.18 to 0.08	FOLATE	0.425
UKU: PSYCHIC (4 WEEK)	8.98 (5.43)	8.38 (5.28)	0.60 (5.36)	–0.40 to 1.61	PLACEBO	0.238
UKU: PSYCHIC (6 WEEK)	7.91 (5.2)	8.09 (5.5)	–0.18 (5.35)	–1.18 to 0.82	FOLATE	0.728
UKU: PSYCHIC (12 week)	6.83 (5.11)	6.72 (5.38)	0.11 (5.24)	–0.87 to 1.09	PLACEBO	0.825
UKU: Neurologic (4 week)[b]	0.63 (0.85)	0.68 (0.83)	–0.04 (0.84)	–0.20 to 0.12	FOLATE	0.605
UKU: Neurologic (12 week)[b]	0.55 (0.78)	0.62 (0.83)	–0.07 (0.80)	–0.22 to 0.08	FOLATE	0.381
UKU: Neurologic (25 week)[b]	0.53 (0.77)	0.57 (0.83)	–0.04 (0.80)	–0.19 to 0.11	FOLATE	0.567
UKU: Autonomic (4 week)	3.04 (2.91)	2.94 (3.15)	0.10 (3.03)	–0.47 to 0.66	PLACEBO	0.74
UKU: Autonomic (12 week)	2.89 (2.85)	2.88 (2.92)	0.00 (2.89)	–0.54 to 0.54	PLACEBO	0.991
UKU: Autonomic (25 week)	2.61 (2.58)	2.44 (2.92)	0.17 (2.75)	–0.35 to 0.68	PLACEBO	0.524
UKU: Other (4 week)	3.74 (3.54)	3.89 (3.58)	–0.15 (3.56)	–0.82 to 0.52	FOLATE	0.66
UKU: Other (12 week)**	**3.42 (3.21)**	**4.12 (4.04)**	**–0.70 (3.64)**	**–1.38 to –0.02**	**FOLATE**	**0.045**
UKU: Other (25 week)	3.36 (3.07)	3.77 (3.81)	–0.41 (3.45)	–1.06 to 0.24	FOLATE	0.213

a After logarithmic transformation.
b After square-root transformation.
* Difference significant at 5% level with effect size = 0.24.
** Difference significant at 5% level with effect size = 0.19.

TABLE 43 Logistic analysis of 'medium-term response to treatment' (50% reduction in BDI-II score) at 25 weeks

Predictors	β	SE (β)	Wald's χ^2	Significance	Odds ratio [Exp(β)]	95% CI for Exp(β)
Unadjusted						
Serum folate (baseline)	0.026	0.026	0.946	0.331	1.026	0.974 to 1.080
Homocysteine (baseline)	0.007	0.019	0.157	0.692	1.007	0.971 to 1.045
Constant	0.332	0.349	0.908	0.341	1.394	
Adjusted by stratification variables						
Serum folate (baseline)	0.027	0.027	0.946	0.331	1.027	0.973 to 1.084
Homocysteine (baseline)	0.008	0.020	0.172	0.679	1.008	0.969 to 1.049
Type of ADM	**0.535**	**0.239**	**5.029**	**0.025**	**1.707**	**1.070 to 2.725**
Previous counselling	0.068	0.216	0.099	0.753	1.071	0.701 to 1.635
Previous treatment	**0.760**	**0.268**	**8.020**	**0.005**	**2.138**	**1.264 to 3.618**
Centre			4.134	0.127		
Centre 1	**0.544**	**0.268**	**4.126**	**0.042**	**1.722**	**1.019 to 2.911**
Centre 2	0.299	0.304	0.964	0.326	1.348	0.743 to 2.448
Gender	0.242	0.231	1.099	0.295	1.273	0.810 to 2.001
Constant	–0.985	0.537	3.361	0.067	0.373	
Adjusted by stratification variables and treatment group						
Serum folate (baseline)	0.027	0.027	0.950	0.330	1.027	0.973 to 1.084
Homocysteine (baseline)	0.008	0.020	0.161	0.688	1.008	0.969 to 1.049
Type of ADM (1)	**0.537**	**0.239**	**5.066**	**0.024**	**1.712**	**1.072 to 2.733**
Previous counselling	0.066	0.216	0.094	0.759	1.069	0.699 to 1.633
Previous treatment	**0.762**	**0.269**	**8.052**	**0.005**	**2.142**	**1.266 to 3.626**
Centre			4.174	0.124		
Centre 1	**0.547**	**0.268**	**4.165**	**0.041**	**1.728**	**1.022 to 2.922**
Centre 2	0.300	0.304	0.973	0.324	1.35	0.744 to 2.451
Gender	0.240	0.231	1.081	0.299	1.271	0.809 to 1.998
Treatment group	0.056	0.211	0.070	0.791	1.057	0.700 to 1.598
Constant	–1.012	0.548	3.418	0.064	0.363	

Predictors significant at 5% level are in bold type.

