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Original Article

Plasma homocysteine levels and risk of vascular dementia: a Mendelian randomization study

Shao-Pu Wu, Jian-Jun Ma, Ya-Wei Qi, Jie-Wen Zhang

Department of Neurology, Zhengzhou University People's Hospital and Henan Provincial People's Hospital, Zhengzhou, Henan, P. R. China

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Abstract: Observational studies have demonstrated an association between elevated homocysteine (Hcy) level and risk of vascular dementia (VaD); however, it remains unresolved whether this relationship is causal. We carried out a Mendelian randomization (MR) study to evaluate whether genetically increased Hcy level influences the risk of VaD. We used the methylene tetra hydro folate reductase (*MTHFR*) C677T polymorphism as an instrumental variable, which affects the plasma Hcy levels. Estimate of its effect on plasma Hcy was based on a recent genome-wide meta-analysis of 44147 individuals, while estimate of its effect on VaD risk was obtained through meta-analysis of case-control studies with 722 cases and 1158 controls. By combining these two estimates, we found that per 1 standard-deviation (SD) increased in natural log-transformed plasma Hcy levels conferred a 4.29-fold increase in risk for VaD (95% CI: 1.11-16.57, P = 0.034). Our study suggests that elevated Hcy levels are causally associated with an increased risk of developing VaD. Whether Hcy-lowering therapy can prevent VaD merits further investigation in long-term randomized controlled trials.

Keywords: Homocysteine, vascular dementia, mendelian randomization, methylene tetra hydro folate reductase

Introduction

Vascular dementia (VaD) is the second leading cause of dementia after Alzheimer's disease by accounting for 15-20 percent of all dementia cases in the world [1]. Vascular dementia is thought to be irreversible and it is caused by cerebrovascular diseases including stroke, diabetes, and hypertension which result in the impairment of specific brain regions involving memory and cognitive function [2]. The incidence of VaD increases with age rapidly, rising from 1.5% at age 70-75 years to 15% at age 80 years and above, which lead to largely irreversible deterioration in patients' quality of life and increased economic burden of their families [3]. Because treatments for VaD are limited, the best approach to reduce mortality and morbidity is primary prevention through modification of acquired risk factors.

Homocysteine (Hcy) is a key metabolite in one-carbon metabolism. Hcy levels could influence several cellular processes including DNA methylation and synthesis of nucleic acids and proteins [4]. A common functional polymorphism, C677T (rs1801133), in the gene encoding methylene tetra hydro folate reductase (*MTHFR*), an enzyme involved in homocysteine metabolism, has been associated with differences in homocysteine concentration [5-7]. The association of this variant with the plasma homocysteine was confirmed by a recent meta-analysis of genome-wide association studies [8].

Data from case-control studies showed a trend for higher Hcy levels in VaD patients as compared to healthy controls and AD patients [9, 10]. However, meta-analysis of four prospective studies, with a total of 2631 participants, did not support a causal relationship between high Hcy level and risk of developing dementia [11]. The discordant results might be caused by the limited period of the trials, confounding factors, or reverse causation.

In the absence of evidence from high-quality randomized controlled trials (RCTs), the principles of Mendelian randomization (MR) can be

utilized to strengthen or refute the causality of biomarkers in disease etiology [12]. Mendelian randomization (MR) is a study design in which genetic variants are used as instrumental variables for estimating the unconfounded effect of an exposure (for example, Hcy) on a disease (for example, stroke) [13]. This approach is based on the principle that genetic variants are assigned randomly when passed from parents to offspring during meiosis, and consequently these genetic variants are independent of many factors that bias observational studies, such as confounding and reverse causation. MR methods have been used previously to investigate the influence of Type 2 Diabetes (T2D) and fasting glucose (FG) on CHD risk, which demonstrate a causal relationship between T2D and CHD [14]. MR approach may be of particular significance for understanding the etiology of VaD since the date of disease onset is often poorly discerned clinically and MR studies assess the effect of lifelong exposures.

MR analyses using *MTHFR* C677T polymorphism as an instrument variable have been carried out in the past. The researchers provided evidence from MR that plasma Hcy level is causally related to stroke, T2D, schizophrenia and offspring birthweight [13, 15-17]. In the present study, we used this polymorphism as an instrumental variable to obtain MR estimate of the effect of Hcy on VaD.

