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MTHFR 677TT genotype and disease risk: is there a modulating role for B-vitamins?

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Methylenetetrahydrofolate reductase (MTHFR) is a critical folate-metabolising enzyme which requires riboflavin as its co-factor. A common polymorphism (677C→T) in the *MTHFR* gene results in reduced MTHFR activity *in vivo* which in turn leads to impaired folate metabolism and elevated homocysteine concentrations. Homozygosity for this polymorphism (TT genotype) is associated with an increased risk of a number of conditions including heart disease and stroke, but there is considerable variability in the extent of excess risk in various reports. The present review will explore the evidence which supports a role for this polymorphism as a risk factor for a number of adverse health outcomes, and the potential modulating roles for B-vitamins in alleviating disease risk. The evidence is convincing in the case which links this polymorphism with hypertension and hypertensive disorders of pregnancy, particularly preeclampsia. Furthermore, elevated blood pressure was found to be highly responsive to riboflavin intervention specifically in individuals with the *MTHFR* 677TT genotype. Future intervention studies targeted at these genetically predisposed individuals are required to further investigate this novel gene–nutrient interaction. This polymorphism has also been associated with an increased risk of neural tube defects (NTD) and other adverse pregnancy outcomes; however, the evidence in this area has been inconsistent. Preliminary evidence has suggested that there may be a much greater need for women with the *MTHFR* 677TT genotype to adhere to the specific recommendation of commencing folic acid prior to conception for the prevention of NTD, but this requires further investigation.

MTHFR 677C→T polymorphism: Folate: Riboflavin: CVD: Neural tube defects: Hypertension

The common 677C→T variant in the gene that encodes the enzyme methylenetetrahydrofolate reductase (MTHFR) is widely recognised as the most important genetic determinant of elevated homocysteine concentration in healthy populations. Emerging evidence links the *MTHFR* 677C→T polymorphism with a greater risk of CHD⁽¹⁾, stroke⁽²⁾ and more recently hypertension⁽³⁾, although there is considerable variability in the extent of excess disease risk in different populations, an observation that is generally explained by differences in folate status. The potential roles of folate and the related B-vitamins in the risk of these and other common diseases have been widely investigated over the past two decades, but relatively few studies have specifically investigated the roles of the relevant gene–nutrient interactions in modulating the risk of disease.

The role of maternal folate status in early pregnancy for the prevention of neural tube defects (NTD) is well established^(4,5). In addition, other pregnancy complications such as gestational hypertension and preeclampsia have been associated with low maternal folate status^(6,7). Although the mechanisms linking low folate status to these pregnancy complications are not fully understood, the identification of genes that may predispose women to adverse pregnancy events has been the focus of much research. There is now strong evidence to support a role for the *MTHFR* 677C→T polymorphism in adverse pregnancy outcomes^(8,9). The present paper will explore the evidence linking this polymorphism with various disease states and the potential modulating role for B-vitamins in alleviating any excess disease risk.

Abbreviations: BP, blood pressure; MTHFR, methylenetetrahydrofolate reductase; NTD, neural tube defect; RCF, red cell folate.
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Table 1. Meta-analyses that investigated the *MTHFR* 677C→T polymorphism and CVD risk by geographical region

Author	Sample size (n)	Region	Odds ratio (95% CI)
Klerk <i>et al.</i> ⁽¹⁾	23920	All	1.16 (1.05, 1.28)
		Europe	1.14 (1.01, 1.28)
		North America	0.87 (0.73, 1.05)
Wald <i>et al.</i> ⁽²¹⁾	16849	All	1.42 (1.11, 1.84)
		Casas <i>et al.</i> ⁽²²⁾	13928
Lewis <i>et al.</i> ⁽²⁰⁾	57 183	North America/Europe	1.21 (1.02, 1.43)
		All	1.14 (1.05, 1.24)
		Middle East	2.61 (1.81, 3.75)
		Asia	1.23 (0.94, 1.62)
		Europe	1.08 (0.99, 1.18)
		North America	0.93 (0.80, 1.10)
		Australia	1.04 (0.73, 1.49)
Holmes <i>et al.</i> ⁽²⁾	45549	All	1.37 (1.25, 1.50)
		Asia	1.68 (1.44, 1.97)
		North America, Australia and New Zealand	1.03 (0.84, 1.25)
		Clarke <i>et al.</i> ⁽²⁴⁾	70474
Clarke <i>et al.</i> ⁽²⁴⁾	70474	Asia	1.49 (1.29, 1.73)
		Europe	1.11 (0.93, 1.22)*
		USA and Australasia	1.04 (0.79, 1.36)*

