Genotypes of the MTHFR (C677T) and MTRR (A66G) genes act independently to reduce migraine disability in response to vitamin supplementation.

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Abstract

Background: Migraine is a chronic disabling neurovascular condition that may in part be caused by endothelial and cerebrovascular disruption induced by hyperhomocysteinemia. We have previous provided evidence that homocysteine lowering by vitamin supplementation can reduce migraine disability in females. The current study examined the genotypic effects of MTHFR and MTRR gene variants on migraine disability in response to vitamin supplementation.

Method: This was a 6-month randomised, double blinded placebo controlled trial of daily B vitamin supplementation (B6, 9 and 12) on homocysteine lowering and reduction of migraine disability in 206 female patients diagnosed with migraine with aura.

Results: Vitamin supplementation significantly reduced homocysteine levels ($P<0.001$), migraine head pain severity ($P=0.017$) and high migraine disability ($P=0.022$) in migraineurs compared to the placebo effect ($P>0.1$). When the vitamin treated group was stratified by genotype, the C allele carriers of the MTHFR C677T variant showed the a higher reduction in homocysteine levels ($P<0.001$), migraine pain severity ($P=0.01$) and percentage of high migraine disability ($P=0.009$) compared to TT genotypes. Similarly the A allele carriers of the MTRR A66G variants showed a higher level of reduction in homocysteine levels ($P<0.001$), migraine pain severity ($P=0.002$) and percentage of high migraine disability ($P=0.006$) compared to GG genotypes. Genotypic analysis for both genes combined indicated that the treatment effect modification of the MTRR vartiant was independent of the MTHFR variant.

Conclusion: This provided further evidence that vitamin supplementation is effective at reducing migraine disability and also that both MTHFR and MTRR gene variants are acting independently to influence treatment response in female migraineurs.

Introduction

Migraine affects about 303 million people in the world according to the World Health Organisation (WHO) and is estimated to affect about 12% of the Caucasian population [1, 2]. Homocysteine related dysfunction of the vascular endothelium may potentially influence migraine susceptibility [3-5]. Hyperhomocysteinemia related endothelial injury may activate trigeminal fibres leading to an inflammatory reaction occurring in the meninges, along with dilation of the large cerebral vessels. It is this reaction that is thought to participate in the characteristic head pain common in patients suffering from migraine with aura (MA)[2, 4, 6]. Various factors determine the levels of circulating plasma homocysteine, in particular dietary deficiencies in the co-factors such as folic acid, vitamin B12 and B6 essential for metabolising homocysteine and mutations in the genes of key enzymes participating in homocysteine metabolism such as cystathionine b-synthase (CBS), methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and methionine synthase reductase (MTRR)[7].

The human MTHFR gene mapped to chromosome 1p36.3, catalyses the nicotinamide adenine dinucleotide phosphate (NADPH) dependent conversion of 5, 10-methylenetetrahydrofolate (CH_2-THF) to 5-methyltetrahydrofolate (CH_3-THF), the principal circulatory form of folate and a cofactor for methylation of homocysteine to methionine [8, 9]. The C667T polymorphism in the MTHFR gene results in an amino acid change at position 222, substituting alanine (Ala) for valine (Val). The TT genotype of the C677T polymorphism is associated with 50% reduction in enzyme activity and can lead to mild hyperhomocysteinemia [6, 10-12]. This effect is exacerbated in combination with low folate levels [13]. Since the first discovery of the MTHFRC677T polymorphism association with migraine in a Japanese population, several studies have replicated the findings in different independent populations [6, 11, 12, 14]. A meta analysis of 2961 migraine patients

has provided further evidence that the TT genotype, specifically increases the risk of MA [15].

The MTRR gene reduces inactive cobalamin II to active cobalamin I and methylates it to methylcobalamin using S-adenosylmethionine as the methyl donor [16]. MTRR therefore plays a pertinent role in maintaining adequate supply of active cobalamin I and may also be a critical determinant of homocysteine concentrations [17]. A common variant, A66G in the MTRR gene results in the replacement of methionine with isoleucine in the enzyme. The MTRR A66G polymorphism is also associated with an increase in plasma homocysteine, with the GG genotype having a greater effect than AG genotype [17]. In addition the coexistence of the MTHFR 677TT genotype and either the AG or GG genotypes for the MTRR A66G polymorphism have been reported to magnify the effect of the MTHFR 677TT genotype alone [18].

A recent study by Lea et al examined whether folic acid (vitamin B_9) and vitamin B_6 and in fifty two MA sufferers [2]. The major findings of this study revealed that vitamin supplementation significantly reduced plasma homocysteine levels and migraine disability, in a small subgroup of migraineurs [2]. The results of this trial were supported by the open-labelled study by Di Rosa et al [10] and provided initial evidence that homocysteine lowering through folic acid coupled vitamins B_6 and B_{12} may reduce migraine disability in a subgroup of patients[2, 10].

Our initial study provided some evidence that the effect of vitamin supplementation on homocysteine-lowering and reduction of migraine disability was influenced by MTHFRC677T genotype, whereby C allele carriers were shown to exhibit a greater response compated to TT genotypes [2]. With multiple genes reported to be involved in the

homocysteine-metabolising pathway, it is plausible that different genotypes may cause varying response to vitamin supplementation. The current study repeated the original trial in an independent and larger sample of migraineurs which allowed us to investigate the genotypic effects of both the MTHFR and the MTRR gene on homocysteine lowering and migraine disability in response to vitamin supplementation.

