

Plasma total homocysteine is associated with abdominal aortic aneurysm and aortic diameter in older men

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Objective: This study was conducted to determine whether plasma total homocysteine (tHcy) and the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism are associated with abdominal aortic aneurysm (AAA) and aortic diameter.

Methods: This was a cross-sectional study set in Western Australia of 4248 community-dwelling men aged 70 to 88 years. Infrarenal aortic diameter was measured using ultrasound scan, tHcy was measured by immunoassay, and MTHFR 677T polymorphism was detected by polymerase chain reaction.

Results: Adjusted multinomial logistic regression analysis showed the odds of having an AAA (aortic diameter ≥ 30 mm) for men with high tHcy (≥ 15 $\mu\text{mol/L}$) compared with those with normal tHcy (< 15 $\mu\text{mol/L}$) was 1.45 (95% confidence interval [CI], 1.10-1.91). Every 5- $\mu\text{mol/L}$ increment in tHcy was associated with 0.15-mm (95% CI, 0.01-0.28 mm) increase in mean aortic diameter. The tHcy concentration was higher in MTHFR TT homozygote individuals than in wild-type CC individuals. There was, however, no apparent association between MTHFR C677T polymorphism with AAA (TT vs CC genotype: odds ratio, 0.97; 95% CI, 0.72-1.31) or aortic diameter (TT vs CC genotype: mean increment of 0.01 mm; 95% CI, -0.63 to 0.65 mm).

Conclusions: Elevated tHcy is associated with the presence of AAA in older men. There is also a positive dose-response relationship between tHcy and abdominal aortic diameter. Longitudinal studies and clinical trials of lowering tHcy are required to assess whether these relationships are causal. (J Vasc Surg 2013;58:364-70.)

Abdominal aortic aneurysm (AAA) affects $\sim 5\%$ of men aged ≥ 65 years.¹ Many of the risk factors for AAA are similar to those for atherosclerosis, including age, family history, smoking, dyslipidemia, hypertension, and obesity.^{2,3} AAA has traditionally been regarded as a consequence of atherosclerosis.⁴ However, epidemiologic differences, such as the inverse association of diabetes with AAA, suggest disparate pathogenesis.^{5,6} Interest in the cause of AAA has been stimulated by the current absence of an effective drug therapy that limits AAA progression.⁷

Homocysteine (Hcy) is a nonprotein amino acid derived from the hepatic metabolism (demethylation) of the essential sulfur-containing amino acid, methionine. It is a substrate for three competing metabolic pathways. Hcy can be remethylated to methionine through (1) a "salvage pathway" (the folate cycle), a process that requires folate and B vitamins as cofactors, and (2) the hepatic betaine-Hcy-methyltransferase reaction. It can also be irreversibly converted via the trans-sulfuration pathway in the liver and kidney to cystathionine and cysteine. Elevated plasma total Hcy (tHcy) has been extensively correlated with vascular events, including myocardial infarction and stroke.^{8,9} Its association with AAA is controversial, however, because previous studies have provided discordant findings.¹⁰⁻¹⁶ The possibility of reverse causality bias and residual confounding in these studies precluded the establishment of a causal link between tHcy and AAA.

Clinical studies have used the principle of Mendelian randomization to explore the nature of the association between tHcy and AAA, and their results have been equivocal.^{10,11,17-19} This is based on the concept that the random assortment of alleles at the time of gamete formation creates a randomized trial of genetic polymorphisms with a greater or lower expression of the relevant protein from birth onward, and hence, the relationship between the genetic variant, the risk factor of interest, and the outcome is not susceptible to the common biases or

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confounding that are prevailing in observational studies.²⁰ It has been well documented that a commonly occurring methylenetetrahydrofolate reductase (MTHFR) gene variant, 677T, encodes a defective enzyme with reduced catalytic activity in the formation of 5-methyltetrahydrofolate. The latter is the methyl donor needed for the conversion of Hcy to methionine, thereby increasing basal plasma tHcy by ~20%.²¹ On the basis of the theory of Mendelian randomization, the MTHFR C677T genotypes should have no direct effect on AAA other than that mediated by tHcy concentration.²²

In this study, we sought to determine whether any relationship exists between tHcy and the presence of AAA and aortic diameter in a large cohort of community-dwelling men aged 70 to 88 years, while accounting for the traditional risk factors for AAA and other comorbidities. In an attempt to further explore the potential causal role of tHcy on AAA, we also investigated the association between the MTHFR C667T polymorphism with AAA and aortic diameter in this cohort of older men.

