The impact of MTHFR 677 C/T genotypes on folate status markers: a meta-analysis of folic acid intervention studies

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Key Words

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Abstract

Purpose: Inadequate folate status has been associated with increased risk of certain cancers, cardiovascular disease, cognitive function and neural tube defect births whereas excessive intake of the folic acid has been linked to the progression of existing cancers. Methylenetetrahydrofolate reductase (MTHFR) is a key folate pathway enzyme with the T variant of the *MTHFR* gene increasing the risk for low folate status, particularly coupled with low folate intake. Food fortification with folic acid has improved folate status and decreased neural tube defect occurrence, but there are concerns regarding potential adverse effects of excessive intake. As genetic variability of *MTHFR* influences folate status, it is important to determine an adequate intake that overrides genetic effects but minimises any adverse effects. Our aim was to assess the influence of *MTHFR* genotype on baseline folate status, and to determine the response to folate supplementation (folic acid and folic acid + food folate) according to *MTHFR* genotype.

Methods: We performed a meta-analysis of folate intervention studies to assess the degree to which *MTHFR* C677T genotype influenced folate status and further to examine any genotype effect after supplementation.

Results: The *MTHFR* 677TT genotype was associated with higher plasma homocysteine and lower serum folate at baseline. Plasma homocysteine and serum folate levels were and less serum folate response to supplementation. After

Conclusions: A daily folic acid intake of 400ug appears to be sufficient to override the effects of *MTHFR* genotype on homocysteine and plasma folate levels. Further research is needed to determine the genetic influence on higher intakes recommended for pregnancy.

Introduction

Adequate folate status reduces the risk of neural tube defect births [1-3], and may protect against certain cancers [4,5] cognitive dysfunction [6-8], and coronary heart disease [9]. Folate status is an important determinant of homocysteine (Hcy) levels [10] which, when elevated, increase the risk of cardiovascular disease [11]. Supplementation with folic acid, the synthetic folate vitamer, has been shown to reduce homocysteine levels [10,12,13] and improve vascular function [14,15], although this does not always translate into reduced risk of disease, for example cardiovascular disease [16] and stroke [17].

While there is considerable evidence to indicate that adequate folate status is associated with reduction in risk of certain diseases, there is some suggestion that a high intake of folic acid may increase disease risk. For example, while high folate status is associated with reduced risk of colorectal cancer [4], an increased risk has been demonstrated with higher circulating folates [18] and higher intake of folic acid [19]. In fact, it has been hypothesised that the increasing rates of colorectal cancer since introduction of folic acid food fortification may be related [20]. Evidence suggests that folate (as dietary and/or supplemental folic acid) may be able to prevent neoplasm [21], however once pre-neoplastic lesions have developed, it may exert the opposite effect [22]. High intake of folic acid is known to mask B₁₂ deficiency and potentially aggravate it [23]. High folate and low B₁₂ levels during pregnancy have been associated with higher adiposity and insulin resistance in offspring [24,25]. Use of a

supplement along with intake from fortified food can result in a chronic high intake. With intakes of folic acid at a level of 400µg per day, the enzymatic reduction to 5-methyl-THF is saturated and measurable levels of unmetabolised folic acid can be detected in blood serum [26]. This has been associated with decreased natural killer cell cytotoxicity, important in the immune response to cancer cells, with a trend toward lower natural killer cell cytotoxicity with greater amounts of folic acid in serum [27] although this was not confirmed in in-vitro studies [28].

The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) plays a key role in folate metabolism converting 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, the main form of circulatory folate and the methyl donor for the conversion of homocysteine to methionine [29]. The 677C-T mutation in the *MTHFR* gene, which results in an alanine to valine (A222V) substitution in exon 4, causes reduced enzyme activity and increased thermolability, particularly in folate deficient states [30]. This likely occurs because folate protects against loss of the essential flavin cofactor in the variant enzyme [31]. Individuals homozygous for the mutation have significantly elevated plasma Hcy levels [32,33] and lower plasma folate levels [34-36]. The frequency of the variant T allele exists across populations of different ethnicities, ranging from 12% in African Americans to 46% in Italians [37]. It has been noted that the biological impact is consistent across the racial boundaries [38]. The *MTHFR* 677T variant has been implicated in several diseases and conditions such as heart disease, vascular disease, type 2 diabetes mellitus, certain cancers, and migraine among others [39-45]. Not surprisingly, meta-analyses have implicated the *MTHFR* 677T variant for involvement in an increased risk of neural tube defects [46,47].

Due to the relationship between neural tube defects and inadequate folate status, many countries including the United States, Canada and Australia have adopted mandatory folic acid fortification of cereal grain products. Some countries have voluntary but not mandatory fortification [48]. Studies in countries with mandatory fortification have reported increased serum folate and reduced Hcy levels at the population level [49,50] and reduced rates of neural tube defects [51,52]. Nevertheless, other studies have reported that despite food fortification with folic acid, certain subgroups of the population, particularly young women of child-bearing age, continue to have sub-optimal intake of folates [53,54]. There is uncertainty about sufficient intake for individuals with the *MTHFR* 677TT genotype, who likely have

increased requirements compared to those with the CC and CT genotypes [36], particularly with low folate status [55]. It has been reported that at both low ($<122\mu\text{g}/1000\text{kcal}$) and high ($>122\mu\text{g}/1000\text{kcal}$) intake of dietary folates, TT individuals had significantly lower plasma folate and higher Hcy than all others (CT + CC). Further, the higher intake did not result in improved Hcy and serum folate [56]. A study in Mexican American men suggested that $400\mu\text{g}$ per day as dietary folate equivalents (DFE), a standardised method to adjust for bioequivalence between natural and synthetic folates [57], was not sufficient for TT individuals [58]. Thus there is a need to establish an adequate minimum intake that incorporates the requirements of genetic variability. It should override any modulatory effect of the deficiency in the MTHFR enzyme and normalise folate status as well as minimise potential side effects of chronic high doses of folic acid. This is particularly important as genetic selection for *MTHFR* variants has been suggested due to increased folic acid intake during pregnancy resulting in protection of embryos with deleterious genotypes [59]. This would increase the number of individuals in the population with *MTHFR* variant genotypes which have been associated with increased risk of disease [39-47]. Intake recommendations for folate vary around the world with some countries recommending an additional intake of $400\mu\text{g}$ of folic acid per day for women who are capable of becoming pregnant or whose diet is inadequate [60].

Thus the aim of our study was firstly to determine the level at which the *MTHFR* C677T genotype influenced folate status as evidenced by baseline measurement of homocysteine and plasma folate levels, and further to determine the impact of folic acid or folic acid + food folate supplementation according to *MTHFR* C677T genotype. Although a number of individual studies have been undertaken to examine this, a single study may lack the power to fully examine the complex nutrigenetic relationship due to small sample size. A meta-analysis may override these limitations. To our knowledge, this is the first meta-analysis of folate intervention studies that has been undertaken to examine the response on folate status in both males and females according to genotype.

Methodology

A systematic literature search of published studies was undertaken. The databases PubMed, Scopus, Cochrane Library, and Cumulative Index to Nursing and Allied Health Literature (CINAHL) were searched for studies published in English up to Jun 2015, using the following Standard Medical Subject Headings (MeSH), without imposing any time limitation: **MTHFR, methylenetetrahydrofolate reductase, 5, 10-methylenetetrahydrofolate reductase and folate or folic acid**. References from all relevant literature were hand searched and used to identify any additional relevant studies. The results of the search strategy are summarised in figure 5. There were 2542 articles initially identified. From those, 2161 were not relevant, leaving 381 articles that required closer inspection by examining the abstract and full text if necessary. From this inspection, there were 28 potential studies for the meta-analysis. Of the 26 studies, 16 were unsuitable because of lack of genotyping data or folate measurements, unsuitability of methodology, potential interaction of other medications/supplements with folate status, supplementation greater than 1µg folic acid/day and data reported came that from another included study. Ten studies were considered potentially suitable for the meta-analysis. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis: The PRISMA Statement were followed as a guideline for conducting this systematic review and meta-analysis [61].

To be eligible for the data to be included in the meta-analysis, studies needed to be human intervention supplementation studies of folic acid or folic acid plus a folic acid fortified diet and/or natural food folates (but not natural food folates alone due to variability in bioavailability due to processing/storage etc.). Studies needed to include both baseline and end measures of plasma or serum Hcy and/or folate categorised by *MTHFR* C677T genotype group. To minimise differences between countries with fortification and those without, studies were excluded if depletion diets for a minimum of 6 weeks were not undertaken prior to intervention in countries in which there was mandatory folic acid fortification. This should better represent baseline folate status from typical diets that are not fortified under mandatory fortification conditions. Studies could be either a randomised controlled trial (parallel or crossover) or a quasi-experimental trial, with or without placebo, of at least a 4 week period. The minimum dose was 400 µg DFEs as folic acid or equivalent folic acid + food daily as this dose is a commonly adopted nutrient intake value, and recommended supplement amount for women capable of becoming pregnant [60]. The maximum dose was 1000 µg of folic acid per day as this is considered the upper tolerable limit [62,63]. Participants needed to be

at least 18 years old without chronic illness that would affect folate status, and not pregnant. Participants could not be taking any supplements or medications that would affect folate status just prior to and during the study. Publications needed to be in English. There was no restriction on ethnicity due to the common effect of the *MTHFR* variant [38].

Two researchers conducted the initial literature screening independently. The decision regarding inclusion or exclusion of articles was made through agreement between the two researchers. A third researcher was involved in the decision making in case of any disagreement. This occurred with one study, which was excluded due to . The next phase involved a review of abstracts and an examination of the full text in terms of the eligibility criteria. The screening process involved review of the title, abstract and full text of literature. A summary of the review is presented in the study flow diagram (Figure 1). Included articles were reviewed to assess their publication bias and extract relevant data.