Methods

Study design and participant group

This study analysed the genotypic effect of the MTHFRC677T and MTRRA66G polymorphisms on daily folic acid and B_6 and B_{12} vitamin treatment for lowering homocysteine, migraine disability, frequency and pain severity by conducting a randomized, double blinded placebo controlled clinical trial over a 6 month period. The trial guidelines were designed using the guidelines for controlled trials of drugs in migraine [19]. The study recruited female Caucasian adult between the ages of 18 and 60, of European ancestry from all over Australia. All participants were interviewed and completed a detailed questionnaire that was administered through Griffith University's Genomics Research Centre (GRC). As migraine is more prevalent in females and the possibility that there may be a difference in migraine susceptibility and response in relation to treatment, the current study only focussed on one gender. Females between the ages of 18 and 60 were recruited and participants were included if they had suffered migraine for more than 5 years and had a current diagnosis of MA (>90% of their migraine attacks were associated with aura), and a 1-year history of severe, long lasting attacks (at least 4 attacks lasting more than 48h), had a family history of migraine. Confirmation of migraine diagnosis was carried out using the IHS criteria. Participants who were currently taking vitamin supplementation, pregnant, or had been diagnosed with a clinically recognised co-morbid disease such as vascular disease, depression or epilepsy were excluded from the trial to reduce clinical and

pathological heterogeneity. Participants that had taken part in another clinical trial or had received any experimental therapy within the last one month were also excluded from the trial. The patient group was not selected on the basis of pre existing folic acid, B12 or B6 deficiency.

Treatment

245 female patients meeting the inclusion criteria were randomly assigned into either the placebo or the treatment group. A blocked random allocation sequence was generated using Microsoft Excel (Microsoft, USA). Participants and everyone involved in this trial were blinded to randomisation and group allocation. Participants received either *Blackmores MigraVit* ^{TM} vitamin tablets containing 2mg of folic acid, 25mg of vitamin B_6 and 400 µg of vitamin B_{12} or the placebo tablet. Participants were instructed to take one tablet daily for 6 months. Both the vitamin and placebo tablets were produced by *Blackmores* and were identical in appearance.

Baseline and Follow-up assessment

Before commencing the trial all participants were assessed for migraine disability using the Migraine Disability Assessment Score (MIDAS) instrument, which provides a measure of productive days lost to migraine headache in previous 3 months (i.e. migraine disability), headache frequency and pain severity [2, 20]. Studies have shown that the MIDAS instrument is a valid and clinically useful instrument for assessing health-related quality of life in migraineurs. Based on the 5-question MIDAS rating, participants were arbitrarily categorized into a ‘low’ disability group if they had a MIDAS rating of 0-10 and into a ‘high’ disability group if they had a MIDAS rating greater than 11[2, 20, 21] . Questions 6 and 7 of the MIDAS instrument were on migraine frequency and head pain severity

respectively. These were measured as number of days with headache (over a 3 month period) and a pain score (based on a scale of 1-10), respectively [2, 20, 21].

Participants were asked to complete a daily diary during the trial period to record the details of their migraine symptoms (duration, frequency and severity) and treatment compliance. Participants were also instructed to take their usual migraine treatment for acute attacks. A blood sample was collected for baseline measurement of plasma homocysteine (μmol/l), folate (nmol/l), vitamin B_6 and B_{12} (pmol/l) concentration. 2ml of venous blood was collected for Genomic DNA extraction and genotyping purposes. Participants were contacted after 3 months of starting the trial for headache diary and compliance checking. At the end of the 6 months trial the patients were reassessed at the GRC clinic. They were questioned about their migraine history in the last 6 months since the start of the trial. A second collection of blood samples was done for measurement of homocysteine, folate, B_6 and B_{12} concentrations. The Plasma homocysteine, folate, B_6 and B_{12} levels were measured in an accredited pathology laboratory.

Dietary consumption

Variation in the participant's dietary intake of folate is a potential confounding factor for this study. Participants were thus required to keep a daily diary of food type, amount and frequency. Each participant was given 2 diary packs, each pack consisting of 7 days of daily diet intake to be recorded. The diet dairy was designed to estimate the usual dietary intake of nutrients such as B_6, B_{12} and dietary folate over a typical week. Participants were asked to complete their diet dairy once a fortnight, on only one day until each day of the week has been recorded. The nutrient intake of participants was analysed using the *NUTTAB* version 2010 database, which is based on the Australian New Zealand food standard code.

Genotyping

The C677T polymorphism was genotyped in the patient group in the GRC laboratory using previously published methods[6]. High resolution melt analysis was used to genotype A66G polymorphisms. Primers used for genotyping the MTRR A66G polymorphism are "Forward 5': GCA AAG GCC ATC GCA GAA GAC AT 3' and "Reverse 5': AAA CGG TAA AAT CCA CTG TAA CGG C 3'. The reaction mixture used HotStarTaq (Qiagen, Hilden Germany) and consisted of 40 ng of genomic DNA, 10× PCR buffer, 25 mM $MgCl_2$, 5uM of each primer, 2.5mM of dNTPs, 50 μM of SYTO 9 (Invitrogen, Carlsbad, USA), 0.5 U of HotStarTaq polymerase and Polymerase Chain Reaction (PCR) grade water in a volume of 25 μL. All PCR reactions were performed in duplicate. PCR cycling and HRM analysis was performed on the Rotor-Gene™ 6000 (Corbett Research, Mortlake, New South Wales, Australia). The PCR cycling conditions for the MTRR A66G were as follows; one cycle of 95°C for 5 minutes; 45 cycles of 95°C for 5 seconds, 45 cycles of 60°C for 10 seconds, 72°C for 20 seconds; one cycle of 95°C for 1 second, 72°C for 90 seconds and a HRM step from 75 to 85°C rising at 0.1°C per second.