* Reported after national folate supplementation in the USA, Canada, Australia, New Zealand and some but not all European countries.

MTHFR 677TT genotype in C₁ metabolism

The *MTHFR* enzyme requires the B-vitamin riboflavin in its co-enzymatic form flavin adenine dinucleotide to catalyse the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. This enzyme plays a key role in regulating the availability of methyl groups required for the remethylation of homocysteine into methionine. The common 677C→T variant results in a less active thermolabile *MTHFR* enzyme typically leading to elevated homocysteine concentration *in vivo*⁽¹⁰⁾. *In vitro* evidence indicates that the reduced activity of the variant enzyme is the result of an increased propensity to dissociate from its FAD co-factor^(11,12). The finding of lower erythrocyte folate concentrations in individuals with the homozygous mutant TT genotype compared with those without this genetic variant, has led to the suggestion that folate requirements may be increased in these individuals⁽¹³⁾. Furthermore, homocysteine concentrations are found to be highest under conditions of suboptimal folate status in combination with the TT genotype^(14,15).

The B-vitamins folate and riboflavin (along with the metabolically related B-vitamins, B₁₂ and B₆) are important nutritional determinants of homocysteine concentrations. Supplementation with folic acid, the synthetic form of folate, has been shown to lower elevated homocysteine concentrations by approximately 25% in the population generally⁽¹⁶⁾. In the case of riboflavin, however, the homocysteine-lowering effects are confined to those with the *MTHFR* 677TT genotype. Riboflavin supplementation specifically in individuals with the TT

genotype has been shown to significantly lower plasma homocysteine by 22%, and by as much as 40% in those with the lowest riboflavin status at baseline⁽¹⁷⁾. This response was not observed in individuals with CC or CT genotypes (even when baseline riboflavin status was suboptimal) despite a significant improvement in riboflavin status post-intervention in both genotype groups. Such evidence confirmed the independent modulating role of riboflavin in determining homocysteine concentrations specifically in individuals with the TT genotype as first suggested by earlier observational studies^(18,19).

Association between the *MTHFR* 677C→T polymorphism and CVD risk

The *MTHFR* 677C→T polymorphism previously received much attention as the main genetic determinant of homocysteine⁽¹⁰⁾ and is also independently associated with an increased risk of CVD and particularly stroke^(1,2,20,21). Several meta-analyses to date have demonstrated an increased risk of CVD in individuals with the TT genotype compared to those without this genetic variant^(1,2,20-22) (Table 1). Those studies which assessed the risk of CHD events estimated a 14–16% higher risk in individuals with the TT genotype compared to those with the CC genotype^(20,23). The most recent meta-analysis considered published and unpublished results in the area and estimated that the *MTHFR* 677TT genotype carried a 15% excess risk of heart disease (based on published results)⁽²⁴⁾.



Differences in B-vitamin status may in some way explain the large geographical variation in the extent of the excess risk, which has previously been reported in relation to this polymorphism (Table 1). Klerk *et al.*⁽¹⁾ and Lewis *et al.*⁽²⁰⁾ found no strong evidence to support an association between the TT genotype and risk of CHD among North American populations; however, the polymorphism was linked to an increased risk of CHD among Asian and Middle Eastern populations. It was concluded that such geographical differences reflected the influence of environmental factors (primarily folate status) on the interaction between the polymorphism and CVD risk^(2,25). In terms of stroke, the TT genotype was associated with an increased risk in regions of low folate only; whereas in populations of optimal folate status or in regions of mandatory folic acid fortification, the effect of the TT genotype on stroke risk was null⁽²⁾. Somewhat surprisingly however, recent prospective evidence from the National Health and Nutrition Examination Survey cohort reported that the *MTHFR* 677TT genotype was associated with a lower rate of CVD mortality (OR 0.69 (95% CI 0.50, 0.95)), although stratified analysis found that this association only occurred after the introduction of mandatory folic acid fortification⁽²⁶⁾.