Data Extraction

The ‘checklist of items to consider in data collection’ from the *Cochrane Handbook for Systematic Review of Interventions* was followed to extract relevant data [64]. Characteristics of the included studies are presented in Table 1. The following information was extracted from each study that was considered eligible: the first author’s name, the year of publication, the sample size, gender and ethnicity of participants, the study design, the genotyping method, the genotype frequency, the folic acid supplementation dose, the length of the intervention, mean and standard deviation (SD) for baseline and post-treatment measures of plasma or serum Hcy and folate in the genotype categories. Where SDs were not available, they were imputed according to the method described by Furakawa et al, 2006 [65]. All plasma Hcy measures were in μ moles per litre (L). All serum folate measures were reported in standard international (SI) units of nmoles per L. Any that were reported in nanograms per L were converted using the formula described in the NHANES [66]: Serum and red cell folate in ng/mL to SI units (nmol/L), multiply by 2.265.

Following the data eligibility criteria, the data were excluded for this meta-analysis study if folate status after intervention according to *MTHFR* C677T genotype was not reported; if taking other supplements along with the folic acid; if the dose was lower than 400 μ g folic acid daily; if supplementation involved only natural folates; if participants were taking

medications or had illnesses that may have affected folate status; if data was from a study already included in the meta-analysis.

After exclusion of collected data, 10 studies were included in the meta-analysis, 6 were randomised controlled trials and 4 were quasi-experimental trials (**see table 1**). Although randomised controlled trials are generally considered the design of choice for the assessment of the effectiveness of health related interventions, there are many instances where non-randomised studies have either been sufficient to demonstrate effectiveness or have arrived at similar results [67]. While the Cochrane methodology for meta-analyses does not recommend combining randomised and non-randomised studies, it can be argued that in order to examine the effect of folate supplementation on the measurable outcomes of folate status, despite obvious differences in the methodology and risk of bias in the two types of studies, it is acceptable to include both in the analysis [68]. Notably, where randomised controlled trials compared naturally occurring food folate to folic acid supplementation, measures for the folic acid supplementation group only were used.

Among the 10 studies, four studies recruited healthy staff and students from local universities [69-72] or a university medical centre [73]. One study recruited hypertensive individuals from hospitals in China [74] while another recruited individuals who had suffered miscarriages [75]. The remaining three studies did not disclose recruitment strategies [76-78]. All but one study [71], described details of screening and exclusion criteria, such as pregnancy, chronic disease, anaemia, B₁₂ deficiency, and alcohol misuse.

Laboratory methods

All studies took fasting blood samples to determine Hcy and folate status. To determine plasma total Hcy, all studies used a version of the high performance liquid chromatography (HPLC) fluorometric method and all reported quality control methods. For those with serum folate data (8 studies), to determine measurements, one study used a radioactive assay techniques [75], one used a competitive protein binding method [72], three used chemiluminescent immunoassay [76,71,74], and three used microbial assays [69,78,73]. All reported quality control methods. Due to the reported variation in blood folate measurements between different assay methods [79], a student *t*-test of measures performed by chemiluminescent immunoassay compared to microbial assay was undertaken for all

genotype groups pre and post supplementation. There were no significant differences in any of the comparisons (Baseline CC $p=0.91$, CT $p=0.32$, TT $p=0.36$; Post supplementation CC $p=0.32$, CT $p=0.61$, TT $p=0.32$) For *MTHFR* genotyping, all but 2 studies used PCR and restriction fragment length polymorphism genotyping using *HinfI*, originally described by Frosst et al. (1995) [32]. Ashfield-Watt used heteroduplex analysis and Crider used allele specific hybridisation [73,72]. Only Crider described genotyping quality control strategies.

Statistical Analysis

Data were analysed using the Cochrane Collaboration's software, Review Manager (Revman) 5.3 [68]. Measures of serum homocysteine and serum or plasma folate were analysed as continuous variables. As we were particularly interested in the effect in the homozygous carriers of the T allele, we applied 2 genetic models comparing TT carriers against either all other carriers (CC + CT) or CC homozygotes. We named these two models as Recessive Model (RM, TT vs CT and CC) and Homozygote Model (HM, TT vs CC), and the names will be used in the rest of this paper. Weighted averages were calculated to produce the comparison groups if not reported directly.

As the units of measurement were the same across the trials, the overall comparison was reported by the mean difference [80]. To account for the heterogeneity of the studies for the comparisons, the DerSimonian-Laird random effects model was applied to the analysis. This random effects model assigns a weight to each study, inversely proportional to the within-study sampling variance. The weights reflect the relative "value" of the information provided in a study, and allows for diversity between the different studies in the meta-analysis [81,82].

To examine if the results of the meta-analysis could have depended on a few studies with special characteristics, sensitivity and subgroup analysis was undertaken. The total effect of each study on the overall results of the meta-analysis was examined, as well as adding and removing female only studies [77,69,78,71,83,73], folic acid doses that differed from 400µg daily [84,76,75], those that chose for mild hyperhomocysteinaemia [85,70], those that supplemented with folic acid for more than 8 weeks [75,72,70,73,76], quasi-experimental trials [69,78,75,71] so that only randomised controlled trials were analysed together according to the method described by Kavoura et al. [86]. Studies in Chinese and Taiwanese populations [74,73] were also analysed in a subgroup due to genetic similarity [87].

Results

Quality Analysis

A methodological quality analysis was developed based on a modified version of Downs and Black's checklist [88], and the American Dietetic Association Research Design and Implementation checklist for primary research [89]. Downs and Black's checklist was modified to include a question on comparison of baseline characteristics as advised by Deeks et al (2003) in their report on evaluating non-randomised intervention studies [67]. This resulted in a 30 question scored checklist examining potential flaws in the design of each study that could bias the overall results of the meta-analysis. The checklist examined several aspects of reporting (description of the research question, protocol, intervention, findings) external validity (whether the treatment group was representative), internal validity (whether there was bias in randomisation, blinding of participants and investigators, compliance, potential for reporting bias), and statistical and genetic analysis methodology. In addition, if genotype selection was not involved, studies were assessed for Hardy Weinberg Equilibrium (HWE) using the goodness of fit chi-squared test (Supplemental table 1). The methodology quality of included articles was also assessed using the Rosendal scale [90]. An overall Rosendal score of 60% was regarded as an excellent methodology quality [91]. A total of twelve studies (1875 participants) were included in qualitative synthesis and quantitative synthesis (Supplemental table 2). Zappacosta et al. had the lowest Rosendal score of 53% [92], and the highest Rosendal score of 86% belonged to Miyaki et al. study [93].

Baseline Plasma Homocysteine

Figure 2 shows that individuals with the TT genotype (n=418) had a statistically significant higher mean of +2.79 $\mu\text{m/L}$ [95% CI 1.70-3.89] for plasma Hcy, compared to those with in the CC/CT genotype group (n=1564) in the dominant genetic model. In the homozygous model individuals with the TT genotype (n=425) had a statistically significant higher mean of +2.93 $\mu\text{m/L}$ [95% CI 1.72-4.15]. Notably, the TT genotype group in 4 of the studies had mean Hcy levels indicative of hyperHcy (>15 $\mu\text{m/L}$) [85,84,74,70], compared to none in the CC/CT genotype group.

The subgroup and sensitivity analysis in table 2 showed that limiting studies to randomised controlled trials had a slightly stronger effect at 3.36 $\mu\text{m/L}$ [2.03-4.69] for the dominant model and 3.88 $\mu\text{m/L}$ [2.17-5.58] for the homozygous model. Results were still significant when limited to only those who supplemented with 400 μg of folic acid per day, and when studies who selected for HyperHcy were removed. The mean difference was notably higher in the Chinese and Taiwanese subgroup analyses at 5.63 $\mu\text{m/L}$ [2.41-8.81] and 6.08 $\mu\text{m/L}$ [2.68-9.48].

Post Intervention Plasma Homocysteine

Figure 3 illustrates combined results of 9 studies, analysed using a dominant genetic model and 8 studies analysed using a homozygous model. Both models showed no significant difference in mean plasma Hcy between the TT genotype group (n=234) and the CC/CT genotype group (n=877) or the CC genotype group (n = 383) after supplementation with a minimum of 400 μg folic acid. No studies in the either genotype group had mean Hcy levels indicative of hyperHcy (>15 $\mu\text{m/L}$).

The subgroup and sensitivity analysis in table 3 showed significant results only in the Chinese and Taiwanese subgroups however, both the dominant and homozygous models weighted the Crider study at 97.8% and 99.8% respectively. Thus this result represents one study only and should be interpreted with caution.

Baseline Serum Folate

Figure 4 shows results of 9 studies, analysed using dominant and homozygous genetic models. The TT group (n = 302) had a statistically significant lower mean of 2.22 nmol/L [95% CI 1.08-3.35] for serum folate, compared to those in the CC or CT group (n=944) and 3.20 nmol/L [95% CI 1.79-4.62] compared to those in the CC group.

The subgroup and sensitivity analysis in table 4 shows that the effect remained for all subgroups except the Chinese and Taiwanese population analysis which weighted the Crider study at 60.8% and 87.1% respectively.

Figure 5 illustrates results of 8 and 9 studies analysing post intervention folate using dominant and homozygous genetic models. Results were significant in both genetic models showing that the TT genotype group (n=275) had lower mean serum folate at 6.24 nmol/L [95% CI 3.78-8.70] when compared to the CT/CC genotype group (n=793) and 7.26 nmol/L [95% CI 4.55-9.97] when compared to the CC genotype group (n=308) after supplementation with folic acid.