Materials and methods

Data on gene association with VaD risk

To estimate the association of the *MTHFR* C677T polymorphism with VaD risk, we performed a meta-analysis of case-control studies. Eligible studies were identified using PubMed, Embase and China Biological Medicine electronic databases before May 1, 2016. The search terms and keywords used were as follows: "methylene tetra hydro folate reductase" or "*MTHFR*", "vascular dementia" or "VaD" or "VD", and "polymorphism" or "variation" or "variant" or "mutation" or "genotype" or "allele" or "SNP", without any restriction on the language. Reference lists of relevant articles were reviewed manually to look for additional studies. For inclusion, studies had to meet the following criteria: (1) evaluation for the association between *MTHFR* C677T polymorphism and VaD; (2) studies were designed as the case-control type; (3) genotype frequencies for both cases and controls were available. Studies were excluded if: (1) no detailed genotype frequency; and (2) case reports, family-based studies, abstracts, editorials and review articles. When multiple publications reported the same population, only the most recent one with the largest sample sets was selected for this meta-analysis. Two authors selected the articles independently according to the above criteria. Any uncertainty regarding the eligibility was adjudged by further joint inspection of the publications.

The following data were independently extracted by two investigators from each eligible article according to a fixed protocol: first author's name, publication year, country and ethnicity of population, genotyping methods, source of control, matching status, number of cases and controls, genotype distributions in cases and controls and the Hardy-Weinberg Equilibrium (HWE) in controls (*P* value). If these were not possible, the authors of the publications were contacted via E-mail for more detailed data.

The methodological quality of the included studies was accessed by two authors respectively according to the Newcastle Ottawa Scale (NOS) (www.ohri.ca/programs/clinical_epidemiology/oxford.asp) [18]. The NOS criteria consist of three aspects: selection, comparability and exposure. Scores ranged from 0 stars (worst) to 9 stars (best) and a score ≥ 7 indicated that a study was of high quality.

Data on gene association with Hcy

Estimate of the effect sizes of the *MTHFR* C677T polymorphism on the plasma Hcy levels was based on the findings of a recent GWAS meta-analysis [8]. The meta-analysis included data from a total of 44,147 white individuals of European ancestry derived from 10 GWAS on Hcy levels.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) of genotypes distribution in the control group was checked by the χ^2-test and $P < 0.05$ was considered as significant disequilibrium. Studies with controls not in HWE were subjected to a sensitivity analysis. The pooled odds ratios (ORs) with their 95% confidence intervals (95%

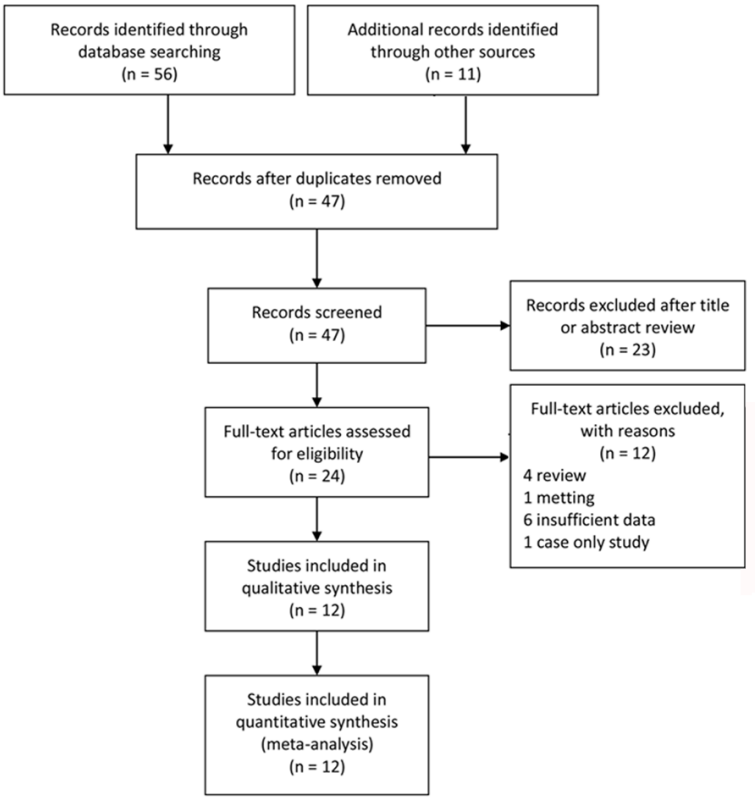

Figure 1. Flow chart of the search strategy and study selection. The terms "N" in the boxes represent the number of corresponding studies.

CIs) were calculated to evaluate the strength of the association between *MTHFR* C677T polymorphism and VaD risk based on different genetic models: allele model (T vs C), homozygous model (TT vs CC), heterozygous model (CT vs CC), dominant model (TT + CT vs CC), and recessive model (TT vs CT + CC). Statistical heterogeneity between eligible studies was evaluated by using the Cochran's Q statistic and I^2 test [19]. $P < 0.1$ and I^2 exceeding 50% indicated substantial heterogeneity across studies, then a random-effects model was chosen to perform meta-analysis, otherwise, the fixed-effects model was selected. Subgroup analyses were performed according to ethnicity (Asian and Caucasian), source of control (population-based and hospital-based), quality score (low quality: score < 7; high quality: score ≥ 7) and matched status. A power calculation was performed using Power and Sample Size Calculation version 3.1.2 (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). Begg's funnel plot and Egger's regression test were used to search for publication bias and a *P* value > 0.05 suggests no significant publication bias have been detected [20]. The fail-safe number (N_{fs} at a significance of 0.05 was also calculated to inspect publication bias, according to the formula $N_{fs0.05} = (\sum Z/1.64)^2$-k, where k is the number of studies included. If the N_{fs} was less than the number of observed studies for a polymorphism, we deemed that there exists a significant publication bias.

