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Homocysteine, the methylenetetrahydrofolate reductase 677C>T polymorphism and hypertension: effect modifiers by lifestyle factors and population subgroups

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Abstract

Evidence linking fasting plasma total homocysteine (tHcy) and methylenetetrahydrofolate reductase (*MTHFR*) 677C>T genotype with hypertension is inconsistent. Differences in B vitamin status, other lifestyle factors or their consideration in analyses might explain this. We investigated these associations in the absence of mandatory fortification with folic acid and B vitamin supplement use. A cross-sectional study was conducted in 788 adults, aged 18–75 years, randomly selected from three Catalonian town population registers. Fasting plasma folate, cobalamin, tHcy, erythrocyte folate, erythrocyte glutathione reductase activation coefficient (EGRAC, functional riboflavin status indicator; increasing EGRAC indicates worsening riboflavin status), *MTHFR* 677C>T and solute carrier family 1 (*SLC19A1*) 80 G>A genotypes were determined. Medical history and lifestyle habits were recorded. Principal tHcy determinants differed between women (age, plasma folate, plasma cobalamin, cigarettes/d) and men (*MTHFR* 677TT genotype, plasma folate, plasma cobalamin and CT genotype). The *MTHFR* 677C>T polymorphism–tHcy association (β standardised regression coefficients) was stronger in male smokers (0·52, $P < 0·001$) compared with non-smokers (0·21, $P = 0·001$) and weaker in participants aged >50 years (0·19, $P = 0·007$) compared with ≤50 years (0·31, $P < 0·001$). Hypertension was more probable in the third tHcy tertile compared with other tertiles (OR 1·9; 95 % CI 1·2, 3·0), and in participants aged ≤50 years, for the *MTHFR* 677TT genotype compared with the CC genotype (OR 4·1; 95 % CI 1·0, 16·9). EGRAC was associated with increased probability of hypertension in participants aged >50 years (OR 6·2; 95 % CI 1·0, 38·7). In conclusion, moderately elevated tHcy and the *MTHFR* 677CT genotype were associated with hypertension. The *MTHFR* 677C>T genotype–hypertension association was confined to adults aged ≤50 years.

Key words: **Homocysteine: Methylenetetrahydrofolate reductase 677C>T polymorphism: B vitamins: Hypertension**

Hypertension affects one in five adults and is a major contributor to mortality and morbidity worldwide[1,2]. The associated healthcare costs are considerable and projected to increase with the current situation of expanding longevity and morbidity in the global population. The current associated socio-economic burden is unsustainable going forward. Public health strategies addressed at lifestyle modification to reduce smoking, salt intake and obesity have proven to be successful at the population level[3] and provide solid grounds for continuing to develop primary and secondary prevention strategies. Established causes of hypertension include genetic factors, sex, age, dietary factors, abdominal obesity, sedentarism, smoking and alcohol consumption. However, 90 % of hypertension cases are idiopathic[4]. The identification of candidate nutrient–gene interactions and novel associated biomarkers are of interest in identifying risk sub-groups to inform new lifestyle prevention and screening protocols going forward. The one-carbon metabolic network has received some attention in this regard.

Abbreviations: EGRAC, erythrocyte glutathione reductase activation coefficient; MTHFR, methylenetetrahydrofolate reductase; SLC19A1, solute carrier family 1; tHcy, total homocysteine.

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Hyperhomocysteinaemia has been proposed to be causally linked with hypertension through various physiopathological mechanisms[5–7]. However, evidence linking moderately elevated fasting plasma total homocysteine (tHcy) with hypertension is inconsistent. It has been positively associated with hypertension prevalence in men and women[7–9] and with incident hypertension in follow-up cohort studies[10,11]. One of these reported a U-shaped relationship[11]. However, other studies reported an association with hypertension risk in men only[12] or in women only[13] or that any association between baseline tHcy in healthy participants and incident hypertension over 4 years was lost on adjustment for multiple factors[10]. Furthermore, a Mendelian randomisation study provided no evidence for a causal association between tHcy and blood pressure in young adults[14]. Whether causally related to hypertension or not, antihypertensive treatment was less effective in lowering blood pressure in patients with elevated tHcy[15]. Studies that successfully achieved homocysteine lowering through intervention have also provided inconsistent evidence regarding its effect on blood pressure, with some reporting no effect[16,17] and others reporting a reducing effect[18,19]. Participant characteristics such as baseline folate status and age vary considerably among these studies, and blood pressure is often a secondary outcome measure. Different consideration is given to established contributors to blood pressure such as BMI or weight change during the interventions, lasting up to 2 years among these studies.

The methylenetetrahydrofolate reductase (*MTHFR*) 677C>T polymorphism has been associated with lower folate status and higher tHcy in the homozygote variant compared with the common genotype[20–22], and its inverse association with folate status is enhanced in the presence of the solute carrier family 1 (*SLC19A1*) 80 G>A polymorphism[23]. Both low folate and riboflavin status have been associated with moderately elevated tHcy[24,25], and the riboflavin–tHcy association in the *MTHFR* 677TT compared with CC genotype is independent of folate status[26]. In fact, elevated tHcy has been reported to be limited to people with the combination of TT genotype and poor riboflavin status[27] and supplementing them with riboflavin, led to a reduction in tHcy[28]. The TT genotype was positively associated with hypertension in case–control Australian[29] and Turkish studies[30] and in women but not in men in a Japanese population study[31]. Diastolic blood pressure was higher in the TT compared with CT and CC genotypes in a Chinese study of patients with essential hypertension[32]. This was true in another Chinese study for diastolic blood pressure in hypertensive males and systolic blood pressure in hypertensive females. However, diastolic blood pressure was lower in the TT compared with the CT genotype in hypertensive females[33]. On the other hand, no association between the variant *MTHFR* 677T allele and essential hypertension was observed in children, but a protective effect was observed in adults, in a Mexican-Mestizo case–control study[34].

Supplementing with folic acid combined with vitamins B_{12} and B_6 for 2 years in a randomised placebo-controlled trial did not affect blood pressure lowering despite lowering tHcy[35]. However, riboflavin supplementation did reduce blood pressure in premature cardiovascular patients with the *MTHFR* 677TT genotype[36]. While the TT genotype remained a determinant of blood pressure after 4 years, supplementation was still associated with lower blood pressure[37].