TABLE 44 Logistic analysis of 'short-term response to treatment (50% reduction in BDI-II score) at 12 weeks

Predictors	β	SE (β)	Wald's χ^2	Significance	Odds ratio [Exp(β)]	95% CI for Exp(β)
Unadjusted						
Serum folate (baseline)	–0.028	0.027	1.10	0.294	0.972	0.923 to 1.025
Homocysteine (baseline)	–0.011	0.018	0.417	0.518	0.989	0.955 to 1.024
Constant	1.295	0.349	13.7	0	3.649	
Adjusted by stratification variables						
Serum folate (baseline)	–0.023	0.028	0.696	0.404	0.977	0.925 to 1.032
Homocysteine (baseline)	–0.014	0.019	0.547	0.459	0.986	0.951 to 1.023
Type of ADM	0.379	0.254	2.228	0.136	1.461	0.888 to 2.403
Previous counselling	0.245	0.228	1.155	0.283	1.277	0.817 to 1.996
Previous treatment	0.252	0.29	0.75	0.386	1.286	0.728 to 2.272
Centre			4.09	0.129		
Centre 1	0.535	0.279	3.664	0.056	1.707	0.987 to 2.952
Centre 2	0.132	0.311	0.181	0.671	1.141	0.621 to 2.098
Gender 1	–0.158	0.246	0.410	0.522	0.854	0.527 to 1.384
Constant	0.644	0.545	1.397	0.237	1.904	
Adjusted by stratification variables and treatment group						
Serum folate (baseline)	–0.023	0.028	0.685	0.408	0.977	0.926 to 1.032
Homocysteine (baseline)	–0.015	0.019	0.603	0.437	0.986	0.950 to 1.022
Type of ADM	0.386	0.254	2.303	0.129	1.471	0.894 to 2.422
Previous counselling	0.240	0.228	1.105	0.293	1.271	0.813 to 1.987
Previous treatment	0.258	0.291	0.788	0.375	1.295	0.732 to 2.29
Centre			4.187	0.123		
Centre 1	0.543	0.280	3.762	0.052	1.722	0.994 to 2.981
Centre 2	0.136	0.311	0.191	0.662	1.146	0.623 to 2.107
Gender	–0.162	0.246	0.432	0.511	0.850	0.525 to 1.379
Treatment group	–0.138	0.223	0.384	0.536	0.871	0.563 to 1.348
Constant	0.715	0.558	1.645	0.200	2.045	

TABLE 45 Logistic analysis of 'medium-term remission from depression' (BDI-II < 8) at 25 weeks

Predictors	β	SE (β)	Wald's χ	Significance	Odds Ratio [Exp(β)]	95% CI
Unadjusted						
Serum folate (baseline)	0.073	0.039	3.526	0.06	1.076	0.997 to 1.161
Homocysteine (baseline)	0.011	0.026	0.191	0.662	1.011	0.961 to 1.064
Constant	1.036	0.479	4.668	0.031	2.817	
Adjusted by stratification variables						
Serum folate (baseline)	0.080	0.041	3.748	0.053	1.083	0.999 to 1.174
Homocysteine (baseline)	0.021	0.031	0.473	0.492	1.022	0.961 to 1.086
Type of ADM (1)	0.540	0.327	2.731	0.098	1.716	0.904 to 3.257
Previous counselling	0.299	0.279	1.144	0.285	1.348	0.780 to 2.331
Previous treatment	0.444	0.339	1.710	0.191	1.559	0.801 to 3.031
Centre			2.407	0.300		
Centre 1	0.477	0.358	1.777	0.182	1.611	0.799 to 3.247
Centre 2	0.017	0.39	0.002	0.964	1.018	0.474 to 2.184
Gender	0.462	0.296	2.431	0.119	1.587	0.888 to 2.834
Constant	–0.313	0.729	0.184	0.668	0.732	
Adjusted by stratification variables and treatment group						
Serum folate (baseline)	**0.080**	**0.041**	**3.770**	**0.052**	**1.084**	**0.999 to 1.175**
Homocysteine (baseline)	0.021	0.032	0.428	0.513	1.021	0.960 to 1.086
Type of ADM (1)	0.548	0.328	2.802	0.094	1.730	0.911 to 3.288
Previous counselling	0.292	0.280	1.093	0.296	1.340	0.774 to 2.318
Previous treatment	0.450	0.341	1.737	0.187	1.568	0.803 to 3.062
Centre			2.558	0.278		
Centre 1	0.496	0.360	1.901	0.168	1.642	0.811 to 3.322
Centre 2	0.020	0.390	0.003	0.960	1.020	0.475 to 2.189
Gender	0.457	0.297	2.370	0.124	1.580	0.883 to 2.827
Treatment group	–0.245	0.275	0.789	0.374	0.783	0.456 to 1.343
Constant	–0.191	0.747	0.065	0.798	0.826	

TABLE 46 Logistic analysis of 'short-term remission from depression' (BDI-II < 8) at 12 weeks