The subgroup and sensitivity analysis in table 5 shows that the effect remained strong for all subgroups, and was even stronger when studies that supplemented for >8 weeks were analysed together at 9.12 nmol/L [95% CI 6.45-11.80] for the recessive model and 11.30 nmol/L [95% CI 8.19-14.41] for the homozygous model. The Chinese and Taiwanese population analysis weighted Crider study at 60.8% and 87.1% respectively.

Discussion

The aim of this meta-analysis was to take a nutrigenetic approach to determine the minimum daily folic acid or DFE intake needed to optimise individual folate status based on *MTHFR* 677 genotype. In particular, we were interested in individuals with the TT genotype which has been associated with reduced folate status [34-36].

We applied two genetic models that were considered appropriate to the *MTHFR* 677 genetic locus; a recessive model where the T allele is completely recessive to the C allele; and a homozygous model where the TT genotype is compared to the CC genotype [94]. These models were chosen as the thermolabile variant is believed to be inherited in an autosomal recessive manner, and individuals with the TT genotype have a greater risk for hyperHcy [95]. The link between hyperHcy and the TT genotype has also been reported in several studies [56,96,58,33], particularly combined with low folate intake.

Overall the baseline results showed that groups with the TT genotype had statistically significant higher plasma Hcy levels compared to the CC and CT genotype (recessive model) at 2.79 $\mu\text{m/L}$ and at 2.93 $\mu\text{m/L}$ when compared to the CC genotype (homozygote model). In four of the studies analysed, the TT groups had Hcy levels >15 $\mu\text{m/L}$ indicating hyperHcy. This was not seen in the other genotype groups. The subgroup analysis showed an even stronger effect in Chinese and Taiwanese populations at differences of 5.63 $\mu\text{m/L}$ (TT vs

CT/CC) and 6.08 $\mu\text{m/L}$ (TT vs CC), with Hcy levels $>15 \mu\text{m/L}$ in TT groups in 3 of the 4 studies. A high prevalence of hyperHcy in Chinese populations has been previously described [97]. In fact, a 2015 meta-analysis of 60,754 subjects reported a prevalence of 27.5%, with low dietary folate proposed as an important determinant [98]. As the MTHFR T variant interacts with dietary folate, this may partly explain our findings in this subgroup. With regard to serum folate, the TT groups had significantly lower levels compared to the CC and CT groups at 2.22 nmol/L (TT vs CT/CC) and 3.20 nmol/L (TT vs CC), although none of the groups had folate levels $<7 \text{ nmol/L}$, which indicates deficiency.

After supplementation with folic acid, there were no longer significant differences in plasma Hcy levels between the genotype groups in both genetic models. Subgroup analysis of studies that supplemented with 400 $\mu\text{g/day}$ of folic acid (or equivalent DFEs) indicated that 400 $\mu\text{g/day}$ was a sufficient supplementary dose to ameliorate any genetic effects of the TT genotype on Hcy levels at baseline. The differences in serum folate remained after supplementation. In fact, they became stronger. This effect was even greater when studies that supplemented for more than 8 weeks were analysed together. This suggested that individuals with the TT genotype did not respond as well to folic acid supplementation even if supplementation was undertaken for more than 8 weeks.

While folate status is an important determinant of Hcy levels, it should be noted that higher levels are associated with as male gender, smoking, coffee consumption, older age, high blood pressure, and unfavourable lipid profile, while physical activity and moderate alcohol consumption, have been associated with lower Hcy levels [99]. Nevertheless, the genetic impact appears to be strong, particularly in younger individuals. A 2007 Danish twin study of 1206 healthy twins determined that Hcy has a heritability of 63% in twins up to 39 years old and 27% in those older and that almost all of the genetic component was attributable to the *MTHFR* gene [100]. Because of the strong genetic architecture influencing Hcy in younger individuals, it would have been interesting to analyse subgroups according to younger age, however only two studies restricted the age of their participants to under 40 years [78,77]. It would also be interesting to examine the influence of a range of supplementation doses on response according to genotype as individuals who are homozygous for the wild-type allele may respond well to a much lesser dose, while individuals with two copies of the T allele may need a minimum of 400 $\mu\text{g/day}$ to obtain acceptable folate status. Notably, this study showed that folate status was acceptable even at baseline in CC and CT groups. As these meta-analyses focussed only on supplementation of 400 $\mu\text{g/day}$ or more, they lacked the

sensitivity to detect response to lesser doses. In fact, genetic architecture may explain why study results are inconsistent with regard to response from daily folate intakes of 200 µg/day or less [101-103].

None of the studies included in these meta-analyses included pregnant women. Whether there is any benefit in taking a nutrigenetic approach to folate intake in the periconceptual period and during pregnancy remains unclear. A recent study gave folic acid supplementation according to risk ranking for folate metabolism based on *MTHFR* C677T, A1298C and methionine synthase reductase (*MTRR*) A66G polymorphisms in 7,812 pregnant women in China, and reported reduced risk of pregnancy complications compared to those without supplementation [104]. However as results were reported as case (supplementation) versus control (no supplementation), it was not possible to determine if the effect was simply due to supplementation instead of risk ranking. Recommended intakes of DFEs for pregnancy vary in different countries from 355-800 µg/day [60]. Some countries have recommendations based on various conditions. For example, in Australia, 600 µg/day of DFE is recommended for pregnancy, but for women who have a higher risk of neural tube defect birth, or who are on anti-folate medication, have type 1 diabetes, or a BMI >30 are recommended to take 5 mg of folate daily [105].

There have been suggestions that high doses of folic acid during pregnancy could have some negative consequences. For example alterations of methylation patterns have been noted due to the role of folate in methylation reactions [106,107]. Abnormal methylation has the potential to promote epimutations. Unmetabolised folic acid has been found in fetal cord blood [108]. Consumption of folic acid supplements by pregnant women after 12 weeks of gestation has been linked to epigenetic changes in fetal cord blood including altered methylation of imprinted genes and repeat elements [109]. The phenotypic effects of these changes are unknown. It has also been suggested that high dose folic acid during pregnancy (>1mg) may disrupt brain development and increase the risk for autism spectrum disorders and other behavioural problems [110]. It has been proposed that maternal folate supplementation may help to reduce the incidence of autism in mothers and children with the *MTHFR* 677T variant that leads to inefficient folate metabolism [111], but for individuals with normal folate metabolism, too much folate may increase the risk for autism [112]. If women supplement with folic acid of 600µg/day as well as consume a diet high in natural folates and fortified foods during pregnancy, intakes of folate greater than 1mg/day are likely. A meta-analysis of randomised controlled trials that assessed the effects of folic acid

supplements on blood Hcy concentrations concluded that doses of folic acid >1mg folic acid/day have no additional homocysteine-lowering effect [113] however, whether homocysteine explains the complete picture with regards to neural tube defects is unknown. Certainly there are other genes implicated in neural tube defect births and not all relate to homocysteine [114]. Nevertheless, because of the important role of MTHFR in homocysteine levels in individuals under 40 years old [100], and the emerging concerns of taking high doses of folate during pregnancy, more research on the benefits of genotype specific folate recommendations for pregnancy planning is warranted. For example, it has been shown that women with the TT genotype have significantly higher Hcy and lower red cell folate if they do not commenced folic acid supplementation before conception, compared to those who do. This does not occur in the other genotype groups [115]. Considering that population frequencies of the *MTHFR* 677TT genotype range from 4 to over 30% worldwide [37], further research into the specialised requirements of this genotype would be valuable. Ultimately, individual recommendations should be able to prevent folate responsive neural tube defects, while lowering the risk of complications due to high folate doses.

There are some limitations of this meta-analysis. There were several differences between the studies and these differences were examined by subgroup and sensitivity analysis. For example, seven studies were randomised controls and five studies were quasi-experimental trials. Three of the studies supplemented with doses greater than 400 µg; and five studies included males and females while others included females only. One study included males only. As males appear to have higher Hcy levels overall, sensitivity analysis was particularly important regarding this aspect [100]. Not all of the studies provided measures for plasma Hcy and serum folate for all genotypes, so analyses did not always involve the twelve studies. Although red cell folate would have been a useful measure of baseline tissue folate status, only four studies provided this data, so only serum folate measures only were used. Nevertheless, serum folate measures were also more appropriate to determine folate status after short term supplementation [116]. It should be noted that there were differences in the methods used for serum folate measures which could impact results. In particular, two studies used radio assay approaches which have been shown to produce lower results that are genotype dependent [117]. All studies had reported that participants were not or had not recently taken folic acid supplementation, but one did not report checking for B₁₂ deficiency [71]. The study also lacked the sensitivity to examine *MTHFR* genotype response to a range of folate doses. Problems inherent to the process of meta-analysis should be acknowledged,

including exclusion of potentially valuable data due to missing information from studies, moderate to high heterogeneity of the included studies, and potential publication bias.

Conclusions

In conclusion, our results show that individuals with the *MTHFR* 677TT genotype have significantly higher Hcy levels and lower serum folate levels at baseline and a lower serum folate response to folic acid supplementation than those with the CT and CC genotypes. A minimum of 400 µg/day of folic acid or fortified food/DFEs ensures adequate folate status and adequate homocysteine lowering in individuals with this genotype. Folic acid fortification policies should consider genetic background when determining fortification levels and carefully monitor potential adverse effects of excess intake. More research is needed into the specialist needs of pregnancy.