Variations in nutrient–gene or gene–gene interactions, as well as control of confounding factors, may lead to differences in reported effects of tHcy or the *MTHFR* 677C>T polymorphism on blood pressure. European countries differ to the USA, Canada and numerous countries across the globe where fortification of flour with folic acid is mandatory. In fact, addition of riboflavin to flour to restore the vitamin lost during milling is also mandatory in the USA and Canada. We hypothesised that moderately elevated tHcy and the *MTHFR* 677C>T polymorphism are associated with hypertension. We set out to investigate whether moderately elevated tHcy and the *MTHFR* 677C>T polymorphism are associated with diagnosed hypertension in a representative population sample of adult women and men unexposed to mandatory fortification with folic acid and non-users of B vitamin supplement use.

Materials and methods

Study sample

This cross-sectional study was carried out by the Unitat de Medicina Preventiva i Salut Pública, Universitat Rovira i Virgili between 1998 and 2002 as previously described[23,26,38]. Participants aged 18–75 years were randomly selected from a representative sample (stratified by age and sex) from the town hall population registers in three towns (representing inland and coastal regions) in Tarragona province, Southern Catalonia. Exclusion criteria included use of B vitamin supplements or of medication affecting folate metabolism, pregnancy, lactation or having given birth in the last 6 months. The study was approved by the Hospital Universitari Sant Joan (Reus) and by the Fundació Jordi Gol Gorina ethics committees. All participants provided their signed informed consent in accordance with the Declaration of Helsinki.

Anthropometric, clinical and lifestyle data

Participants attended a medical check-up in which weight, height and blood pressure were measured. Blood pressure was measured by the clinicians using a mercury column sphygmomanometer (Riester) and standardised protocol. Participants were seated for at least 15 min before the measurement. Their back was supported, feet on the floor and arm resting palm up in the arm rest of the chair so that the cubital fossa was at heart level. An adjustable cuff (encircling at least 80 % of the upper arm) was fitted by the clinician. The average of two measurements (2 min apart) was recorded. Participants were also interviewed on lifestyle habits (including smoking habits, alcohol intake and drug use). B vitamin supplement users were initially excluded during the recruitment phone call. Participants were further interrogated at the check-up to confirm that they were not using B vitamin supplements.

Medical history

Current illnesses and medication were recorded and classified using the Spanish Ministry of Health, Consumer Affairs and

Social Welfare 'Clasificación Internacional de Enfermedades, 9ª Revisión, Modificación Clínica'[39]. The frequency of diagnosed hypertension was recorded (previous diagnosis of hypertension based on blood pressure ≥140/90 mmHg, being treated or monitored by their clinician). Following 15 min rest, two readings (2 min apart) of systolic and diastolic blood pressure were measured by the clinicians in the left arm, while sitting, using a standard mercury sphygmomanometer and standardised protocol. Participants never diagnosed previously with hypertension and with normal blood pressure at the check-up were classified as normotensive.

Blood samples

Fasting blood samples were collected from the antecubital vein in EDTA-K_3 vacutainers and kept at 4°C until processing, in <2 h of collection, as previously described for erythrocyte folate, and plasma tHcy, creatinine, folate, cobalamin, determinations[23], as well as erythrocyte glutathionine reductase activation coefficient (EGRAC) (functional measurement of riboflavin status)[26]. Plasma total cholesterol and TAG were measured by standard techniques (ITC diagnostics). The *MTHFR* 677C>T (rs1801133)[20] and *SLC19A1* 80 G>A (rs 1051266)[23] polymorphisms were determined as previously described from leucocyte-extracted DNA[23].

Statistical analysis

Descriptive data are reported as means and 95 % CI for normally distributed variables and as geometric means and 95 % CI when variables with skewed distributions were ln-transformed for the application of parametric statistical tests. Means were compared between groups by ANOVA. Categorical variables are reported as percentages and 95 % CI, calculated using the Confidence Interval Analysis program (University of Southampton, UK), and compared between groups with the χ^2 test. Hardy–Weinberg distributions of allele frequencies were tested as previously described[23]. Predictors of tHcy were assessed using multiple linear regression analysis. The probability of having hypertension for tHcy in the third compared with first and second tHcy tertiles (sex and age group, 50 years or younger and over 50 years, specific) was explored in multiple logistic regression models (basic model). Further models were adjusted for sex, age, socio-economic status, BMI, alcohol intake, smoking and total plasma cholesterol. The probability of having hypertension with the *MTHFR* 677CT and TT compared with CC genotypes was also investigated using logistic regression (basic model) and further models adjusted for sex, age, BMI, plasma creatinine, *SLC19A1* 80 GA *v.* GG genotype, *SLC19A1* 80 AA *v.* GG genotype, plasma folate, plasma cobalamin, EGRAC, socio-economic status, alcohol intake, smoking and plasma total cholesterol.

We based our sample size calculation on data from a previous population-based study[33] in which the OR for hypertension was 1·7 for people with the *MTHFR* 677CT genotype and 3·0 for those with the TT compared with CC genotype. In the same study, 49 and 9·3 % of the non-hypertensive group had CT and TT genotypes, respectively. Accepting an α risk of 0·05 and a β risk of 0·2 in a one-sided test, 134 hypertensive and 482 non-hypertensive participants were necessary to detect a statistically significant, lowest expected OR of hypertension. These calculations were carried out using the Poisson approximation available on an online Sample Size and Power calculator designed by the Institut Municipal d'InvestigacióMèdica, Barcelona[40].

Significance was accepted at $P < 0{\cdot}05$, and SPSS version 23.0 was used for statistical analyses.

Results

The prevalence of diagnosed hypertension in the population was 16·2 (95 % CI 13·5, 19·1) %. It was 4·5 (95 % CI 3·0, 6·8) % in participants aged 50 years or less and 42·6 (95 % CI 36·0, 49·5) % in participants aged over 50 years.