We calculated a MR estimate of the effect of the plasma Hcy levels on the risk of VaD ($OR_{VaD/Hcy}$) as Log $OR_{VaD/Hcy}$ = (log $OR_{VaD/per\ T\text{-}allele}$)/$beta_{Hcy/per\ T\text{-}allele}$, as in previous studies [21, 22]. Log $OR_{VaD/Hcy}$ is the (log) increase of VaD risk by SD unit increase in the natural log-transformed plasma Hcy (MR estimate). Log $OR_{Hcy/per\ T\text{-}allele}$ is the (log) increase in VaD risk per allele (gene-VaD association). $Beta_{Hcy/per\ t\text{-}allele}$ is the number of SD differences in the natural log-transformed plasma Hcy levels per allele (SD/allele) (gene-Hcy association). The standard error of the MR estimate was derived using the Delta method [23]. The MR estimate is presented in terms of OR, by exponentiatingthe Log $OR_{VAD/Hcy}$ All *P* values were two sided. All above statistical analyses were performed using STATA software version 12.0 (STATA Corporation,College Station, TX, USA).

Results

Summary statistics

The process of literature retrieval and exclusion was shown in **Figure 1**. The initial comprehensive search generated a total of 67 potentially relevant articles, 20 articles were excluded for duplication, and 23 additional articles were excluded for their unmatched titles or abstracts. After reading the full text of the remaining 24 articles, 12 articles were removed due to review, meeting abstract, study with insufficient data and case only study. Finally, 12 stud-

Table 1. Main characteristics of studies included in the meta-analysis

First Author	Year	Country	Ethnicity	Geno-typing Methods	Source of control	Matched Variables	Sample Size (Case/ Control)	Case			Control			HWE (*P* value)	Quality
								TT	CT	CC	TT	CT	CC		
Bottiglieri	2001	Italy	Caucasian	PCR-SSCP	HB	NA	6/36	2	3	1	8	17	11	0.769	6
Chapman	1998	Israel	Caucasian	PCR-SSCP		Age	41/40	7	20	14	9	16	15	0.251	8
McIlroy	2002	Ireland	Caucasian	PCR	PB	NA	76/71	8	37	31	2	19	50	0.904	6
Pollak	2000	Israel	Caucasian	PCR-SSCP	PB	Ethnicity	85/82	10	41	34	16	37	29	0.501	8
Wehr	2006	Poland	Caucasian	PCR-SSCP	PB	NA	65/141	5	26	34	12	66	63	0.360	6
Zuliani	2001	Italy	Caucasian	PCR-SSCP	PB	NA	60/54	14	26	20	12	25	17	0.627	6
Nishiyama	2000	Japan	Asian	PCR-SSCP	PB	NA	35/33	9	17	9	5	15	13	0.845	7
Pandey	2009	India	Asian	PCR-SSCP	PB	Age	80/170	2	29	49	7	45	118	0.315	8
Sun	2014	China	Asian	PCR-RFLP	PB	NA	52/56	10	31	11	8	21	27	0.254	6
Wu	2006	China	Asian	PCR-RFLP	PB	NA	29/138	9	12	8	24	73	41	0.383	7
Yoo	2000	Korea	Asian	PCR-RFLP	HB	Age, gender	143/217	36	58	49	26	114	77	0.099	8
Mansoori	2012	India	Asian	PCR-SSCP	HB	Age, gender	50/120	1	14	35	2	29	89	0.836	8

NA = not available, PCR = polymerase chain reaction, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, PCR-SSCP = polymerase chain reaction-single strand conformation polymorphism, HWE = Hardy-Weinberg equilibrium, PB = population-based, HB = hospital-based.

ies with a total of 722 cases and 1158 controls, were included in our meta-analysis [24-35]. Detailed characteristics and genotype distributions of included studies were summarized in **Table 1**. The distribution of the genotypes in the control group was consistent with HWE. The number of cases among all selected studies varied from 6 to 143, while the numbers of controls varied from 33 to 217. All the studies included met quality criteria ranging from 6 to 8.