METHODS

The Human Research Ethics Committee of the University of Western Australia approved the protocol for the Health in Men Study (HIMS), which was conducted in accordance with the Helsinki Declaration for Human Rights.

Study design and participants. We conducted a cross-sectional study of participants from the HIMS, which has been described in detail elsewhere.²³ In brief, 12,203 community-dwelling men aged 65 to 83 years participated in a trial of screening for AAA. Each man completed a health assessment between 1996 and 1999 (HIMS Wave 1). In 2001 to 2004, 5154 men responded to the second phase of this study (HIMS Wave 2), and blood samples were collected from 4249 of these individuals.

Outcome of interest. The abdominal aortic diameter was measured during HIMS Wave 1. The greatest transverse and anteroposterior diameter of the infrarenal aorta was measured using a Toshiba Capasee ultrasound machine with a 3.75-MHz probe (Toshiba Australia, North Ryde, NSW, Australia). The reproducibility of these ultrasound measurements has been previously reported.²⁴ An AAA was considered present if the abdominal aortic diameter was ≥ 30 mm.

Biochemical analyses. Blood samples were collected during HIMS Wave 2 between 0800 and 1030. Plasma was separated from the blood samples ≤ 1 hour of collection and stored at -80°C until assayed. Presence of tHcy was measured by fluorescence polarization immunoassay on an IMx analyzer (Abbott Laboratories, Abbott Park, Ill).²⁵ The interassay coefficient of variation was 4%. Genomic DNA was isolated from nucleated blood cells by using the Triton X-100 method, and the nt677C \rightarrow T mutation was determined using polymerase chain reaction. HinfI restriction enzyme digestion was performed directly in the polymerase chain reaction tube at 37°C for 4 hours

before analysis of restriction fragments by polyacrylamide gel electrophoresis, as previously described.²⁶ Allele frequencies were estimated by gene counting, and the genotype distribution was compared with those expected under Hardy-Weinberg equilibrium.

Serum high-sensitivity C-reactive protein was measured with assay on a BNII analyzer (Dade Behring, Birmingham, UK). Serum creatinine, glucose, cholesterol, low-density lipoprotein, and triglycerides were measured with a Roche Hitachi 917 analyzer (Roche Diagnostics, Indianapolis, Ind).

Other explanatory variables. Variables available from the Wave 2 assessment were age, smoking status (current, former, or never smoker), and taking B-vitamin supplements or not. Data from Waves 1 and 2 were used to determine the prevalence of cardiovascular disease (CVD; self-reported history of angina, myocardial infarction, heart failure, coronary artery bypass grafting, coronary angioplasty, carotid endarterectomy, aortic bypass surgery, peripheral arterial surgery or stroke, or use of antiplatelet medications for these conditions), hypertension (self-reported diagnosis, use of antihypertensive medications, or measured blood pressure $\geq 140/90$ mm Hg), diabetes (self-reported diagnosis or use of glucose-lowering medication, or fasting glucose of ≥ 7 mmol/L or nonfasting glucose of ≥ 11 mmol/L) and dyslipidemia (self-reported diagnosis, use of lipid-lowering medication, or fasting low-density lipoprotein ≥ 3.4 mmol/L, high-density lipoprotein < 0.9 mmol/L, triglycerides ≥ 1.8 mmol/L, or total cholesterol ≥ 5.5 mmol/L).

To calculate the weighted Charlson Comorbidity Index (CCI),²⁷ we obtained the health records and death certificates (until the end of Wave 2) from the Western Australian Data Linkage System. This database provides electronic linkage to the state's use of health services and medical morbidities.²⁸ Seventeen common medical conditions that predict 1-year mortality were accounted for: myocardial infarction, congestive heart failure, peripheral arterial disease, cerebrovascular disease, dementia, chronic pulmonary disease, connective tissue disease, ulcers, liver disease, diabetes (including diabetes with end-organ damage), hemiplegia, renal disease, leukemia, lymphoma, other tumors, metastatic tumors, and acquired immunodeficiency syndrome. The number and seriousness of these comorbid diseases were evaluated by assigning integer weights to these conditions using adjusted relative risks and developing a composite index score that ranged from 0 to 37.²⁷

Height, weight, and waist circumference were measured during Wave 2 in accordance with guidelines of the International Society for the Advancement of Kinanthropometry.²⁹ Height and weight were used to calculate body mass index (BMI, kg/m^2).