References

1. Peake JN, Copp AJ, Shawe J (2013) Knowledge and periconceptional use of folic acid for the prevention of neural tube defects in ethnic communities in the United Kingdom: systematic review and meta-analysis. *Birth defects research Part A, Clinical and molecular teratology* 97 (7):444-451. doi:10.1002/bdra.23154
2. Jian M, Wang J, Sun H (2009) [Meta-analysis of effect of intervention with folic acid on neural tube defects]. *Wei sheng yan jiu = Journal of hygiene research* 38 (6):682-684
3. Kim YI (2005) Nutritional epigenetics: impact of folate deficiency on DNA methylation and colon cancer susceptibility. *The Journal of nutrition* 135 (11):2703-2709
4. Sanjoaquin MA, Allen N, Couto E, Roddam AW, Key TJ (2005) Folate intake and colorectal cancer risk: a meta-analytical approach. *International journal of cancer Journal international du cancer* 113 (5):825-828. doi:10.1002/ijc.20648
5. Kato I, Dnistrian AM, Schwartz M, Toniolo P, Koenig K, Shore RE, Akhmedkhanov A, Zeleniuch-Jacquotte A, Riboli E (1999) Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. *British journal of cancer* 79 (11-12):1917-1922. doi:10.1038/sj.bjc.6690305
6. Smach MA, Jacob N, Golmard JL, Charfeddine B, Lammouchi T, Ben Othman L, Dridi H, Bennamou S, Limem K (2011) Folate and homocysteine in the cerebrospinal fluid of patients with Alzheimer's disease or dementia: a case control study. *European neurology* 65 (5):270-278. doi:10.1159/000326301
7. Gilbody S, Lightfoot T, Sheldon T (2007) Is low folate a risk factor for depression? A meta-analysis and exploration of heterogeneity. *Journal of epidemiology and community health* 61 (7):631-637. doi:10.1136/jech.2006.050385
8. Payne ME, Jamerson BD, Potocky CF, Ashley-Koch AE, Speer MC, Steffens DC (2009) Natural food folate and late-life depression. *Journal of nutrition for the elderly* 28 (4):348-358. doi:10.1080/01639360903417181
9. Wang ZM, Zhou B, Nie ZL, Gao W, Wang YS, Zhao H, Zhu J, Yan JJ, Yang ZJ, Wang LS (2012) Folate and risk of coronary heart disease: a meta-analysis of prospective studies. *Nutrition, metabolism, and cardiovascular diseases : NMCD* 22 (10):890-899. doi:10.1016/j.numecd.2011.04.011
10. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH (1993) Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *Jama* 270 (22):2693-2698
11. Wald DS, Wald NJ, Morris JK, Law M (2006) Folic acid, homocysteine, and cardiovascular disease: judging causality in the face of inconclusive trial evidence. *BMJ* 333 (7578):1114-1117. doi:10.1136/bmj.39000.486701.68
12. Bonna KH, Njolstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, Wang H, Nordrehaug JE, Arnesen E, Rasmussen K (2006) Homocysteine lowering and cardiovascular events after acute myocardial infarction. *The New England journal of medicine* 354 (15):1578-1588. doi:10.1056/NEJMoa055227
13. Brouwer IA, van Dusseldorp M, Thomas CM, Duran M, Hautvast JG, Eskes TK, Steegers-Theunissen RP (1999) Low-dose folic acid supplementation decreases plasma homocysteine concentrations: a randomized trial. *The American journal of clinical nutrition* 69 (1):99-104
14. Shirodaria C, Antoniadou C, Lee J, Jackson CE, Robson MD, Francis JM, Moat SJ, Ratnatunga C, Pillai R, Refsum H, Neubauer S, Channon KM (2007) Global improvement of vascular function and redox state with low-dose folic acid: implications for folate therapy in patients with coronary artery disease. *Circulation* 115 (17):2262-2270. doi:10.1161/CIRCULATIONAHA.106.679084
15. Quinlivan EP, McPartlin J, McNulty H, Ward M, Strain JJ, Weir DG, Scott JM (2002) Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease. *Lancet* 359 (9302):227-228

16. Bazzano LA, Reynolds K, Holder KN, He J (2006) Effect of folic acid supplementation on risk of cardiovascular diseases: a meta-analysis of randomized controlled trials. *Jama* 296 (22):2720-2726. doi:10.1001/jama.296.22.2720
17. Miller ER, 3rd, Juraschek S, Pastor-Barriuso R, Bazzano LA, Appel LJ, Guallar E (2010) Meta-analysis of folic acid supplementation trials on risk of cardiovascular disease and risk interaction with baseline homocysteine levels. *The American journal of cardiology* 106 (4):517-527. doi:10.1016/j.amjcard.2010.03.064
18. Van Guelpen B, Hulthén J, Johansson I, Hallmans G, Stenling R, Riboli E, Winkvist A, Palmqvist R (2006) Low folate levels may protect against colorectal cancer. *Gut* 55 (10):1461-1466. doi:10.1136/gut.2005.085480
19. Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS, McKeown-Eyssen G, Summers RW, Rothstein RI, Burke CA, Snover DC, Church TR, Allen JI, Robertson DJ, Beck GJ, Bond JH, Byers T, Mandel JS, Mott LA, Pearson LH, Barry EL, Rees JR, Marcon N, Saibil F, Ueland PM, Greenberg ER, Polyp Prevention Study G (2007) Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. *Jama* 297 (21):2351-2359. doi:10.1001/jama.297.21.2351
20. Mason JB, Dickstein A, Jacques PF, Haggarty P, Selhub J, Dallal G, Rosenberg IH (2007) A temporal association between folic acid fortification and an increase in colorectal cancer rates may be illuminating important biological principles: a hypothesis. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 16 (7):1325-1329. doi:10.1158/1055-9965.EPI-07-0329
21. Roswall N, Olsen A, Christensen J, Dragsted LO, Overvad K, Tjønneland A (2010) Micronutrient intake and risk of colon and rectal cancer in a Danish cohort. *Cancer epidemiology* 34 (1):40-46. doi:10.1016/j.canep.2009.12.012
22. Ulrich CM, Potter JD (2006) Folate supplementation: too much of a good thing? *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology* 15 (2):189-193. doi:10.1158/1055-9965.EPI-152CO
23. Johnson MA (2007) If high folic acid aggravates vitamin B12 deficiency what should be done about it? *Nutrition reviews* 65 (10):451-458
24. Krishnaveni GV, Veena SR, Karat SC, Yajnik CS, Fall CH (2014) Association between maternal folate concentrations during pregnancy and insulin resistance in Indian children. *Diabetologia* 57 (1):110-121. doi:10.1007/s00125-013-3086-7
25. Yajnik CS, Deshpande SS, Jackson AA, Refsum H, Rao S, Fisher DJ, Bhat DS, Naik SS, Coyaji KJ, Joglekar CV, Joshi N, Lubree HG, Deshpande VU, Rege SS, Fall CH (2008) Vitamin B12 and folate concentrations during pregnancy and insulin resistance in the offspring: the Pune Maternal Nutrition Study. *Diabetologia* 51 (1):29-38. doi:10.1007/s00125-007-0793-y
26. Sweeney MR, McPartlin J, Scott J (2007) Folic acid fortification and public health: report on threshold doses above which unmetabolised folic acid appear in serum. *BMC public health* 7:41. doi:10.1186/1471-2458-7-41
27. Troen AM, Mitchell B, Sorensen B, Wener MH, Johnston A, Wood B, Selhub J, McTiernan A, Yasui Y, Oral E, Potter JD, Ulrich CM (2006) Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. *The Journal of nutrition* 136 (1):189-194
28. Hirsch S, Miranda D, Munoz E, Montoya M, Ronco AM, de la Maza MP, Bunout D (2013) Natural killer cell cytotoxicity is not regulated by folic acid in vitro. *Nutrition* 29 (5):772-776. doi:10.1016/j.nut.2012.10.006
29. Lim U, Wang SS, Hartge P, Cozen W, Kelemen LE, Chanock S, Davis S, Blair A, Schenk M, Rothman N, Lan Q (2007) Gene-nutrient interactions among determinants of folate and one-carbon metabolism on the risk of non-Hodgkin lymphoma: NCI-SEER case-control study. *Blood* 109 (7):3050-3059. doi:10.1182/blood-2006-07-034330