Overall, the evidence supports a modulating role of folate on CVD risk in this genetically at-risk group. However caution is required when interpreting the results from some observational studies. Further large-scale genetic studies, particularly among populations with sub-optimal folate status, are required to fully investigate the extent of CVD risk associated with this polymorphism and to consider the role of metabolically related B-vitamins linked with folate.

MTHFR 677C→T polymorphism and blood pressure

Hypertension, defined as a blood pressure (BP) of 140/90 mmHg or greater, is a major risk factor for CVD, with uncontrolled hypertension associated with an almost threefold greater risk of developing CVD⁽²⁷⁾. Furthermore, elevated BP is recognised as an even stronger predictor of stroke. A modest lowering of systolic BP by 2-mmHg is estimated to decrease cardiovascular risk by 10%, whereas a lowering of 10-mmHg is associated with a 40% reduction in stroke mortality⁽²⁸⁾.

Approximately 30–60% of BP variability is considered to be inherited^(29,30) and several studies have focused on investigating genetic variants associated with elevated BP. Recent genome-wide association studies have investigated associations between single-nucleotide polymorphisms and elevated BP^(3,31). One such study, which included data from 34433 individuals, reported that the *MTHFR* loci was one of eight loci related to BP; however, the authors noted that there may be other genetic variants linked to the BP located in this region which have not been considered or are still unknown⁽³⁾. Several subsequent investigations of a genome-wide association studies nature have confirmed the

association between the *MTHFR* gene and BP variability^(32–34).

The evidence from case-control studies suggests that there is a graded association between the *MTHFR* 677T allele and BP risk among hypertensive populations across different ethnic groups. An early case-control study in a Caucasian–Australian population reported a significantly higher frequency of the TT genotype in cases with hypertension compared to controls (after adjustment for BMI)⁽³⁵⁾. Within an Asian–Indian population, a nearly fourfold increased risk of hypertension in individuals with the TT genotype was reported⁽³⁶⁾. Interestingly, in a large population-based study of over 3000 Japanese people, the TT genotype was associated with a 42% increased risk of hypertension in women; however no significant association was shown in men⁽³⁷⁾. As the evidence accumulated to support an association between the *MTHFR* 677C→T polymorphism and hypertension risk, a number of meta-analyses were carried out. One meta-analysis of thirteen case-control studies⁽³⁸⁾ confirmed a significant association between the TT genotype and hypertension among the Asian and the Caucasian populations, albeit significant heterogeneity was reported (OR 1.24 (95% CI 1.02, 1.50)). The most recent meta-analysis, which attempted to address the issue of heterogeneity by focusing specifically on Chinese population studies only, reported a 87% increased risk of hypertension in individuals with the TT genotype compared to those with the CC genotype⁽⁸⁾. Similarly, case-control studies carried out in hypertensive patients with various co-morbidities such as diabetes⁽³⁹⁾, coronary artery disease⁽⁴⁰⁾ and chronic renal failure⁽⁴¹⁾ have also reported significant associations between this polymorphism and hypertension.