Statistical analysis. Data were analyzed using Stata 11.1 software (StataCorp, College Station, Tex). Descriptive statistics were calculated for the demographic, lifestyle, and clinical variables according to the presence or absence of AAA. Concentration of tHcy was dichotomized into high

tHcy (≥ 15 $\mu\text{mol/L}$) and normal tHcy (< 15 $\mu\text{mol/L}$), as determined by the laboratory's reference range. Aortic diameter was divided into intervals of < 19 , 19 to 22, 23 to 29, and ≥ 30 mm, based on standard deviations (SDs) of logarithmic diameter.⁴ tHcy was modeled as categorical and continuous variables according to whether tHcy was ≥ 15 $\mu\text{mol/L}$, per 5- $\mu\text{mol/L}$ increment in tHcy, and doubling of tHcy concentration (by dividing the natural logarithm of tHcy by the natural logarithm of 2).

Multinomial logistic regression analyses were performed to assess for any dose-response relationship between tHcy and aortic diameter intervals, using a reference interval of 19 to 22 mm. Linear regression analyses were performed with aortic diameter modeled as a continuous variable. Analyses were also repeated after logarithmic transformations of the aortic diameter due to its positively skewed distribution. Adjustments were made for age, smoking, CVD, diabetes, hypertension, dyslipidemia, CCI, BMI, and serum creatinine as potential confounders. The results are reported as odds ratios (ORs) with 95% confidence intervals (95% CIs). $P < .05$ was considered statistically significant.

Descriptive statistics were used to calculate the distribution of the MTHFR genotypes according to AAA. Analysis of variance and linear regression analysis were used to explore the association of the MTHFR genotypes with tHcy. Linear and multinomial logistic regression analyses were performed to investigate the relationship between the MTHFR polymorphism with aortic diameter and AAA, using the CC genotype and aortic diameter interval of 19 to 22 mm as references.

RESULTS

Sociodemographic, clinical, and biochemical characteristics of the study population, according to the presence or absence of AAA, are reported in Table I. Complete tHcy and aortic diameter measurements were available for 4248 men, aged 70 to 88 years; of these, 1120 men (26.3%) had high tHcy (≥ 15 $\mu\text{mol/L}$) and the mean \pm SD tHcy concentration for the cohort was 13.4 ± 5.6 $\mu\text{mol/L}$. None of the participants had homocystinuria. AAA (aortic diameter ≥ 30 mm) was present in 318 men (7.5%), and the mean \pm SD aortic diameter for the cohort was 22.8 ± 4.9 mm (range, 15.9-79.2 mm).

Association of tHcy with AAA. An incremental association was found between tHcy and aortic diameter intervals ($P < .001$; Table II). Adjustments were made for age, smoking, CVD, diabetes, hypertension, dyslipidemia, CCI, BMI, and serum creatinine. At a reference interval of 19 to 22 mm, the OR of having an AAA (aortic diameter ≥ 30 mm) for men with high tHcy (≥ 15 $\mu\text{mol/L}$) compared with those with normal tHcy (< 15 $\mu\text{mol/L}$) was 1.45 (95% CI, 1.10-1.91). The association persisted when tHcy was modeled as continuous variables. The Fig demonstrates the OR of having an AAA with changing tHcy concentrations, with tHcy entered into the models as restricted cubic splines.³⁰

Table I. Demographic, lifestyle, and clinical characteristics of the study population according to the presence or absence of abdominal aortic aneurysm (AAA)

Variable ^a	AAA (n = 318)	No AAA (n = 3930)	P
Age, years	77.7 \pm 4.1	76.5 \pm 3.6	<.001
Smoking			
Current smoker	37 (11.7)	179 (4.6)	<.001
Former smoker	240 (75.7)	2371 (60.3)	
Never smoked	41 (12.6)	1380 (35.1)	
Taking B-vitamin supplement	15 (4.7)	231 (5.9)	.395
Cardiovascular disease	208 (65.4)	1625 (41.3)	<.001
Diabetes	67 (21.1)	596 (15.2)	.005
Hypertension	307 (96.5)	3452 (87.8)	<.001
Dyslipidemia	262 (82.4)	2740 (69.7)	<.001
CCI ≥ 5	29 (9.1)	146 (3.7)	<.001
BMI, kg/m ²	27.1 \pm 3.8	26.5 \pm 3.6	.006
Aortic diameter, mm	36.4 \pm 7.5	21.8 \pm 2.6	<.001
Plasma tHcy, $\mu\text{mol/L}$	15.1 \pm 6.1	13.2 \pm 5.1	<.001
hsCRP, mg/L	4.9 \pm 8.4	3.7 \pm 7.3	.008
Creatinine, $\mu\text{mol/L}$	104.8 \pm 40.9	92.8 \pm 30.6	<.001

