Title: Homocysteine, the *MTHFR* 677C>T polymorphism and hypertension: effect modifiers by lifestyle factors and population subgroups.

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Short title: Homocysteine, *MTHFR* 677C>T and hypertension.

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ABSTRACT

Evidence linking fasting plasma total homocysteine (tHcy), 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 was conducted in 788 adults, aged 18-75 years, randomly selected from 3 Catalonian town population registers. Fasting plasma folate, cobalamin, total homocysteine (tHcy), red blood cell folate, erythrocyte glutathione reductase activation coefficient (EGRAC, functional riboflavin status indicator; increasing EGRAC indicates worsening riboflavin status), *MTHFR* 677 C>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/day) 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 to nonsmokers (0.21, $P = 0.001$) and weaker in participants >50 (0.19, $P = 0.007$) compared to ≤50 years (0.31, $P < 0.001$). Hypertension was more probable in the 3rd tHcy tertile compared to the other tertiles [OR 1.9 (1.2, 3.0)], and in participants ≤50 years, for the *MTHFR* 677TT genotype compared to the CC genotype [OR 4.1 (1.0, 16.9)]. EGRAC was associated with increased probability of hypertension in participants >50 years [OR 6.2 (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 ≤50 years.

INTRODUCTION

Hypertension affects 1 in 5 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 socioeconomic 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.

Hyperhomocysteinemia 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 with 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 *MTHFR* 677C>T polymorphism has been associated with lower folate status and higher tHcy in the homozygote variant compared to the common genotype[20-22] and its inverse association with folate status is enhanced in the presence of the *SLC19A1* 80G>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* 677 TT compared to 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 to 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 to 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 B12 and B6 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* 677 C>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* 677 C>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.

MATERIAL AND METHODS

Study sample

This cross-sectional study was carried out by the *Unitat de Medicina Preventiva i Salut Pública,* Universitat Rovira i Virgili (URV) 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 3 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, Germany) and standardised protocol. Participants were seated for at least 15 minutes 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 (two minutes apart) was recorded. Partcipants 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*" (CIE-9-MC)[39]. The frequency of diagnosed hypertension was recorded (previous diagnosis of hypertension based on blood pressure greater than or equal to 140/90 mmHg, being treated or monitored by their clinician). Following 15 minutes rest, 2 readings (2 minutes 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 4ºC until processing, in less than 2 h of collection, as previously described for red blood cell 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 triglycerides were measured by standard techniques (ITC diagnostics). The methylenetetrahydrofolate reductase (*MTHFR*) 677C>T (rs1801133) and solute reduced carrier 1 (*SLC19A1*) 80G>A (rs 1051266) polymorphisms were determined as previously described from leukocyte-extracted DNA[23].

Statistical analysis

Descriptive data is reported as mean (95% CI) for normally distributed variables and as geometric means (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 % (95% CI), calculated using the Confidence Interval Analysis program (University of Southampton, UK), and compared between groups with the chi-square 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 3rd compared to 1st and 2nd 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, socioeconomic status, BMI, alcohol intake, smoking and total plasma cholesterol. The

probability of having hypertension with the *MTHFR* 677 CT and TT compared to CC genotypes was also investigated using logistic regression (basic model) and further models adjusted for sex, age, BMI, plasma creatinine, *SLC19A1* 80 GA vs GG genotype, *SLC19A1* 80 AA vs GG genotype, plasma folate, plasma cobalamin, EGRAC, socioeconomic 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* 677 CT genotype and 3.0 for those with the TT compared to CC genotype. In the same study, 49% and 9.3% of the non-hypertensive group had CT and TT genotypes respectively. Accepting an alpha risk of 0.05 and a beta 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 odds ratio 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 $P < 0.05$ and SPSS version 23.0 used for statistical analyses.

RESULTS

The prevalence (95% CI) of diagnosed hypertension in the population was 16.2 % (13.5, 19.1). It was 4.5% (3.0, 6.8) in participants aged 50 years or less and 42.6% (36.0, 49.5) in participants aged over 50.

The characteristics of the studied population, stratified by tHcy tertiles, are reported in **Table 1** and **Supplemental Table 1**. The allele frequencies for the *MTHFR* 677 C>T and *SLC19A1* 80 G>A polymorphisms were in Hardy Weinberg equilibrium. In the 3rd 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, Supplemental Table 1), the *MTHFR* 677TT genotype and the combination of *MTHFR* 677TT + *SLC19A1* 80AA genotypes were more prevalent, compared to the other tertiles. Specifically, women had higher plasma creatinine concentrations and more of them were hypertensive and more men had low socioeconomic status compared to those in the other tertiles. Globally, plasma folate (nmol/L) status (geometric mean [95% CI]) was higher in women (12.2

[11.5, 12.9]) than in men (10.9 [10,4, 11.5]), *P*=0.006. Participant characteristics are reported by age group and sex in **Supplemental Table 2.**