30. Ogino S, Wilson RB (2003) Genotype and haplotype distributions of MTHFR677C>T and 1298A>C single nucleotide polymorphisms: a meta-analysis. *Journal of human genetics* 48 (1):1-7. doi:10.1007/s100380300000
31. Guenther BD, Sheppard CA, Tran P, Rozen R, Matthews RG, Ludwig ML (1999) The structure and properties of methylenetetrahydrofolate reductase from *Escherichia coli* suggest how folate ameliorates human hyperhomocysteinemia. *Nature structural biology* 6 (4):359-365. doi:10.1038/7594
32. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, et al. (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nature genetics* 10 (1):111-113. doi:10.1038/ng0595-111
33. Malinow MR, Nieto FJ, Kruger WD, Duell PB, Hess DL, Gluckman RA, Block PC, Holzgang CR, Anderson PH, Seltzer D, Upson B, Lin QR (1997) The effects of folic acid supplementation on plasma total homocysteine are modulated by multivitamin use and methylenetetrahydrofolate reductase genotypes. *Arteriosclerosis, thrombosis, and vascular biology* 17 (6):1157-1162
34. Nishio K, Goto Y, Kondo T, Ito S, Ishida Y, Kawai S, Naito M, Wakai K, Hamajima N (2008) Serum folate and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism adjusted for folate intake. *Journal of epidemiology / Japan Epidemiological Association* 18 (3):125-131
35. de Bree A, Verschuren WM, Bjorke-Monsen AL, van der Put NM, Heil SG, Trijbels FJ, Blom HJ (2003) Effect of the methylenetetrahydrofolate reductase 677C-->T mutation on the relations among folate intake and plasma folate and homocysteine concentrations in a general population sample. *The American journal of clinical nutrition* 77 (3):687-693
36. Yang QH, Botto LD, Gallagher M, Friedman JM, Sanders CL, Koontz D, Nikolova S, Erickson JD, Steinberg K (2008) Prevalence and effects of gene-gene and gene-nutrient interactions on serum folate and serum total homocysteine concentrations in the United States: findings from the third National Health and Nutrition Examination Survey DNA Bank. *The American journal of clinical nutrition* 88 (1):232-246
37. Wilcken B, Bamforth F, Li Z, Zhu H, Ritvanen A, Renlund M, Stoll C, Alembik Y, Dott B, Czeizel AE, Gelman-Kohan Z, Scarano G, Bianca S, Ettore G, Tenconi R, Bellato S, Scala I, Mutchinick OM, Lopez MA, de Walle H, Hofstra R, Joutchenko L, Kavteladze L, Bermejo E, Martinez-Frias ML, Gallagher M, Erickson JD, Vollset SE, Mastroiacovo P, Andria G, Botto LD (2003) Geographical and ethnic variation of the 677C>T allele of 5,10 methylenetetrahydrofolate reductase (MTHFR): findings from over 7000 newborns from 16 areas world wide. *Journal of medical genetics* 40 (8):619-625
38. Ioannidis JP, Ntzani EE, Trikalinos TA (2004) 'Racial' differences in genetic effects for complex diseases. *Nature genetics* 36 (12):1312-1318. doi:10.1038/ng1474
39. Zhang D, Zhou Y, Han L, Ji H, Li J (2014) The effect of MTHFR C677T polymorphism on type 2 diabetes mellitus with vascular complications in Chinese Han population: a meta-analysis. *Endocrine journal* 61 (7):717-726
40. Zhang MJ, Li JC, Yin YW, Li BH, Liu Y, Liao SQ, Gao CY, Zhang LL (2014) Association of MTHFR C677T polymorphism and risk of cerebrovascular disease in Chinese population: an updated meta-analysis. *Journal of neurology* 261 (5):925-935. doi:10.1007/s00415-014-7300-4
41. Zhang P, Gao X, Zhang Y, Hu Y, Ma H, Wang W, Wang H, Zhang J, Xu H, Lu Z (2014) Association Between MTHFR C677T Polymorphism and Venous Thromboembolism Risk in the Chinese Population: A Meta-Analysis of 24 Case-Controlled Studies. *Angiology*. doi:10.1177/0003319714546368
42. Zhao M, Ren Y, Shen L, Zhang Y, Zhou B (2014) Association between MTHFR C677T and A1298C polymorphisms and NSCL/P risk in Asians: a meta-analysis. *PloS one* 9 (3):e88242. doi:10.1371/journal.pone.0088242
43. Yan Y, Han F, Fu H, Xia W, Qin X (2014) Association between MTHFR C677T polymorphism and thyroid cancer risk: a meta-analysis. *Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine* 35 (8):7707-7712. doi:10.1007/s13277-014-2038-2

44. Liu R, Geng P, Ma M, Yu S, Yang M, He M, Dong Z, Zhang W (2014) MTHFR C677T polymorphism and migraine risk: a meta-analysis. *Journal of the neurological sciences* 336 (1-2):68-73. doi:10.1016/j.jns.2013.10.008
45. Badiga S, Johanning GL, Macaluso M, Azuero A, Chambers MM, Siddiqui NR, Piyathilake CJ (2014) A Lower Degree of PBMC L1 Methylation in Women with Lower Folate Status May Explain the MTHFR C677T Polymorphism Associated Higher Risk of CIN in the US Post Folic Acid Fortification Era. *PloS one* 9 (10):e110093. doi:10.1371/journal.pone.0110093
46. Zhang T, Lou J, Zhong R, Wu J, Zou L, Sun Y, Lu X, Liu L, Miao X, Xiong G (2013) Genetic variants in the folate pathway and the risk of neural tube defects: a meta-analysis of the published literature. *PloS one* 8 (4):e59570. doi:10.1371/journal.pone.0059570
47. Yadav U, Kumar P, Yadav SK, Mishra OP, Rai V (2014) "Polymorphisms in folate metabolism genes as maternal risk factor for neural tube defects: an updated meta-analysis". *Metabolic brain disease*. doi:10.1007/s11011-014-9575-7
48. To QG, Chen TT, Magnussen CG, To KG (2013) Workplace physical activity interventions: a systematic review. *American journal of health promotion : AJHP* 27 (6):e113-123. doi:10.4278/ajhp.120425-LIT-222
49. Pfeiffer CM, Caudill SP, Gunter EW, Osterloh J, Sampson EJ (2005) Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999-2000. *The American journal of clinical nutrition* 82 (2):442-450
50. Hickling S, Hung J, Knuiman M, Jamrozik K, McQuillan B, Beilby J, Thompson P (2005) Impact of voluntary folate fortification on plasma homocysteine and serum folate in Australia from 1995 to 2001: a population based cohort study. *Journal of epidemiology and community health* 59 (5):371-376. doi:10.1136/jech.2004.027078
51. Williams LJ, Mai CT, Edmonds LD, Shaw GM, Kirby RS, Hobbs CA, Sever LE, Miller LA, Meaney FJ, Levitt M (2002) Prevalence of spina bifida and anencephaly during the transition to mandatory folic acid fortification in the United States. *Teratology* 66 (1):33-39. doi:10.1002/tera.10060
52. De Wals P, Tairou F, Van Allen MI, Uh SH, Lowry RB, Sibbald B, Evans JA, Van den Hof MC, Zimmer P, Crowley M, Fernandez B, Lee NS, Niyonsenga T (2007) Reduction in neural-tube defects after folic acid fortification in Canada. *The New England journal of medicine* 357 (2):135-142. doi:10.1056/NEJMoa067103
53. Mallard SR, Gray AR, Houghton LA (2012) Periconceptional bread intakes indicate New Zealand's proposed mandatory folic acid fortification program may be outdated: results from a postpartum survey. *BMC pregnancy and childbirth* 12:8. doi:10.1186/1471-2393-12-8
54. Yang QH, Carter HK, Mulinare J, Berry RJ, Friedman JM, Erickson JD (2007) Race-ethnicity differences in folic acid intake in women of childbearing age in the United States after folic acid fortification: findings from the National Health and Nutrition Examination Survey, 2001-2002. *The American journal of clinical nutrition* 85 (5):1409-1416
55. Nagele P, Meissner K, Francis A, Fodinger M, Saccone NL (2011) Genetic and environmental determinants of plasma total homocysteine levels: impact of population-wide folate fortification. *Pharmacogenetics and genomics* 21 (7):426-431. doi:10.1097/FPC.0b013e32834741ff
56. Messika AH, Kaluski DN, Lev E, Iakobishvili Z, Shohat M, Hasdai D, Mager A (2010) Nutrigenetic impact of daily folate intake on plasma homocysteine and folate levels in patients with different methylenetetrahydrofolate reductase genotypes. *European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology* 17 (6):701-705. doi:10.1097/HJR.0b013e32833a1cb5
57. Bailey LB (1998) Dietary reference intakes for folate: the debut of dietary folate equivalents. *Nutrition reviews* 56 (10):294-299
58. Solis C, Veenema K, Ivanov AA, Tran S, Li R, Wang W, Moriarty DJ, Maletz CV, Caudill MA (2008) Folate intake at RDA levels is inadequate for Mexican American men with the methylenetetrahydrofolate reductase 677TT genotype. *The Journal of nutrition* 138 (1):67-72