The characteristics of the studied population, stratified by tHcy tertiles, are reported in Table 1 and online Supplementary Table S1. The allele frequencies for the *MTHFR* 677C>T and *SLC19A1* 80 G>A polymorphisms were in Hardy–Weinberg equilibrium. In the third tHcy tertile (women > 9·6 μmol/l; men > 11·1 μmol/l), participants were older, had lower plasma folate, red cell folate and plasma cobalamin concentrations, more of them had suboptimal riboflavin status (based on EGRAC category, online Supplementary Table S1), the *MTHFR* 677TT genotype and the combination of *MTHFR* 677TT + *SLC19A1* 80AA genotypes were more prevalent, compared with the other tertiles. Specifically, women had higher plasma creatinine concentrations and more of them were hypertensive and more men had low socio-economic status compared with those in the other tertiles. Globally, plasma folate status was higher in women (geometric mean 12·2; 95 % CI 11·5, 12·9 nmol/l) than in men (geometric mean 10·9; 95 % CI 10·4, 11·5 nmol/l) ($P = 0{\cdot}006$). Participant characteristics are reported by age group and sex in online Supplementary Table S2.

Multiple linear regression analysis, testing the associations between non-modifiable factors and lifestyle factors with tHcy, is summarised in Table 2. In the complete model in women, age group followed by *MTHFR* 677TT genotype, plasma cobalamin, folate, creatinine and smoking was most strongly associated with tHcy. In men, the strongest predictor of tHcy was the *MTHFR* 677TT genotype, followed by plasma folate, age group and plasma cobalamin. There was a significant interaction between *MTHFR* 677C>T genotype and age group ($P = 0{\cdot}030$) in the overall population and between *MTHFR* 677C>T genotype and smoking in men ($P = 0{\cdot}028$). The interaction is illustrated in Fig. 1. The effect sizes of the associations (β-coefficients) between the *MTHFR* 677TT genotype *v.* CC genotype and tHcy were greater in smokers than in non-smokers in all of the models. In a stratified analysis by age and sex, the *MTHFR* 677TT–tHcy association was confined to women aged 50 years or less (β: 0·20, $P < 0{\cdot}001$; in women >50, β: 0·09, $P = 0{\cdot}19$) but in men, it was observed in both age groups (aged ≤50 years or less: β: 0·29, $P < 0{\cdot}001$; >50 years, β: 0·14, $P = 0{\cdot}020$).

A stratified analysis by *MTHFR* 677C>T genotype showed some differences in predictors of tHcy among genotypes (Table 3). The strongest associations with tHcy were observed for sex, age group and plasma folate (in that order) in participants with the CC genotype. In the case of the CT genotype,

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Table 1. Characteristics of the study population according to sex-specific fasting plasma total homocysteine (tHcy) tertiles (μmol/l)†
(Median values and 25th, 75th percentiles; mean values and 95 % confidence intervals)

	Women (tHcy (μmol/l) tertiles)						Men (tHcy (μmol/l) tertiles)					
	1 (<7·7)		2 (7·7–9·6)		3 (>9·6)		1 (<9·3)		2 (9·3–11·1)		3 (>11·1)	
	n		*n*		*n*		*n*		*n*		*n*	
Age (years)	125		125		121		117		118		117	
Median		39·6		42·3		46·3**		39·2		43·4		46·1**
P25, P75		37·3, 41·9		39·7, 45·0		43·2, 49·4		36·7, 41·7		40·9, 45·9		43·0, 49·1
BMI (kg/m^2)	125		123		116		116		116		116	
Arithmetic mean		25·8		26·7		27·5		27·2		27·7		27·2
95 % CI		24·9, 26·8		25·7, 27·6		26·3, 28·7		26·4, 28·1		26·9, 28·4		26·4, 28·0
Smokers	39		35		41		51		41		45	
%		31·2		28·2		34·2		43·6		34·7		38·8
95 % CI		23·7, 39·8		21·1, 36·7		27·2, 44·4		34·9, 52·6		26·8, 43·7		30·4, 47·9
Alcohol consumption‡	125		125		121		117		118		117	
Low to moderate	10		21		23		39		42		43	
%		8·0		16·8		19·0*		33·3		35·6		36·8
95 % CI		4·4, 14·1		11·3, 24·3		13·0, 26·9		25·4, 34·1		27·5, 44·6		28·6, 45·8
High	9		3		6		33		27		36	
%		7·2		2·4		4·9		28·2		22·9		30·8
95 % CI		3·8, 13·1		0·8, 6·8		2·3, 10·4		20·8, 37·1		16·2, 31·2		23·1, 39·6
Diagnosed hypertension	11		10		31		10		15		17	
%		8·8		8·0		25·6***		8·5		12·7		14·4
95 % CI		5·0, 15·1		4·4, 14·1		18·7, 34·1		4·7, 15·0		7·9, 19·9		9·2, 21·9
Low socio-economic status	53		61		61		13		30		40	
%		42·4		48·8		50·4		15·4		25·4		34·2**
95 % CI		34·1, 51·2		40·2, 57·5		41·6, 59·2		10·0, 23·0		18·4, 34·0		26·2, 43·2
Plasma folate (nmol/l)	125		125		121		117		118		117	
Geometric mean		14·3		11·5		11·0***		12·6		11·3		9·1***
95 % CI		13·1, 15·6		10·4, 12·7		9·8, 12·3		11·6, 13·6		10·3, 12·4		8·2, 10·1
Red cell folate (nmol/l)	125		125		121		117		118		118	
Geometric mean		899		781		733***		952		852		721***
95 % CI		846, 954		734, 830		676, 796		302, 1004		795, 913		673, 773
Plasma cobalamin (pmol/l)	124		125		121		117		118		117	
Geometric mean		377		352		322**		385		343		317***
95 % CI		355, 401		331, 375		298, 348		363, 408		321, 367		299, 336
EGRAC	123		125		121		116		117		113	
Geometric mean		1·41		1·33		1·36*		1·39		1·34		1·34
95 % CI		1·37, 1·45		1·30, 1·37		1·31, 1·40		1·36, 1·43		1·30, 1·37		1·30, 1·38
tHcy (μmol/l)	125		125		121		117		118		118	
Geometric mean		6·5		8·6		11·8***		7·9		10·1		13·6***
95 % CI		6·4, 6·7		8·5, 8·7		11·4, 12·2		7·7, 8·1		10·0, 10·2		13·1, 14·1
Plasma creatinine (μmol/l)	125		125		121		116		118		118	
Arithmetic mean		70·4		64·5		67·3		81·3		81·7		81·9
95 % CI		58·2, 82·5		63·0, 66·0		65·7, 69·0		79·2, 83·4		79·8, 83·7		79·7, 81·4
Plasma total cholesterol (mmol/l)	125		125		120		117		117		118	
Arithmetic mean		5·1		5·3		5·3		5·3		5·3		5·4
95 % CI		5·0, 5·3		5·1, 5·5		5·1, 5·5		5·1, 5·5		5·1, 5·5		5·2, 5·6
MTHFR CC	50		38		44		53		43		30	
%		40·0		30·4		36·4		45·3		36·8		25·6
95 % CI		31·8, 48·8		23·0, 38·9		28·3, 45·2		36·6, 54·3		28·6, 45·8		18·6, 34·2