Association of MTHFR C677T polymorphism with VaD risk

The main results of meta-analysis and heterogeneity test were summarized in **Table 2**. Overall, the pooled results showed a significant association between *MTHFR* C677T polymorphism and the risk of VaDunder allele model (T vs C: OR = 1.26, 95% CI = 1.02-1.56), homozygous model (TT vs CC: OR = 1.48, 95% CI = 1.08-2.02) and recessive model (TT vs CT + CC: OR = 1.41, 95% CI = 1.06-1.87) (**Figure 2**). If we set $\alpha = 0.05$, based on the data set for 677T allele, we have a 90.6% power to detect an OR of 1.26. When stratified by ethnicity, a significant association was also found in Asian population (T vs C: OR = 1.40, 95% CI = 1.15-1.70; TT vs CC: OR = 2.03, 95% CI = 1.33-3.11; TT vs CT + CC: OR = 1.93, 95% CI = 1.31-2.82; TT + CT vs CC: OR = 1.37, 95% CI = 1.05-1.79), while a null result was noted in the Caucasian population under all genetic models. In the subgroup analysis str-atified by control source and matched status, similar trends with overall results were observed in HB and no matched subgroups. With regard to quality score, there were no significant findings observed under any genetic models in low-quality studies (quality score < 7) or in high-quality studies (quality score ≥ 7). Moderate heterogeneity was observed under allele, heterozygous and dominant models ($I^2 = 47.4\%$, $I^2 = 47.5\%$, $I^2 = 48.1\%$, respectively). In the subgroup analysis, heterogeneity vanished in HB subgroup as well as dramatically decreased in Asian subgroup, high-quality studies and matched subgroup.

Mendelian randomization analysis for the association of Hcy with VaD risk

Under the principles of Mendelian randomization, we observed that each 1-SD increase in the natural log-transformed plasma Hcy level was significantly associated with a 4.29-fold increased risk of VaD (95% CI: 1.11-16.57, $P = 0.034$) (**Figure 3**). Considering that the null hypothesis value of unity was not covered by derived 95% CIs for predicted estimate, it is safe to the reject the null hypothesis of none causal relationship between plasma Hcy level and VaD.

Sensitivity analysis and publication bias

The leave-one-out sensitivity analysis showed that no single study qualitatively changed the summary ORs (data not shown). Begg's funnel plot and Egger's test were performed to evaluate the potential publication bias of literatures.

Table 2. Meta-analysis of the association between *MTHFR* C677T polymorphism and VaD risk

Groups	N	Allele model		Homozygous model		Heterozygous model		Recessive model		Dominant model	
		OR (95% CI)	P^{het}	OR (95% CI)	P^{het}	OR (95% CI)	P^{het}	OR (95% CI)	P^{het}	OR (95% CI)	P^{het}
Overall	12	**1.26 (1.02-1.56)**	0.034	**1.48 (1.08-2.02)**	0.160	1.27 (0.93-1.74)	0.034	**1.41 (1.06-1.87)**	0.179	1.32 (0.98-1.78)	0.031
Ethnicity											
Caucasian	6	1.12 (0.75-1.70)	0.009	1.01 (0.63-1.62)	0.169	1.22 (0.73-2.02)	0.058	0.95 (0.62-1.47)	0.353	1.20 (0.70-2.08)	0.017
Asian	6	**1.40 (1.15-1.70)**	0.826	**2.03 (1.33-3.11)**	0.769	1.33 (0.86-2.05)	0.069	**1.93 (1.31-2.82)**	0.643	**1.37 (1.05-1.79)**	0.249
Control source											
HB	3	**1.33 (1.02-1.74)**	0.891	**2.14 (1.20-3.82)**	0.898	0.93 (0.62-1.38)	0.528	**2.33 (1.39-3.91)**	0.811	1.12 (0.77-1.63)	0.788
PB	9	1.25 (0.94-1.66)	0.009	1.26 (0.87-1.84)	0.111	1.38 (0.94-2.02)	0.028	1.13 (0.80-1.60)	0.295	1.38 (0.93-2.05)	0.010
Quality score											
High	7	1.18 (0.99-1.42)	0.430	1.38 (0.94-2.03)	0.196	1.08 (0.83-1.41)	0.635	1.27 (0.72-2.23)	0.057	1.15 (0.89-1.47)	0.836
Low	5	1.44 (0.87-2.39)	0.007	1.67 (0.97-2.88)	0.151	1.64 (0.76-3.51)	0.005	1.36 (0.82-2.24)	0.597	1.70 (0.78-3.67)	0.002
Matched											
Yes	5	1.13 (0.93-1.38)	0.330	1.23 (0.80-1.90)	0.124	1.07 (0.81-1.42)	0.477	0.99 (0.44-2.22)	0.026	1.11 (0.85-1.45)	0.764
NR	7	**1.45 (1.02-2.06)**	0.025	**1.80 (1.14-2.84)**	0.309	1.48 (0.84-2.61)	0.014	**1.54 (1.02-2.33)**	0.710	1.60 (0.92-2.80)	0.008

N = Number of studies; P^{het} = *P* value for heterogeneity test. The OR values with statistical significance were shown in bold.