Predictors	β	SE (β)	Wald's χ^2	Significance	Odds Ratio [Exp(β)]	95% CI for Exp(β)
Homocysteine (baseline)	–0.008	0.023	0.121	0.728	0.992	0.949 to 1.038
Constant	2.051	0.478	18.389	0	7.774	
Adjusted by stratification variables						
Serum folate (baseline)	0.018	0.042	0.193	0.661	1.019	0.938 to 1.106
Homocysteine (baseline)	–0.008	0.025	0.096	0.757	0.992	0.946 to 1.042
Type of ADM	0.440	0.375	1.376	0.241	1.553	0.744 to 3.242
Previous counselling	0.298	0.322	0.856	0.355	1.347	0.716 to 2.534
Previous treatment	0.200	0.399	0.251	0.616	1.221	0.559 to 2.667
Centre			0.352	0.838		
Centre 1	0.119	0.413	0.083	0.773	1.127	0.501 to 2.533
Centre 2	–0.121	0.453	0.072	0.789	0.886	0.365 to 2.151
Gender	0.111	0.340	0.107	0.744	1.117	0.574 to 2.177
Constant	1.512	0.758	3.983	0.046	4.538	
Adjusted by stratification variables and treatment group						
Serum folate (baseline)	0.019	0.042	0.199	0.656	1.019	0.938 to 1.107
Homocysteine (baseline)	–0.009	0.025	0.144	0.704	0.991	0.943 to 1.040
Type of ADM (1)	0.453	0.377	1.448	0.229	1.574	0.752 to 3.293
Previous counselling	0.288	0.323	0.796	0.372	1.334	0.709 to 2.510
Previous treatment	0.208	0.401	0.27	0.604	1.232	0.561 to 2.705
Centre			0.394	0.821		
Centre 1	0.139	0.416	0.111	0.739	1.149	0.509 to 2.594
Centre 2	–0.115	0.453	0.065	0.799	0.891	0.367 to 2.165
Gender	0.102	0.341	0.089	0.765	1.107	0.567 to 2.162
Treatment group	–0.319	0.318	1.004	0.316	0.727	0.389 to 1.357
Constant	1.691	0.784	4.651	0.031	5.424	

TABLE 47 Sensitivity analysis – comparison of those without follow-up with their nearest neighbours

Participant characteristic	Nearest neighbours ($n = 35$)	No follow-up ($n = 35$)	Significance test
Age in years			
Range	20–68	20–66	
Mean (SD)	42 (13)	39 (14)	$F(1,68) = 0.98$, $p = 0.33$
Marital status, no. (%)			
Single	12 (48)	13 (52)	
Had a partner	13 (54)	11 (46)	
Have a partner	10 (48)	11 (52)	χ^2 with 2 df = 0.25, $p = 0.88$
Number of dependent children, no. (%)			
0	21 (49)	22 (51)	
1	6 (55)	5 (45)	
2	1 (17)	5 (83)	
3 or more	7 (70)	3 (30)	Fisher's $p = 0.24$
Employment, no. (%)			
Full time employed	10 (48)	11 (52)	
Part time or in education	12 (63)	7 (37)	
Inactive	13 (43)	17 (57)	χ^2 with 2 df = 1.90, $p = 0.39$
Alcohol units per week, no. (%)			
None	13 (57)	10 (43)	
Within safe limit*	17 (52)	16 (48)	
Above safe limit*	5 (36)	9 (64)	χ^2 with 2 df = 1.56, $p = 0.46$
BDI-II screening			
Range	24–56	24–57	
Mean (SD)	39 (09)	42 (09)	$F(1,68) = 1.68$, $p = 0.20$
BDI-II baseline			
Range	20–61	18–57	
Mean (SD)	37 (11)	40 (11)	$F(1,68) = 1.00$, $p = 0.321$

*Difference significant at 5% level with effect size = 0.24.

TABLE 48 Repeated measures analysis adjusting for stratification variables and baseline biochemistry

		Difference (folate minus placebo)				Baseline prediction of outcome per biochemical unit			
Outcome	Covariate	Mean	(SE)	95% CI	Significance	Mean	(SE)	95% CI	Significance
BDI-II	Serum folate	0.682	(0.833)	–0.955 to 2.320	0.413	–0.070	(0.088)	–0.243 to 0.104	0.431
	Red cell folate	–0.045	(0.990)	–1.993 to 1.904	0.964	0.003	(0.003)	–0.003 to 0.009	0.344
	B_{12}	0.437	(1.012)	–1.554 to 2.427	0.666	–0.004	(0.004)	–0.012 to 0.004	0.279
	Homocysteine	0.703	(0.850)	–0.968 to 2.373	0.409	0.000	(0.071)	–0.139 to 0.139	0.999
MADRS	Serum folate	0.530	(0.649)	–0.746 to 1.806	0.415	0.057	(0.069)	–0.077 to 0.192	0.404
	Red cell folate	0.351	(0.744)	–1.113 to 1.815	0.638	0.003	(0.002)	–0.001 to 0.008	0.140
	B_{12}	0.528	(0.814)	–1.073 to 2.128	0.517	–0.003	(0.003)	–0.010 to 0.003	0.323
	Homocysteine	0.353	(0.662)	–0.948 to 1.654	0.594	0.035	(0.055)	–0.073 to 0.143	0.520
EQ-5D	Serum folate	0.012	(0.038)	–0.063 to 0.086	0.763	0.002	(0.002)	–0.002 to 0.006	0.315
	Red cell folate	0.351	(0.744)	–1.113 to 1.815	0.638	0.001	(0.002)	–0.003 to 0.005	0.683
	B_{12}	0.066	(0.044)	–0.021 to 0.153	0.137	0.000	(0.000)	0.000 to 0.000	0.107
	Homocysteine	0.013	(0.039)	–0.063 to 0.089	0.739	–0.002	(0.001)	–0.005 to 0.001	0.192
EQ-VAS	Serum folate	1.019	(0.850)	–0.651 to 2.689	0.231	0.134	(0.091)	–0.045 to 0.312	0.142
	Red cell folate	–0.026	(1.016)	–2.026 to 1.973	0.979	0.001	(0.003)	–0.005 to 0.007	0.713
	B_{12}	0.739	(1.044)	–1.314 to 2.791	0.480	–0.006	(0.004)	–0.015 to 0.002	0.130
	Homocysteine	0.997	(0.865)	–0.704 to 2.697	0.250	0.015	(0.072)	–0.127 to 0.158	0.833