Table 1. *(Continued)*

	Women (tHcy (μmol/l) tertiles)						Men (tHcy (μmol/l) tertiles)					
	1 (<7·7)		2 (7·7–9·6)		3 (>9·6)		1 (<9·3)		2 (9·3–11·1)		3 (>11·1)	
	n		*n*		*n*		*n*		*n*		*n*	
MTHFR CT	64		63		49		55		59		47	
%		51·2		50·4		40·5		47·0		50·4		40·2
95 % CI		42·5, 59·8		41·8, 59·0		32·2, 49·4		38·2, 56·0		41·5, 59·3		31·7, 49·2
MTHFR TT	11		24		28		9		15		40	
%		8·8		19·2		23·1*		7·7		12·8		34·2***
95 % CI		5·0, 15·1		13·3, 27·0		16·5, 31·4		4·1, 14·0		7·9, 20·1		26·2, 43·2
SLC19A1 GG	30		21		28		33		35		35	
%		24·2		17·4		23·3		28·2		29·9		30·4
95 % CI		17·5, 32·4		11·6, 25·1		16·7, 31·7		20·8, 37·0		22·4, 38·7		22·8, 39·4
SLC19A1 GA	58		77		55		58		56		55	
%		46·8		63·6		45·8		49·6		47·9		47·8
95 % CI		38·2, 55·5		54·8, 71·7		37·2, 54·7		40·7, 58·5		39·0, 56·8		38·9, 56·9
SLC19A1 AA	36		23		37		26		26		25	
%		29·0		19·0		30·8*		22·2		22·2		21·7
95 % CI		21·8, 37·6		13·0, 26·9		23·3, 39·6		15·6, 30·6		15·6, 30·6		15·2, 30·1

EGRAC, erythrocyte glutathione reductase activation coefficient; *MTHFR*, methylenetetrahydrofolate reductase 677C>T polymorphism; *SLC19A1*, solute carrier family 19A member 1 80 G>A polymorphism.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (χ^2 test comparing categorical variables and ANOVA comparing continuous variables between tHcy tertiles).
† Twenty-four participants were excluded after the medical check-up due to declared B vitamin supplement use. A further fifty-one participants were excluded from all analyses involving tHcy because their blood samples were not processed within 2 h of collection and five participants because they had suspected altered renal function (plasma creatinine >97 mmol/l for women and >124 mmol/l for men).
‡ Category of habitual alcohol intake: moderate (<16 g/d in women and <24 g/d in men) and high (≥16 g/d in women and ≥24 g/d in men).

these were plasma cobalamin, sex, plasma folate and number of cigarettes smoked/d for the CT genotype and plasma cobalamin and folate only in the case of the TT genotype.

In the models exploring the predictors of hypertension, we excluded the participants that were initially classified as 'non-hypertensive', based on the absence of diagnosed hypertension, but that had a point blood pressure reading of systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg at the study check-up, or with missing blood pressure readings. Thus, the prevalence of hypertension among the participants included in the final models was 21·8 %. The final ratio of non-hypertensive:hypertensive participants in these models was 3·6.

The probability of having hypertension when tHcy is in the third tertile *v.* the first is reported in Table 4. Age and BMI were significant predictors of hypertension in all of the models.

Being in the third tertile of tHcy was associated with increased probability of hypertension in the population as a whole (1·9; 95 % CI 1·2, 3·2) and this association was sustained after adjusting for multiple confounding variables in all of the models.A stratified analysis by age group showed that the association was confined to participants aged >50 years (2·8; 95 % CI 1·1, 5·6).

No association between either of the variant *MTHFR* 677C>T genotypes and diagnosed hypertension was observed in the overall population (Table 5). The TT genotype was associated with greater probability of having hypertension than the CC genotype (4·1; 95 % CI 1·0, 16·9), in participants aged ≤50 years. This association was sustained in all of the models. In the final model in this age group, the strongest predictors of hypertension were low compared with mid-high socio-economic status (9·5; 95 % CI 2·4, 27·9) and sex (male *v.* female) (8·8; 95 % CI 1·8, 43·2), followed by the *MTHFR* 677TT *v.* CC genotype. No association between genotype and hypertension was observed in participants older than 50 years. The strongest predictors of hypertension were EGRAC (6·2; 95 % CI 1·0, 38·7), low compared with mid-high socio-economic status (2·7; 95 % CI 1·1, 6·6) and BMI (1·2; 95 % CI 1·1, 1·3).

Discussion

Principal findings

Age interacted with the *MTHFR* 677TT genotype in its association with tHcy and smoking interacted with the genotype in men. Moderately elevated tHcy was associated with increased probability of hypertension in the overall population and specifically in people over 50 years of age. The association in this older age group may have been driving that observed in the overall population. On the other hand, the *MTHFR* 677TT genotype was associated with increased probability of hypertension compared with the CC genotype in participants of 50 years of age or under. Worsening riboflavin status was associated with increased probability of hypertension in people over 50 years of age.