Statistical analysis

The analysis for the current trial was conducted on a modified intention-to-treat (ITT) principle. The modified ITT population was composed of all randomised participants who started the trial and consumed study supplements on at least one occasion, excluding those who withdrew from the trial after the randomisation process had taken place but before the commencement of study supplement consumption.

At baseline, the unpaired samples *t*-tests were used to test the group means. The median were compared using the Mann Whitney *U* tests and proportions were compared using the

χ^2 test of independence. The primary hypothesis of this trial, that vitamin supplementation reduced migraine disability was tested by comparing proportions of high disability migraineurs before and after the 6 month trial in both the vitamin and placebo groups. The χ^2 test of independence was used to compare the proportion changes. Mean changes were compared before and after treatment using paired samples *t*-tests and median changes were compared using nonparametric Wilcoxin signed rank tests for related samples. The relationships among the baseline biochemical variables were assessed using the Pearson's correlation tests. Post treatment means for treatment and placebo groups were compared at 6 months using the unpaired samples *t*-tests. The significance threshold was set at α level of 0.05.

Linear regression analysis was performed using the "successive steps" method, to determine the independent predictors of the difference in homocysteine levels before and after the trial, allowing the introduction of a new variable if the P value of the new model was less than 0.05, and excluding those yielding a P value higher than 0.10 in each step. The independent variables were age, genotype, treatment group (placebo vs vitamin), and dietary intake of B6, B12 and folate. All analyses were performed using the Statistical Package for Social Sciences (SPSS version 18.0).

Results

Figure 1 illustrates the patient flow through the trial from January 2009 to January 2010. Six hundred and twenty nine migraine patients were assessed for eligibility prior to enrolment into trial. 384 migraine patients were excluded from enrolment due to reasons such as not meeting inclusion criteria, refusal to participate in placebo controlled trial and other reasons. 245 participants were initially enrolled in the trial and were randomly assigned to either the placebo group or the vitamin treated group but 3 participants dropped

out before the commencement of the trial and the remaining 242 participants received baseline assessment and commenced the trial. Hundred and nineteen participants were on the vitamin treated group and the remaining 123 participants were in the placebo treated group. Forty four participants were lost to follow up due to lack of compliance and 162 participants completed the trial (76 vitamin: 86 placebo).

Baseline analysis

Table 1 shows the baseline clinical characteristics of the participant group. For the total migraine group (n= 206), mean folate concentration was 30.1 nmol/l, which is above the average for a general Caucasian population replete for folate (13.7nmol/l)[2]. The mean plasma homocysteine concentration for the migraine group was 11.5 µmol/l, which is also above the average for a general Caucasian population (8.9 µmol/l)[2]. The mean levels of B_6 and B_{12} at baseline fell within the normal range for this patient group. For the total group, plasma homocysteine concentration was negatively correlated with plasma folate (Pearson's r = - 0.057, P = 0.438). Vitamin B_6 (Pearson's r = -0.212, P = 0.05), vitamin B_{12} (Pearson's r = - 0.279, P= 0.000). The percentage of participants with high migraine disability did not differ significantly between the placebo and the vitamin treated group (P=0.18). Similarly the migraine attack frequency (P=0.41) and migraine pain severity (P= 0.38) did not differ significantly between the placebo and the vitamin treated group. There were no statistically significant differences between the vitamin and placebo groups for the teat variables at baseline.

Six-month follow-up analysis

A total of 162 participants completed the trial. 86 of them were on placebo and 76 of them were on vitamin supplementation. After 6 months of treatment, the vitamin treated group had marked increases in folate, B_6, B_{12} concentration compared with baseline and the

placebo group (P< 0.001). In the placebo group, the mean folate levels increased by 11.8% after 6 months (28.7 – 32.1 nmol/I, P= 0.852). The B_{12} levels decreased by 6.5% (328.6-307.1 pmol/I, P= 0.059) and the B_6 levels decreased by 7.8% (80.8- 74.5 pmol/I, P= 0.292). In the vitamin treated group, the median homocysteine levels reduced by 20% after 6 months (11.5-9.2 pmol/I, P<0.001) compared with 4.8% reduction observed for the placebo group (P=0.121). The effect of treatment on the reduction of homocysteine levels remained significant after correction for confounding factors such as age, genotype and dietary consumption of vitamin B_6, B_{12} and dietary folate (r^2=0.042; P = 0.019). The dietary consumption of B_6, B_{12} and dietary folate between the vitamin and placebo groups were not significantly different at the 6 month follow-up analysis (B_6, P=0.721; B_{12}, P= 0.891; Dietary folate, P= 0.373).

In the vitamin treated group the frequency of high migraine disability decreased after 6 months of supplementation from 74% to 56.9% (P=0.022). The reduction in the placebo group was not statistically significant (65.3% to 53.2% (P=0.098). Headache frequency did not decrease from a median of 2 for both the vitamin (P=0.46) and placebo (P=0.147) groups after 6 months. The vitamin treated group reported a decrease in pain severity from a median score of 7 to 6 (P= 0.017), whereas the placebo group reported no change (P>0.1).