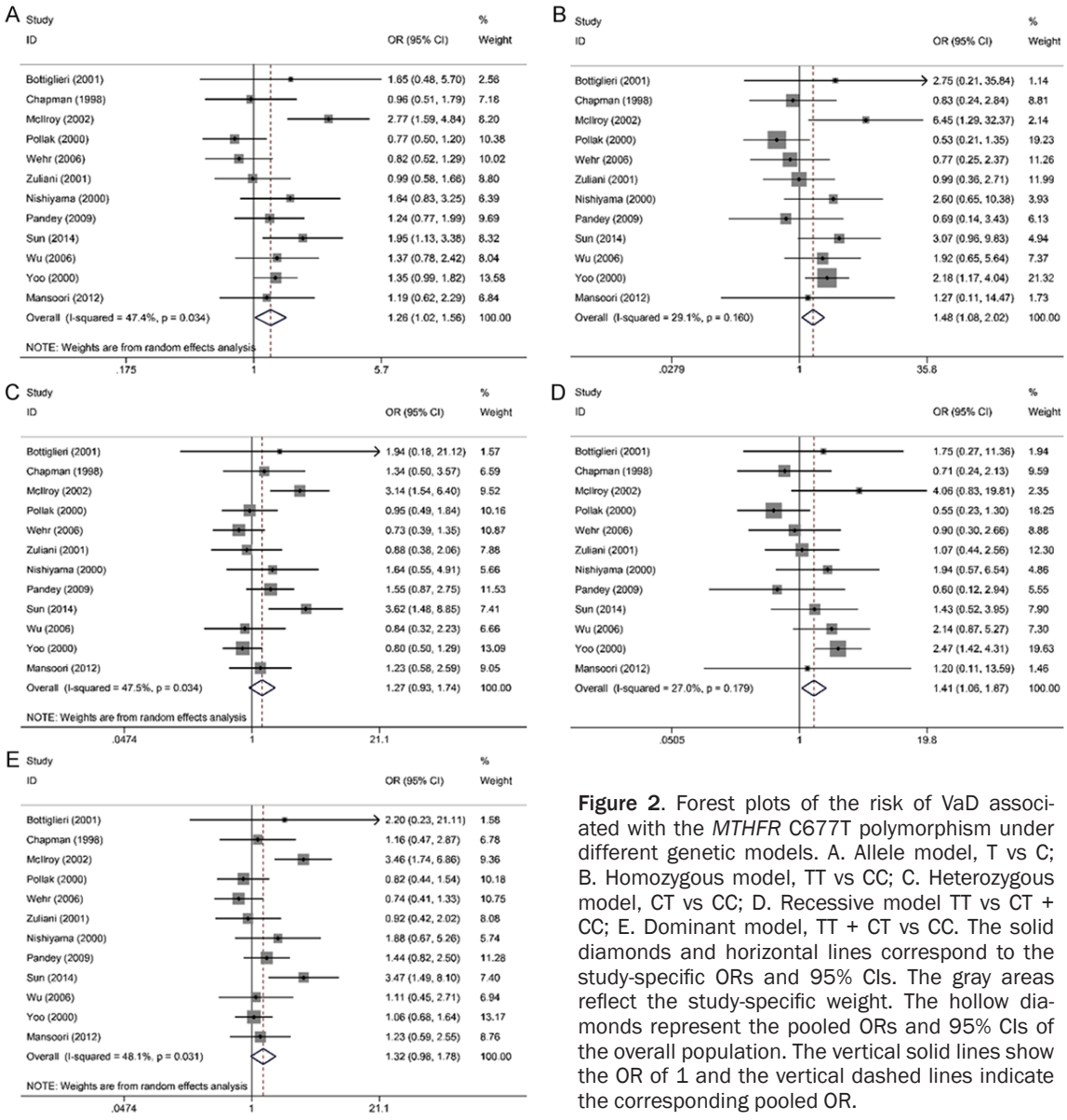

Figure 2. Forest plots of the risk of VaD associated with the *MTHFR* C677T polymorphism under different genetic models. A. Allele model, T vs C; B. Homozygous model, TT vs CC; C. Heterozygous model, CT vs CC; D. Recessive model TT vs CT + CC; E. Dominant model, TT + CT vs CC. The solid diamonds and horizontal lines correspond to the study-specific ORs and 95% CIs. The gray areas reflect the study-specific weight. The hollow diamonds represent the pooled ORs and 95% CIs of the overall population. The vertical solid lines show the OR of 1 and the vertical dashed lines indicate the corresponding pooled OR.

The shape of the funnel plot showed no evidence of obvious asymmetry (**Figure 4**). The Egger's test result did not support the existence of publication bias (allele model, P = 0.565). The $N_{fs0.05}$ value was 68, which is greater than the number of studies included in this meta-analysis, implying a low probability of publication bias.