Comparisons with other studies

The models explained up to 26 % of the variability of tHcy. The prevalence of the homozygote variant genotype at 17·9 %

Table 2. Multiple linear regression analysis of factors associated with fasting plasma total homocysteine in all participants and separately by sex (Adjusted R^2 values and β-coefficients)

Model	Adjusted R^2†	Independent variables	Standardised β‡	*P*
All participants (*n* 687)				
Model 1 (non-modifiable factors)§	0·184***	Age group (≤50, >50 years)	0·206	<0·001
		Sex	0·305	<0·001
		MTHFR TT *v.* CC genotype	0·358	<0·001
		MTHFR CT *v.* CC genotype	0·095	0·025
		Interaction *MTHFR* genotype × age group		0·056
Model 2 (model 1 + modifiable lifestyle factors)§‖	0·194***	Age group (≤50, >50 years)	0·196	0·001
		Sex	0·237	<0·001
		MTHFR TT *v.* CC genotype	0·348	<0·001
		MTHFR CT *v.* CC genotype	0·084	0·048
		Interaction *MTHFR* genotype × age group		0·071
		Cigarettes/d	0·079	0·030
		Plasma creatinine (μmol/l)	0·097	0·033
Model 3 (model 2 + 1CM nutrient status)§‖¶	0·259***	Age group (≤50, >50 years)	0·266	<0·001
		Sex	0·198	<0·001
		MTHFR TT *v.* CC genotype	0·325	<0·001
		MTHFR CT *v.* CC genotype	0·069	0·089
		Interaction *MTHFR* genotype × age group		0·030
		Cigarettes/d	0·053	0·138
		Plasma creatinine (μmol/l)	0·111	0·011
		Plasma cobalamin (pmol/l)	−0·198	<0·001
		Plasma folate (nmol/l)	−0·173	<0·001
		EGRAC	−0·051	0·143
Women (*n* 349)				
Model 1 (non-modifiable factors)§	0·090***	Age group (≤50, >50 years)	0·263	0·001
		MTHFR TT *v.* CC genotype	0·308	<0·001
		MTHFR CT *v.* CC genotype	0·095	0·134
		Interaction *MTHFR* genotype × age group		0·314
Model 2 (model 1 + modifiable lifestyle factors)§‖	0·137***	Age group (≤50, >50 years)	0·281	0·001
		MTHFR TT *v.* CC genotype	0·287	<0·001
		MTHFR CT *v.* CC genotype	0·089	0·156
		Interaction *MTHFR* genotype × age group		0·341
		Cigarettes/d	0·165	0·002
		Plasma creatinine (μmol/l)	0·133	0·008
Model 3 (model 2 + 1CM nutrient status)§‖¶	0·211***	Age group (≤50, >50 years)	0·371	<0·001
		MTHFR TT *v.* CC genotype	0·271	<0·001
		MTHFR CT *v.* CC genotype	0·068	0·258
		Interaction *MTHFR* genotype × age group		0·162
		Cigarettes/d	0·117	0·025
		Plasma creatinine (μmol/l)	0·161	0·001
		Plasma cobalamin (pmol/l)	−0·251	0·003
		Plasma folate (nmol/l)	−0·161	<0·001
		EGRAC	−0·086	0·094
Men (*n* 337)				
Model 1 (non-modifiable factors)§	0·128***	Age group (≤50, >50 years)	0·161	0·040
		MTHFR TT *v.* CC genotype	0·453	<0·001
		MTHFR CT *v.* CC genotype	0·106	0·085
		Interaction *MTHFR* genotype × age group		0·089
Model 2 (model 1 + modifiable lifestyle factors)§‖	0·128***	Age group (≤50, >50 years)	0·136	0·106
		MTHFR TT *v.* CC genotype	0·454	<0·001
		MTHFR CT *v.* CC genotype	0·100	0·109
		Interaction *MTHFR* genotype × age group		0·066
		Cigarettes/d	0·015	0·784
		Plasma creatinine (μmol/l)	0·013	0·810
Model 3 (model 2 + 1CM nutrient status)§‖¶	0·129***	Age group (≤50, >50 years)	0·217	0·011
		MTHFR TT *v.* CC genotype	0·422	<0·001
		MTHFR CT *v.* CC genotype	0·091	0·080
		Interaction *MTHFR* genotype × age group		0·025
		Cigarettes/d	−0·009	0·868
		Plasma creatinine (μmol/l)	0·008	0·870
		Plasma cobalamin (pmol/l)	−0·175	0·001
		Plasma folate (nmol/l)	−0·227	<0·001
		EGRAC	−0·002	0·974

MTHFR, methylenetetrahydrofolate reductase; 1CM, 1C metabolism; EGRAC, erythrocyte glutathione reductase activation assay; *SLC19A1*, solute carrier family 19 A member.
*** $P < 0·001$.
† Corresponding with each model.
‡ From the complete models.
§ Adjusted for *SLC19A1* 80GA *v.* GG and *SLC19A1* 80AA *v.* GG genotypes.
‖ Adjusted for the same variables as model 1 plus low *v.* mid-high socio-economic status, BMI, moderate (<16 g/d in women, <24 g/d in men) *v.* no alcohol consumption, high (≥16 g/d in women, ≥24 g/d in men) *v.* no alcohol consumption, number of cigarettes smoked/d and plasma creatinine.
¶ Adjusted for the same variables as model 3. Missing data are due to some incomplete lifestyle questionnaires or insufficient blood sample for all of the determinations. Only data relating to blood samples processed in <2 h of collection were included in the models.