Treatment response by MTHFRC677T and MTRRA66G genotype

When the vitamin treated group was stratified by MTHFRC677T genotype, the mean homocysteine reduction was 20% for both the CC and the CT group (P<0.001) compared to 13.3% for the TT carriers (P=0.095) (Figure 2). For further analysis the genotypes (CC and CT) that showed the largest decrease in homocysteine levels after vitamin supplementation were grouped and their effect on migraine frequency, pain severity and disability were analysed. There was no significant reduction in migraine frequency in both CC/CT and the

TT genotype groups after the trial. The CC/CT genotype group showed a significant reduction in migraine pain severity (P= 0.01) with the mean pain scores decreasing from 7 to 6. This significant reduction was not observed in the TT genotype group (P=0.278) with the mean pain scores remaining at 6 before and after the trial. When migraine disability was analysed in the CC/CT and TT genotype groups, the percentage of highly disabled migraineurs decreased from 77% to 57% after the trial in CC/CT group (P= 0.009) and by 6.3% in the TT genotype group (P=0.568).

When the vitamin - treated group was stratified by MTRRA66G genotype, the mean homocysteine reduction was 27% for the AA carriers (P<0.001), 16% for the AG carriers (P= 0.001) compared to 13% for the GG carriers (P=0.03) (Figure 3). For further analysis the AA and the AG genotypes of the A66G variant, which showed the largest decrease in homocysteine levels after vitamin supplementation were grouped and their effect on migraine frequency, pain severity and disability were analysed. Again there was no significant change in migraine frequency between the two groups. The A allele carriers of the MTRRA66G variant showed a significant reduction in migraine pain severity with the pain scores decreasing from 7.1 to 6.2 after the trial (P=0.002). In the GG genotype group however, the pain scores increased from 6.3 to 7 (P=0.05). When the percentage of high migraine disability was analysed in the two genotype groups, the percentage of highly disabled migraineurs decreased from 75.3% to 53.8% in the A allele carriers (P= 0.006), while it only decreased from 68.8% to 66.7% in the GG genotype group (P=0.45).

The C allele carriers of the MTHFRC677T polymorphism and the A allele carriers of the MTRRA66G polymorphism were compared with the homozygote mutant allele carriers for both genotypes (CC/CT/AA/AG vs TT/GG). There was no significant decrease in migraine frequency in the two groups. Migraine head pain severity was however significantly

decreased after the 6 months of vitamin supplementation in the CC/CT /AA/AG group from a pain score of 7 to 6.2 (P=0.002) compared to the TT/GG group, which remained at a constant score of 6 (P=0.5) (Figure 4). Similarly migraine disability was significantly reduced after the trial in the CC/CT/AA/AG group with the percentage of highly disabled migraineurs decreasing from 74.6% to 52.2% (P=0.015) compared to the TT/GG group, where the percentage of highly disabled migraineurs decreased from 50% to 33.3% (P=0.64) (Figure 5).

Discussion

Migraine is a chronic and often disabling condition has an enormous impact on both the individual sufferer and on society at large [22]. There are several acute care therapies and medications currently available that have been successful in either treating migraine symptoms or decreasing migraine attacks and several other medications and therapies in various stages of development [23]. However current medications and therapies work with differing efficacy in migraineurs and are often associated with adverse effects [2]. Thus the search for effective, safe and inexpensive migraine therapies to combine with or replace current therapies still continues [2]. One of the most significant findings in recent times is the involvement of genetics in migraine aetiology, which has added further complexity to understanding the pathophysiology underlying migraine. This has made migraine diagnosis and treatment options varied and continuously improved.

Mild hyperhomocysteinemia has been reported to increase the risk of artherosclerotic vascular disease [24]. MA sufferers have been linked to increased risk of vascular brain lesions and ischaemic stroke [25-27]. It is still not clear if homocysteine levels are raised in migraineurs; however there is evidence to suggest that the CSD phenomenon observed in MA can also occur during a stroke episode [28]. Based on the potential role homocysteine

may play on the cerebrovascular system and the co morbidity of migraine and stroke, it is plausible that homocysteine levels may be involved in the underlying pathophysiology of both MA and stroke[29]. The reasons for hyperhomocysteinemia may be varied: mutations in the genes for MTHFR, MTRR and CBS or possible nutritional deficiencies in cofactors in the homocysteine metabolism [30]. Alternatively, migraineurs may be hyper sensitive to folate at "normal" levels.

The current study examined the genotypic effects of the MTHFR and MTRR gene on folate and vitamin B treatment response in migraineurs. Vitamin supplementation in the current trial was well tolerated by the study group with no reports of adverse reactions. At baseline, the homocysteine levels were mildly elevated in the migraine group compared to the general Caucasian population with some but not all studies of homocysteine in migraine [2, 10, 28]. Results of this trial showed the vitamin treatment compared with the placebo significantly decreases the homocysteine levels at six months by an average of 2.2 µmol/l, an effect size in Australian Caucasian female MA sufferers. This decrease in homocysteine levels remained significant after correcting for dietary consumption of folate, B6 and B12.

The 2.2 µmol/l reduction in the homocysteine levels at 6 months in the current trial approximates the 2.0 µmol/l reduction observed in the "Vitamin Intervention for Stroke Prevention (VISP) trial, which randomised 3680 stroke survivors of primarily white origin from North America to receive high-dose (folic acid 2.5mg; B_6 25mg: B_{12} 0.4 mg) or low dose folic acid 20 µg; B_6 200ug; B_{12} 0.6 µg)[31]. However the 2.2 µmol/l reduction in homocysteine levels observed in the current trial is lower compared to the 4.0 µmol/l reduction observed in a study by Lea et al in 2009 that investigated the effect of vitamin supplementation and MTHFR (C677T) genotype on homocysteine- lowering and migraine disability in 52 Australian Caucasians [2]. The difference in decrease of homocysteine

levels in the vitamin groups of both trials was not statistically significant (Lea et al 2009 trial- 39% vs 20% - current trial : P= 0.475).