BMI, Body mass index; CCI, Charlson Comorbidity Index; hsCRP, high-sensitivity C-reactive protein; tHcy, total homocysteine.

^aContinuous data are presented as mean \pm standard deviation and categorical data as number (%).

Association of tHcy with aortic diameter. Multivariate linear regression analyses showed men with high tHcy had a 0.41-mm (95% CI, 0.06-0.77 mm) larger mean aortic diameter than those with normal tHcy concentrations. Every 5- $\mu\text{mol/L}$ increment in tHcy was associated with 0.15-mm (95% CI, 0.01-0.28 mm) larger mean aortic diameter. Doubling of tHcy was associated with 0.39-mm (95% CI, 0.05-0.73 mm) larger mean aortic diameter. These associations persisted when aortic diameter was log-transformed (data not shown).

Sensitivity analyses. We performed a sensitivity analysis by excluding men who reported taking B vitamin supplements. When this was done, the odds of having an AAA for men with high tHcy and not taking B vitamin supplements were not altered to a great extent compared with those with normal tHcy (OR, 1.48; 95% CI, 1.11-1.96). When high-sensitivity C-reactive protein was included in the model of tHcy and AAA, the effect estimate remained similar (OR, 1.44; 95% CI, 1.09-1.90).

Association of MTHFR polymorphism with AAA and aortic diameter. MTHFR polymorphism data were available for 4130 men. The distribution of the MTHFR genotypes was similar for participants with and without AAA: CC, 281 (45.5%); CT, 267 (43.3%); and TT, 69 (11.2%) for men with AAA, and CC, 1549 (44.1%); CT, 1566 (44.6%); and TT, 398 (11.3%) for the remaining men ($P = .795$). The genotype distribution was in Hardy-Weinberg equilibrium (exact test, $P = .773$). Mean \pm SD aortic diameters were 24.1 ± 6.5 , 23.8 ± 6.1 , and 24.1 ± 6.2 mm for men with the CC, CT, and TT genotypes, respectively ($F = 1.08$, $P = .341$). As reported previously,³¹ The mean (\pm SD) tHcy concentration

Table II. Multinomial logistic regression models^a evaluating the association of plasma total homocysteine (*tHcy*) with aortic diameter intervals in community-dwelling older men

Variables	Aortic diameter intervals			
	<19 mm (n = 440)	19-22 mm (n = 2427)	23-29 mm (n = 1063)	≥30 mm (n = 318)
tHcy, μmol/L ^b	12.8 ± 5.0	13.1 ± 5.1	13.8 ± 5.1	15.1 ± 6.1
tHcy ≥15 μmol/L ^c	0.76 (0.58-1.00)	Reference	1.20 (1.00-1.43)	1.45 (1.10-1.91)
Per 5-μmol/L increment in tHcy ^c	0.90 (0.79-1.02)	Reference	1.06 (0.99-1.14)	1.12 (1.02-1.23)
Doubling of tHcy ^c	0.76 (0.58-0.97)	Reference	1.14 (0.96-1.36)	1.50 (1.14-1.99)

BMI, Body mass index; CCI, Charlson Comorbidity Index; CI, confidence interval; OR, odds ratio.

^aAdjusted for age, smoking status, cardiovascular disease, hypertension, dyslipidemia, diabetes, CCI score, BMI, and serum creatinine.

^bData shown as mean ± standard deviation or the OR (95% CI).

^cOR (95% CI) presented for high tHcy (≥15 μmol/L) relative to normal tHcy (<15 μmol/L), and per 5-μmol/L increment in tHcy and doubling of tHcy.