Multiple linear regression analysis, testing the associations between nonmodifiable factors and lifestyle factors with tHcy, are summarised in **Table 2**. In the complete model in women, age group followed by *MTHFR* 677 TT genotype, plasma cobalamin, folate, creatinine and smoking were the 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*=0.030) in the overall population and between *MTHFR* 677C>T genotype and smoking in men (*P*=0.028). The interaction is illustrated in Figure 1. The effect sizes of the associations (β coefficients) between the *MTHFR* 677TT genotype versus 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*<0.001; in women >50, β: 0.09, *P*=0.19) but in men it was observed in both age groups (aged ≤50 or less: β: 0.29, *P*<0.001; >50, β: 0.14, *P*= 0.020).

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, these were plasma cobalamin, sex, plasma folate and number of cigarettes smoked/ day 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 (OR (95% CI)), of having hypertension when tHcy is in the 3rd tertile versus the 1st is reported in **Table 4**. Age and BMI were significant predictors of hypertension in all of the models.

Being in the 3rd tertile of tHcy was associated with increased probability of hypertension in the population as a whole, 1.9 (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 (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 (1.0, 16.9), in participants ≤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 to mid-high socioeconomic status (9.5 [2.4, 27.9]) and sex (male versus female) (8.8 [1.8, 43.2]), followed by the *MTHFR* 677TT versus CC genotype. No association between genotype and hypertension was observed in participants older than 50. The strongest predictors of hypertension were EGRAC (6.2 (1.0, 38.7)), low compared to mid-high socioeconomic status (2.7 [1.1, 6.6]) and BMI (1.2 [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 to 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.

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%, 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 nonsmokers. 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 in, was women older than 50 years of age.

The *MTHFR* 677TT genotype was associated with hypertension in people younger than 50, but moderately elevated tHcy was not. On the other hand, moderately elevated tHcy was associated with hypertension in people over 50. 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 cardiovascular diseases 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 3rd tertile of diastolic blood pressure compared to the 1st and 2nd 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 nonsmokers. 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 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, the *MTHFR* 677TT genotype was associated with a greater risk of diagnosed hypertension compared to 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 3rd tertile, in participants under 50, tHcy in the 3rd 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. After low socioeconomic status and sex, the *MTHFR* 677C>T polymorphism was most strongly associated with hypertension in this age group. On the other hand, EGRAC, low socioeconomic 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, red blood cell folate and riboflavin status were all higher in the older compared to 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 2 age groups, impaired one carbon metabolism due to low folate or riboflavin status and/or *MTHFR* 677 C>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.

Hyperhomocysteinemia may be marking each of these "different" groups of risk factors. If folate protects against hypertension, when hyperhomocysteinemia 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, hyperhomocysteinemia due to renal impairment or aging 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* 677 C>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 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.

CONCLUSIONS

The probability of hypertension was increased with the *MTHFR* 677TT genotype in adults under 50 and with moderately elevated tHcy in people over 50. The strengths of the factors predicting hypertension and their order of importance were different between younger and older adults. Different underlying origins of hyperhomocysteinemia 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 risk of hypertension and that these associations differ in subgroups of the population.

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Conflicts of Interest: None.

REFERENCES

1. World Health Organization. Q&As on hypertension.https://www.who.int/features/qa/82/en/ (accessed July 2019)
2. Athanasakis K. (2017) The Socioeconomic Effects of Uncontrolled Hypertension. *Curr Vasc Pharmacol* 16: 5–9.
3. Partridge L, Deelen J, Slagboom PE. (2018) Facing up to the global challenges of ageing. *Nature* 561:45–56.
4. Rossier BC, Bochud M, Devuyst O. (2017= The Hypertension Pandemic: An Evolutionary Perspective. *Physiology* 32:112–25.
5. Symons JD, Mullick AE, Ensunsa JL *et al*. (2002) Hyperhomocysteinemia evoked by folate depletion: effects on coronary and carotid arterial function. *Arterioscler Thromb Vasc Biol* 22:772–80.
6. Rodrigo R, Passalacqua W, Araya J *et al*. (2003) Homocysteine and Essential Hypertension. *J Clin Pharmacol* 43:1299–306.
7. Tawakol A, Omland T, Gerhard M *et al*. (1997) Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilation in humans. *Circulation.* 95:1119–21.
8. Nygård O, Vollset SE, Refsum H *et al*. (1995) Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study. *JAMA* 274:1526–33.
9. Yang B, Fan S, Zhi X *et al*. (2017) Interactions of homocysteine and conventional predisposing factors on hypertension in Chinese adults. *J Clin Hypertens* 19:1162–70.
10. Sundström J, Sullivan L, D'Agostino RB *et al*. (2003) Plasma homocysteine, hypertension incidence, and blood pressure tracking: the Framingham Heart Study. *Hypertension* 42:1100–5.
11. Wang Y, Chen S, Yao T *et al*. (2014) Homocysteine as a Risk Factor for Hypertension: A 2-Year Follow-Up Study. *PLoS One* 9:e108223.
12. Rodríguez-Esparragón F, Hernández-Perera O, Rodríguez-Pérez JC *et al*. (2003) The effect of methylenetetrahydrofolate reductase C677T common variant on hypertensive risk is not solely explained by increased plasma homocysteine values. *Clin Exp Hypertens* 25:209–20.
13. Lim U, Cassano PA (2002) Homocysteine and blood pressure in the Third National Health and Nutrition Examination Survey, 1988-1994. *Am J Epidemiol*

