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RESEARCH PAPER

Hydrogen sulphide-related thiol metabolism and nutrigenetics in relation to hypertension in an elderly population

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Abstract Hydrogen sulphide (H_2S) is a gaseous signalling molecule that regulates blood flow and pressure. It is synthesised from cysteine via cystathionine β-synthase and cystathionine γ-lyase. We examined whether thiol precursors of H_2S, transsulphuration pathway gene variants (CBS-844ins68 and CTH-G1364T) and key B-vitamin cofactors might be critical determinants of hypertension in an elderly Australian population. An elderly Australian retirement village population ($n = 228$; age 65–96 years, 91 males and 137 females) was assessed for the prevalence of two transsulphuration pathway–related variant genes associated with cysteine synthesis and hence H_2S production. Thiols were determined by HPLC, genotypes by PCR and dietary intake by food frequency questionnaire. Homocysteine levels were statistically higher in the hypertensive phenotype ($p = 0.0399$), but there was no difference for cysteine or glutathione. Using nominal logistic regression, cysteine, CTH-G1364T genotype, dietary synthetic folate and vitamin B_6 predicted clinical phenotype (determined as above/below 140/90 mm Hg) and then only in female subjects ($p = 0.0239$, 0.0178, 0.0249 and 0.0371, respectively). Least-squares regression supports cysteine being highly inversely predictive of diastolic blood pressure: p and r^2 values <0.0001 and 0.082; 0.0409 and 0.046; and <0.0001 and 0.113 for all subjects, males and females, respectively. Additionally, CTH-G1364T genotype predicts diastolic blood pressure in males ($p = 0.0217$; $r^2 = 0.083$), but contrasts with observations for females. Overall, analyses, including stepwise regression, suggest cysteine, dietary natural and synthetic folate, vitamins B_6 and B_{12}, and both genetic variants (CTH-C1364T and CBS-844ins68) are all aetiologically relevant in the regulation of blood pressure. Hydrogen sulphide is a vasorelaxant gasotransmitter with characteristics similar to nitric oxide. Cysteine and the G1364T and 844ins68 variants of the cystathionine γ-lyase and cystathionine β-synthase genes, respectively, are the biological determinants of H_2S synthesis, and all three are shown here to influence the hypertensive phenotype. Additionally, B-vitamin cofactors for these three enzymes may also be important determinants of blood pressure.

Keywords Hydrogen sulphide · Cystathionine γ-lyase · Cysteine · Homocysteine · Hypertension · Cystathionine β-synthase · B-vitamins

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Introduction

Globally, the hypertensive phenotype affects 25 % of adults (Kearney et al. 2005) and is therefore a major concern, and if undiagnosed, it contributes to cardiovascular disease (CVD) and chronic kidney disease (Reynolds et al. 2007). Indeed, there is a clear association between occurrence of stroke (Inoue et al. 2007), myocardial infarction (Elliott 2005), heart failure (Schultz et al. 2007) and kidney disease (Paoletti et al. 2006), and the degree of hypertension. Despite this, the pathoaetiology of hypertension has still not been fully elucidated. One area of emerging importance is thiol metabolism, particularly where

vasculotoxic homocysteine (Hcy) and the related vasorelaxant hydrogen sulphide (H_2S) are involved.

It is well recognised that Hcy acts as an independent risk factor for CVD, including hypertension (Parnetti et al. 2004; Zylberstein et al. 2004; Boushey et al. 1995; Zhou et al. 2001; Sutton-Tyrell et al. 1997; Nygard et al. 1997). In this context, much work has been published in relation to the role of folic acid nutrition and genetics in lowering Hcy, including the beneficial effects of vitamins B_{12} and B_6 (Lucock 2006). However, an emerging area of interest involves the role of thiol metabolism in the endogenous production of the vasorelaxant gaseous signalling molecule, H_2S, which regulates arterial diameter, blood flow and leucocyte adhesion and may modulate inflammation and apoptosis (Wagner 2009). Its character is therefore similar to the better-known gasotransmitter, nitric oxide (NO), although its origins differ; it is synthesised from cysteine (Cys), involving the enzymes cystathionine β-synthase and cystathionine γ-lyase (see Fig. 1). Animal models lacking this latter enzyme exhibit lower H_2S levels with reduced endothelium-mediated vasorelaxation (Yang et al. 2008). Given the importance of this gaseous signal molecule as a newly emerging regulator of vascular blood flow and blood pressure, it is possible that thiol precursors of H_2S and genetic variation in transsulphuration pathway genes may be critical determinants of hypertension. Furthermore, since vitamin B_6 is an important cofactor for both cystathionine β-synthase and cystathionine γ-lyase and since dietary folic acid and vitamin B_{12} regulate the flux of Hcy into the transsulphuration pathway, dietary intake of these B-vitamins may additionally regulate H_2S-related hypertension, opening up the possibility for novel nutrigenetic relationships to exist.