continued

TABLE 48 Repeated measures analysis adjusting for stratification variables and baseline biochemistry (*continued*)

		Difference (folate minus placebo)				Baseline prediction of outcome per biochemical unit			
Outcome	Covariate	Mean	(SE)	95% CI	Significance	Mean	(SE)	95% CI	Significance
MCS	Serum folate	–1.848	(0.832)	–3.483 to –0.213	0.027	–0.046	(0.088)	–0.219 to 0.127	0.602
	Red cell folate	–0.610	(0.991)	–2.560 to 1.339	0.538	–0.007	(0.003)	–0.013 to –0.001	0.019
	B_{12}	–1.820	(1.055)	–3.895 to 0.254	0.085	0.001	(0.004)	–0.008 to 0.009	0.871
	Homocysteine	–1.627	(0.855)	–3.308 to 0.053	0.058	0.125	(0.071)	–0.015 to 0.264	0.080
PCS	Serum folate	0.657	(0.635)	–0.591 to 1.905	0.302	0.029	(0.068)	–0.104 to 0.163	0.666
	Red cell folate	0.996	(0.743)	–0.466 to 2.457	0.181	0.001	(0.002)	–0.003 to 0.005	0.683
	B_{12}	0.718	(0.765)	–0.786 to 2.221	0.349	0.005	(0.003)	–0.001 to 0.011	0.090
	Homocysteine	0.583	(0.656)	–0.706 to 1.872	0.375	0.052	(0.055)	–0.057 to 0.160	0.348
CGI: Severity	Serum folate	0.003	(0.082)	–0.157 to 0.163	0.971	0.002	(0.009)	–0.015 to 0.019	0.804
	Red cell folate	–0.085	(0.095)	–0.271 to 0.101	0.368	0.000	(0.000)	0.000 to 0.001	0.160
	B_{12}	–0.009	(0.103)	–0.210 to 0.193	0.933	0.000	(0.000)	–0.001 to 0.001	0.473
	Homocysteine	–0.014	(0.083)	–0.176 to 0.149	0.870	–0.004	(0.007)	–0.018 to 0.009	0.523
CGI: Improvement	Serum folate	0.059	(0.109)	–0.154 to 0.273	0.586	0.007	(0.012)	–0.016 to 0.030	0.533
	Red cell folate	–0.022	(0.124)	–0.265 to 0.221	0.859	0.001	(0.000)	0.000 to 0.002	0.026
	B_{12}	0.038	(0.110)	–0.177 to 0.253	0.728	0.000	(0.000)	–0.001 to 0.001	0.891
	Homocysteine	0.075	(0.110)	–0.142 to 0.292	0.498	0.052	(0.012)	0.029 to 0.075	<0.0001

TABLE 49 Repeated measures analysis adjusting for stratification variables and baseline on treatment