In addition to lifestyle and dietary modifications, improvements in the management of BP are largely considered to be due to the development of effective antihypertensive medications. Such medications are broadly categorised into five main classes (diuretics, β -blockers, calcium channel blockers, angiotension converting enzyme inhibitors and angiotension II receptor blockers), all of which have slightly different mechanisms of action. Only a limited number of studies, however, have investigated whether the BP-lowering effect of antihypertensive medications is influenced by the *MTHFR* 677C→T polymorphism. Jiang *et al.*⁽⁴²⁾ reported an increased diastolic BP response to treatment with an angiotension converting enzyme inhibitor in hypertensive patients with the TT genotype compared to those without the polymorphism. More recently, in a larger cohort of hypertensive patients with the TT genotype (who expressed the phenotype of elevated BP at baseline), an increased diastolic BP response to treatment with an angiotension converting enzyme inhibitor was again reported ($P=0.038$)⁽⁴³⁾. Similarly, short-term treatment with a calcium channel blocker resulted in a significantly higher pulse wave velocity response in patients with the TT genotype compared with those with CC and CT genotypes ($P=0.018$); the polymorphism however did not affect the antihypertensive effects of the calcium channel blocker treatment⁽⁴⁴⁾. In contrast, other studies (reviewed later)

suggest that the response to antihypertensive medication is generally suboptimal in individuals with the TT genotype compared to those with the CC or CT genotype. Given the limited evidence to date, further work is needed to explore potential interactions between the *MTHFR* 677C→T polymorphism and the antihypertensive effects of various BP medications.

***MTHFR* genotype and the modulating potential of B-vitamins in blood pressure control**

Although strong evidence shows an association between the *MTHFR* 677C→T polymorphism and hypertension, the interrelationships between *MTHFR* genotypes, B-vitamin status and hypertension risk are not generally appreciated. In one small placebo-controlled, cross-over design study (*n* 41), Williams *et al.*⁽⁴⁵⁾ reported a decreased pulse pressure and arterial stiffness following short-term folic acid supplementation (5 mg/d); an effect that was reported to be independent of homocysteine concentration or *MTHFR* genotype, but the lack of statistical power (i.e. only five subjects with the TT genotype were available for investigation) makes such conclusions questionable. A subsequent intervention study reported no BP response to short-term folic acid supplementation, but did observe an association between *MTHFR* T allele frequency and hypertension risk⁽⁴⁶⁾. Owing to the opportunistic nature of such studies (and therefore the lack of genotype-driven recruitment) such inconsistent results are not surprising. Similarly, in the large-scale trials of CVD risk involving folic acid intervention with or without other B-vitamins, no response of BP has been reported to date; however, no trial has analysed the results of BP (or other CVD outcomes) by *MTHFR* genotype^(47,48).

Given the emerging evidence linking hypertension with this polymorphism⁽³⁾ and the established genotype-specific response of homocysteine to riboflavin supplementation⁽¹⁷⁾, recent research from our Centre has focused on investigating the BP-lowering potential of riboflavin in hypertensive individuals with the TT genotype. In a placebo-controlled trial, premature CVD patients (pre-screened for the *MTHFR* 677C→T polymorphism) were randomised within all three genotype groups to receive either riboflavin (1.6 mg/d) or placebo over a 16-week intervention period. Baseline BP was significantly higher in patients with the TT genotype compared to those with other genotypes; an effect that was most pronounced in those with the lowest baseline riboflavin status (as determined by the biomarker erythrocyte glutathione reductase activation co-efficient). Systolic and diastolic BP decreased significantly (by 13 and 8 mmHg, respectively) in those individuals with the TT genotype but no response was observed in the other genotype groups⁽⁴⁹⁾. A follow-up, cross-over, randomised controlled trial reinvestigated the effect of riboflavin on BP in the same cohort of high-risk CVD patients 4 years after the original investigation⁽⁵⁰⁾. Although there were marked changes in prescribed antihypertensive therapy

over the 4-year period, the mean BP of those with the TT genotype (who originally received riboflavin supplementation for the 16-week period) had returned to pre-intervention levels. When the original treatment groups were reversed, the genotype-specific BP-lowering effect of riboflavin was again confirmed⁽⁵⁰⁾ with reductions of 9.2 (SD 12.8) mmHg (*P*=0.001) and 6.0 (SD 9.9) mmHg (*P*=0.003) in systolic and diastolic BP, respectively. Most recently, the responsiveness of BP to riboflavin supplementation in a separate cohort of treated hypertensive individuals (without overt CVD) with the TT genotype (*n* 91) was investigated⁽⁵¹⁾. At baseline, 60% of the participants had failed to achieve target BP levels ($\leq 140/90$ mmHg), despite taking three or more antihypertensive medications. Following riboflavin intervention (1.6 mg/d for 16 weeks), systolic BP decreased significantly, a finding which confirmed that the BP-lowering effects of riboflavin in individuals with the *MTHFR* 677TT genotype were not confined to high-risk CVD patients but also applied to hypertensive individuals generally⁽⁵¹⁾.