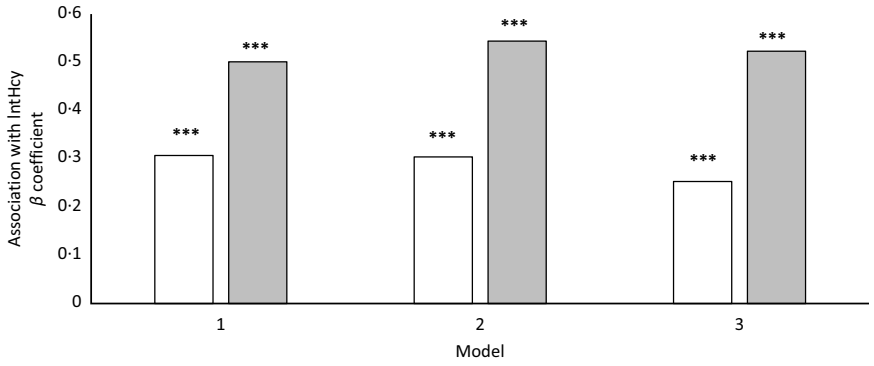

Fig. 1. Interaction between smoking and the methylenetetrahydrofolate reductase (*MTHFR*) 677TT *v.* CC genotype in its association with fasting plasma total homocysteine in men. Columns represent the difference in ln tHcy for *MTHFR* 677TT compared with the CC genotype in non-smokers (white columns) and smokers (shaded columns), determined by multiple linear regression analysis. Dependent variable natural log-transformed tHcy. All models were significant ($P < 0{\cdot}001$). R^2 (n) for each model: model 1, non-smokers: 0·093 (214); smokers: 0·216 (122); model 2, non-smokers: 0·084 (214); smokers: 0·212 (122); model 3, non-smokers: 0·183 (214); smokers: 0·276 (122). Model 1: adjusted for age group (≤50, >50 years), solute carrier family 19A member 1 80 G>A polymorphism (*SLC19A1*) 80GA *v.* GG and *SLC19A1* 80AA *v.* GG genotypes; model 2: adjusted for the same variables as model 1 plus low *v.* mid-high socio-economic status, BMI, moderate (<16 g/d in women, <24 g/d in men) *v.* no alcohol consumption, high (≥16 g/d in women, ≥24 g/d in men) *v.* no alcohol consumption, number of cigarettes smoked/d and plasma creatinine; model 3: adjusted for the same variables as model 2 plus plasma folate, plasma cobalamin and erythrocyte glutathionine reductase activation coefficient. Missing data are due to some incomplete lifestyle questionnaires or insufficient blood sample for all of the determinations. Only data relating to blood samples processed in <2 h of collection were included in the models. *** $P < 0{\cdot}001$.

was higher than the 11·8 % previously reported for Spanish Caucasians[41].

We confirm findings from previous studies[24,42] that both folate and cobalamin status are the most influential modifiable determinants of tHcy. Age and sex[24] or sex, age, folate intake, smoking status, and coffee consumption[43] were also reported to be the strongest determinants of tHcy. We add to these findings with the observation that the *MTHFR* 677C>T genotype is the strongest determinant of tHcy in men and the next strongest after age in women. The strength of the *MTHFR* 677TT–tHcy association is stronger in male smokers than non-smokers. Our results disagree with the finding that the association between the *MTHFR* 677TT genotype and tHcy is confined to men under 55 years of age[44]. The only group that we did not observe this association was women older than 50 years of age.

The *MTHFR* 677TT genotype was associated with hypertension in people younger than 50 years of age, but moderately elevated tHcy was not. On the other hand, moderately elevated tHcy was associated with hypertension in people over 50 years of age. The results support previous findings of a positive association between moderately elevated tHcy and hypertension in adults[13]. Another study reported a positive association between tHcy and diastolic blood pressure, mostly in young adults[8]. We did not test the association between tHcy and diastolic blood pressure but observed no association between moderately elevated tHcy and hypertension in young adults.

A B-vitamin intervention trial in elderly adult New Zealanders, with high baseline tHcy, lowered tHcy but did not affect blood pressure[16]. The results from this and other trials were inconsistent[17–19]. It is possible that the elevated tHcy observed in older adults is marking age-related processes that also contribute to blood pressure or cardiovascular risk in general. These processes are independent of tHcy reduction achieved by B vitamin supplementation. This may explain why there is little apparent benefit of tHcy lowering to the outcomes of interest if the same exposure persists to other underlying risk factors. It is well established that CVD and stroke are caused by exposure to multifactorial factors that interact with each other over a lifetime. Timing of the tHcy reduction relevant to the development/progression of the biological lesion would be essential to changing the outcome, if it is causally involved. However, this is an extremely difficult component to control and to replicate between trials that are already compounded by a wide diversity of exposures to biological, lifestyle and environmental risks.

A Chinese study reported that the *MTHFR* 677TT genotype was most prevalent in the third tertile of diastolic blood pressure compared with the first and second tertiles in hypertensive patients but this was not true for systolic blood pressure[32]. Another study reported that the association between the *MTHFR* 677TT genotype and hypertension was modulated by riboflavin status and riboflavin supplementation was effective in reducing blood pressure in patients with the TT genotype only[36].

The results also support the observations that the association between the *MTHFR* TT genotype and hypertension did not appear to be mediated by tHcy concentration[12] or those previously mentioned in the Mendelian randomisation study in young adults[14]. However, folate[12] and riboflavin[26] status modulate the effect of the polymorphism on tHcy but were not considered in the Mendelian randomisation study. Here, we report an interaction between smoking and the *MTHFR* 677TT genotype, in its association with tHcy, in men. The genotype–tHcy association is stronger in smokers than in non-smokers. Furthermore, folate[45] and riboflavin[36] may modulate the association between the polymorphism and hypertension.

Interpretation

Globally, plasma folate status was higher in women than in men so this may explain why plasma cobalamin is a stronger determinant of tHcy than plasma folate in women. Cobalamin status

Table 3. Multiple linear regression analysis of factors associated with fasting plasma total homocysteine in all participants and separately according to methylenetetrahydrofolate reductase (*MTHFR*) 677C>T genotype
(Adjusted R^2 values and β-coefficients)