156:1105–13.

14. Borges MC, Hartwig FP, Oliveira IO *et al*. (2016) Is there a causal role for homocysteine concentration in blood pressure? A Mendelian randomization study. *Am J Clin Nutr* 103:39–49.
15. Qin X, Li Y, Sun N *et al*. (2017) Elevated Homocysteine Concentrations Decrease the Antihypertensive Effect of Angiotensin-Converting Enzyme Inhibitors in Hypertensive Patients. *Arterioscler Thromb Vasc Biol* 37:166–72.
16. McMahon JA, Skeaff M, Williams SM *et al.* (2007). Lowering homocysteine with B vitamins has no effect on blood pressure in older adults. *J Nutr* 137: 1183–1187.
17. Koutatsu Maruyama K, Eshak ES, Kinuta M, *et al.* (2019). Association between vitamin B group supplementation with changes in % flow- mediated dilatation and plasma homocysteine levels: a randomized controlled trial. *J Clin Biochem Nutr* 64: 243–249.
18. van Dijk RA, Rauwerda JA, Steyn M *et al.* (2001) Long term homocysteine-lowering treatment with folic acid plus pyridoxine is associated with decreased blood pressure but not with improved brachial artery endothelium-dependent vasodilation or carotid artery stiffness: a 2-year, randomized, placebo-controlled trial. *Arterioscler Thromb Vasc Biol* 21, 2072–2079.
19. Mangoni AA, Sherwood RA, Swift CG *et al.* (2002) Folic acid enhances endothelial function and reduces blood pressure in smokers: a randomized controlled trial. *J Intern Med* 252, 497–503.
20. Frosst P, Blom HJ, Milos R *et al*. (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 10:111–3.
21. Jacques PF, Bostom AG, Williams RR *et al*. (1996) Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 93:7–9.
22. Crider KS, Zhu J-H, Hao L *et al*. (2011) MTHFR 677CT genotype is associated with folate and homocysteine concentrations in a large, population-based, double-blind trial of folic acid supplementation. *Am J Clin Nutr* 93:1365–72.
23. Bueno O, Molloy AM, Fernandez-Ballart JD *et al*. (2016) Common Polymorphisms That Affect Folate Transport or Metabolism Modify the Effect of the MTHFR 677C T Polymorphism on Folate Status. *J Nutr* 146:1–8.

24. Jacques PF, Bostom AG, Wilson PW *et al*. (2001) Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. *Am J Clin Nutr* 73:613–21.

25. Selhub J, Jacques PF, Bostom AG *et al*. (2000) Relationship between plasma homocysteine and vitamin status in the Framingham study population. Impact of folic acid fortification. *Public Health Rev* 28:117–45.

26. García-Minguillán CJ, Fernandez-Ballart JD, Ceruelo S *et al*. (2014) Riboflavin status modifies the effects of methylenetetrahydrofolate reductase (MTHFR) and methionine synthase reductase (MTRR) polymorphisms on homocysteine. *Genes Nutr* 9:435.

27. McNulty H, McKinley MC, Wilson B *et al*. (2002) Impaired functioning of thermolabile methylenetetrahydrofolate reductase is dependent on riboflavin status: implications for riboflavin requirements. *Am J Clin Nutr* 76:436–41.

28. McNulty H, Dowey LRC, Strain JJ *et al*. (2006). Riboflavin lowers homocysteine in individuals homozygous for the MTHFR 677C>T polymorphism. *Circulation* 113:74–80.

29. Heux S, Morin F, a. Lea R *et al*. (2004) The Methylentetrahydrofolate Reductase Gene Variant (C677T) as a Risk Factor for Essential Hypertension in Caucasians. *Hypertens Res* 27:663–7.

30. Ilhan N, Kucuksu M, Kaman D *et al*. (2008) The 677 C/T MTHFR polymorphism is associated with essential hypertension, coronary artery disease, and higher homocysteine levels. *Arch Med Res* 39:125–30.

31. Inamoto N, Katsuya T, Kokubo Y *et al*. (2003) Association of methylenetetrahydrofolate reductase gene polymorphism with carotid atherosclerosis depending on smoking status in a Japanese general population. *Stroke* 34:1628–33.