Although accumulating evidence highlights the BP-lowering effects of riboflavin in hypertensive individuals with the *MTHFR* 677C→T polymorphism, the mechanism to explain the role of this gene–nutrient interaction in BP remains unclear. It is possible however that the potent vasodilator nitric oxide may be involved. Decreased vascular concentrations of 5-methyltetrahydrofolate, a nitric oxide regulator, have been reported in those with the *MTHFR* 677TT genotype⁽⁵²⁾. By stabilising the variant enzyme via riboflavin supplementation, it is possible that normal concentrations of 5-methyltetrahydrofolate would be restored in vascular cells, thus improving the bioavailability of nitric oxide and resulting in BP-lowering specifically in those with the TT genotype. Further investigations are required to explore this and other potential mechanisms as well as the role of other B-vitamins involved in C₁ metabolism. Given the known metabolic interdependencies between the relevant B-vitamins, work is underway at this Centre to investigate the independent and combined effects of riboflavin and folic acid on BP in hypertensive individuals with the TT genotype in a factorial study design. In addition, although convincing evidence has demonstrated the BP-lowering effects of low-dose riboflavin over a relatively short period of time (1.6 mg/16 weeks)^(49–51), it remains to be seen how variations in dose and duration would influence the BP-lowering effects of riboflavin in these genetically at-risk patients.

***MTHFR* genotype, B-vitamins and pregnancy outcomes**

Well-established evidence has accumulated to suggest a link between the *MTHFR* 677C→T polymorphism and the risk of certain adverse pregnancy outcomes including NTD as well as hypertensive disorders such as preeclampsia and gestational hypertension; some of which will be the focus of the next section of this review.



The link between the B-vitamins and neural tube defects

NTD are of multi-factorial origin and involve both genetic and environmental factors. Landmark studies in the early 1990s confirmed the protective effects of periconceptional folic acid supplementation against the first occurrence⁽⁴⁾ and reoccurrence⁽⁵⁾ of NTD. Of note, Daly *et al.*⁽⁵³⁾ illustrated a dose–response inverse relationship between maternal red cell folate (RCF) and NTD risk and identified maternal RCF concentrations of ≥ 906 nmol/l as the level associated with the lowest risk of an NTD affected pregnancy. Subsequently, folic acid supplementation levels of 400, 200 or 100 $\mu\text{g}/\text{d}$ were reported to produce RCF concentrations, which predicted reductions in NTD by 47, 41 and 22%, respectively⁽⁵⁴⁾.

Present recommendations, in place in most countries worldwide, are that women planning a pregnancy should take a folic acid supplement of 400 $\mu\text{g}/\text{d}$ for at least 1 month before conception and during the first trimester of pregnancy⁽⁵⁵⁾. Reports have confirmed, however, that the uptake of this recommendation among women of child bearing age is typically poor^(56,57) and so it is not surprising that the NTD rates have changed very little across several European countries as a result of these folic acid recommendations⁽⁵⁸⁾. In contrast, the introduction of mandatory fortification of food with folic acid in the USA has successfully increased both serum and RCF concentrations among women of childbearing age⁽⁵⁹⁾ which has coincided with a 27–50% reduction in the reported prevalence of NTD in the USA and Canada^(60,61). At a global level, folic acid fortification of food in countries worldwide has led to an estimated 46% reduction in the prevalence of NTD since 2008⁽⁶²⁾.