59. Reyes-Engel A, Munoz E, Gaitan MJ, Fabre E, Gallo M, Dieguez JL, Ruiz M, Morell M (2002) Implications on human fertility of the 677C-->T and 1298A-->C polymorphisms of the MTHFR gene: consequences of a possible genetic selection. *Molecular human reproduction* 8 (10):952-957
60. Stamm RA, Houghton LA (2013) Nutrient intake values for folate during pregnancy and lactation vary widely around the world. *Nutrients* 5 (10):3920-3947. doi:10.3390/nu5103920
61. Moher D, Liberati A, Tetzlaff J, Altman DG (2009) Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of internal medicine* 151 (4):264-269
62. Australian Government Department of Health Nutrient Reference Values 2006. Available <http://www.nrv.gov.au/home>.
63. European Food Safety Authority. Folic Acid: An update on scientific developments. 21-22 January 2009, Uppsala, Sweden. ISBN: 978-92-9199-178-5.
64. Higgins J, Deeks JJ, Altman DG (2008) Special topics in statistics. *Cochrane handbook for systematic reviews of interventions: Cochrane book series*:481-529
65. Furukawa TA, Barbui C, Cipriani A, Brambilla P, Watanabe N (2006) Imputing missing standard deviations in meta-analyses can provide accurate results. *Journal of clinical epidemiology* 59 (1):7-10. doi:10.1016/j.jclinepi.2005.06.006
66. Wong JY, Gilson ND, van Uffelen JG, Brown WJ (2012) The effects of workplace physical activity interventions in men: a systematic review. *American journal of men's health* 6 (4):303-313. doi:10.1177/1557988312436575
67. Deeks JJ, Dinnes J, D'Amico R, Sowden AJ, Sakarovich C, Song F, Petticrew M, Altman DG (2003) Evaluating non-randomised intervention studies. *Health Technol Assess* 7 (27):iii-x, 1-173
68. Higgins J, Green S *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.1.0 [updated March 2011]. The Cochrane Collaboration, 2011. Available from www.cochrane-handbook.org.
69. Guinotte CL, Burns MG, Axume JA, Hata H, Urrutia TF, Alamilla A, McCabe D, Singgih A, Cogger EA, Caudill MA (2003) Methylenetetrahydrofolate reductase 677C-->T variant modulates folate status response to controlled folate intakes in young women. *The Journal of nutrition* 133 (5):1272-1280
70. Zappacosta B, Mastroiacovo P, Persichilli S, Pounis G, Ruggeri S, Minucci A, Carnovale E, Andria G, Ricci R, Scala I, Genovese O, Turrini A, Mistura L, Giardina B, Iacoviello L (2013) Homocysteine lowering by folate-rich diet or pharmacological supplementations in subjects with moderate hyperhomocysteinemia. *Nutrients* 5 (5):1531-1543. doi:10.3390/nu5051531
71. Hiraoka M, Kato K, Saito Y, Yasuda K, Kagawa Y (2004) Gene-nutrient and gene-gene interactions of controlled folate intake by Japanese women. *Biochemical and biophysical research communications* 316 (4):1210-1216. doi:10.1016/j.bbrc.2004.02.174
72. Ashfield-Watt PA, Pullin CH, Whiting JM, Clark ZE, Moat SJ, Newcombe RG, Burr ML, Lewis MJ, Powers HJ, McDowell IF (2002) Methylenetetrahydrofolate reductase 677C-->T genotype modulates homocysteine responses to a folate-rich diet or a low-dose folic acid supplement: a randomized controlled trial. *The American journal of clinical nutrition* 76 (1):180-186
73. Crider KS, Zhu JH, Hao L, Yang QH, Yang TP, Gindler J, Maneval DR, Quinlivan EP, Li Z, Bailey LB, Berry RJ (2011) MTHFR 677C->T genotype is associated with folate and homocysteine concentrations in a large, population-based, double-blind trial of folic acid supplementation. *The American journal of clinical nutrition* 93 (6):1365-1372. doi:10.3945/ajcn.110.004671
74. Qin X, Li J, Cui Y, Liu Z, Zhao Z, Ge J, Guan D, Hu J, Wang Y, Zhang F, Xu X, Wang X, Huo Y (2012) Effect of folic acid intervention on the change of serum folate level in hypertensive Chinese adults: do methylenetetrahydrofolate reductase and methionine synthase gene polymorphisms affect therapeutic responses? *Pharmacogenetics and genomics* 22 (6):421-428. doi:10.1097/FPC.0b013e32834ac5e8
75. Nelen WL, Blom HJ, Thomas CM, Steegers EA, Boers GH, Eskes TK (1998) Methylenetetrahydrofolate reductase polymorphism affects the change in homocysteine and folate

concentrations resulting from low dose folic acid supplementation in women with unexplained recurrent miscarriages. *The Journal of nutrition* 128 (8):1336-1341

76. Miyaki K, Murata M, Kikuchi H, Takei I, Nakayama T, Watanabe K, Omae K (2005) Assessment of tailor-made prevention of atherosclerosis with folic acid supplementation: randomized, double-blind, placebo-controlled trials in each MTHFR C677T genotype. *Journal of human genetics* 50 (5):241-248. doi:10.1007/s10038-005-0247-7

77. Fohr IP, Prinz-Langenohl R, Bronstrup A, Bohlmann AM, Nau H, Berthold HK, Pietrzik K (2002) 5,10-Methylenetetrahydrofolate reductase genotype determines the plasma homocysteine-lowering effect of supplementation with 5-methyltetrahydrofolate or folic acid in healthy young women. *The American journal of clinical nutrition* 75 (2):275-282

78. Shelnutt KP, Kauwell GP, Chapman CM, Gregory JF, 3rd, Maneval DR, Browdy AA, Theriaque DW, Bailey LB (2003) Folate status response to controlled folate intake is affected by the methylenetetrahydrofolate reductase 677C-->T polymorphism in young women. *The Journal of nutrition* 133 (12):4107-4111

79. Nakazato M, Maeda T, Emura K, Maeda M, Tamura T (2012) Blood folate concentrations analyzed by microbiological assay and chemiluminescent immunoassay methods. *Journal of nutritional science and vitaminology* 58 (1):59-62

80. Takeshima N, Sozu T, Tajika A, Ogawa Y, Hayasaka Y, Furukawa TA (2014) Which is more generalizable, powerful and interpretable in meta-analyses, mean difference or standardized mean difference? *BMC medical research methodology* 14:30. doi:10.1186/1471-2288-14-30

81. DerSimonian R, Laird N (1986) Meta-analysis in clinical trials. *Controlled clinical trials* 7 (3):177-188

82. DerSimonian R, Kacker R (2007) Random-effects model for meta-analysis of clinical trials: an update. *Contemporary clinical trials* 28 (2):105-114. doi:10.1016/j.cct.2006.04.004

83. Hung J, Yang TL, Urrutia TF, Li R, Perry CA, Hata H, Cogger EA, Moriarty DJ, Caudill MA (2006) Additional food folate derived exclusively from natural sources improves folate status in young women with the MTHFR 677 CC or TT genotype. *The Journal of nutritional biochemistry* 17 (11):728-734. doi:10.1016/j.jnutbio.2005.11.009

84. Kasiman K, Eikelboom JW, Hankey GJ, Lee SP, Lim JP, Lee JH, Chang HM, Wong MC, Chen CP (2009) Ethnicity does not affect the homocysteine-lowering effect of B-vitamin therapy in Singaporean stroke patients. *Stroke; a journal of cerebral circulation* 40 (6):2209-2211. doi:10.1161/STROKEAHA.108.535237

85. Liu CS, Chiang HC, Chen HW (2004) Methylenetetrahydrofolate reductase polymorphism determines the plasma homocysteine-lowering effect of large-dose folic acid supplementation in patients with cardiovascular disease. *Nutrition* 20 (11-12):974-978. doi:10.1016/j.nut.2004.08.004

86. Kavvoura FK, Ioannidis JP (2008) Methods for meta-analysis in genetic association studies: a review of their potential and pitfalls. *Human genetics* 123 (1):1-14. doi:10.1007/s00439-007-0445-9

87. Trejaut JA, Poloni ES, Yen JC, Lai YH, Loo JH, Lee CL, He CL, Lin M (2014) Taiwan Y-chromosomal DNA variation and its relationship with Island Southeast Asia. *BMC genetics* 15:77. doi:10.1186/1471-2156-15-77

88. Downs SH, Black N (1998) The feasibility of creating a checklist for the assessment of the methodological quality both of randomised and non-randomised studies of health care interventions. *Journal of epidemiology and community health* 52 (6):377-384

89. Guize L, Thomas F, Pannier B, Bean K, Danchin N, Benetos A (2006) [Metabolic syndrome: prevalence, risk factors and mortality in a French population of 62 000 subjects]. *Bulletin de l'Academie nationale de medecine* 190 (3):685-697; discussion 697-700

90. van Rosendal SP, Osborne MA, Fassett RG, Coombes JS (2010) Guidelines for glycerol use in hyperhydration and rehydration associated with exercise. *Sports Medicine* 40 (2):113-139

91. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJM, Gavaghan DJ, McQuay HJ (1996) Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 17 (1):1-12

92. Zappacosta B, Mastroiacovo P, Persichilli S, Pounis G, Ruggeri S, Minucci A, Carnovale E, Andria G, Ricci R, Scala I (2013) Homocysteine lowering by folate-rich diet or pharmacological supplementations in subjects with moderate hyperhomocysteinemia. *Nutrients* 5 (5):1531-1543
93. Miyaki K, Murata M, Kikuchi H, Takei I, Nakayama T, Watanabe K, Omae K (2005) Assessment of tailor-made prevention of atherosclerosis with folic acid supplementation: randomized, double-blind, placebo-controlled trials in each MTHFR C677T genotype. *Journal of human genetics* 50 (5):241-248
94. Salanti G, Higgins JP (2008) Meta-analysis of genetic association studies under different inheritance models using data reported as merged genotypes. *Statistics in medicine* 27 (5):764-777. doi:10.1002/sim.2919
95. Kang SS, Wong PW, Zhou JM, Sora J, Lessick M, Ruggie N, Grcevic G (1988) Thermolabile methylenetetrahydrofolate reductase in patients with coronary artery disease. *Metabolism: clinical and experimental* 37 (7):611-613
96. Agodi A, Barchitta M, Valenti G, Quattrocchi A, Marchese AE, Oliveri Conti G, Fallico R, Sciacca S, Ferrante M (2013) Dietary folate intake and blood biomarkers reveal high-risk groups in a Mediterranean population of healthy women of childbearing potential. *Annals of nutrition & metabolism* 63 (3):179-185. doi:10.1159/000346962
97. Hao L, Ma J, Zhu J, Stampfer MJ, Tian Y, Willett WC, Li Z (2007) High prevalence of hyperhomocysteinemia in Chinese adults is associated with low folate, vitamin B-12, and vitamin B-6 status. *The Journal of nutrition* 137 (2):407-413
98. Yang B, Fan S, Zhi X, Wang Y, Wang Y, Zheng Q, Sun G (2015) Prevalence of hyperhomocysteinemia in China: a systematic review and meta-analysis. *Nutrients* 7 (1):74-90. doi:10.3390/nu7010074
99. Refsum H, Nurk E, Smith AD, Ueland PM, Gjesdal CG, Bjelland I, Tverdal A, Tell GS, Nygard O, Vollset SE (2006) The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. *The Journal of nutrition* 136 (6 Suppl):1731S-1740S
100. Bathum L, Petersen I, Christiansen L, Konieczna A, Sorensen TI, Kyvik KO (2007) Genetic and environmental influences on plasma homocysteine: results from a Danish twin study. *Clinical chemistry* 53 (5):971-979. doi:10.1373/clinchem.2006.082149
101. Alfthan G, Laurinen MS, Valsta LM, Pastinen T, Aro A (2003) Folate intake, plasma folate and homocysteine status in a random Finnish population. *European journal of clinical nutrition* 57 (1):81-88. doi:10.1038/sj.ejcn.1601507
102. Bart S, Sr., Marr J, Diefenbach K, Trummer D, Sampson-Landers C (2012) Folate status and homocysteine levels during a 24-week oral administration of a folate-containing oral contraceptive: a randomized, double-blind, active-controlled, parallel-group, US-based multicenter study. *Contraception* 85 (1):42-50. doi:10.1016/j.contraception.2011.05.013
103. Schorah CJ, Devitt H, Lucock M, Dowell AC (1998) The responsiveness of plasma homocysteine to small increases in dietary folic acid: a primary care study. *European journal of clinical nutrition* 52 (6):407-411
104. Li X, Jiang J, Xu M, Xu M, Yang Y, Lu W, Yu X, Ma J, Pan J (2015) Individualized supplementation of folic acid according to polymorphisms of methylenetetrahydrofolate reductase (MTHFR), methionine synthase reductase (MTRR) reduced pregnant complications. *Gynecologic and obstetric investigation* 79 (2):107-112. doi:10.1159/000367656
105. Encouraging periconceptional use of folic acid supplement. National Health and Medical Research Council National Institute of Clinical Studies Evidence Practice Gaps Report Volume 2 2005. Available https://www.nhmrc.gov.au/files/nhmrc/file/nics/material_resources/folic_acid_supplements.pdf.
106. Hoyo C, Murtha AP, Schildkraut JM, Jirtle RL, Demark-Wahnefried W, Forman MR, Iversen ES, Kurtzberg J, Overcash F, Huang Z, Murphy SK (2011) Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. *Epigenetics : official journal of the DNA Methylation Society* 6 (7):928-936