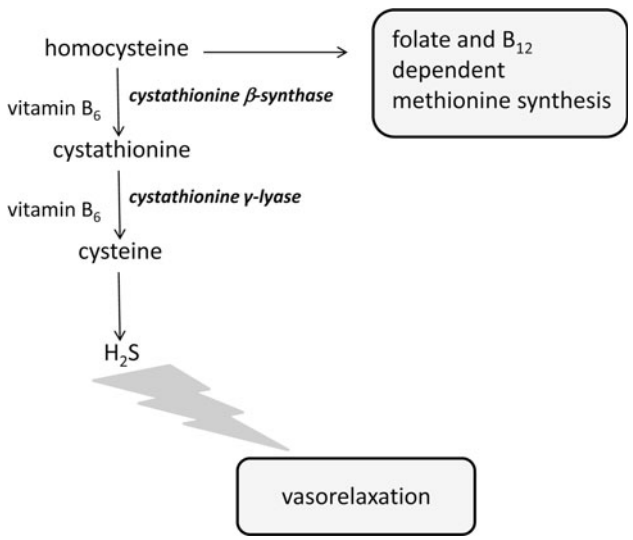

Fig. 1 Simplified transsulphuration pathway showing the various components of relevance to this study

In order to evaluate this, we examined the effect of dietary vitamin B_{12}, B_6 and both synthetic and natural folic acid, plasma Hcy and Cys, and common polymorphisms in cystathionine β-synthase (CBS-844ins68) and cystathionine γ-lyase (CTH-G1364T) sometimes referred to as (CTH-G1208T) on risk for hypertension in an elderly Australian retirement village population sample. Key dietary micronutrients, transsulphuration metabolites and gene variants were determined in 228 subjects (186 normotensive and 42 hypertensive) with an age range of 65–96 years.

Methods

Study design

Subjects

A total of 228 participants (65–96 years, 91 males and 137 females) from Central Coast retirement villages, New South Wales, were assessed for the prevalence of two transsulphuration-related variant genes associated with Cys and hence H_2S production. The average recumbent systolic and diastolic blood pressures were measured, and 186 individuals were determined to be normotensive (age range 65–96; 72 males and 114 females), and 42 were hypertensive (age range 66–89; 19 male and 23 female). Within the 228 individuals, 153 (67 %) were on one or more antihypertensive agents. Use of an antihypertensive agent did not differ significantly between the normotensive and hypertensive phenotype.

Ethics approval

University of Newcastle Human Research Ethics Committee approval—H-782-0304 and Northern Sydney Central Coast Health Human Research Ethics Committee approval—04/19 apply.

Blood analysis

HPLC analysis of thiols

Following derivatisation with the fluorogenic reagent SBDF, step-gradient HPLC with fluorescence detection was used to measure plasma total Hcy, Cys and glutathione (GSH) levels according to our established method (Dufficy et al. 2006).

PCR and SNP scoring for genotype

All subjects were scored for CTH-G1364T and CBS-844ins68 polymorphisms using the polymerase chain

reaction (PCR), followed by restriction enzyme digestion when necessary. Gel electrophoresis was used to visualise the results.

CTH-G1364T

Primers (sense-5′-AGG GAG CTC AGT CAA AGT GC-3′ and antisense-5′-CAC CTC CTT CAG AGG CAA AC-3′) were designed by online software Primer3 (http://frodo.wi.mit.edu/primer3/) and used to amplify a 401-bp amplicon. After digestion with the restriction enzyme EcoRI (NEB), the 1364GG wild type was uncut, leaving a single 401-bp band. The 1364TT homozygous recessive genotype was digested to yield two fragments of 265 and 136 bp (Li et al. 2008).

CBS-844ins68

Primers (sense-5′-CTGGCCTTGAGCCCTGAA-3′ and antisense-5′-GGCCGGGCTCTGGACTC-3′) were used to amplify a 184-bp amplicon. DNA from individuals without the insertion show a 184-bp product, whereas DNA from individuals heterozygous for the insertion show a 252-bp band in addition to the expected 184-bp product (Tsai et al. 1996).

Blood pressure determination

Blood pressure measurements were taken on three discrete occasions over 6 months, and the average was taken as the subject's usual blood pressure. At each clinic visit, an individual's blood pressure was measured while they were in a recumbent position after they had been resting for at least 5 min (recumbent blood pressure). A standard mercury sphygmomanometer was used, and the first (systolic) and fifth (diastolic) Korotkoff sounds were recorded to the nearest 2 mm Hg. Two sets of readings were taken and averaged for each visit. Hypertension was diagnosed at an average systolic reading of >140 and/or diastolic reading of >90, and these were the values used to define clinical phenotype in this study (National Cholesterol Education Program Expert Panel 2002).