Model	Adjusted R^2†	Independent variables	Standardised β‡	*P*
MTHFR 677CC genotype (*n* 241)				
Model 1 (non-modifiable factors)§	0·117***	Sex	0·298	<0·001
		Age group (≤50, >50 years)	0·200	0·001
Model 2 (model 1 + modifiable lifestyle factors)§‖	0·100***	Sex	0·290	0·002
		Age group (≤50, >50 years)	0·162	0·033
		Cigarettes/d	0·007	0·920
Model 3 (model 2 + 1CM nutrient status)§‖¶	0·140***	Sex	0·268	0·004
		Age group (≤50, >50 years)	0·227	0·004
		Cigarettes/d	–0·002	0·974
		Plasma cobalamin (pmol/l)	–0·064	0·301
		Plasma folate (nmol/l)	–0·217	0·001
		EGRAC	–0·069	0·279
MTHFR 677CT genotype (*n* 322)				
Model 1 (non-modifiable factors)§	0·126***	Sex	0·327	<0·001
		Age group (≤50, >50 years)	0·174	0·001
Model 2 (model 1 + modifiable lifestyle factors)§‖	0·167***	Sex	0·181	0·017
		Age group (≤50, >50 years)	0·125	0·048
		Cigarettes/d	0·151	0·006
Model 3 (model 2 + 1CM nutrient status)§‖¶	0·239***	Sex	0·157	0·032
		Age group (≤50, >50 years)	0·055	0·322
		Cigarettes/d	0·151	0·006
		Plasma cobalamin (pmol/l)	–0·235	<0·001
		Plasma folate (nmol/l)	–0·156	0·005
		EGRAC	–0·073	0·160
MTHFR 677TT genotype (*n* 122)				
Model 1 (non-modifiable factors)§	0·087**	Sex	0·340	<0·001
		Age group (≤50, >50 years)	–0·028	0·752
Model 2 (model 1 + modifiable lifestyle factors)§‖	0·083*	Sex	0·246	0·043
		Age group (≤50, >50 years)	0·067	0·532
		Cigarettes/d	0·088	0·344
Model 3 (model 2 + 1CM nutrient status)§‖¶	0·266***	Sex	0·146	0·187
		Age group (≤50, >50 years)	0·104	0·292
		Cigarettes/d	0·088	0·344
		Plasma cobalamin (pmol/l)	–0·417	<0·001
		Plasma folate (nmol/l)	–0·191	0·032
		EGRAC	–0·008	0·924

1CM, 1C metabolism; EGRAC, erythrocyte glutathione reductase activation coefficient; *SLC19A1*, solute carrier family 19A member.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Corresponding with each model.
‡ From the complete models.
§ Adjusted for *SLC19A1* 80 GA *v.* GG and *SLC19A1* 80 AA *v.* GG genotypes.
‖ Adjusted for the same variables as model 1 plus low *v.* mid-high socio-economic status, BMI, moderate (<16 g/d in women, <24 g/d in men) *v.* no alcohol consumption, high (≥16 g/d in women, ≥24 g/d in men) *v.* no alcohol consumption, number of cigarettes smoked/d and plasma creatinine.
¶ Adjusted for the same variables as model 3. Missing data are due to some incomplete lifestyle questionnaires or insufficient blood sample for all of the determinations. Only data relating to blood samples processed in <2 h of collection were included in the models.

Table 4. Association between moderately elevated fasting plasma total homocysteine (tHcy) and diagnosed hypertension†
(Odds ratios and 95 % confidence intervals)

	All participants				Aged ≤50 years				Aged >50 years			
Model	*n*	R^2‡	OR§	95 % CI	*n*	R^2	OR	95 % CI	*n*	R^2	OR	95 % CI
1	583	0·024*	1·9	1·2, 3·0	418	0·006	1·5	0·6, 3·5	165	0·079**	2·8	1·5, 5·5
2		0·202***	1·9	1·2, 3·0		0·083**	1·5	0·6, 3·7		0·108**	2·5	1·3, 4·9
3		0·492***	1·8	1·0, 3·3		0·372***	1·2	0·4, 3·5		0·351***	2·5	1·2, 5·4

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† Multiple logistic regression analysis was used. Cut-offs for the third tertiles were ≥9·09 μmol/l in women ≤50 years, ≥10·60 μmol/l in women >50, ≥10·88 μmol/l in men ≤50 years, ≥11·59 μmol/l in men >50. Participants without diagnosed hypertension but with point blood pressure measurements >140/90 mm Hg, at the study check-up, were referred for blood pressure monitoring and excluded from the analysis (*n* 77). A further forty-one participants without diagnosed hypertension but with no point blood pressure measurement and BMI > 30 kg/m^2 as well as five participants with possible impaired renal function (plasma creatinine concentration >124 mmol/l in men and >97 mmol/l in women) were also excluded. Only tHcy determinations performed in samples processed in less than 2 h of collection were included. Model 1: (basic model) having tHcy in the thirdtertile compared with tHcy in the first and secondtertiles. Model 2: included the same variables as model 1 as well as low *v.* mid-high socio-economic status. Model 3: included the same variables as model 2 as well as BMI, category of regular alcohol intake (moderate (<16 g/d in women and <24 g/d in men) *v.* none; high *v.* none (≥16 g/d in women and ≥24 g/d in men)), current smoking (cigarettes/d) and total plasma cholesterol (mmol/l).
‡ Nagelkerke R^2.
§ OR and 95 % CI for diagnosed hypertension in participants in the third *v.* the first and secondage and sex-specific tHcy tertiles are shown.

Table 5. Association between methylenetetrahdyrofolate reductase (*MTHFR*) 677C>T genotype and diagnosed hypertension† (Odds ratios and 95 % confidence intervals)

	All participants						Aged ≤50 years						Aged >50 years					
			CT *v.* CC§		TT *v.* CC				CT *v.* CC		TT *v.* CC				CT *v.* CC		TT *v.* CC	
Model	*n*	R^2‡	OR‖	95 % CI	OR‖	95 % CI	*n*	R^2	OR‖	95 % CI	OR‖	95 % CI	*n*	R^2	OR‖	95 % CI	OR‖	95 % CI
1	573	0·003	1·2	0·7, 1·9	1·4	0·7, 2·6	410	0·037	3·3	0·9, 11·7	4·1	1·0, 16·9	163	0·002	1·1	0·6, 2·2	1·3	0·5, 3·1
2		0·433***	1·5	0·8, 2·8	1·5	0·7, 3·4		0·160***	3·2	0·9, 11·6	4·0	0·9, 17·0		0·059	1·1	0·5, 2·1	1·2	0·5, 3·0
3		0·585***	1·2	0·6, 2·6	1·7	0·7, 4·4		0·472***	3·8	0·7, 20·3	8·2	1·3, 53·9		0·348***	1·0	0·4, 2·2	1·2	0·4, 3·7

*** $P < 0·001$.

† Participants that did not have diagnosed hypertension but point blood pressure measurements greater than 140/90, at the study check-up, were referred for blood pressure monitoring and excluded from the analysis (*n* 77). A further forty-one participants with no point blood pressure measurement and BMI > 30 kg/m^2 and five participants with plasma creatinine concentration >124 mmol/l in men and >97 mmol/l in women (indicating possible impaired renal function) were also excluded. Model 1: (basic model) including the predictor variables *MTHFR* 677CT *v.* CC and *MTHFR* 677TT *v.* CC genotypes. Model 2: included the same variables as model 1 as well as sex, age and BMI. Model 3: included the same variables as model 2 as well as plasma folate, plasma cobalamin, erythrocyte glutathionine reductase activation coefficient (functional indicator of riboflavin status) low *v.* mid-high socio-economic status, category of regular alcohol intake (moderate (<16 g/d in women and <24 g/d in men) *v.* none; high (≥16 g/d in women and ≥24 g/d in men)) *v.* none, current smoking (cigarettes/d) and serum total cholesterol.