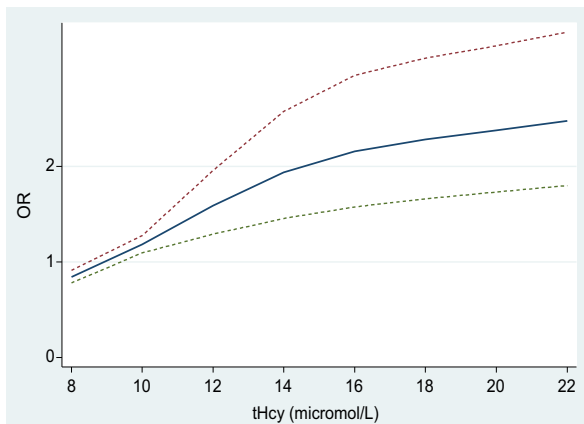


Fig. Odds ratio (OR; solid line) of an abdominal aortic aneurysm (AAA) (aortic diameter ≥30 mm) with changing plasma total homocysteine (*tHcy*) levels is shown. Levels of tHcy are entered into the models as restricted cubic splines (3 knots) with a reference value of 9 μmol/L (median of the lowest quartile of tHcy concentrations). The tHcy scale of 8 to 22 μmol/L relates to the range between the 5th and 95th percentiles of values (n = 3823). The dashed lines denote 95% confidence interval (CI).

was 13.1 ± 5.1, 13.5 ± 4.9, and 14.4 ± 9.7 μmol/L for men with the CC, CT and TT genotypes, respectively ($F = 10.16, P < .001$).

Linear and multinomial logistic regression analyses showed no apparent association between MTHFR polymorphism with aortic diameter and AAA (Table III). The OR of having an AAA was 0.95 (95% CI, 0.79-1.15) for men with the CT genotype and 0.97 (95% CI, 0.72-1.31) for men with the TT genotype compared with those with the CC genotype. Men with the CT genotype had a reduction of 0.29 mm (95% CI, -0.69 to 0.12 mm) in mean aortic diameter; however, men with the TT genotype had an increment of 0.01 mm (95% CI, -0.63 to 0.65 mm) in diameter.

DISCUSSION

Our results provide further evidence for an association between elevated tHcy and the presence of AAA,

independent of the traditional CVD risk burden and other comorbidities. The risk of having an AAA for men with high tHcy is 45% higher compared with those with normal tHcy concentrations. tHcy is also correlated with abdominal aortic diameter in a positive dose-response relationship, with every 5-μmol/L increment in tHcy being associated with 0.15-mm larger mean aortic diameter. These findings suggest that elevated tHcy could be an epiphenomenon or have a role in the development of an AAA in aging men.

The strengths of this study include the large sample size of robustly characterized, population-based, community-dwelling older men. We were able to comprehensively adjust for potential confounders in our multivariate analyses, including age, smoking, CVD, and risk factors that have established associations with tHcy and AAA. The addition of other variables that might modify the relationship, including statin and antiplatelet therapies, did not significantly alter the effect estimates. Measurement of the aortic diameter was performed in a standardized procedure using ultrasound imaging.

A major limitation would be that blood samples were obtained from the participants several years (5.7 ± 0.9 years) after the baseline aortic diameter measurements. It is possible that the aortic diameters in our cohort could have increased by a small margin in the time interval.³² However, it is unlikely that the small number of interval cases of AAA would have substantially altered the results. The cross-sectional design of our study and the lack of serial assessments of tHcy concentration or aortic diameter also preclude determination of causality. Our findings could reflect reverse causality, with higher comorbidities in men with an AAA at baseline leading to less physical activity and poorer diet, and consequently, higher tHcy concentrations.

Our findings are conservative due to a possible “healthy survivor” effect. Participants who had responded for the Wave 2 assessment were younger ($P < .001$), more physically active ($P < .001$), and had fewer self-reported comorbidities ($P < .001$) during Wave 1 compared with the nonrespondents of the follow-up study. This self-selection of participants might have biased our findings toward lower tHcy concentrations in our cohort compared

Table III. Multinomial logistic regression models evaluating the association of the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism with aortic diameter intervals in community-dwelling older men

MTHFR C677T polymorphisms ^a	Aortic diameter intervals			
	<19 mm	19-22 mm	23-29 mm	≥30 mm
CT genotype	1.18 (0.94-1.48)	Reference	0.99 (0.84-1.16)	0.95 (0.79-1.15)
TT genotype	0.91 (0.62-1.33)	Reference	1.10 (0.86-1.41)	0.97 (0.72-1.31)

CI, Confidence interval; OR, odds ratio.