Food frequency questionnaire for intake of B-vitamins

Estimated daily intake of nutrients was assessed by interviewer-administered food frequency questionnaire (FFQ). The questionnaire was extensive and covered 225 food items and every food group. Subjects also provided a list of all supplements they were taking and were asked about these during the FFQ interview.

The FFQs were analysed using Foodworks™ 2.10.146 (Xyris Software, Brisbane, QLD, Australia). The package uses food databases covering the majority of foods consumed by Australians. These include AusFoods (brands), AUSNUT (base foods) and the New Zealand—Vitamin and Mineral Supplements 1999 databases. A fuller description of the FFQ methodology is given in Lucock et al. (2012). Foodworks™ does not contain vitamin B_6 or B_{12} values; therefore, data were evaluated by matching food items from the United States Department of Agricultural (USDA) National Nutrient Database for Standard References, which does include vitamin B_6 and B_{12} values. While vitamin B_6 dietary values relate to food intake only, the other micronutrients include supplemental intake values in the final estimate.

Statistics

Statistical analysis was performed using JMP (version 8.0; SAS Institute Inc., Cary, NC, USA). Associations between key variables and related parameters were examined using either standard least-squares analysis or, where nominal data were examined, logistic regression analysis that fits the cumulative response probabilities to the logistic distribution function of a linear model using maximum likelihood; the Wald χ^2 test p value ($p < 0.05$) acted as a significant indicator for screening effects. Descriptive statistics and odds ratios (OR) with 95 % confidence intervals (CI) have been calculated with data tabulated and presented as appropriate.

Stepwise regression analysis was performed in a mixed direction with significant probability (0.250) for a parameter to be considered as a forward step and entered into the model or considered as a backward step and removed from the model. Mallow's Cp criterion was used for selecting the model where Cp first approaches p variables.

Results

Table 1 shows descriptive statistics for the blood thiol metabolites, Hcy, Cys and GSH, dietary B-vitamins (×4), and both systolic and diastolic blood pressure (mean and SEM). The table gives these data for all subjects, normotensive subjects and hypertensive subjects. A simple comparison of sample means following $\log_{10}$ transformation of data shows that only Hcy exhibits a statistically significant difference between clinical phenotypes ($p = 0.0399$). Table 2 provides genotype prevalence, allele frequency and polymorphic allele carriage frequency for the entire population sample and for each clinical phenotype. Table 3 presents the OR with associated 95 % CI, indicating the degree and significance of the two polymorphic alleles under investigation as risk factors for hypertension. Clearly, neither CTH-G1364T (OR = 0.72:

95 % CI; 0.43–1.21) nor CBS-844ins68 (OR = 0.68: 95 % CI; 0.28–1.66) modifies risk of hypertension in the population as a whole despite apparently low OR. Furthermore, standard least-squares analysis shows that neither of these polymorphisms predict Hcy, Cys or GSH ($p > 0.05$).

However, when nominal logistic regression analysis is used to see whether any blood thiol–related variables predict hypertensive phenotype (Table 4), it is clear that while Hcy is approaching significance for the population as a whole, only Cys and CTH-G1364T genotype predict clinical phenotype, and then only in female subjects ($p = 0.0239$ and 0.0178, respectively). The trend is for females with the hypertensive phenotype to have lower Cys levels than females with the normotensive phenotype (mean Cys 245.12 and 262.39 μmol/L, respectively). By comparison, males exhibit little difference in Cys level between the two clinical phenotypes (mean hypertensive Cys 256.47 and normotensive Cys 255.74 μmol/L). The trend for the CTH-G1364T polymorphic allele is towards a reduced frequency in females with the hypertensive phenotype (0.152) compared with that in the normotensive phenotype (0.376), although this effect does appear to be gender specific (see below). This suggests the possibility that Cys level and variant CTH-G1364T may protect against hypertension in females.

Nominal regression analysis also shows that dietary B-vitamins predict hypertensive phenotype: dietary synthetic folic acid and dietary vitamin B_6 are both associated with the hypertensive phenotype in females ($p = 0.0249$ and 0.0371, respectively), but not in males. The mean female intake of synthetic folic acid in the normotensive and hypertensive phenotype was 126.22 and 38.34 μg/day, respectively. The same indices in males were 124.50 and 170.62 μg/day, respectively. For vitamin B_6 intake, consumption in the female normotensive and hypertensive phenotype was 3.98 and 3.24 mg/day, respectively. The same indices in males were 4.10 and 4.14 mg/day, respectively. Clearly, both B-vitamins are lower in hypertensive female subjects than in normotensive ones.

No interactive effect was seen between vitamin B_6 and the two gene variants with respect to hypertensive phenotype. This is a valid analysis given that vitamin B_6 acts as a cofactor for the expression products of both genes.