		Folate – placebo				On treatment prediction of outcome per biochemical unit			
Outcome	Covariate	Mean	(SE)	95% CI	Significance	Mean	(SE)	95% CI	Significance
BDI-II	Serum folate	0.107	(1.093)	–2.041 to 2.255	0.922	0.032	(0.034)	–0.034 to 0.099	0.337
	Red cell folate	–1.794	(1.490)	–4.728 to 1.141	0.230	0.003	(0.002)	–0.002 to 0.007	0.247
	B_{12}	0.503	(1.012)	–1.488 to 2.493	0.620	–0.001	(0.003)	–0.008 to 0.006	0.823
	Homocysteine	1.154	(1.044)	–0.899 to 3.206	0.270	0.334	(0.095)	0.148 to 0.520	0.0005
MADRS	Serum folate	0.260	(0.894)	–1.498 to 2.017	0.772	0.026	(0.031)	–0.034 to 0.086	0.398
	Red cell folate	–0.623	(1.188)	–2.963 to 1.717	0.601	0.002	(0.002)	–0.002 to 0.006	0.299
	B_{12}	0.578	(0.813)	–1.021 to 2.177	0.477	0.001	(0.003)	–0.005 to 0.007	0.701
	Homocysteine	1.025	(0.824)	–0.595 to 2.645	0.214	0.265	(0.081)	0.106 to 0.423	0.001
EQ-5D	Serum folate	0.021	(0.023)	–0.025 to 0.067	0.363	0.000	(0.001)	–0.002 to 0.001	0.738
	Red cell folate	0.059	(0.031)	–0.002 to 0.121	0.060	0.000	(0.000)	0.000 to 0.000	0.150
	B_{12}	0.018	(0.021)	–0.024 to 0.060	0.397	0.000	(0.000)	0.000 to 0.000	0.966
	Homocysteine	0.003	(0.022)	–0.041 to 0.046	0.904	–0.006	(0.002)	–0.010 to –0.002	0.007
EQ-VAS	Serum folate	0.107	(1.093)	–2.041 to 2.255	0.922	0.032	(0.034)	–0.034 to 0.099	0.337
	Red cell folate	–1.794	(1.490)	–4.728 to 1.141	0.230	0.003	(0.002)	–0.002 to 0.007	0.247
	B_{12}	0.503	(1.012)	–1.488 to 2.493	0.620	–0.001	(0.003)	–0.008 to 0.006	0.823
	Homocysteine	1.154	(1.044)	–0.899 to 3.206	0.270	0.334	(0.095)	0.148 to 0.520	0.0005

continued

TABLE 49 Repeated measures analysis adjusting for stratification variables and baseline on treatment (*continued*)

		Folate – placebo				On treatment prediction of outcome per biochemical unit			
Outcome	Covariate	Mean	(SE)	95% CI	Significance	Mean	(SE)	95% CI	Significance
MCS	Serum folate	–1.172	(1.146)	–3.424 to 1.081	0.307	–0.053	(0.038)	–0.127 to 0.020	0.155
	Red cell folate	0.096	(1.565)	–2.986 to 3.178	0.951	–0.002	(0.002)	–0.006 to 0.003	0.522
	B_{12}	–1.834	(1.052)	–3.903 to 0.235	0.082	–0.001	(0.004)	–0.008 to 0.007	0.812
	Homocysteine	–2.148	(1.098)	–4.307 to 0.011	0.051	–0.140	(0.104)	–0.344 to 0.064	0.178
PCS	Serum folate	1.136	(0.825)	–0.485 to 2.757	0.169	–0.042	(0.025)	–0.091 to 0.007	0.090
	Red cell folate	1.502	(1.114)	–0.693 to 3.696	0.179	–0.003	(0.002)	–0.006 to 0.000	0.081
	B_{12}	0.634	(0.761)	–0.863 to 2.131	0.405	0.003	(0.003)	–0.002 to 0.008	0.255
	Homocysteine	0.357	(0.784)	–1.184 to 1.899	0.649	0.012	(0.071)	–0.126 to 0.151	0.862
CGI: Severity	Serum folate	–0.042	(0.112)	–0.261 to 0.177	0.706	0.003	(0.004)	–0.004 to 0.010	0.391
	Red cell folate	–0.233	(0.144)	–0.517 to 0.051	0.107	0.000	(0.000)	0.000 to 0.001	0.339
	B_{12}	–0.004	(0.103)	–0.205 to 0.198	0.972	0.000	(0.000)	–0.001 to 0.001	0.686
	Homocysteine	0.040	(0.105)	–0.166 to 0.247	0.702	0.029	(0.010)	0.010 to 0.049	0.003
CGI: Improvement	Serum folate	–0.063	(0.119)	–0.298 to 0.172	0.596	0.009	(0.004)	0.000 to 0.017	0.038
	Red cell folate	–0.116	(0.165)	–0.440 to 0.207	0.480	0.000	(0.000)	0.000 to 0.001	0.481
	B_{12}	0.041	(0.109)	–0.173 to 0.256	0.704	0.000	(0.000)	0.000 to 0.001	0.308
	Homocysteine	0.055	(0.108)	–0.158 to 0.268	0.610	0.042	(0.011)	0.020 to 0.063	0.0001

Appendix 11 Recruitment into clinical trials

Introduction

Randomised controlled trials have been seen in recent decades as the 'gold standard' for clinical research.[173] They are accepted as generally the best way to estimate the effectiveness and cost-effectiveness of interventions, as they make strong causal connections between interventions and their effects.[174] However more than two thirds of published trials do not achieve their recruitment targets.[175] McDonald *et al.* looked at 114 trials funded by the MRC or the NIHR HTA programme and found that 54% required an extension, while 41% experienced delays in starting recruitment.[176] This has large cost implications for funders, resulting in trial extensions, and even complete failure. Why some trials recruit well and other suffer problems remains unclear.[177]

Several systematic reviews have sought to improve recruitment in trials. Prescott *et al.*[177] attempted to identify factors that affected the effective running of trials, suggesting that recruitment problems may be reduced by piloting, using multiple recruitment strategies, making contingency plans for slow recruitment and using recruitment coordinators. However none of these approaches had been rigorously evaluated. Watson and Torgerson concluded that recruitment interventions were both sparse and often of poor methodological quality.[178]

Campbell *et al.* set out to identify factors associated with good and poor recruitment in multi-centred trials from a cohort of studies, a selection of case studies and a single in-depth case study.[179] The main themes associated with success were flexibility, adaptability to unexpected issues and better training. The study suggested that the complex nature of multi-centred trials generated unexpected difficulties.