Discussion

Using summary-level data for VaD and Hcy levels, our study demonstrated that a genetic increase in natural log-transformed plasma Hcy by 1 SDwas associated with a 4.29-fold increased risk of VaD, providing strong evidence in support of a causal role of Hcy in VaD susceptibility. Since genetic effects on Hcy levels represent differences that generally persist throughout adult life, the estimate of our MR study reflects an effect of Hcyover the course of a lifetime. These findings are consistent with evidence from observational studies that have showed that high levels of plasma Hcy influence risk of VaD [9, 10]. To the authors' knowledge, this report is the first to provide evidence for putative causal nature of the association between plasma Hcy and VaD.

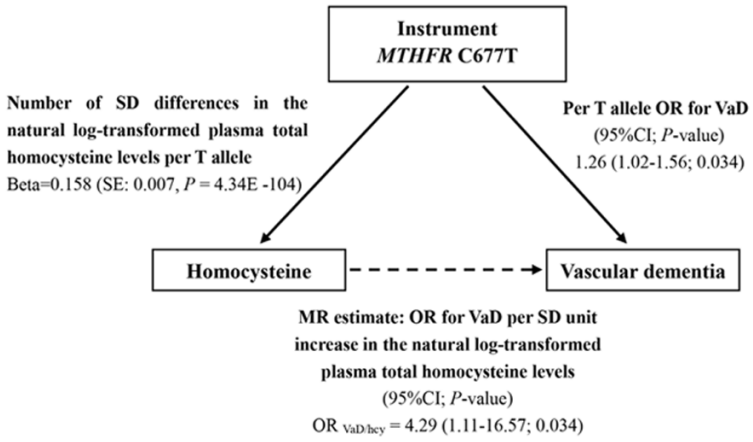

Figure 3. Graphical representation of the Mendelian randomization design. The risk estimate for the association between *MTHFR* C677T polymorphism and VaD risk was obtained from the present meta-analysis. The effect of *MTHFR* C677T polymorphism on the SD change in natural log-transformed plasma Hcy levels was obtained from a recent meta-analysis of GWA studies. SE = standard error, SD = standard deviation.

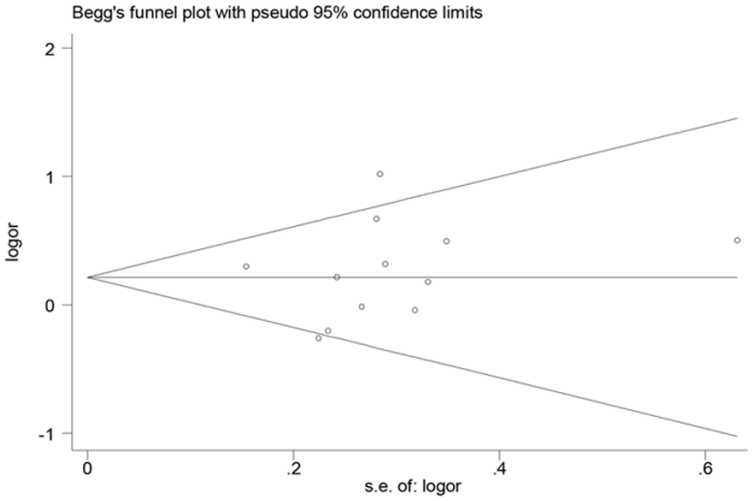

Figure 4. Begg's funnel plot for the *MTHFR* C677T polymorphism and VaD risk under allele model. Each circle represents a separate study for the indicated association. Logor = natural logarithm of OR, s.e. = standard error.

In the present study, risk estimates of *MTHFR* C677T variant with VaD were heterozygous between Asians and Caucasians. Considering the multifactorial nature of VaD, divergent genetic backgrounds or linkage disequilibrium patterns might be the most likely explanation for such divergence. The finding suggests a potential implication for genotyping *MTHFR* C677T variant in VaD risk appraisal among Asians.

A reasonable first step to understanding the role of Hcy therapy in delaying the onset or severity of VaD would be to treat high Hcy level in those most at risk of developing VaD. VaD is the type of dementia caused by problems in the supply of blood to the brain, typically by a series of stroke [36]. The symptoms of VaD may progress gradually or step-wise after each stroke, thereby providing a therapeutic window and rationale for intervening with Hcy reduction. Future RCTs are required to validate the therapeutic approach (for example, supplementation with folic acid and vitamin B12) to prevent VaD, and may therefore provide needed insights into the role of Hcy reduction. An important difference between MR studies and RCTs is that MR studies describe the effect of lifetime exposure to Hcy-increasing allele in the general population, whereas RCTs provide insights from intervention for shorter periods (generally less than a decade) in individuals at risk. Thus, it is possible that RCTs designed to test Hcy lowering may need considerably long-term follow-up to adequately assess the effect of these interventions on VaD.