The role of the MTHFR 677C→T polymorphism in neural tube defects

To date, no single gene or genetic variant has been identified as a risk factor for NTD. Given the established protective effects however, of folic acid supplementation on the occurrence and reoccurrence of NTD, polymorphisms in genes encoding the proteins that are directly involved in folate metabolism, uptake and transport have been investigated. The reduced activity of the MTHFR enzyme in individuals with the MTHFR 677TT genotype, decreases the availability of 5-methyltetrahydrofolate which plays a key role in methylation⁽¹⁰⁾ and decreases global DNA methylation^(63,64); therefore, it is no surprise that this specific mutant gene has been extensively investigated as a genetic risk factor for NTD^(65,66).

Over the past two decades, several reports have shown a 2 to 4-fold increased risk of NTD if the infant or the mother has the MTHFR 677TT genotype^(65,67–70). van der Put *et al.*⁽⁷¹⁾ reported an even higher risk of an NTD pregnancy if both the mother and the fetus were homozygous for the MTHFR 677C→T polymorphism. The large meta-analysis conducted by Botto and Yang⁽⁹⁾ considered the frequency of the MTHFR

677C→T polymorphism and the risk of NTD across a number of different countries and ethnic groups. Overall, a 1.8 to 2-fold increased risk of spina bifida was established regardless of whether the infant or the mother was homozygous for the MTHFR 677C→T polymorphism⁽⁹⁾. However, other reports have indicated that the presence of the MTHFR 677TT genotype in the infant, and not in the mother, was the relevant consideration in NTD risk⁽⁷²⁾. Although Shaw *et al.*⁽⁷³⁾ established an association between the TT genotype and spina bifida risk, only a weak interaction between the infant genotype and maternal folic acid usage in the occurrence of spina bifida was reported, possibly suggesting that other contributing factors may also need to be considered. Studies focusing on maternal rather than infant MTHFR genotype have found that maternal folate status and/or maternal homozygosity for the MTHFR 677C→T genotype are important determinants of NTD risk^(74,75). Evidence from the aforementioned meta-analysis suggested that the excess NTD risk associated with the TT genotype may depend on folate status and/or a variant of other folate-related genes⁽⁹⁾. Christensen *et al.*⁽⁷⁶⁾ demonstrated a 13-fold increased risk for spina bifida where the TT genotype was combined with RCF concentrations within the lowest quartile, a finding that strongly supports a gene–nutrient interactive effect being implicated in NTD risk, and also may explain some of the inconsistencies within the literature in this area.

Limited studies have investigated the influence of the MTHFR TT genotype in relation to present folic acid recommendations for the prevention of NTD. Preliminary evidence from a study conducted by our group reported that among women with the TT genotype, sampled at the fourteenth gestational week, significantly increased homocysteine and lower RCF concentrations were observed if folic acid supplementation (400 $\mu\text{g}/\text{d}$) was commenced prior to conception (i.e. as recommended) compared with post-conception (i.e. more typical practice); such differences were not evident in the other genotype groups⁽⁷⁷⁾. Furthermore, a large randomised controlled trial reported that despite 6 months supplementation with 4000 $\mu\text{g}/\text{d}$ folic acid, women of childbearing age with the TT genotype achieved lower serum folate concentrations than did those with the CC genotype⁽⁷⁸⁾. Such evidence strongly suggests that folate requirements are higher in women with the MTHFR TT genotype and highlights the much greater need for women with this genotype to adhere to the specific recommendation of commencing folic acid prior to conception in order to optimise folate status for the prevention of NTD, although these findings need to be confirmed in larger studies among different populations.