Recent methods to improve recruitment have achieved some success. One such method is the business model using marketing strategies; however these require further research to establish effectiveness and develop useable tools for medical research where these approaches, concepts and terminology are unfamiliar.[180]

Barnard *et al.* looked at the recruitment of participants into trials from a different perspective and aimed to identify different models that may be useful to RCTs when estimating the recruitment of participants.[181] They noted that most trials use an unconditional model of recruitment and suggested that a new model was needed to predict recruitment to clinical trials which takes account of both centre and patient recruitment, recognising that one drives the other.

Health services research often recruits from primary care. However Bower *et al.,*[175] exploring recruitment difficulties, responses to recruitment problems and the relationship between trial characteristics and recruitment, found that recruitment methods requiring GPs to consent patients into trials were particularly problematic. In a review of current literature, Bower also concludes that recruitment of patients into health research from primary care continues to be a major hurdle.[182]

Goodyear-Smith *et al.* sought to identify barriers to recruitment in primary care,[183] including lack of time (exacerbated by the annual influenza vaccinations campaign in general practice), the need to identify staff responsible for decision making, the need to clarify the nature of the study, and the need to be flexible in accommodating practices. Strategies to improve recruitment included providing incentives to practices (both material and educational), using a personal approach, ensuring practices feel engaged, minimising disruption, streamlining processes, and using doctors to recruit doctors. They also suggested that smaller practices were easier to recruit than larger practices.

In the UK in recent years, national research networks have tried to improve some of the difficulties in recruiting to clinical trials and running them. These include specialised networks in key areas of NHS research such as cancer, mental health and stroke. Overarching networks also support research in areas like primary care and children's research. The aim of these research networks is to improve the process of regulatory approvals, provide infrastructure and research support to trials and facilitate the recruitment of patients into trials.[184] In Wales the NISCHR Clinical Research Centre (NISCHR CRC) was established in 2010 to provide research workforce to support and develop research activity within health and social care.[185] However few reported trials have assessed the effectiveness of research networks. Those that have report that they have been successful in improving recruitment rates into trials.[185–187]

So we aimed to identify barriers to recruitment and factors that facilitated recruitment in the FolATED trial.

Recruitment methods

We recruited participants over three years in three centres across Wales. Recruitment methods included direct referrals from GPs, psychiatrists and other healthcare professionals, clinical database searches and self-referral. We reported the number of participants recruited by each method in each centre to identify differences.

Recruitment into the trial from primary care was facilitated by research staff employed directly by the trial or by NISCHR CRC. Their Clinical Studies Officers played a pivotal role in recruitment, including practice recruitment through visits and presentations, development and editing of regular newsletters to recruiting practices, and contributing to local and national meetings and conferences. NISCHR CRC staff also helped with computer searches in practices and invitation mailings from practices.

We collected data from each centre on recruitment, including whether from secondary care, general practices or community mental health services, and method of recruitment, for example whether participants were directly referred by healthcare professionals or as a result of invitations from computer searches in practices.

We sought qualitative feedback from centres through a reflective recruitment tool providing written accounts of recruitment strategies, barriers and facilitators in each site (*Table 50*). We analysed these data using a thematic approach. We also held monthly recruitment meetings and annual training events to identify recruitment difficulties and develop new strategies.

TABLE 50 Recruitment strategies: questions for qualitative analysis

Recruitment and retention issues and supporting data
1. General recruitment Issues. Describe any recruitment issues in your area. Below are some suggested categories but you can alter to your own experiences. What difficulties arose? What worked well?
1.1 Geographical
1.2 Population demographics
1.3 Participant attributes
1.4 The GP–patient relationship
1.5 Appointment booking strategies
1.6 Other issues – e.g. staffing issues, recruiting surgeries and psychiatrists, maintaining psychiatric cover, centre approaches and structure, physical environment and travel.
2. Patient recruitment strategies. What strategies were adopted in your centre? How well did they work? Below are suggested categories; please add or omit as appropriate to your centre.
2.1 Posters, leaflets, newsletters
2.2 Traditional GP referral
2.3 Psychiatrist referral
2.4 Computer searches
2.5 Other strategies
3. Increasing recruitment and retention rates. Other methods used to improve patient recruitment and retention rates. How did these work in practice?

Recruitment performance

We used a wide variety of strategies to help with the recruitment and retention of both recruiters to the trial such as general practices and secondary care services, and trial participants (*Table 51*). We adopted a flexible approach to recruitment allowing the three centres to identify and deploy the strategies found to be successful in their area. However some strategies were universal such as the use of trial posters, payment of general practices, feedback to GPs of BDI-II scores, regular training events and research team meetings. Local variations included use of research networks such as NISCHR CRC, access to direct referrals to secondary care through flagging in referral notes, different methods of referral and participant reminders for appointments and venues.