The conclusions made for blood thiol–related variables may also be drawn from Table 5, which shows results from standard least-squares regression analysis designed to see which thiol-related variables might predict recumbent systolic and diastolic blood pressure without reference to the standard categoric >140/90 mm Hg index. Clearly, Cys is once again highly predictive of blood pressure, particularly diastolic blood pressure: p and r^2 values are <0.0001 and 0.082; 0.0409 and 0.046; and <0.0001 and 0.113 for all subjects, males and females, respectively. In all cases, these are inverse linear relationships, again possibly indicating a potentially protective association between Cys and elevated blood pressure. Table 5 also shows that CTH-G1364T genotype predicts diastolic blood pressure in males ($p = 0.0217$; $r^2 = 0.083$) with a mean diastolic pressure of 66.8, 72.0 and 69.4 mm of Hg for GG, GT and TT individuals, respectively. This compares to corresponding values of 69.3, 70.3 and 68.8 mm of Hg in the population as a whole. This effect of CTH genotype on numeric blood pressure in males (Table 5) contrasts with that shown for a categoric normotensive/hypertensive outcome in females (Table 4). To illustrate this dichotomy, Fig. 2 shows the percentage of hypertensive individuals

Table 1 Descriptive statistics for blood thiols, dietary micronutrients and both systolic and diastolic blood pressure (mean and SEM)

	Homocysteine (μmol/L)	Cysteine (μmol/L)	Glutathione (μmol/L)	Synthetic folic acid (μg/day)	Natural folic acid (μg/day)	Vitamin B_{12} (μg/day)	Vitamin B_6 (mg/day)	Recumbent systolic average (mm Hg)	Recumbent diastolic average (mm Hg)
All subjects	9.39	258.06	10.40	120.15	327.48	14.47	3.96	131.40	69.64
SEM	0.18	2.15	0.25	9.94	6.87	1.64	0.11	0.83	0.54
n	228	228	228	228	228	228	228	228	228
Normotensive	9.24	259.82	10.24	125.55	326.89	15.42	4.03	127.37	68.20
SEM	0.20	2.47	0.26	11.04	7.65	1.91	0.12	0.71	0.54
n	186	186	186	186	186	186	186	186	186
Hypertensive	10.11	250.26	11.07	98.18	330.05	10.27	3.65	149.24	76.05
SEM	0.40	3.88	0.77	22.83	15.85	2.67	0.24	1.05	1.32
n	42	42	42	42	42	42	42	42	42
Comparison of clinical phenotypes (*t* test of $\log_{10}$ transformed data)	$p = 0.0399$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	$p > 0.05$	na	na

Table 2 Genotype prevalence, allele frequency and polymorphic allele carriage frequency for CBS-844ins68 and CTH-G1364T according to clinical phenotype

Genotype	CTH-G1364T					CBS-844ins68				
	Wild type	Heterozygote	Recessive	Allele frequency	Carriage frequency	Wild type	Heterozygote	Recessive	Allele frequency	Carriage frequency
All subjects										
n	99	100	28	0.34	0.57	186	40	2	0.10	0.18
Percentage	43.6	44.1	12.3			81.6	17.5	0.9		
Normotensive										
n	77	84	24	0.36	0.58	150	34	2	0.10	0.24
Percentage	41.6	45.4	13.0			80.6	18.3	1.1		
Hypertensive										
n	22	16	4	0.29	0.48	36	6	0	0.07	0.17
Percentage	52.4	38.1	9.5			85.7	14.3	0		

Table 3 Odds ratio with 95 % confidence interval examining the polymorphic alleles in CBS-844ins68 and CTH-G1364T as risk factors for hypertension

Genotype	CTH-G1364T			CBS-844ins68		
	Mutant allele	Wild-type allele	OR, 95 % CI	Mutant allele	Wild-type allele	OR, 95 % CI
Normotensive	132	238	OR = 0.72: 95 % CI; 0.43–1.21	38	334	OR = 0.68: 95 % CI; 0.28–1.66
Hypertensive	24	60		6	78	

Table 4 Nominal logistic regression analysis to examine whether thiol metabolites and genes, along with related micronutrient variables, predict hypertensive phenotype. Data show the effect Wald test *p* (r^2 and slope estimate)

Variable	All subjects	Males	Females
Homocysteine	0.0604	0.4383	0.0877
Cysteine	0.0866	0.9296	0.0239 ($r^2 = 0.0454$, 0.0177)
Glutathione	0.2126	0.0944	0.9641
Dietary natural folate	0.8583	0.5532	0.6287
Dietary synthetic folate	0.2887	0.2558	0.0249 ($r^2 = 0.0847$, 0.0090)
Dietary vitamin B_{12}	0.2370	0.6567	0.1759
Dietary vitamin B_6	0.1816	0.9276	0.0371 ($r^2 = 0.0408$, 0.3835)
CTH-G1364T	0.4408	0.3499	0.0178 ($r^2 = 0.0757$)
CBS-844ins68	0.8046	0.7252	0.9851

within each of the CTH-C1364T genotypes by gender. Clearly, the percentage of the population with a hypertensive phenotype increases with carriage of mutant allele in males but decreases in females.