Our analysis has several strengths. First, because of the random allocation of genotype in advance of disease development, these results indicate that the relation between Hcy concentration and VaD is not subject topotential confounding or reverse causality bias. Second, using data from the largest GWAS meta-analysis for Hcy level (n = 44147), and from the current meta-analysis for VaD risk (up to 722 cases and 1158 controls) have enabled us to more precisely examine our study hypothesis than if we had employed individual-level data from a small study. Thirdly, the findings from this study represent the relationship of a lifetime exposure to elevated Hcy levels with VaD

in the general population, and, in the absence of long-term RCT data, our findings provide strong evidence for a causal role of high Hcy levels in VaD susceptibility.

A few limitations of our study merit consideration. Firstly, MR estimates which utilize instrumental variables accounting for little variance in a trait tend to be biased towards the null [37]. In this study, we used only one genetic variant as the instrumental variable that influences the plasma Hcy levels. However, such bias does not seem to have affected either the direction or significance of the results of this study since our MR analysis suggests a positive association between Hcy and VaD. Secondly, canalization, the process by which compensatory feedback mechanisms attenuate the phenotypic consequences of genetic variation, has been extensively investigated in the context of MR [12, 38, 39]. Although compensatory feedback interactions tend to bias results towards the null, the presence of this mechanism would not alter the statistical significance or direction of the effects we identify through MR. Thirdly, it seems impractical for us to exclude the pleiotropy of *MTHFR* C677T polymorphism since data on other clinical parameters across C677T genotypes are rarely provided from most qualified literatures, requiring further confirmation. Finally, considering the differences in minor allele frequencies between populations and other demographic characteristics in the included studies, it is hard to ignore an impact of population stratification, but significant results in the meta-analysis argue against stratification.

In summary, our MR study suggests that genetically increased Hcy level is causally associated with an increased risk of developing VaD. These findings provide rationale for further exploring the potential therapeutic benefits of Hcy-lowering in preventing the onset and progression of VaD.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jie-Wen Zhang, Department of Neurology, Zhengzhou University People's Hospital and Henan Provincial People's Hospital, 7 Wei Wu Rd., Zhengzhou 450003, Henan, P. R. China. E-mail: jiewenzh@126.com

References

[1] Jellinger KA. The enigma of vascular cognitive disorder and vascular dementia. Acta Neuropathol 2007; 113: 349-388.

[2] Wu L, Feng XT, Hu YQ, Tang N, Zhao QS, Li TW, Li HY, Wang QB, Bi XY and Cai XK. Global gene expression profile of the hippocampus in a rat model of vascular dementia. Tohoku J Exp Med 2015; 237: 57-67.

[3] Roman GC. The Epidemiology of vascular dementia. Handb Clin Neurol 2008; 89: 639-658.

[4] Selhub J. Public health significance of elevated homocysteine. Food Nutr Bull 2008; 29: S116-125.

[5] Wald DS, Law M and Morris JK. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. BMJ 2002; 325: 1202.

[6] Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ and Schouten EG. MTHFR 677C-->T polymorphism and risk of coronary heart disease: a meta-analysis. JAMA 2002; 288: 2023-2031.

[7] Brattstrom L, Wilcken DE, Ohrvik J and Brudin L. Common methylene tetra hydro folate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis. Circulation 1998; 98: 2520-2526.

[8] van Meurs JB, Pare G, Schwartz SM, Hazra A, Tanaka T, Vermeulen SH, Cotlarciuc I, Yuan X, Malarstig A, Bandinelli S, Bis JC, Blom H, Brown MJ, Chen C, Chen YD, Clarke RJ, Dehghan A, Erdmann J, Ferrucci L, Hamsten A, Hofman A, Hunter DJ, Goel A, Johnson AD, Kathiresan S, Kampman E, Kiel DP, Kiemeney LA, Chambers JC, Kraft P, Lindemans J, McKnight B, Nelson CP, O'Donnell CJ, Psaty BM, Ridker PM, Rivadeneira F, Rose LM, Seedorf U, Siscovick DS, Schunkert H, Selhub J, Ueland PM, Vollenweider P, Waeber G, Waterworth DM, Watkins H, Witteman JC, den Heijer M, Jacques P, Uitterlinden AG, Kooner JS, Rader DJ, Reilly MP, Mooser V, Chasman DI, Samani NJ and Ahmadi KR. Common genetic loci influencing plasma homocysteine concentrations and their effect on risk of coronary artery disease. Am J Clin Nutr 2013; 98: 668-676.

[9] Koseoglu E and Karaman Y. Relations between homocysteine, folate and vitamin B12 in vascular dementia and in Alzheimer disease. Clin Biochem 2007; 40: 859-863.