Methods used in recruitment

We recruited participants in three centres – North East Wales, North West Wales and Swansea (*Table 52*). Although several methods of recruitment were adopted by all centres, different methods were used in different centres. North West Wales, the most successful recruiting centre, acquired their referrals mainly from direct referrals to the psychiatric services, supplemented by computer searches at general practices and other referrals through secondary care. North East Wales acquired the majority of their referrals through computer searches at general practices and direct GP referrals supplemented by other mental health referrals. Swansea acquired the majority of their referrals through direct GP referrals, supplementing this with computer searches and other referrals from secondary care (*Figure 19*).

TABLE 51 Strategies for maintaining recruitment and retention

Recruiting practices
Raise awareness of practices
Advertising in local health board bulletin
Presentations at local GP meetings
Stands at mental health practitioner network meetings
Posters at NISCHR CRC primary care research events
Letters of invitation to general practices
Direct phone calls to general practices
Raise awareness of patients
Posters in general practices
Participant information leaflets in waiting rooms
Incentives for primary care
Pay £50 per patient consented to cover administration costs
Provide GP with BDI-II scores and blood results for Quality & Outcomes Framework (QOF) assessments
Provide 'fast track' access to psychiatric assessment
Use NISCHR CRC
Provide primary care recruitment assistance and research support in North East Wales
Provide research staff for recruitment in Swansea
Flag triage to secondary care in North West Wales
Training event e.g. recruitment 'brainstorming'
Monthly research team meetings to discuss recruitment issues across centres
Maintaining recruitment
Monthly newsletter to recruiting practices and mental health professionals
GP feedback events
Regular personal contact with practices and feedback from researchers
Maintenance of participation in trial
Appointment reminder letters and telephone calls
Continuity of researcher for participant
Reduction in assessment at screening visit
Introduction of home visits when beneficial to participants

TABLE 52 Referral method by recruitment centre

Referral method	North East Wales	North West Wales	Swansea
Direct GP referrals	97 (24.3%)	429 (61.5%)	314 (80.5%)
Computer search in general practice	261 (65.4%)	129 (18.5%)	32 (8.2%)
Direct psychiatrist referral	21 (5.3%)	106 (15.1%)	12 (3%)
Other secondary care professional (e.g. CMHT)	16 (4%)	32 (4.6%)	1 (0.3%)
Other primary care professional (e.g. Mental Health Practitioner)	4 (1%)	2 (0.3%)	28 (7.2%)
Unknown	0 (0%)	0 (0%)	3 (0.8%)
Total referrals	399 (100%)	698 (100%)	390 (100%)

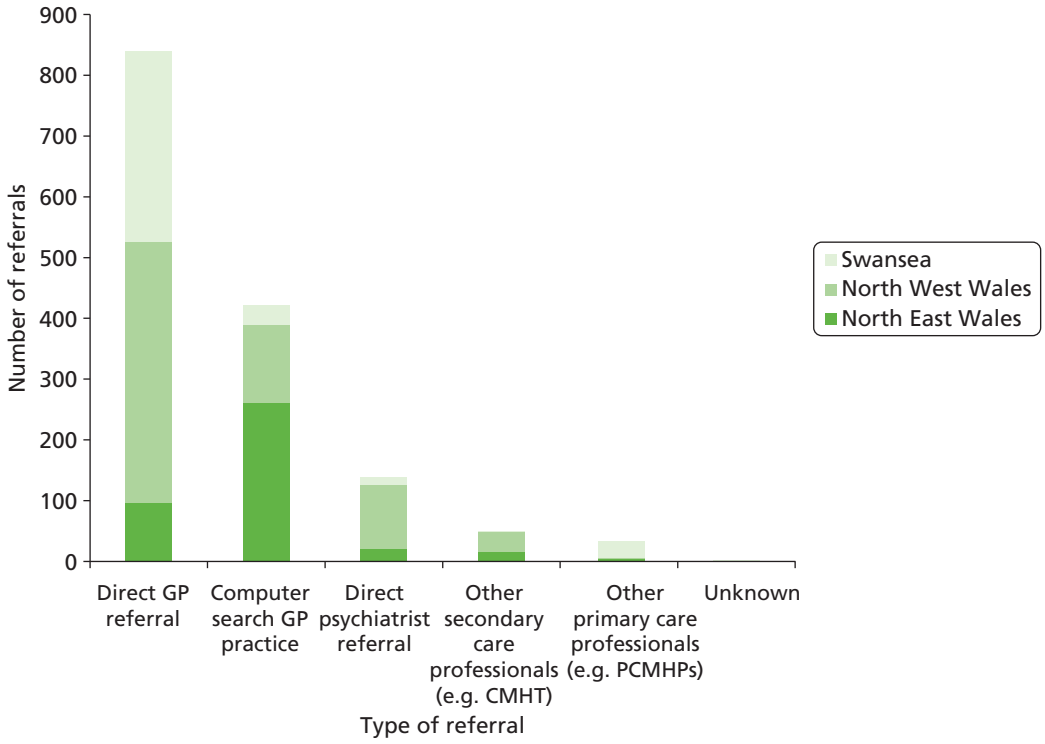

FIGURE 19 Number and type of referrals.