Role of the MTHFR genotype in determining the risk of hypertensive disorders of pregnancy

Hypertensive disorders of pregnancy, such as gestational hypertension and preeclampsia affect 5–7% of all low-risk pregnancies worldwide and are the

Table 2. Studies that investigated the association between the *MTHFR* 677C→T polymorphism and preeclampsia risk

Author	Sample size	Country/population	Odds ratio (95% CI)
Sohda <i>et al.</i> ⁽⁸⁷⁾	425	Japan	2.5 (1.3, 4.8)
Grandone <i>et al.</i> ⁽⁸⁸⁾	225	Italy	1.8 (1.0, 3.5)
Kupferminc ⁽⁹⁶⁾	144	Israel	2.9 (1.0, 8.5)
Powers <i>et al.</i> ⁽⁹⁷⁾	237	Caucasian women	1.28 (0.58, 2.79)
Kobashi <i>et al.</i> ⁽⁹⁸⁾	316	Japan	0.68 (0.30, 1.55)
Laivuori <i>et al.</i> ⁽⁹⁹⁾	216	Finland	0.50 (0.14, 1.77)
Rigo <i>et al.</i> ⁽¹⁰⁰⁾	221	Caucasian women	1.13 (0.38, 3.37)
Morrison <i>et al.</i> ⁽¹⁰¹⁾	404	Scotland	1.00 (0.55, 1.82)
Prasmusinto <i>et al.</i> ⁽¹⁰²⁾	112	Germany and Croatia	0.28 (0.03, 2.47)
Pegoraro <i>et al.</i> ⁽¹⁰³⁾	609	South African (Black)	0.62 (0.06, 6.90)
Perez-Mutul <i>et al.</i> ⁽⁹¹⁾	325	Mexico	0.94 (0.59, 1.49)
Williams <i>et al.</i> ⁽¹⁰⁴⁾	304	Peru	1.6 (0.7, 3.8)
Yilmaz <i>et al.</i> ⁽¹⁰⁵⁾	111	Turkey	0.84 (0.26, 2.67)
Also-Rallo <i>et al.</i> ⁽⁷⁹⁾	165	Spain	0.73 (0.31, 1.76)
Hernandez-Diaz <i>et al.</i> ⁽⁸⁶⁾	154	USA/Canada	3.0 (1.2, 7.7)
Stiefel <i>et al.</i> ⁽¹⁰⁶⁾	584	Spain	0.92 (0.50, 1.71)

leading cause of maternal and fetal morbidity and mortality⁽⁷⁹⁾. Gestational hypertension occurs without proteinuria (a predominant feature of preeclampsia), but increases the risk of developing preeclampsia by 15–26%⁽⁸⁰⁾. Historical investigations reported an association between gestational hypertension and folate deficiency^(81–84).

In the past two decades, a plethora of studies has investigated the effect of B-vitamin supplementation on hypertensive disorders of pregnancy. The evidence, however, remains inconsistent. Bodnar *et al.*⁽⁶⁾ and Hernandez *et al.*⁽⁷⁾ reported that regular users of a multi-vitamin containing folic acid compared with non-users, had a reduced risk of developing preeclampsia and gestational hypertension by 45 and 55%, respectively. However, Ray and Mamdani⁽⁸⁵⁾ compared the rate of preeclampsia before and after population-wide fortification of food with folic acid in Canada and reported only a small reduction in the rate of preeclampsia (OR 0.96 (95% CI 0.94, 0.98)). It is possible that such inconsistencies may be explained to some extent by different frequencies of the *MTHFR* 677C→T genotype among study populations.

Hernandez-Diaz *et al.*⁽⁸⁶⁾ found a significant association between the *MTHFR* 677TT genotype and risk of gestational hypertension and noted that this association may be weakened among women taking folic acid supplementation. Convincing evidence from meta-analyses has also reported a link between this polymorphism and the risk of hypertensive disorders of pregnancy. Qian *et al.*⁽³⁸⁾ conducted a meta-analysis among Caucasian and Asian populations and reported a 1.66-fold increased risk of hypertension-in-pregnancy in T allele carriers; however, significant heterogeneity was noted. Subgroup analysis found that the T allele was associated with an increased risk of hypertension in Asians only, whereas no significant association was found in Caucasians. In general agreement with these findings, a subsequent meta-analysis that included studies in Chinese populations only, reported a significant

association between the *MTHFR* 677C→T polymorphism and risk of hypertension-in-pregnancy⁽⁸⁾.