107. Penailillo R, Guajardo A, Llanos M, Hirsch S, Ronco AM (2015) Folic acid supplementation during pregnancy induces sex-specific changes in methylation and expression of placental 11beta-hydroxysteroid dehydrogenase 2 in rats. *PloS one* 10 (3):e0121098. doi:10.1371/journal.pone.0121098
108. Sweeney MR, McPartlin J, Weir DG, Daly S, Pentieva K, Daly L, Scott JM (2005) Evidence of unmetabolised folic acid in cord blood of newborn and serum of 4-day-old infants. *The British journal of nutrition* 94 (5):727-730
109. Haggarty P, Hoad G, Campbell DM, Horgan GW, Piyathilake C, McNeill G (2013) Folate in pregnancy and imprinted gene and repeat element methylation in the offspring. *The American journal of clinical nutrition* 97 (1):94-99. doi:10.3945/ajcn.112.042572
110. Beard CM, Panser LA, Katusic SK (2011) Is excess folic acid supplementation a risk factor for autism? *Medical hypotheses* 77 (1):15-17. doi:10.1016/j.mehy.2011.03.013
111. Leeming RJ, Lucock M (2009) Autism: Is there a folate connection? *Journal of inherited metabolic disease* 32 (3):400-402. doi:10.1007/s10545-009-1093-0
112. Rogers EJ (2008) Has enhanced folate status during pregnancy altered natural selection and possibly Autism prevalence? A closer look at a possible link. *Medical hypotheses* 71 (3):406-410. doi:10.1016/j.mehy.2008.04.013
113. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Homocysteine Lowering Trialists' Collaboration (1998). *BMJ* 316 (7135):894-898
114. Imbard A, Benoist JF, Blom HJ (2013) Neural tube defects, folic acid and methylation. *International journal of environmental research and public health* 10 (9):4352-4389. doi:10.3390/ijerph10094352
115. Reilly R, McNulty H, Pentieva K, Strain JJ, Ward M (2014) MTHFR 677TT genotype and disease risk: is there a modulating role for B-vitamins? *The Proceedings of the Nutrition Society* 73 (1):47-56. doi:10.1017/S0029665113003613
116. Farrell CJ, Kirsch SH, Herrmann M (2013) Red cell or serum folate: what to do in clinical practice? *Clinical chemistry and laboratory medicine : CCLM / FESCC* 51 (3):555-569. doi:10.1515/cclm-2012-0639
117. Fazili Z, Pfeiffer CM, Zhang M, Jain RB, Koontz D (2008) Influence of 5,10-methylenetetrahydrofolate reductase polymorphism on whole-blood folate concentrations measured by LC-MS/MS, microbiologic assay, and bio-rad radioassay. *Clinical chemistry* 54 (1):197-201. doi:10.1373/clinchem.2007.096545

Table 1. Characteristics of studies included in the meta-analyses

Study/ Year	Design/ Location	Intervention / Control	Sample Size	Total Duration (weeks)	Source	Daily dose	Participants/ Ethnicity	Age (years)/ Gender
Ashfield-Watt /2002 [72]	RCT, DB/ UK	FA/ Placebo	108	16	Food/Supplement	575 DFE (FA fortified foods and natural food folates)	Healthy/ British	20-63/ M&F
Fohr /2002 [77]	RCT, DB/ Germany	FA/ Placebo	51	8	Capsules	667 DFE (400µg/ FA)	Nonpregnant healthy/ German	19-39/ F
Nelen/1998 [75]	B-A Quasi- experimental trial/ Netherlands	NA	49	16	Supplement	833DFE (500µg/ FA)	unexplained recurrent miscarriages/ Caucasian	22-41/ F
Shelnutt/2003 [78]	B-A Quasi- experimental trial/ USA	NA	41	7	Supplement	400 DFE (168 µg FA + 115 µg food folate after 7 weeks depletion)	Nonpregnant healthy/ White, non-Hispanic American	20-30/ F
Hiraoka/2004 [71]	B-A Quasi- experimental trial/ Japan	NA	100	4	Supplement	333 DFE (200µg/ for 7 weeks) then 667 DFE (400µg/ FA for 7 weeks)	Healthy & Outpatients/ Japanese	19-81/ F
Miyaki/2005 [76]	RCT, DB/ Japan	FA/ Placebo	203	12	Capsule	1667 DFE (1mg/ FA)	Healthy	45.8±11.5/ M

Guinotte/2003 [69]	B-A Quasi-experimental trial/ USA	NA	43	7	Supplement	667 DFE (400µg/ FA after 7 weeks depletion)	Healthy/ Mexican	18-44/ F
Crider/ 2011 [73]	RCT, DB/China	NA	338	24	Supplement	667 DFE (400µg/ FA)	Healthy, and mother of 2-4 years kid/Chinese	childbearing age/ F
Qin/ 2012 [74]	RCT, DB/ China	FA/ Placebo	480	8	Tablet	667 DFE (400µg/ FA + Enalapril for Htn)	Hypertensive/ Chinese	18-75/ M&F
Zappacosta/2013 [70]	RCT, DB/ Italy	FA/ Placebo	37	13	Capsule	533 DFE (200µg/ FA + 200 µg/ Food folate)	Mild Hyperhomocytinemia/ Italian	18-60/ M&F

Abbreviation used in this table: **RCT**: randomised controlled trial; **M**: male; **F**: female; **FA**: folic acid; **Htn**: hypertension; **DB**: Double Blind; **NA**: Not Applicable; **CVD**: cardiovascular disease; **B-A**: before and after; **CHD**: coronary heart disease

*Conversion factor for 1 µg dietary folate equivalent (DFE) = 1 µg food folate, 0.6 µg folic acid with meals or as fortified foods [62]

Table 1. Subgroup and sensitivity analyses of baseline Hcy

DOMINANT MODEL		
Included Studies		
Baseline Hcy	Mean Difference	Overall Effect
RCT only n = 1588	3.36 [2.03-4.69]	Z = 4.96, P = 0.00001
HyperHcy selection removed n = 1922	2.83 [1.65-4.00]	Z = 4.72, P = 0.00001
F only n = 848	1.65 [-0.01-3.31]	Z = 1.95, P = 0.05
400µg only n = 1406	2.76 [1.14-4.39]	Z = 3.24, P = 0.0008
Chinese & Taiwanese population n = 1186	5.63 [2.41-8.84]	Z = 3.43, P = 0.0006
RCT only n = 864	3.88 [2.17-5.58]	Z = 4.46, P = 0.00001
HyperHcy selection removed n = 1092	2.90 [1.67-4.12]	Z = 4.64, P = 0.00001
F only n = 482	1.75 [0.24, 3.26]	Z = 2.27, P = 0.02
400µg only n = 763	2.96 [1.19-4.73]	Z = 3.28, P = 0.001
Chinese & Taiwanese population n = 654	6.08 [2.68-9.48]	Z = 3.50, P = 0.0005

Table 2. Subgroup and sensitivity analysis of plasma Hcy levels post intervention.