^aOR (95% CI) presented for CT and TT genotypes relative to CC genotype.

with the nonrespondents, and therefore, move our results toward the null hypothesis, leading to an underestimation of the magnitude of the association between tHcy with AAA.

Finally, our data for B vitamin supplementation was based on self-reported history. By not having access to B vitamin concentrations, we were therefore unable to definitively account for possible effects due to the prevailing folate concentration.

Hcy influences a range of molecular pathways of potential relevance to the pathogenesis of AAA, some of which may be different from those implicated in atherosclerosis. Experimentally, Hcy induces endothelial dysfunction and matrix remodeling in part by stimulating the secretion of matrix metalloproteases and release of reactive oxygen species, which are implicated in AAA pathogenesis.³³⁻³⁵ Immune mechanisms may also be important in AAA formation.^{36,37} Hcy stimulates chemokine and cytokine secretion from cultured human monocytes and has been implicated in suppressing regulatory T-cell function.^{38,39} Homocysteinylated, which is a manifestation of Hcy toxicity, is characterized by a nonenzymatic chemical reaction that can cause structural protein inactivation, with consequent destruction of the aortic wall.⁴⁰ A recent animal study demonstrated that hyperhomocysteinemia contributes to the development of AAA through aortic adventitial inflammation, a process that is reversible with folic acid supplementation.⁴¹

Although we have demonstrated a statistically significant quantitative association between elevated tHcy with the enlargement of the abdominal aortic diameter by mere fractions of a millimeter, these small differences are of limited clinical significance for an individual person. Despite this, findings from previous experimental studies have indicated that this association is biologically plausible, suggesting the possibility that Hcy-lowering strategies could limit AAA formation in humans.

To our knowledge, this is the largest epidemiologic study to demonstrate an independent association between elevated tHcy with the presence of AAA and aortic diameter. Brunelli et al¹¹ were the first to conduct a case-control study of 58 men and reported a sixfold increase in the likelihood of having AAA with hyperhomocysteinemia (OR, 6.0; 95% CI, 1.2-29.6). Subsequent studies further implicated tHcy in the pathophysiology of AAA.^{10,14,42-44} Researchers also found that patients with

AAA had lower levels of vitamin B₁₂, which is important in the regulation of tHcy concentrations.¹³ However, discrepant results have emerged from other studies that may be limited by patient selection and residual confounding.^{12,15,16} Further exploration of this relationship with adequately powered longitudinal studies and clinical trials would be required to have an improved understanding of any role homocysteine metabolism plays in AAA pathogenesis.

In our study, tHcy concentrations were elevated with the MTHFR 677T variant compared with the 677C or "wild" allele. However, no obvious association was found between the T allele and the presence of AAA and aortic diameter. We performed a retrospective power calculation (80% power with an $\alpha = .05$) and deduced that the study would require >1 million participants (~300,000 men with the TT genotype) to detect an effect size of 0.03-mm increase in mean aortic diameter associated with a 1- μ mol/L increment in tHcy (effect size derived from our multivariate linear regression analysis). Similarly, other studies would have been clearly underpowered to establish an association.^{10,11,17-19} When a meta-analysis of these studies was conducted (~2300 individuals), an increased risk of AAA associated with the T allele variant (risk ratio, 1.14, 95% CI, 1.08-1.21) was obtained.⁴⁵ This is supportive of a causal link between hyperhomocysteinemia and AAA.

CONCLUSIONS

Elevated tHcy is associated with the presence of AAA in older men, independent of the traditional CVD risk burden and other comorbidities. There is also a positive dose-response relationship between tHcy and abdominal aortic diameter, suggesting a role of tHcy in AAA development in aging men. However, Mendelian randomization to the MTHFR TT genotype is not associated with AAA or aortic diameter, despite higher tHcy. The discordance in these findings may reflect confounding or random error. Longitudinal studies and clinical trials of lowering tHcy are required to assess whether these relationships are causal.

AUTHOR CONTRIBUTIONS

Conception and design: YW, LF, PN

Analysis and interpretation: YW, JG, LF, KM, GH, FB, BY, PN

Data collection: LF, GH, FB, BY, PN

Writing the article: YW

Critical revision of the article: JG, LF, KM, GH, FB, BY, PN

Final approval of the article: YW, JG, LF, KM, GH, FB, BY, PN

Statistical analysis: YW, KM

Obtained funding: LF, GH, PN

Overall responsibility: YW

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