Facilitators to recruitment

Although we adopted different methods of recruitment across sites, we identified several factors as aiding recruitment. A major facilitator was a good relationship with recruiting practices and services. This was seen as a key factor in North West Wales: they had a history of working with general practices and had established good working relationships with them. Elements important in building and maintaining a good relationship included regular contact with practices, fast responses to their queries, regular updates by newsletters and personal contact, and small tokens of appreciation like cards at Christmas.

A perception that participation conferred benefits on patients was also seen as a facilitator. Many GPs were pleased to offer something extra to long-term patients and saw quick access to a psychiatrist as beneficial, and time with the researcher as an alternative or supplement to counselling, which was often difficult to access. Some GPs felt this would be an alternative to patients visiting the surgery.

Relationships were a recurrent theme. A good patient–doctor relationship was viewed as a facilitator to direct referrals, as small surgeries had higher referral rates. Good relationships between participants and researchers were also seen as important in retaining participants. Follow-ups were face to face, helping relationships to develop. Continuity also contributed to the success of relationships with researchers encouraged to follow participants from beginning to end. Also important was personal contact through reminder letters and phone calls.

Awareness raising and networking with gatekeepers like practice managers were important in gaining access to recruiters. Initial contact with Local Health Boards was also seen as a facilitator by providing advice about key contacts, monthly meetings and identifying research-active practices. Furthermore aides-memoire facilitated recruitment by providing reminders to health professionals; these included pens, note paper and trolley tokens with the FolATED logo.

Barriers to recruitment

One common theme was the competing demands on general practice time and resources. Examples of commitments that preoccupied practices included preparing for QOF and the contemporaneous vaccination campaign. Also changes in staff and surgery relocation affected recruitment in some centres.

Barriers included perceptions that research might compromise practice, including concerns about increased visits to GPs, access to the practice database, confidentiality, disruption, expense, inappropriate referrals, consequences of participation for patients, in particular those found not suitable at screening, increased workload, availability of space in surgeries and even the validity of the study.

North East Wales reported that some practices were already taking part in other studies and did not feel they could recruit to another study. Also previous demands by researchers for information had made several practices reluctant to participate, fearing our study was connected in some way.

Staffing problems also reduced recruitment. The Swansea centre lost several research staff within a short time, thus halting participant recruitment during staff recruitment and training. North East Wales had only one researcher for several months before NISCHR CRC could help. North West Wales also had to restrict computer searches and mailings to focus on direct referrals.

Inclement weather also affected recruitment and retention during the winter of 2010. Participants and researchers were often unable to attend appointments, particularly in rural areas.

Discussion

This study has identified facilitators of, and barriers to, recruitment and retention in the FolATED trial. Facilitators included the importance of building good relationships with psychiatric services, practices and participants through the interpersonal skills of researchers and continuing feedback to recruiters. Also important was the potential benefit of the intervention, and participation in the trial, to patients, as perceived both by those recruiting and the participants themselves. Thus raising awareness and networking with gatekeepers like practice managers reportedly improved recruitment.

Barriers included the high demand on practitioners' time, disruption to the surgery, consequences for ineligible patients, and worries about confidentiality. It is therefore vital that researchers design trials that minimise impact on surgeries and provide reassurance about effects on patients and their confidentiality. It is important that, when dealing with recruiters, researchers tread carefully. If future research is to be successful they need to nurture relationships and not make undue demands. As staffing levels often posed a threat to recruitment, researchers and funders need to be realistic when designing trials. Finally the FolATED trial experienced unforeseen disruption, notably from the volcano eruption in Iceland and the inclement weather of the winter of 2010.

This study also examined the strategies and methods used in the FolATED trial for the recruitment and retention of both those recruiting into the trial and participants in the trial. Recommendations include the need for a flexible approach to such recruitment and retention. An explicit recruitment strategy is essential in any trial; however multi-centred complex interventions present extra challenges which require a flexible and often creative approach. However we suffered from the slow regulatory systems and long waits to implement much needed changes. For example we waited several months to get approval for an improved poster and information leaflet for GP surgeries.

Monthly research team meetings provided the platform for sharing problems and exploring ideas with colleagues. This also encouraged the trial centres to work as a team. Annual training sessions also assisted in exploring and sharing ideas and experiences across centres.

The resources required to recruit and run a complex intervention should not be underestimated. There is a need to acknowledge that recruiting into trials and the day-to-day running of trials is laborous, and that the recruitment phase of a trial is particularly so. Alongside this, trials that employ labour-intensive methods like interviews must be costed accordingly. The competitive nature of bidding for trial funding often results in underfunded studies risking failure of trials owing to a lack of researchers on the ground to coordinate and perform the research. In the FolATED trial assistance from NHS staff and the emerging NISCHR CRC was crucial in centres with limited resources, where even the most successful recruiting centre could have handled more participants but for the limitation of research staff to provide follow-up interviews.

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