Analysis weighted Crider study at *97.8% and **99.8%

RECESSIVE MODEL		
Included Studies		
Post Intervention Hcy	Mean Difference	Overall Effect
RCT only n = 1017	0.98 (-0.35-2.31)	Z = 1.44, P = 0.15
HyperHcy selection removed n = 1051	0.76 (-0.54-2.05)	Z = 1.15, P = 0.25
F only n = 439	0.41 (-1.49-2.30)	Z = 0.42, P = 0.67
400µg only n = 360	0.8 (-0.66-2.27)	Z = 1.07, P = 0.28
*Chinese & Taiwanese population n = 593	2.20 (1.95-2.25)	Z = 17.3, P = 0.00001
HOMOZYGOUS MODEL		
RCT only n = 498	1.23 [-1.14-3.19]	Z = 0.92, P = 0.36
HyperHcy selection removed n = 577	0.92 [-0.58-2.43]	Z = 1.20, P = 0.23
F only n = 274	0.93 [-0.90-2.42]	Z = 0.99, P = 0.32
400µg only n = 248	0.93 [-0.90-2.76]	Z = 0.99, P = 0.32
**Chinese & Taiwanese population n = 431	2.90 [2.55-3.24]	Z = 16.45, P = 0.00001

Table 4. Subgroup and sensitivity analysis of baseline serum folate levels

RECESSIVE MODEL		
Baseline folate		
Included Studies	Mean Difference	Overall Effect
RCT only n = 852	2.04 [0.70-3.38]	Z = 2.98, P = 0.003
F only n = 848	2.82 [0.90-4.73]	Z = 2.89, P = 0.004
400µg only n = 1072	2.50 [1.10-3.90]	Z = 3.49, P = 0.0005
Chinese & Taiwanese population n = 487	1.16 [-0.07-2.39]	Z = 1.85, P = 0.06
HOMOZYGOUS MODEL		
Included Studies	Mean Difference	Overall Effect
RCT only n = 458	2.46 [0.96-3.96]	Z = 3.22, P = 0.001
F only n = 445	3.95 [1.55-6.34]	Z = 3.23, P = 0.001
400µg or 500 µg only n = 575	3.82 [2.03- 5.59]	Z = 4.20, P = 0.0001
Chinese & Taiwanese population n = 248	1.36 [0.61- 2.12]	Z = 3.54, P = 0.04

Table 5. Subgroup and sensitivity analysis of post-intervention serum folate levels

RECESSIVE MODEL		
Post intervention folate		
Included Studies	Mean Difference	Overall Effect
RCT only n = 674	7.53 [5.62-9.44]	Z = 7.73, P = 0.00001
F only n = 688	7.08 [3.83-10.34]	Z = 4.27, P = 0.0001
400µg only n = 894	5.53 [3.13-7.94]	Z = 4.51, P = <0.00001
>8/52 supplementation n = 400	9.12 [6.45-11.80]	Z = 6.68, P = <0.00001
Chinese & Taiwanese population n = 487	4.12 [-1.05-9.29]	Z = 1.56, P = 0.12
HOMOZYGOUS MODEL		
Included Studies	Mean Difference	Overall Effect
RCT only n = 361	6.84 [3.20-10.49]	Z = 3.22, P = 0.001
F only n = 397	8.36 [4.90-11.81]	Z = 4.74, P = 0.00001
400µg only n = 511	6.28 [3.11- 9.54]	Z = 3.88, P = 0.0001
>8/52 supplementation n = 166	11.30 [8.19-14.41]	Z = 7.11, P = <0.00001
Chinese & Taiwanese population n = 248	7.13 [2.11-12.15]	Z = 2.78, P = 0.005

Figure 1. Study Flow Diagram

Figure 2. Baseline plasma Hcy using a dominant model TT vs CC/CT (top) and homozygous model CC vs TT (bottom)

Figure 3. Post-intervention plasma Hcy using a dominant model TT vs CC/CT (top) and homozygous model TT vs CC (bottom).

Figure 4. Baseline serum folate using a dominant model CC/CT vs TT (top) and homozygous model CC vs TT (bottom)

Figure 5. Post-intervention serum folate using a dominant model CC/CT vs TT (top) and homozygous model (CC vs TT) bottom

Supplemental table 1. Methodology assessment summary based on modified version of Downs and Black's checklist of studies included in systematic review for the Nutrigenetic impact of MTHFR 677 C/T genotypes on folate status

Authors (References)

**Crider et al./
2011**

**Guinotte et al./
2003**

**Nelen et al./
1998**

**Shelnutt et al./
2003**

**Fohr et al./
2002**

**Miyaki et al./
2005**

**Ashfield-Wat et al./
2002**

**Zappacosta et al./
2013**

**Kasiman et al./
2009**

**Liu et al./
2004**

**Hiraoka et al./
2004**

**Qin et al./
2012**

Reporting

Is the hypothesis/aim/objective of the study clearly described?	1	1	1	1	1	1	1	1	1	1	1	1
Are the main outcomes to be measured clearly described in intro or methods?	1	1	1	1	1	1	1	1	1	1	1	1
Are the characteristics of the patients included in the study clearly described?	1	1	1	1	1	1	1	1	1	1	1	1
Are the interventions of interest clearly described?	1	1	1	1	1	1	1	1	1	1	1	1
Are biases and limitations clearly described?	0	1	1	1	0	0	1	1	1	1	0	1
Are the main findings of the study clearly described?	1	1	1	1	1	1	1	1	1	1	1	1
Have the characteristics of patients lost to follow up been described?	0	1	1	0	1	1	0	0	1	NA	NA	1
Have actual probability values been reported except where <0.001?	1	1	1	1	0	0	1	1	1	1	1	1
Was intensity and duration sufficient to produce an effect?	1	1	1	1	1	1	1	1	1	1	1	1
Were protocols of the intervention described?	1	1	1	1	1	1	1	1	1	1	1	1
Were other factors accounted for that could affect results?	1	1	1	1	1	1	1	1	1	1	0	1

External Validity

Were the subjects asked to participate in the study representative of the entire population from which they were recruited?	1	0	0	0	1	1	0	0	1	0	0	0
Were those subjects who were prepared to participate in the study representative of the entire population from which they were recruited?	1	0	0	0	1	1	0	0	1	0	0	0
Were the staff, places, and facilities where the patients were treated representative of the treatment the	0	1	1	1	1	1	1	1	0	0	1	0

majority of patients would receive?												
Was there no apparent conflict of interest?	1	1	1	1	NA	1	1	1	1	1	1	1
Internal Validity-bias												
Was an attempt made to blind the subjects to the treatment? If RCT	1	0	NA	NA	1	1	1	1	1	NA	NA	1
Was an attempt made to blind those measuring the main outcomes? If RCT	1	0	NA	NA	1	1	1	1	1	NA	NA	1
Were baseline characteristics compared?	1	1	1	1	1	1	1	1	1	1?	1	1
Was compliance reliable?	0	1	1	0	1	1	1	1	0	1	0	0
Were all outcomes reported?	1	1	1	1	1	1	1	1	1	1	1	1
Were the main outcome measures used accurate (valid and reliable)?	1	1	1	1	1	1	1	0?	1	1	1	1
Internal Validity-selection bias												
Were the patients in different intervention groups (trials) or where case and controls recruited from the same population?	1	1	0	0	1	1	1	1	1	0	1	1
Were the patients in different intervention groups (trials) or where case and controls recruited over the same period of time?	1	1	0	0	1	1	1	1	1	0	1	1
Were study subjects randomised to intervention groups?	1	1	NA	NA	1	1	1	1	1	NA	NA	1
Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?	1	NA	NA	NA	1	1	1	1	1	NA	NA	1
Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?	1	1	1	1	1	1	1	1	1	1	0	1
Were losses of patients to follow-up taken into account?	0	1	1	1	1	1	1	1	1	NA	NA	1
Statistics												
Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?	1	0	0	0	1	1	0	0	1	0	0	0
Was the statistical analysis appropriate?	1	1	1	1	1	1	1	1	1	1	1	1
Genetic Methodology												

Were the genotyping methods described?	1	1	1	1	1	1	1	1	1	1	1	1
Were the genotyping methods adequate?	1	1	1	1	1	1	1	1	1	1	1	1
Were genotyping results in Hardy Weinberg Equilibrium? If appropriate	1	NA	1	NA	1	1	NA	NA	1	NA	1	1

Supplemental table 2. Methodology assessment summary and Rosendal score of studies included in systematic review for the Nutrigenetic impact of MTHFR 677 C/T genotypes on folate status

Authors (References)	Eligibility	Randomization ¹	Method for Randomization ²	Sample Size Calculated ³	Pre-trial Conditions ⁴	Baseline Measures ⁵	Blinding of Subjects ⁶	Blinding of Investigators ⁷	Method and Evaluation of Blinding ⁸	Non Completers Described ⁹	Stats Described ¹⁰	Measures and Variability Described ¹¹	Between Group Stats Comparisons ¹²	Adverse Effects Described ¹³	Reproducibility Reported ¹⁴	% Score
Crider et al./ 2011	1	1	0	1	0	1	1	1	0	0	1	1	1	0	0	60%
Guinotte et al./ 2003	1	1	0	0	1	1	0	0	NA	1	1	1	1	0	0	57%
Nelen et al./ 1998	1	NA	NA	0	NA	1	NA	NA	NA	1	1	1	1	0	0	66%
Shelnutt et al./ 2003	1	NA	NA	0	NA	1	NA	NA	NA	0	1	1	1	0	1	55%
Fohr et al./ 2002	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	80%
Miyaki et al./ 2005	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	86%
Ashfield-Wat et al./ 2002	1	1	1	0	0	1	1	1	0	0	1	1	1	0	0	60%
Zappacosta et al./ 2013	1	1	0	0	0	1	1	1	1	0	1	0	1	0	0	53%
Hiraoka et al./ 2004	1	NA	NA	0	1	1	NA	NA	NA	NA	1	1	1	0	0	66%
Qin et al./ 2012	1	1	1	0	0	1	1	1	1	1	1	1	1	0	0	73%

1- A clear description of the inclusion and exclusion criteria was provided

- 2- The trials were randomized
- 3- The method used to generate the random allocation sequence, including details of any restrictions (e.g. blocking, stratification) was described
- 4- Sample size was justified (e.g. by power calculation)
- 5- Attempts were made to control and/or monitor pre-trial condition (e.g. diet, exercise)
- 6- Design incorporated measures of important baseline variables
- 7- There was blinding of all subjects
- 8- There was blinding of all investigators involved in the trials
- 9- Both the method of blinding and the evaluation of the successfulness of blinding were described
- 10- Details were provided regarding the inability of subjects to complete study requirements