32. Cheng J, Tao F, Liu Y *et al*. (2018) Associations of methylenetetrahydrofolate reductase C677T genotype with blood pressure levels in Chinese population with essential hypertension. *Clin Exp Hypertens* 40:207–12.

33. Yin R-X, Wu J-Z, Liu W-Y *et al*. (2012) Association of several lipid-related gene polymorphisms and blood pressure variation in the Bai Ku Yao population. *Am J Hypertens* 25:927-936.

34. Pérez-Razo JC, Cano-Martínez LJ, Vargas Alarcón G *et al*. (2015) Functional Polymorphism rs13306560 of the MTHFR Gene Is Associated With Essential

Hypertension in a Mexican-Mestizo Population. *Circ Cardiovasc Genet* 8:603–9.

35. McMahon JA, Skeaff CM, Williams SM *et al*. (2007) Lowering Homocysteine with B Vitamins Has No Effect on Blood Pressure in Older Adults. *J Nutr* 137:1183–7.
36. Horigan G, McNulty H, Ward M *et al*. (2010) Riboflavin lowers blood pressure in cardiovascular disease patients homozygous for the 677C→T polymorphism in MTHFR. *J Hypertens* 28:478–86.
37. Wilson CP, Ward M, McNulty H *et al*. (2012) Riboflavin offers a targeted strategy for managing hypertension in patients with the MTHFR 677TT genotype: a 4-y follow-up. *Am J Clin Nutr* 95:766–72.
38. Berrocal-Zaragoza MI, Murphy MM, Ceruelo S *et al*. (2009) High Milk Consumers Have an Increased Risk of Folate Receptor Blocking Autoantibody Production but This Does Not Affect Folate Status in Spanish Men and Women. *J Nutr* 139:1037–41.
39. Ministerio de sanidad consumo y bienestar social (2014). eCIE9MC Edición electrónica de la Clasificación Internacional de Enfermedades 9ª Edición, Modificación Clínica. https://eciemaps.mscbs.gob.es/ecieMaps/browser/index_9_mc.html (accessed 2019).
40. Institut Municipal d'Investigació Mèdica, Barcelona (2012). Sample size and power calculator, version 7.12. https://www.imim.cat/ofertadeserveis/software-public/granmo/ (accessed August 2019).
41. Wilcken B, Bamforth F, Li Z *et al*. (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. *J Med Genet* 40:619–25.
42. Selhub J, Jacques PF, Rosenberg IH *et al*. (1999) Serum total homocysteine concentrations in the third National Health and Nutrition Examination Survey (1991-1994): population reference ranges and contribution of vitamin status to high serum concentrations. *Ann Intern Med* 131:331–9.
43. Nygård O, Refsum H, Ueland PM *et al*. (1998). Major lifestyle determinants of plasma total homocysteine distribution: the Hordaland Homocysteine Study. *Am J Clin Nutr* 67:263–70.
44. Russo GT, Friso S, Jacques PF *et al*. (2003) Age and Gender Affect the Relation

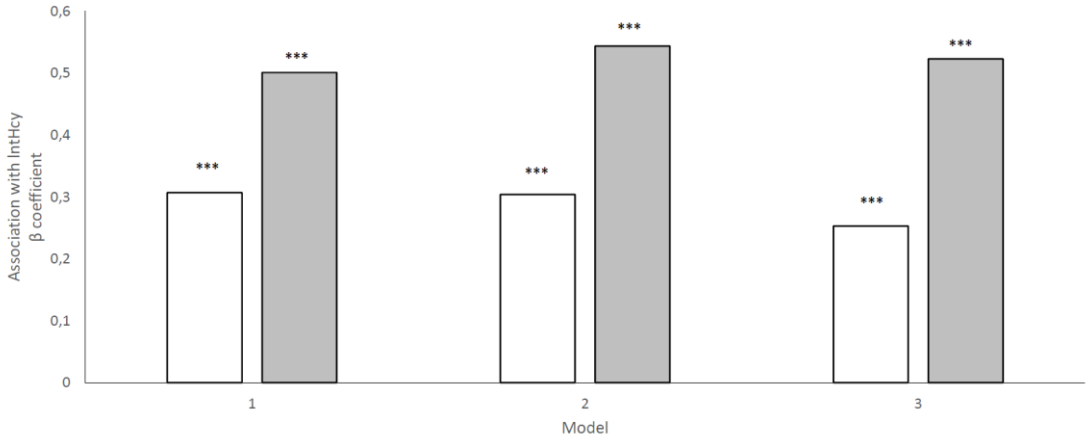
between Methylenetetrahydrofolate Reductase C677T Genotype and Fasting Plasma Homocysteine Concentrations in the Framingham Offspring Study Cohort. *J Nutr* 133:3416–21.