The smaller than expected treatment effect of the prescribed dosage of vitamins seen in the current trial in comparison to the earlier trial by Lea et al in 2009 , is most likely attributable to the implementation on September 2009, of fortification of wheat flour for bread making with folic acid in Australia, which coincided with the conduct of the trial. The mean folate levels of the participants in the vitamin group at baseline in the current trial (31.4nmol/l) was significantly higher (P<0.001) than those in the Lea et at 2009 trial (11.2nmol/l). While there was no statistically significant difference between the B_{12} and B_6 levels at baseline between the two trials (P<0.4). It has been evidenced in clinical trials in cardiovascular disease, that mandatory folic acid fortification has influenced the homocysteine-lowering effects of folic acid [31]. Moreover it has been observed that trials conducted in countries with mandatory folate fortification show narrower differences in homocysteine reduction for study groups than trials conducted in countries without folate fortification [32, 33].

Participants may have failed to take a "*MigraVit*^{TM} " pill everyday of the 6 months trial period and although participants were asked to record details of their daily diet and days where they had missed taking the prescribed "*MigraVit*^{TM}" pill, this may not have been accurately reported or detailed in the migraine diary they were provided with. These may also be reasons that could have contributed to the lower levels of homocysteine reduction observed in the current trial in comparison to the previous trial by Lea et al 2009[2].

There was significant reduction in migraine pain severity and disability according to the MIDAS instrument scores in the vitamin treated group. This decrease was greater overall

compared with the placebo effect, which was not statistically significant (Figure 3). However the frequency of migraine headache did not decrease from a median of 2 in the vitamin treated group, which is inconsistent to that observed in the pilot study by Lea et al that reported a decrease from a median of 4 to 1 in migraine headache frequency in the vitamin treated group[2]. The absence of significant reduction in migraine headache frequency observed in this trial may be attributable to the fact that the trial population only had a median headache frequency of 2 at baseline. A population of median headache frequency of 4 and more at baseline may have yielded a significant reduction in headache frequency after the prescribed vitamin treatment.

When the effects of the MTHFRC677T variant on treatment effect in migraineurs was assessed in the current study, a significant reduction in homocysteine levels in the C allele carriers compared to the mutant homozygote TT genotype carriers was observed. Further analysis found the C allele carriers to respond better to vitamin supplementation in lowering migraine pain severity and high migraine disability compared to the TT genotype carriers. These results are supported by similar findings reported in the pilot study by Lea et al [2] and adds to the idea that individuals with TT genotypes, having a 50% reduction in their enzymatic rate, are genetically slower in homocysteine metabolism and thus would require an increased dosage of vitamins compared with the C allele carriers to experience the same reduction in homocysteine levels and consequent migraine symptoms. Similarly when the analysis was stratified by the MTRRA66G variant, the A allele carriers showed the largest reduction in homocysteine levels and migraine pain severity and high migraine disability under vitamin supplementation compared to the mutant homozygote GG genotype carriers.. The combined effects of the C and A allele carriers of the C677T and A66G variants respectively, showed significant reduction in migraine pain severity and migraine disability. However the combined effect of the two variants was not significantly different from the

independent effect of the two variants on migraine pain severity and disability. This suggests that both the MTHFRC677T and MTRRA66G appear to be acting independently from one another in affecting vitamin treatment response in migraineurs.

The MTHFR product, 5-methyl-THF, donates a methyl group for the remethylation of homocysteine to methionine, which is catalysed by MTR in a vitamin B_{12} dependent reaction. MTR may become inactive due to oxidation of its vitamin B_{12} cofactor and restoration of MTR activity is dependent on reductive remethylation of vitamin B_{12} by MTRR [34]. The functional effects of the MTRR A66G variant have not been fully understood, however in vivo experiments suggest that the A66G variant MTRR enzyme restores MTR activity less efficiently than wild-type [35], and has also been shown to increase plasma homocysteine levels in humans [36, 37]. When the MTHFR 677TT genotype occurred with either the homozygous or heterozygous genotype for the MTRR A66G variant, it may exacerbate the effect of the MTHFR variant alone [18].

Genes involved in the homocysteine pathway play a crucial role in the amount of homocysteine in the extracellular media such as plasma [38]. The allele groups of MTHFR and MTRR that showed the largest reduction in homocysteine levels showed the most significant reduction in migraine pain severity and disability under vitamin treatment. There is an undeniable relationship between homocysteine levels and migraine disability. The homozygote mutant allele carriers of the MTHFR and MTRR variant may need a higher dose of vitamin supplementation to experience the same effect as the wild type allele carriers of the variants, in migraine pain severity and disability reduction. Further clinical trials of higher doses of vitamin supplementation are required to make an evidence based argument of this idea. The effects of hyperhomocysteinemia may be a partial determinant for the neuro and/or vascular pathologies underlying MA and stroke [2]. Other genes and

functional variants associated with the homocysteine metabolism cascade other than the MTHFR and MTRR have to be investigated in relation to vitamin treatment response in migraineurs.

Conclusion

The current study has added further evidence that homocysteine reduction through vitamin supplementation may reduce migraine disability in a subgroup of patients and that the MTHFR and MTRR variants both contribute an independent effect on migraine treatment response.