Of the dietary factors that were examined in relation to recumbent diastolic and systolic blood pressure, only natural folate and vitamin B_6 showed a significant association (Table 5). Natural folate was associated with both diastolic and systolic blood pressure in males (but not in females): *p*, r^2 and slope values were 0.0024, 0.0990 and 0.0237; and 0.0256, 0.0548 and 0.0267, respectively. There was a more general association for vitamin B_6. In males, *p*, r^2 and slope values were 0.0052, 0.0846 and 1.3547, respectively, for diastolic blood pressure (systolic approaching significance), and for females, *p*, r^2 and slope values were 0.0178, 0.0409 and −1.6171, respectively, for systolic blood pressure.

Therefore, dietary B-vitamins, thiol metabolites and genes all appear to be factors associated with blood pressure. For completion, a stepwise regression model was used to examine all dietary, biochemical and genetic variables as predictors of (1) diastolic and (2) systolic blood pressure.

Table 5 Standard least-squares regression analysis to examine whether transsulphuration pathway–related variables predict recumbent diastolic and systolic blood pressure (mm Hg). Data are examined without reference to the standard >140/90 mm Hg index for defining hypertension. Data show p (r^2 and slope estimate) values for all subjects, males and females

Variable	All subjects		Males		Females	
	Diastolic	Systolic	Diastolic	Systolic	Diastolic	Systolic
Homocysteine	NS	NS	NS	NS	NS	NS
Cysteine	<0.0001 ($r^2 = 0.082$, −0.0719)	0.0407 ($r^2 = 0.018$, −0.0523)	0.0409 ($r^2 = 0.046$, −0.0568)	NS	<0.0001 ($r^2 = 0.113$, −0.0819)	0.0463 ($r^2 = 0.029$, −0.0640)
Glutathione	NS	NS	NS	NS	NS	NS
Dietary natural folate	0.0513 ($r^2 = 0.0167$, 0.0102)	NS	0.0024 ($r^2 = 0.0990$, 0.0237)	0.0256 ($r^2 = 0.0548$, 0.0267)	NS	NS
Dietary synthetic folate	NS	NS	NS	NS	NS	NS
Dietary vitamin B_{12}	NS	NS	NS	NS	NS	NS
Dietary vitamin B_6	0.0860 ($r^2 = 0.0130$, 0.5603)	NS	0.0052 ($r^2 = 0.0846$, 1.3547)	0.0722 ($r^2 = 0.0359$, 1.3398)	NS	0.0178 ($r^2 = 0.0409$, −1.6171)
CTH-G1364T	NS	NS	0.0217 ($r^2 = 0.083$)	NS	NS	NS
CBS-844ins68	NS	NS	NS	NS	NS	NS

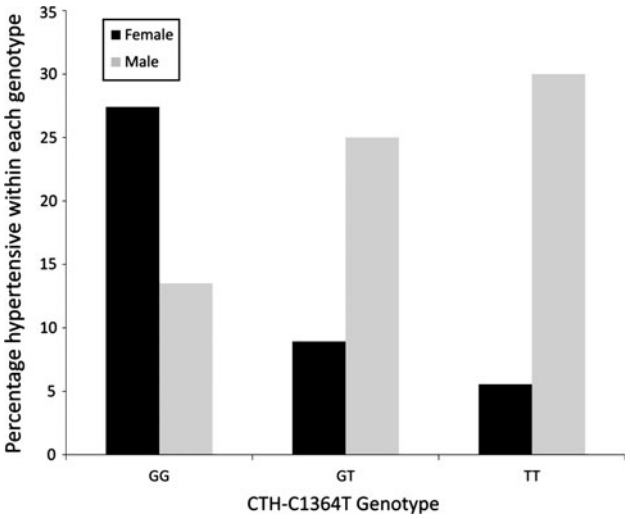

Fig. 2 Percentage of hypertensive individuals within each of the CTH-C1364T genotypes by gender

Cys and CBS-844ins68 were significant predictors of diastolic pressure, and Cys, dietary natural and synthetic folate, vitamin B_{12} and CBS-844ins68 were significant predictors of systolic pressure (Table 6). These stepwise linear models support the former analyses with respect to Cys, but not dietary vitamin B_6. They additionally suggest that vitamin B_{12} and CBS-844ins68 may be aetiologically relevant factors in the regulation of blood pressure.