45. Ng X, Boyd L, Dufficy L , *et al*. (2009) Folate nutritional genetics and risk for hypertension in an elderly population sample. *J Nutrigenet Nutrigenomics* 2:1–8.
46. Quinlivan EP, McPartlin J, McNulty H *et al*. (2002) Importance of both folic acid and vitamin B12 in reduction of risk of vascular disease. *Lancet* 359:227–8.
47. Tallova J, Bicikova M, Hill M *et al*. (2003) Homocysteine during the menstrual cycle in depressive women. *Eur J Clin Invest* 33:268–73.
48. Rasmussen LB, Ovesen L, Bülow I *et al*. (2000) Folate intake, lifestyle factors, and homocysteine concentrations in younger and older women. *Am J Clin Nutr* 72:1156–63.
49. Morris MS, Jacques PF, Selhub J *et al*. (2000) Total homocysteine and estrogen status indicators in the Third National Health and Nutrition Examination Survey. *Am J Epidemiol* 152:140–8.
50. Refsum H, Nurk E, Smith AD *et al*. (2006) The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. *J Nutr* 136(6 Suppl):1731S–1740S.
51. Doshi SN, McDowell IFW, Moat SJ *et al*. (2002) Folic acid improves endothelial function in coronary artery disease via mechanisms largely independent of homocysteine lowering. *Circulation* 105:22–6.
52. Williams C, Kingwell BA, Burke K *et al*. (2005) Folic acid supplementation for 3 wk reduces pulse pressure and large artery stiffness independent of MTHFR genotype. *Am J Clin Nutr* 82:26–31.
53. Forman JP, Stampfer MJ, Curhan GC (2009) Diet and lifestyle risk factors associated with incident hypertension in women. *JAMA* 302:401–11.

Figure

Title:

Interaction between smoking and the *MTHFR* 677TT versus CC genotype in its association with fasting plasma total homocysteine in men.



Legend:

Columns represent the difference in lntHcy for *MTHFR* 677TT compared to the CC genotype in nonsmokers (white columns) and smokers (shaded columns), determined by multiple linear regression analysis. Dependent variable natural log transformed tHcy. All models were significant (P <0.001). R^2 (n) for each model:

Models 1, nonsmokers: 0.093 (214); smokers: 0.216 (122).

Models 2, nonsmokers: 0.084 (214); smokers: 0.212 (122).

Models 3, nonsmokers: 0.183 (214); smokers: 0.276 (122).

Models 1: adjusted for age group (≤ 50 y, > 50 y), *SLC19A1* 80GA versus GG and *SLC19A1* 80AA versus GG genotypes.
Models 2: adjusted for the same variables as model 1 plus low versus mid-high socioeconomic status, BMI, moderate (<16 g/d in women, <24 g/d in men) versus no alcohol consumption, high (≥16 g/d in women, ≥24 g/d in men) versus no alcohol consumption, number of cigarettes smoked/ d and plasma creatinine.

Models 3. adjusted for the same variables as model 2 plus plasma folate, plasma cobalamin and erythrocyte glutathionine reductase activation coefficient.

Missing data is 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. $^{***}P$<0.001

Table 1. Characteristics of the study population according to sex specific fasting plasma total homocysteine (tHcy) tertiles (µmol/L).

	Women						**Men**					
	tHcy (µmol/L) tertiles						*tHcy (µmol/L) tertiles*					
	1		2		3		1		2		3	
	(<7.7)		(7.7-9.6)		(>9.6)		(<9.3)		(9.3-11.1)		(>11.1)	
	N		*N*		*N*		*N*		*N*		*N*	
Age (years)[†]	125	39.6 (37.3, 41.9)	125	42.3 (39.7, 45.0)	121	46.3 (43.2, 49.4)**	117	39.2 (36.7, 41.7)	118	43.4 (40.9, 45.9)	117	46.1 (43.0, 49.1)**
BMI (kg/m^2)[‡]	125	25.8 (24.9, 26.8)	123	26.7 (25.7, 27.6)	116	27.5 (26.3, 28.7)	116	27.2 (26.4, 28.1)	116	27.7 (26.9, 28.4)	116	27.2 (26.4, 28.0)
Smokers[§]	39	31.2 (23.7, 39.8)	35	28.2 (21.1, 36.7)	41	34.2 (27.2, 44.4)	51	43.6 (34.9, 52.6)	41	34.7 (26.8, 43.7)	45	38.8 (30.4, 47.9)
Alcohol consumption[§,¶]	125		125		121		117		118		117	
Low to moderate	10	8.0 (4.4, 14.1)	21	16.8 (11.3, 24.3)	23	19.0 (13.0, 26.9)*	39	33.3 (25.4, 34.1)	42	35.6 (27.5, 44.6)	43	36.8 (28.6, 45.8)
High	9	7.2 (3.8, 13.1)	3	2.4 (0.8, 6.8)	6	4.9 (2.3, 10.4)	33	28.2 (20.8, 37.1)	27	22.9(16.2, 31.2)	36	30.8 (23.1, 39.6)
Diagnosed hypertension[§]	11	8.8 (5.0, 15.1)	10	8.0 (4.4, 14.1)	31	25.6 (18.7, 34.1)***	10	8.5 (4.7, 15.0)	15	12.7 (7.9, 19.9)	17	14.4 (9.2, 21.9)
Low socioeconomic status[§]	53	42.4 (34.1, 51.2)	61	48.8 (40.2, 57.5)	61	50.4 (41.6, 59.2)	13	15.4(10.0, 23.0)	30	25.4 (18.4, 34.0)	40	34.2 (26.2, 43.2)**