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Tables

Table 1:

Variable	Total	Vitamin	Placebo	*P*-value*
No.patients	206	103	103	
Age in years, mean(SD)[b]	44(13)	42(13)	45(13)	0.65
Female	206			
Folate, mean (SD)[b]	30.1(12.5)	31.4(12.8)	28.7(12.2)	0.56
Vitamin B_{12}, mean (SD)[b]	322.7(148.5)	315.7(114.9)	328.6(175.7)	0.15
Vitamin B_6, mean (SD)[b]	78.8(12.6)	77(11.9)	80.8(13.5)	0.82
Homocysteine, mean (SD)[b]	11.5(3.4)	11.6(3.6)	11.5(3.3)	0.7
B12ug consumption, mean (SD)[b]	1.01(0.5)	1.1(0.5)	0.9(0.5)	0.9
B6mg consumption, mean (SD)[b]	1.7(0.6)	1.7(0.7)	1.7(0.6)	0.13
Folate ug consumption, median (range)[a]	428.7(225.6)	433(239)	424(212)	0.74
High migraine disability (%)[a]	69.7	74%	65.30%	0.18
Attack frequency median (range)[a]	2 (1-6)	2(1-6)	2(1-4)	0.41
Head pain score median (range)[a]	7(3.5-10)	7(1-10)	7(3.5-10)	0.38
MTHFR C677T (TT) genotype %	15.3	18.3	12.4	0.46
MTRR A66G (GG) genotype %	25.4	19.6	30.9	0.15

MTHFR C677T, methlenetetrahydrofolate gene
MTRR A66G Methionine synthase reductase gene

[a] Based on a 3-month history.

[b] Average daily consumption.

* All *P* values are two-tailed.