Discussion

Relatively little work has been done on H_2S and its relationship to hypertension in human populations. Given the importance of this clinical phenotype and our present findings, the current study may offer new insight into the molecular origins of elevated blood pressure. While endothelial NO is now well established as a critical component of endothelium-derived relaxation factor (EDRF), it is not the only component. H_2S is also a vital component of EDRF and shares certain signalling modalities such as regulation via vasorelaxative hormones through the calmodulin and IP_3 pathways (Wagner 2009). Both the enzymes studied here are vitamin B_6 (pyridoxal-5′-phosphate) dependent, and so consideration should be given to this B-vitamin along with both folate and vitamin B_{12}, which regulate the provision and utilisation of methyl groups for remethylation of Hcy. Precise control of the remethylation versus the transsulphuration of Hcy is under careful allosteric regulation by *S*-adenosylmethionine and *S*-adenosylhomocysteine (SAM/SAH) at the level of 5,10-methylenetetrahydrofolate reductase and cystathionine β-synthase. However, control at this nexus is also subject to dietary B-vitamin availability and genetic variation in key enzymes (Lucock 2000). Indeed, it has been shown that at equimolar concentrations of cystathionine β-synthase and cystathionine γ-lyase, the former enzyme is predicted to yield around 25–70 % of the total H_2S produced by

Table 6 Effect of transsulphuration pathway–related dietary, biochemical and genetic variables on diastolic and systolic blood pressure: p has been determined using a stepwise regression model that takes account of Hcy, Cys, GSH, dietary natural and synthetic folate, dietary vitamin B_6 and B_{12}, and CTH-G1364T and CBS-844ins68 genotypes. Values provided are p (r^2 for whole model and slope estimate)

Variable	All subjects	
	Diastolic	Systolic
Homocysteine	NS	NS
Cysteine	<0.0001 ($r^2 = 0.1368, -0.0745$)	0.0107 ($r^2 = 0.0508, -0.0680$)
Glutathione	NS	NS
Dietary natural folate	0.0567 ($r^2 = 0.1368, 0.0098$)	NS
Dietary synthetic folate	0.0548 ($r^2 = 0.1368, 0.0081$)	NS
Dietary vitamin B_{12}	0.0095 ($r^2 = 0.1368, -0.0687$)	NS
Dietary vitamin B_6	NS	NS
CTH-G1364T	NS	NS
CBS-844ins68	0.0267 ($r^2 = 0.1368, 6.1198$)	0.0228 ($r^2 = 0.0508, 10.4235$)

transsulphuration, depending on the level of allosteric activation by SAM (Singh et al. 2009). The contribution of this haem-containing enzyme to H_2S generation likely decreases under conditions of hyperhomocysteinaemia; gasotransmitter synthesis is relatively insensitive to Hcy, indicating that cystathionine γ-lyase is largely responsible for enhancing H_2S generation under conditions of hyperhomocysteinaemia. These findings, it has been suggested, point to an important new role for cystathionine γ-lyase in the thiol metabolome and Hcy management (Singh et al. 2009) and, as the present data indicate, in contributing to a significant clinical phenotype associated with substantial rates of morbidity and mortality (Kearney et al. 2005; Reynolds et al. 2007; Inoue et al. 2007; Elliott 2005; Schultz et al. 2007; Paoletti et al. 2006; Parnetti et al. 2004; Zylberstein et al. 2004; Boushey et al. 1995; Zhou et al. 2001; Sutton-Tyrell et al. 1997; Nygard et al. 1997). Since H_2S is highly reactive and has long been considered as toxic, its impact on various tissues is well characterised, but with recent advances in our knowledge, implicating H_2S in Alzheimer's disease, epilepsy and stroke (Gadalla and Snyder 2010; Gupta et al. 2010) as well as hypertension, future development of drugs specifically modulating H_2S levels is likely to prove beneficial (Gupta et al. 2010).

Our data suggest an interesting effect of gender on the relationship between CTH-C1364T genotype and hypertension (Fig. 2). This clear dichotomy may reflect hormonal regulatory control. Indeed, it has been demonstrated by others that testosterone elicits a nongenomic vasodilator effect that involves H_2S and that this androgen modulates H_2S levels by increasing the enzymatic conversion of Cys to form this gasotransmitter (Bucci et al. 2009). However, a precise explanation for the effect shown in Fig. 2 and Tables 4, 5, 6 remain unclear, but presumably, it must relate to some kind of hormonal effect linked to gender-specific gonadal steroids. Certainly, in males, as carriage of the mutant allele increases, so does the proportion of individuals with hypertension—an observation that would be consistent with progressively decreasing H_2S production. However, beyond this obvious deduction, the counter-observation in females requires further explanation. The combination of regression approaches used suggests that Cys, dietary natural and synthetic folate, vitamins B_6 and B_{12}, and both genetic variants (CTH-C1364T and CBS-844ins68) are aetiologically relevant in the regulation of blood pressure. Further work is required to move beyond these associations to better understand the mechanisms involved.

Overall, the data from this study support a significant correlation between the thiol metabolome and the hypertensive phenotype. The key metabolite, Hcy, is significantly increased in hypertensive subjects, Cys is protective against elevated blood pressure, and CTH-G1364T genotype predicts the hypertensive phenotype in female subjects in a fashion that may be protective, but given the gender differences observed for this polymorphism, there is a possibility of a major hormonal component to this effect. This may arise from a putative interaction between androgen/oestrogen, H_2S and functional changes in the enzyme protein conferred by the G1364T transition.