Plasma folate (nmol/L)[|]	125	14.3 (13.1, 15.6)	125	11.5 (10.4, 12.7)	121	11.0 (9.8, 12.3)***	117	12.6 (11.6, 13.6)	118	11.3 (10.3, 12.4)	117	9.1 (8.2, 10.1)***
Red cell folate (nmol/L)[|]	125	899 (846, 954)	125	781 (734, 830)	121	733 (676, 796)***	117	952 (302, 1004)	118	852 (795, 913)	118	721 (673, 773)***
Plasma cobalamin (pmol/L)[|]	124	377 (355, 401)	125	352 (331, 375)	121	322 (298, 348)**	117	385 (363, 408)	118	343 (321, 367)	117	317 (299, 336)***
EGRAC[|]	123	1.41 (1.37, 1.45)	125	1.33 (1.30, 1.37)	121	1.36 (1.31, 1.40)*	116	1.39 (1.36, 1.43)	117	1.34 (1.30, 1.37)	113	1.34 (1.30, 1.38)
tHcy (µmol/L)[|]	125	6.5 (6.4, 6.7)	125	8.6 (8.5, 8.7)	121	11.8 (11.4, 12.2)***	117	7.9 (7.7, 8.1)	118	10.1 (10.0, 10.2)	118	13.6 (13.1, 14.1)***
Plasma creatinine (µmol/L)[‡]	125	70.4 (58.2, 82.5)	125	64.5 (63.0, 66.0)	121	67.3 (65.7, 69.0)	116	81.3 (79.2, 83.4)	118	81.7 (79.8, 83.7)	118	81.9 (79.7, 81.4)
Plasma total cholesterol (mmol/L)[‡]	125	5.1 (5.0. 5.3)	125	5.3 (5.1, 5.5)	120	5.3 (5.1, 5.5)	117	5.3 (5.1, 5.5)	117	5.3 (5.1, 5.5)	118	5.4 (5.2, 5.6)
MTHFR CC[§]	50	40.0 (31.8, 48.8)	38	30.4 (23.0, 38.9)	44	36.4 (28.3, 45.2)	53	45.3 (36.6, 54.3)	43	36.8 (28.6, 45.8)	30	25.6 (18.6, 34.2)
MTHFR CT[§]	64	51.2 (42.5, 59.8)	63	50.4 (41.8, 59.0)	49	40.5 (32.2, 49.4)	55	47.0 (38.2, 56.0)	59	50.4 (41.5, 59.3)	47	40.2 (31.7, 49.2)
MTHFR TT[§]	11	8.8 (5.0, 15.1)	24	19.2 (13.3, 27.0)	28	23.1 (16.5, 31.4)*	9	7.7 (4.1, 14.0)	15	12.8 (7.9, 20.1)	40	34.2 (26.2, 43.2)***
SLC19A1 GG[§]	30	24.2 (17.5, 32.4)	21	17.4 (11.6, 25.1)	28	23.3 (16.7, 31.7)	33	28.2 (20.8, 37.0)	35	29.9 (22.4, 38.7)	35	30.4 (22.8, 39.4)
SLC19A1 GA[§]	58	46.8 (38.2, 55.5)	77	63.6 (54.8, 71.7)	55	45.8 (37.2, 54.7)	58	49.6 (40.7, 58.5)	56	47.9 (39.0, 56.8)	55	47.8 (38.9, 56.9)
SLC19A1 AA[§]	36	29.0 (21.8 , 37.6)	23	19.0 (13.0, 26.9)	37	30.8 (23.3, 39.6)*	26	22.2 (15.6, 30.6)	26	22.2 (15.6, 30.6)	25	21.7 (15.2, 30.1)

Abbreviations: BMI, Body Mass Index; EGRAC, Erythrocyte Glutathione Reductase Activation Coefficient; *MTHFR*, Methylene Tetrahydrofolate Reductase 677C>T polymorphism; *SLC19A1*, Solute Carrier family 19A member 1 80G>A polymorphism. 24 participants were excluded after the medical checkup due to declared B vitamin supplement use. A further 59 participants were excluded from all analyses involving tHcy because their blood samples were not processed within 2 h of collection and 5 participants because they had suspected altered renal function (plasma creatinine >97 mmol/L for women and >124 mmol/L for men).