Figures

Figure 1: Patient flow chart for the trial

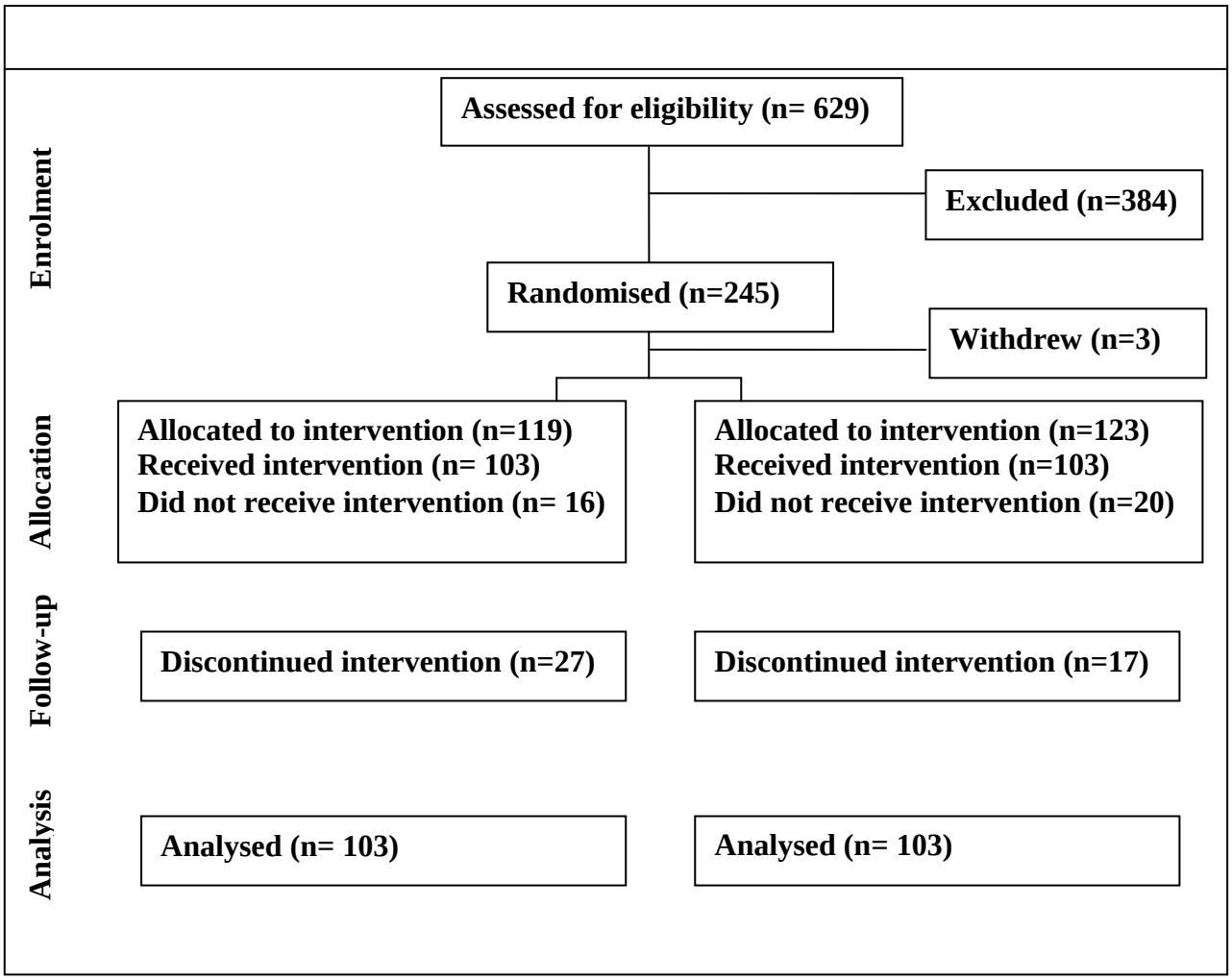
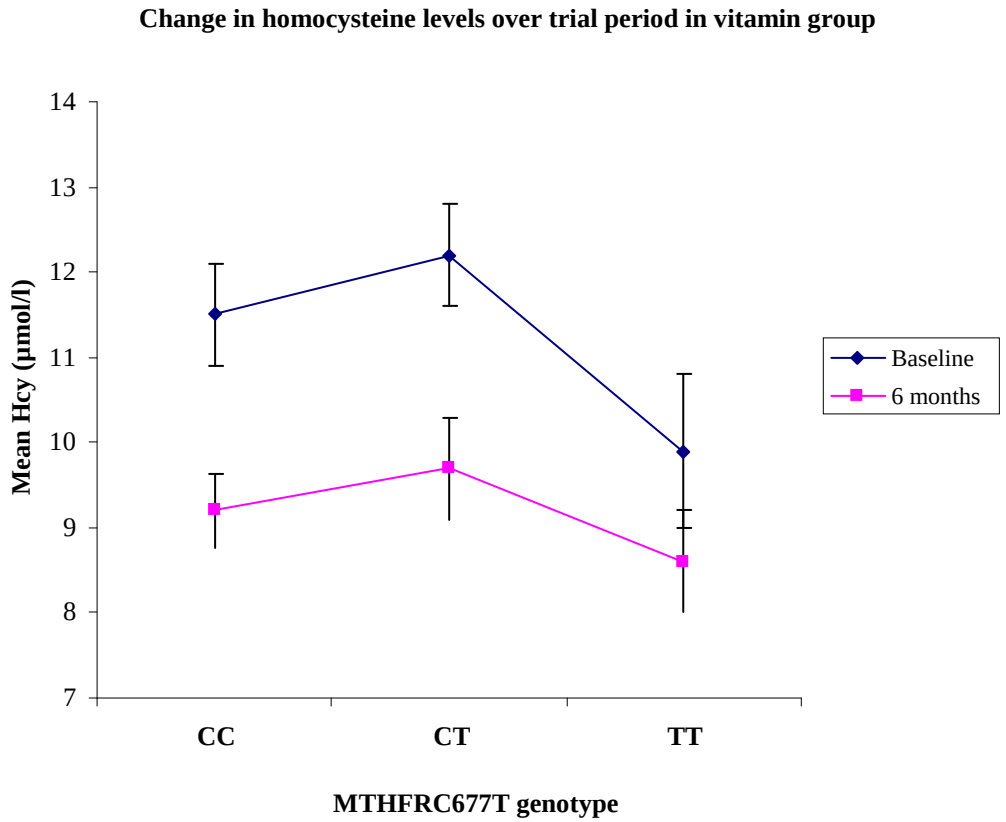
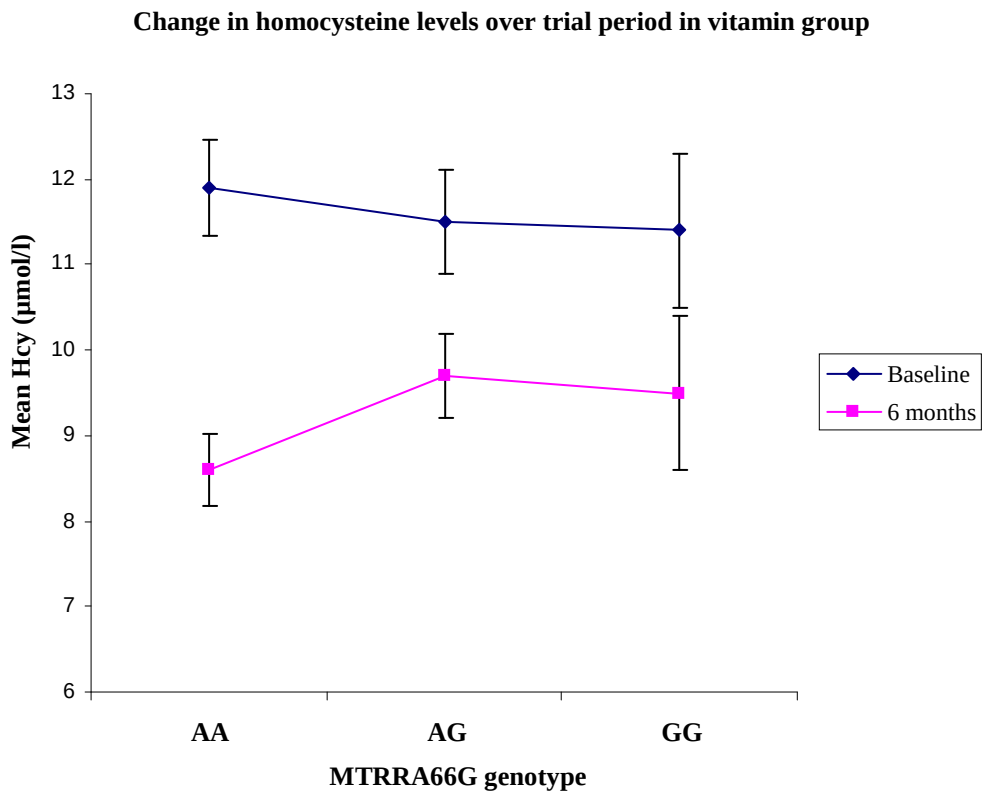