Apart from gestational hypertension, the closely related hypertensive-disorder of pregnancy, preeclampsia, has separately been investigated in relation to the *MTHFR* 677C→T polymorphism. In 1997, two separate case-control studies independently reported a significant association between the TT genotype and preeclampsia^(87,88). Since then, numerous studies have investigated this association; however, conflicting results have emerged (Table 2). Worldwide, Mexico has the highest frequency of the TT genotype⁽⁸⁹⁾ as well as a high prevalence of preeclampsia⁽⁹⁰⁾. Within populations from two regions of Mexico, however, Perez-Mutul *et al.*⁽⁹¹⁾ and Davalos *et al.*⁽⁹²⁾ reported no significant association between the polymorphism and preeclampsia in pregnant women. In contrast, Canto *et al.*⁽⁹³⁾ reported from another group of pregnant Mexican women, that the TT genotype (compared to the CC and CT genotypes) was associated with a decreased risk of preeclampsia, following adjustment for age and BMI. Such discrepancies between studies may suggest that the effect of the *MTHFR* 677TT genotype may be modulated by relevant dietary and environmental factors that could vary considerably among different populations.

The association between the *MTHFR* 677C→T polymorphism and preeclampsia has been addressed in several meta-analyses. Kosmas *et al.*⁽⁹⁴⁾ reported that the TT genotype compared with the CC genotype was associated with a 33% increased risk of preeclampsia, however significant between-study heterogeneity was found. Most recently, the meta-analysis by Xia *et al.*⁽⁹⁵⁾ reported that the TT genotype compared with the CC and CT genotypes carried a significantly greater risk of preeclampsia (by 76%) among Asian women only, whereas in Caucasian women this increased risk was not evident. It is worth noting however that a much greater number of large-scale studies in this area have been conducted within Asian populations with far fewer studies conducted in Caucasian populations; a factor that limits



the extent to which different populations can be compared. An additional limitation is that genotype-driven recruitment is generally not undertaken in these studies, a feature that is reflected by the relatively small numbers of pregnant women with the TT genotype being investigated and these raised the concern that many such studies may be statistically underpowered. It is not surprising therefore that in some cases the association between the *MTHFR* polymorphism and gestational hypertension is found to be relatively weak. In addition, the modulating effects of the polymorphism in relation to hypertension may be masked by the presence of other risk factors, such as reduced physical activity, increased BMI and gestational diabetes, which can develop during pregnancy and increase BP.

Conclusion

Overall, convincing evidence supports an independent role for the *MTHFR* 677C→T polymorphism as a risk factor for a number of adverse health outcomes, but there are considerable inconsistencies among individual studies. The *MTHFR* 677TT genotype has been shown in several populations to be an important determinant of hypertension and this may provide a possible explanation for the excess risk of CVD generally linked with this polymorphism as reported in numerous meta-analyses. In addition to the widely acknowledged modulating role of folate, riboflavin (which has been largely overlooked to date) is emerging as an important nutrient that drives the phenotype and potentially the disease risk associated with the *MTHFR* 677C→T polymorphism. Given the genotype-specific responsiveness of BP to riboflavin intervention demonstrated in trials from this Centre, it is possible that the large geographical variations in the extent of disease risk may relate not only to comprised folate status, as commonly suggested, but may also be the result of differences in prevailing riboflavin status among different populations. Large genotype-driven intervention trials (involving pre-screening to select individuals with the TT genotype) conducted within unfortified regions or among populations with sub-optimal B-vitamin status are required to confirm this. Overall, there is convincing evidence of the potential for a personalised approach to disease prevention or treatment, whereby B-vitamin intervention may be targeted at those individuals sharing this common genetic factor.

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Conflicts of Interest

There is a patent granted in Europe and pending elsewhere by H. McN., J. J. S. and M. W. on the use of riboflavin in the treatment of hypertension.

Authorship

R. R. drafted the manuscript. M. W., H. McN., K. P. and J. J. S. critically revised the manuscript for important intellectual content. All the authors have read and approved the final manuscript.

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