[†]median (P25, P75), [‡]arithmetic mean (95% CI), [§]% (95% CI), [¶]category of habitual alcohol intake (moderate <16 g/d in women and <24 g/d in men), high (≥16 g/d in women and ≥24 g/d in men); [|]geometric mean (95% CI).

Chi-square test comparing categorical variables and ANOVA comparing continuous variables between tHcy tertiles, $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$.

Table 2. Multiple linear regression analysis of factors associated with fasting plasma total homocysteine in all participants and separately by sex.

Model	adjusted $R^{2\dagger}$	Independent variables	Standardized $\beta^{\ddagger}$	*P* value
All participants (*N* = 687)	0.184***	Age group§	0.206	<0.001
Model 1 (Non modifiable factors)¶		Sex	0.305	<0.001
		MTHFR TT vs CC genotype	0.358	<0.001
		MTHFR CT vs CC genotype	0.095	0.025
		*Interaction MTHFR genotype*age group*		0.056
Model 2 (model 1 + modifiable lifestyle factors)¶,ǀ	0.194***	Age group§	0.196	0.001
		Sex	0.237	<0.001
		MTHFR TT vs CC genotype	0.348	<0.001
		MTHFR CT vs CC genotype	0.084	0.048
		*Interaction MTHFR genotype*age group*		0.071
		Cigarettes per day	0.079	0.030
		Plasma creatinine (µmol/l)	0.097	0.033
Model 3 (Model 2 + 1CM nutrient status)¶,ǀ,ǁ	0.259***	Age group3	0.266	<0.001
		Sex	0.198	<0.001
		MTHFR TT vs CC genotype	0.325	<0.001

		MTHFR CT vs CC genotype	0.069	0.089
		*Interaction MTHFR genotype*age group*		0.030
		Cigarettes per day	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)	0.090***	Age group	0.263	0.001
Model 1 (Non modifiable factors)¶		*MTHFR* TT vs CC genotype	0.308	<0.001
		MTHFR CT vs CC genotype	0.095	0.134
		*Interaction MTHFR genotype*age group*		0.314
Model 2 (model 1 + modifiable lifestyle factors)¶,ǀ	0.137***	Age group	0.281	0.001
		MTHFR TT vs CC genotype	0.287	<0.001
		MTHFR CT vs CC genotype	0.089	0.156
		*Interaction MTHFR genotype*age group*		0.341
		Cigarettes per day	0.165	0.002
		Plasma creatinine (µmol/l)	0.133	0.008

Model 3 (Model 2 + 1CM nutrient status)[¶,ǂ,ǁ]	0.211***	Age group	0.371	<0.001
		MTHFR TT vs CC genotype	0.271	<0.001
		MTHFR CT vs CC genotype	0.068	0.258
		*Interaction MTHFR genotype*age group*		0.162
		Cigarettes per day	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)	0.128***	Age group	0.161	0.040
Model 1 (Non modifiable factors)[¶]		*MTHFR* TT vs CC genotype	0.453	<0.001
		MTHFR CT vs CC genotype	0.106	0.085
		*Interaction MTHFR genotype*age group*		0.089
Model 2 (model 1 + modifiable lifestyle factors)[¶,ǂ]	0.128***	Age group	0.136	0.106
		MTHFR TT vs CC genotype	0.454	<0.001
		MTHFR CT vs CC genotype	0.100	0.109
		*Interaction MTHFR genotype*age group*		0.066

		Cigarettes per day	0.015	0.784
		Plasma creatinine (µmol/l)	0.013	0.810
Model 3 (Model 2 + 1CM nutrient status)[¶,|,‡]	0.129[***]	Age group	0.217	0.011
		MTHFR TT vs CC genotype	0.422	<0.001
		MTHFR CT vs CC genotype	0.091	0.080
		*Interaction MTHFR genotype*age group*		0.025
		Cigarettes per day	-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

1CM, 1C metabolism; EGRAC, Erythrocyte Glutathione Reductase Activation assay; *MTHFR*, Methylenetetrahydrofolate Reductase, *SLC19A1*, Solute Carrier family 19A member [†]Corresponding with each model; [‡]From the complete models; [§]≤ 50 y, > 50 y; [¶]adjusted for *SLC19A1* 80GA versus GG and *SLC19A1* 80AA versus GG genotypes; [|]adjusted for the same variables as Model 1 plus low versus mid-high socioeconomic status, BMI, moderate (<16 g/d in women, <24 g/d in men) versus no alcohol consumption, high (≥16 g/d in women, ≥24 g/d in men) versus no alcohol consumption, number of cigarettes smoked/ d and plasma creatinine; [‖]adjusted for the same variables as Model 3. Missing data is 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*<0.001

Table 3. Multiple linear regression analysis of factors associated with fasting plasma total homocysteine in all participants and separately according to *MTHFR* 677C>T genotype.