Figure 2



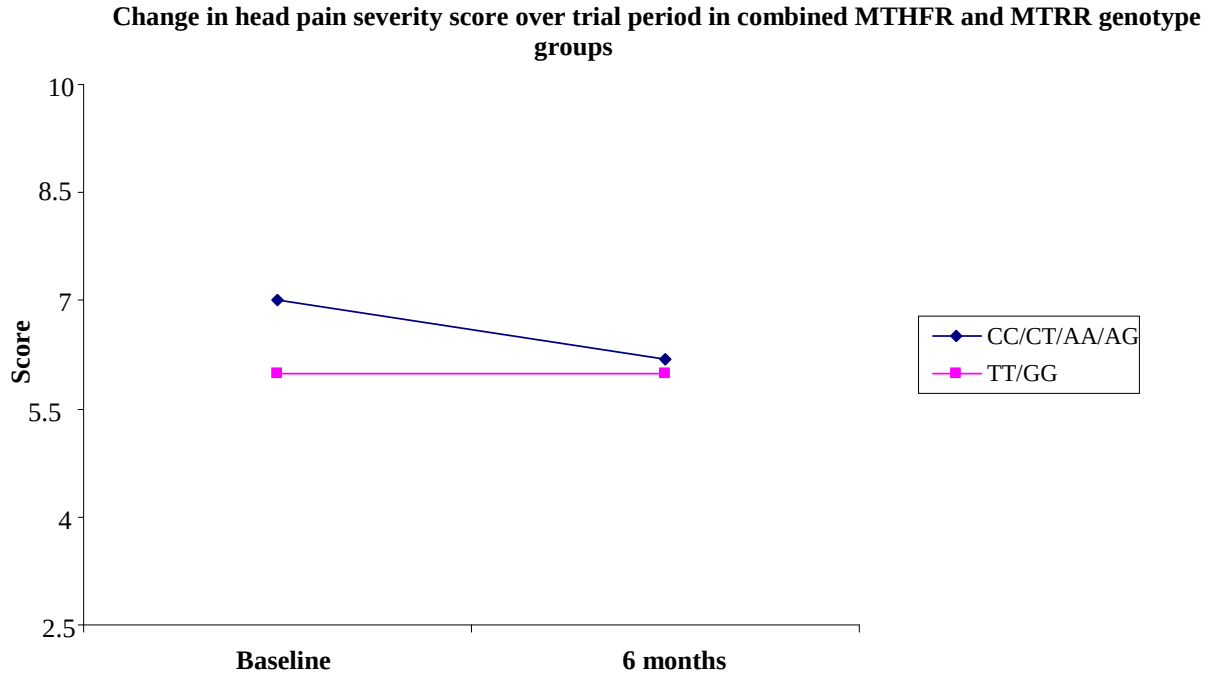
Change in homocysteine levels over the treatment period in vitamin treated group stratified by the MTHFRC677T variant. Values are represented as mean ± SEM.

Figure 3



Change in homocysteine levels over the treatment period in vitamin treated group stratified by the MTRRA66G variant. Values are represented as mean ± SEM.

Figure 4

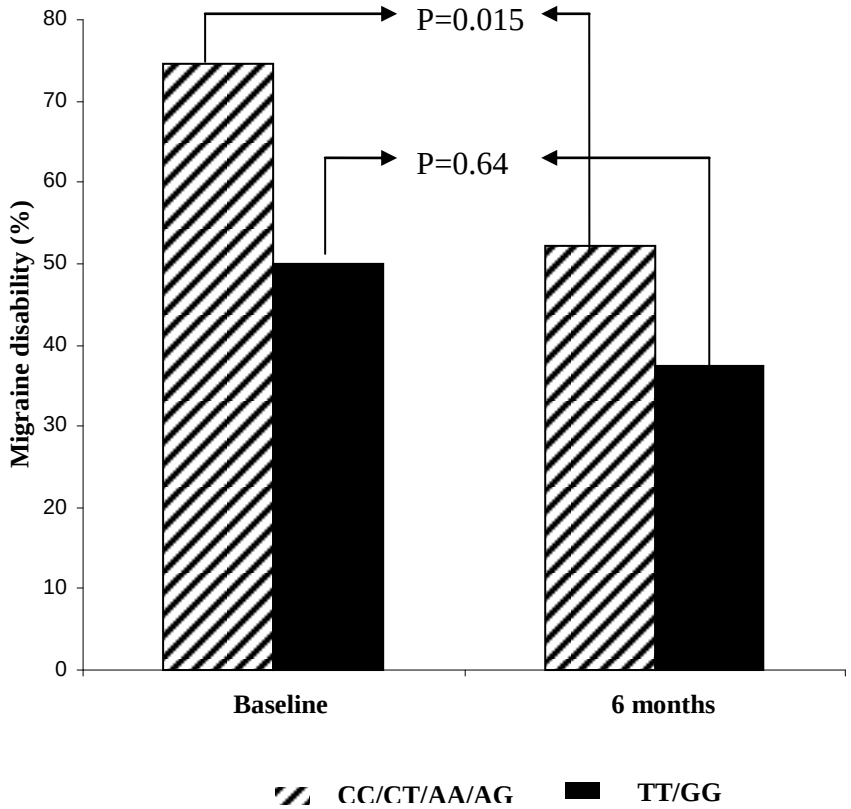


Change in average pain score over treatment period for vitamin treated group with the MTHFR677, CC and CT genotypes combined with the MTRR66 AA and AG genotypes group and the MTHFR667 TT genotype combined with the MTRR 66 GG genotype group. Quartiles are not shown but reductions are statistically significant ($P<0.05$).

5.4
Change in head pain severity score after trial in

igure 5

Change in high migraine disability in MTHFR and MTRR genotype groups



Change in frequency of high-level of migraine disability as measured by the Migraine Disability Score (MIDAS) instrument (MIDAS> 11) over the treatment period in the MTHFR677, CC and CT genotypes combined with the MTRR66 AA and AG genotypes group and the MTHFR667 TT genotype combined with the MTRR 66 GG genotype group.