‡ Nagelkerke R^2 from multiple logistic regression analysis.

§ *MTHFR* 677C>T genotype.

‖ OR and 95 % CI for diagnosed hypertension in participants with the CT *v.* CC genotype and TT *v.* CC genotype, globally and according age group.

has been shown to be the next limiting factor in determining tHcy after folate[46].

We did not measure female hormones but, based on previous evidence that female hormones are inversely associated with tHcy, we suggest that the strong determining effect of age on tHcy in women may reflect the effects of changes in hormonal status during different stages of life[47–49]. Female hormones may also influence the differences in the determining factors of tHcy between women and men.

In participants under 50 years, the *MTHFR* 677TT genotype was associated with a greater risk of diagnosed hypertension compared with the CC genotype. This confirms previous reports of an association between the variant T allele and hypertension[36]. Our data do not directly support that the mechanism linking the *MTHFR* genotype to hypertension is via elevated tHcy. Although more participants with the TT genotype (in both age groups) had tHcy in the third tertile, in participants under 50 years, tHcy in the third tertile was not associated with hypertension. Other factors, such as loss in renal function, may also lead to increasing tHcy with age[50]. This age itself and elevated BMI were less prevalent in the participants under 50 years. After low socio-economic status and sex, the *MTHFR* 677C>T polymorphism was most strongly associated with hypertension in this age group. On the other hand, EGRAC, low socio-economic status and BMI were the strongest predictors of hypertension in the older age groups. These risk factors for hypertension may be more important in older people than in younger people, thus overriding the underlying *MTHFR* 677C>T polymorphism effect. Regarding riboflavin status (indicated by EGRAC), worsening status was associated with greater probability of hypertension in the older age group only. The reason for this is unclear but plasma folate, erythrocyte folate and riboflavin status were all higher in the older compared with the younger age group, as we reported previously[26]. We can speculate that the EGRAC–hypertension association becomes evident when folate status is replete. Folic acid supplementation has been shown to improve flow-mediated dilatation in blood vessels in coronary artery disease patients independently of tHcy[51] and improved artery stiffness independently of *MTHFR* genotype[52]. Riboflavin supplementation has been shown to reduce systolic blood pressure in *MTHFR* 677TT homozygotes[36]. Folic acid supplement use has been reported to protect against incident hypertension[53]. Regarding the differences in predictors of hypertension between the two age groups, impaired one-carbon metabolism due to low folate or riboflavin status and/or *MTHFR* 677C>T genotype may be more important in younger people where the risk factors associated with ageing are of lower prevalence. In older people, these established age-related risk factors may be more important causes of hypertension. Hyperhomocysteinaemia may be marking each of these 'different' groups of risk factors. If folate protects against hypertension, when hyperhomocysteinaemia is due to impairment in the folate cycle (for genetic or dietary reasons) rather than renal impairment or ageing, it might be linked with hypertension via the same impaired vascular function process. On the other hand, hyperhomocysteinaemia due to renal impairment or ageing may be a biomarker of alternative processes leading to hypertension.

Strengths and limitations

Associations between folate, cobalamin and riboflavin status as well as the *MTHFR* 677C>T polymorphism with tHcy and hypertension were explored without the influence of B vitamin supplement use and mandatory fortification of staple foods. These factors are likely contributors to the inter-study discrepancies in the effects of tHcy or the *MTHFR* 677C>T genotype previously reported.

Reverse causation cannot be ruled out in the observed associations between tHcy and hypertension in a study of this design. However, this potential limitation does not affect the association between the *MTHFR* 677C>T polymorphism and hypertension. Unknown causes, to date, are likely to explain a relatively large number of hypertension cases. Regarding the known causes, they are diverse and precise control of the intensity of exposures is difficult. Such sources of residual confounding are potential limitations to the study. Previously diagnosed hypertension was the designated outcome of the models. Study point blood pressure measurements were only used to categorise

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participants with normal readings and no previous diagnosis or suspicion of hypertension, as the normal blood pressure group. To avoid misclassification to either group, participants with high blood pressure detected for the first time at the study check-up were excluded. Changes in lifestyle habits in response to medical advice may have affected tHcy or other predictor variables included in the hypertension models, and blood pressure itself may also have been affected. However, the expected predictors of tHcy were confirmed in the models, and the categorisation of diagnosed hypertension was maintained regardless of whether it had normalised due to treatment. Established predictors of hypertension such as age and BMI were also confirmed in the hypertension models. The study was of an ostensibly low-risk adult population and only 4·2 % of participants under 50 years of age had hypertension. Nevertheless, a significant association between the *MTHFR* 677TT genotype and probability of hypertension was observed in this group.

Conclusion

The probability of hypertension was increased with the *MTHFR* 677TT genotype in adults under 50 years and with moderately elevated tHcy in people over 50 years of age. The strengths of the factors predicting hypertension and their order of importance were different between younger and older adults. Different underlying origins of hyperhomocysteinaemia may explain differences in its links with hypertension with age. This study in a representative sample of an adult population, unexposed to mandatory folic acid fortification or B vitamin supplement use, adds to the evidence that both moderately elevated tHcy and the *MTHFR* 677C>T polymorphism are associated with the risk of hypertension and that these associations differ in subgroups of the population.

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G. O.-M., M. M. M. and J. D. F.-B. designed the research. S. C., L. R., G. O.-M., M. M. M. and J. D. F.-B. conducted the research. P. M. U. and K. M. were responsible for the rs 1051266 determinations. G. O.-M., M. M. M. and J. D. F.-B. analysed the data. G. O.-M. and M. M. M. wrote the manuscript. M. M. M. had primary responsibility for the final content. All authors read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material referred to in this article, please visit https://doi.org/10.1017/S0007114520000793

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