Model	adjusted $R^{2\dagger}$	Independent variables	Standardized β‡	*P* value
***MTHFR* 677CC genotype (*N*= 241)**	0.117***	Sex	0.298	<0.001
Model 1 (Non modifiable factors)¶		Age group§	0.200	0.001
Model 2 (model 1 + modifiable lifestyle factors)¶,ǀ	0.100***	Sex	0.290	0.002
		Age group§	0.162	0.033
		Cigarettes/ day	0.007	0.920
Model 3 (Model 2 + 1CM nutrient status)¶, ǀ,ǁ	0.140***	Sex	0.268	0.004
		Age group§	0.227	0.004
		Cigarettes/ day	-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)**	0.126***	Sex	0.327	<0.001
Model 1 (Non modifiable factors)¶		Age group§	0.174	0.001
Model 2 (model 1 + modifiable lifestyle factors)¶,ǀ	0.167***	Sex	0.181	0.017

		Age group[§]	0.125	0.048
		Cigarettes/ day	0.151	0.006
Model 3 (Model 2 + 1CM nutrient status)[¶,ǀ,ǁ]	0.239[***]	Sex	0.157	0.032
		Age group[§]	0.055	0.322
		Cigarettes/ day	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)**	0.087[**]	Sex	0.340	<0.001
Model 1 (Non modifiable factors)[¶]		Age group[§]	-0.028	0.752
Model 2 (model 1 + modifiable lifestyle factors)[¶,ǀ]	0.083[*]	Sex	0.246	0.043
		Age group[§]	0.067	0.532
		Cigarettes/ day	0.088	0.344
Model 3 (Model 2 + 1CM nutrient status)[¶,ǀ,ǁ]	0.266[***]	Sex	0.146	0.187
		Age group[§]	0.104	0.292
		Cigarettes/ day	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; *MTHFR*, Methylenetetrahydrofolate Reductase, *SLC19A1*, Solute Carrier family 19A member [†]Corresponding with each model; [‡]From the complete models; [§]≤ 50 y, > 50 y; [¶]adjusted for *SLC19A1* 80GA versus GG and *SLC19A1* 80AA versus GG genotypes*;* [|]adjusted for the same variables as Model 1 plus low versus mid-high socioeconomic status, BMI, moderate (<16 g/d in women, <24 g/d in men) versus no alcohol consumption, high (≥16 g/d in women, ≥24 g/d in men) versus no alcohol consumption, number of cigarettes smoked/ d and plasma creatinine; [‖]adjusted for the same variables as Model 3. Missing data is 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. [***]$P<0.001$, [**]$P<0.01$, [*]$P<0.05$.

Table 4. Association between moderately elevated fasting plasma total homocysteine and diagnosed hypertension.

	All participants			Aged ≤50 years			Aged >50 years		
Model	N	$R^{2\dagger}$		N	R^2		N	R^2	
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)

Multiple logistic regression analysis was used. †Nagelkerke R^2; ‡OR (95% CI) for diagnosed hypertension in participants in the 3rd versus the 1st and 2nd age and sex specific tHcy tertiles are shown. Cut offs for the 3rd 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 41 participants without diagnosed hypertension but with no point blood pressure measurement and BMI >30 as well as 5 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 hours of collection were included. Model 1: (basic model) having tHcy in the 3rd tertile compared to thcy in the 1st and 2nd tertiles. Model 2: Included the same variables as model 1 as well as low versus mid-high socioeconomic 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] versus none; high versus none [≥16 g/d in women and ≥24 g/d in men]), current smoking (cigarettes/ d) and total plasma cholesterol (mmol/L). $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$.

Table 5. Association between *MTHFR* 677C>T genotype and diagnosed hypertension.

	All participants				Aged ≤50 years				Aged >50 years			
Model	N	$R^{2\dagger}$	CT vs CC‡	TT vs CC	N	R^2	CT vs CC	TT vs CC	N	R^2	CT vs CC	TT vs CC
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)

†Nagelkerke R^2 from multiple logistic regression analysis; ‡Methylenetetrahdyrofolate reductase (*MTHFR* 677C>T) genotype. §OR (95% CI) for diagnosed hypertension in participants with the CT vs CC genotype and TT vs CC genotype, globally and according age group. 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 41 participants with no point blood pressure measurement and BMI > 30 and 5 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* 677 CT versus CC and *MTHFR* 677 TT versus 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 versus mid-high socioeconomic status, category of regular alcohol intake (moderate [<16 g/d in women and <24 g/d in men] versus none; high [≥16 g/d in women and ≥24 g/d in men]) versus none, current smoking (cigarettes/d) and serum total cholesterol. ***P<0.001.