It is not surprising that vitamin B_6 may be relevant to this clinical phenotype given its role as cofactor at both the transsulphuration pathway enzymes—cystathionine β-synthase and cystathionine γ-lyase. Folates drive synthesis of methyl groups needed to remethylate Hcy, and control the SAM/SAH ratio; so again, it is not too surprising that dietary folate has an impact on blood pressure. Indeed, methylfolate itself may have a direct effect on blood pressure via a mechanism independent of transsulphuration metabolites such as Hcy. It has been suggested that reduced

folates may interact synergistically with tetrahydrobiopterin metabolism in the synthesis of NO by endothelial nitric oxide synthase (Hayden and Tyagi 2004; Moat et al. 2004; Stroes et al. 2000; Hyndman et al. 2002; Doshi et al. 2003) and as such help to maintain vascular wall elasticity.

Taken collectively, the dietary, metabolite and genetic findings suggest a clear influence of H_2S-related transsulphuration parameters on blood pressure and warrant further studies to better characterise this important new area of clinical significance.

Study limitations

A number of possible limitations have been identified and should be discussed for completion. These include a relatively small ($n = 228$) data set, although it is a fairly homogeneous one. Despite our small sample size, this retirement village population has generated interesting findings that would be worthy of further study in a larger cohort with more power to better tease out these and similarly related, novel nutrigenetic associations. The second obvious limitation is that while we provide a focus on H_2S metabolism, we do not have any direct measure of this gaseous signalling molecule. Despite this, the biochemical correlation remains valid given the increasing evidence linking thiol metabolism to H_2S and its subsequent regulation of blood flow and pressure. The final point to make is that while we measured native food folate (as methylfolate), we also measured synthetic folate (as pteroylmonoglutamate), which is variously added to the diet on a mandatory basis as well as being taken as a supplement via discretionary use. The wide-scale use of synthetic folate (see Table 1) justifies an examination of supplemental as well as food intake since increasingly there is a blurring in the way in which we receive this specific synthetic vitamer of folic acid. In the context of this study, methylfolate is entirely natural and not found in supplements. Vitamin B_{12} is a nutrient of particular clinical concern in the elderly, giving increased problems relating to the vitamins bioavailability, and for this reason, food and supplemental use has been recorded. Vitamin B_6 data relate only to food intake and do not take account of supplemental use. This vitamin is not used as a supplement to the same extent as the former vitamins, and given the critical role of the vitamin as a cofactor for transsulphuration enzymes, it was felt that food intake represents the most stable estimate to examine as a variable in the present context.

The data presented link diet, genes and phenotype in a novel way. It is hoped that future studies will support and expand upon these findings.

Conclusion

The recent discovery of H_2S as a new gaseous signalling component of EDRF that regulates vascular pressure has opened up a fresh avenue of research in pharmaceutical development, including, quite possibly, pharmacogenomic interventions. The present study has shown that key factors in the transsulphuration pathway that generate H_2S are predictive of both hypertensive phenotype and diastolic/systolic blood pressure. This study therefore furthers knowledge in this novel area, but also raises interesting questions in relation to gender-specific and wider nutritional effects.

Conflict of interest The authors declare that there is no conflict of interest.

References

Boushey CJ, Beresford SA, Omenn GS, Motulsky AG (1995) A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes. JAMA 274:1049–1057

Bucci M, Mirone V, Di Lorenzo A, Vellecco V, Roviezzo F, Brancaleone V, Ciro I, Cirino G (2009) Hydrogen sulphide is involved in testosterone vascular effect. Eur Urol 56:378–383

Doshi S, McDowell I, Moat S, Lewis M, Goodfellow J (2003) Folate improves endothelial function in patients with coronary heart disease. Clin Chem Lab Med 41:1505–1512

Dufficy L, Naumovski N, Ng X, Blades B, Yates Z, Travers C, Lewis P, Sturm J, Veysey M, Roach PD, Lucock MD (2006) G80A reduced folate carrier SNP influences the absorption and cellular translocation of dietary folate and its association with blood pressure in an elderly population. Life Sci 79:957–966

Elliott WJ (2005) Cardiovascular events in hypertension trials of Angiotensin-converting enzyme inhibitors. J Clin Hypertens 7:2–4

Gadalla MM, Snyder SH (2010) Hydrogen sulfide as a gasotransmitter. J Neurochem 113:14–26

Gupta YK, Dahiya AK, Reeta KH (2010) Gaso-transmitter hydrogen sulphide: potential new target in pharmacotherapy. Indian J Exp Biol 48:1069–1077

Hayden MR, Tyagi SC (2004) Homocysteine and reactive oxygen species in metabolic syndrome, type 2 diabetes mellitus, and atheroscleropathy: the pleiotropic effects of folate supplementation. Nutr J 3:4

Hyndman ME, Verma S, Rosenfeld RJ, Anderson TJ, Parsons HG (2002) Interaction of 5-methyltetrahydrofolate and tetrahydrobiopterin on endothelial function. Am J Physiol Heart Circ Physiol 282:2167–2172

Inoue R, Ohkubo T, Kikuya M, Metoki H, Asayama K, Obara T, Hirose T, Hara A, Hoshi H, Hashimoto J, Totsune K, Satoh H, Kondo Y, Imai Y (2007) Stroke risk in systolic and combined systolic and diastolic hypertension determined using ambulatory blood pressure the ohasama study. Am J Hypertens 20:1125–1131

Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J (2005) Global burden of hypertension: analysis of worldwide data. Lancet 365:217–223

Li Y, Zhao Q, Liu XL, Wang LY, Lu XF, Li HF, Chen SF, Huang JF, Gu DF (2008) Relationship between cystathionine gamma-lyase

gene polymorphism and essential hypertension in Northern Chinese Han population. Chin Med J 121:716–720

Lucock M (2000) Folic acid: nutritional biochemistry, molecular biology, and role in disease processes. Mol Genet Metab 71:121–138

Lucock MD (2006) Synergy of genes and nutrients: the case of homocysteine. Curr Opin Clin Nutr Metab Care 9:748–756

Lucock M, Yates Z, Boyd L, Naylor C, Choi JH, Ng X, Skinner V, Wai R, Kho J, Tang S, Roach P, Veysey M (2012) Vitamin C-related nutrient–nutrient and nutrient-gene interactions that modify folate status. Eur J Nutr (In Press)

Moat SJ, Lang D, McDowell IFW, Clarke ZL, Madhavan AK, Lewis MJ, Goodfellow J (2004) Folate, homocysteine, endothelial function and cardiovascular disease. J Nutr Biochem 15:64–79

National Cholesterol Education Program Expert Panel (2002) Expert panel on detection, evaluation and treatment of high blood cholesterol in adults: third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). Circulation 106:3143–3421

Nygard O, Nordrehaug JE, Refsum H, Ueland PM, Farstad M, Vollset SE (1997) Plasma homocysteine levels and mortality in patients with coronary artery disease. N Engl J Med 337:230–236

Paoletti E, Bellino D, Amidone M, Rolla D, Cannella G (2006) Relationship between arterial hypertension and renal damage in chronic kidney disease: insights from ABPM. J Nephrol 19:778–782

Parnetti L, Caso V, Santucci A, Corea F, Lanari A, Floridi A, Conte C, Bottiglieri T (2004) Mild hyperhomocysteinemia is a risk-factor in all etiological subtypes of stroke. Neurol Sci 25:13–17

Reynolds K, Gu D, Muntner P, Kusek JW, Chen J, Wu X et al (2007) A population-based, prospective study of blood pressure and risk for end-stage renal disease in china. J Am Soc Nephrol 18:1928–1935

Schultz HD, Li YL, Ding Y (2007) Arterial chemoreceptors and sympathetic nerve activity: implications for hypertension and heart failure. Hypertension 50:6–13

Singh S, Padovani D, Leslie RA, Chiku T, Banerjee R (2009) Relative contributions of cystathionine beta-synthase and gamma-cystathionase to H2S biogenesis via alternative trans-sulfuration reactions. J Biol Chem 284:22457–22466

Stroes ES, van Faassen EE, Yo M, Martasek P, Boer P, Govers R, Rabelink TJ (2000) Folic acid reverts dysfunction of endothelial nitric oxide synthase. Circ Res 86:1129–1134

Sutton-Tyrrell K, Bostom A, Selhub J, Zeigler-Johnson C (1997) High homocysteine levels are independently related to isolated systolic hypertension in older adults. Circulation 96:1745–1749

Tsai MY, Bignell M, Schwichtenberg K, Hanson NQ (1996) High prevalence of a mutation in the cystathionine beta-synthase gene. Am J Hum Genet 59:1262–1267

Wagner CA (2009) Hydrogen sulfide: a new gaseous signal molecule and blood pressure regulator. J Nephrol 22:173–176

Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, Wang R (2008) H_2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. Science 322:587–590

Zhou J, Moller J, Danielson CC, Bentzon J, Ravn HB, Austin RC, Falk E (2001) Dietary supplementation with methionine and homocysteine promotes early atherosclerosis but not plaque rupture in ApoE-deficient mice. Arterioscler Thromb Vasc Biol 21:1470–1476

Zylberstein DE, Bengtsson C, Bjorkelund C, Landaas S, Sundh V, Thelle D, Lissner L (2004) Serum homocysteine in relation to mortality and morbidity from coronary heart disease: a 24-year follow-up of the population study of women in Gothenburg. Circulation 109:601–606