[10] Lehmann M, Gottfries CG and Regland B. Identification of cognitive impairment in the elderly: homocysteine is an early marker. Dement Geriatr Cogn Disord 1999; 10: 12-20.
[11] Ho RC, Cheung MW, Fu E, Win HH, Zaw MH, Ng A and Mak A. Is high homocysteine level a risk factor for cognitive decline in elderly? A systematic review, meta-analysis, and meta-regression. Am J Geriatr Psychiatry 2011; 19: 607-617.
[12] Lawlor DA, Harbord RM, Sterne JA, Timpson N and Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med 2008; 27: 1133-1163.
[13] Casas JP, Bautista LE, Smeeth L, Sharma P and Hingorani AD. Homocysteine and stroke: evidence on a causal link from Mendelian randomisation. Lancet 2005; 365: 224-232.
[14] Ahmad OS, Morris JA, Mujammami M, Forgetta V, Leong A, Li R, Turgeon M, Greenwood CM, Thanassoulis G, Meigs JB, Sladek R and Richards JB. A Mendelian randomization study of the effect of type-2 diabetes on coronary heart disease. Nat Commun 2015; 6: 7060.
[15] Huang T, Ren J, Huang J and Li D. Association of homocysteine with type 2 diabetes: a meta-analysis implementing Mendelian randomization approach. BMC Genomics 2013; 14: 867.
[16] Numata S, Kinoshita M, Tajima A, Nishi A, Imoto I and Ohmori T. Evaluation of an association between plasma total homocysteine and schizophrenia by a Mendelian randomization analysis. BMC Med Genet 2015; 16: 54.
[17] Yajnik CS, Chandak GR, Joglekar C, Katre P, Bhat DS, Singh SN, Janipalli CS, Refsum H, Krishnaveni G, Veena S, Osmond C and Fall CH. Maternal homocysteine in pregnancy and offspring birthweight: epidemiological associations and Mendelian randomization analysis. Int J Epidemiol 2014; 43: 1487-1497.
[18] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010; 25: 603-605.
[19] Higgins JP and Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21: 1539-1558.
[20] Egger M, Davey Smith G, Schneider M and Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315: 629-634.
[21] Nishi A, Numata S, Tajima A, Kinoshita M, Kikuchi K, Shimodera S, Tomotake M, Ohi K, Hashimoto R, Imoto I, Takeda M and Ohmori T. Meta-analyses of blood homocysteine levels for gender and genetic association studies of the MTHFR C677T polymorphism in schizophrenia. Schizophr Bull 2014; 40: 1154-1163.
[22] Pichler I, Del Greco MF, Gogele M, Lill CM, Bertram L, Do CB, Eriksson N, Foroud T, Myers RH, Nalls M, Keller MF, Benyamin B, Whitfield JB, Pramstaller PP, Hicks AA, Thompson JR and Minelli C. Serum iron levels and the risk of Parkinson disease: a Mendelian randomization study. PLoS Med 2013; 10: e1001462.
[23] Greco MF, Minelli C, Sheehan NA and Thompson JR. Detecting pleiotropy in Mendelian randomisation studies with summary data and a continuous outcome. Stat Med 2015; 34: 2926-2940.
[24] Chapman J, Wang N, Treves TA, Korczyn AD and Bornstein NM. ACE, MTHFR, factor V Leiden, and APOE polymorphisms in patients with vascular and Alzheimer's dementia. Stroke 1998; 29: 1401-1404.
[25] Nishiyama M, Kato Y, Hashimoto M, Yukawa S and Omori K. Apolipoprotein E, methylene tetra hydro folate reductase (MTHFR) mutation and the risk of senile dementia--an epidemiological study using the polymerase chain reaction (PCR) method. J Epidemiol 2000; 10: 163-172.
[26] Pollak RD, Pollak A, Idelson M, Bejarano-Achache I, Doron D and Blumenfeld A. The C677T mutation in the methylene tetra hydro folate reductase (MTHFR) gene and vascular dementia. J Am Geriatr Soc 2000; 48: 664-668.
[27] Wehr H, Bednarska-Makaruk M, Łojkowska W, Graban A, Hoffman-Zacharska D, Kuczyńska-Zardzewiały A, Mrugała J, Rodo M, Bochyńska A, Sułek A and Ryglewicz D. Differences in risk factors for dementia with neurodegenerative traits and for vascular dementia. Dement Geriatr Cogn Disord 2006; 22: 1-7.
[28] Zuliani G, Ble A, Zanca R, Munari MR, Zurlo A, Vavalle C, Atti AR and Fellin R. Genetic polymorphisms in older subjects with vascular or Alzheimer's dementia. Acta Neurol Scand 2001; 103: 304-308.
[29] McIlroy SP, Dynan KB, Lawson JT, Patterson CC and Passmore AP. Moderately elevated plasma homocysteine, methylene tetra hydro folate reductase genotype, and risk for stroke, vascular dementia, and Alzheimer disease in Northern Ireland. Stroke 2002; 33: 2351-2356.
[30] Mansoori N, Tripathi M, Luthra K, Alam R, Lakshmy R, Sharma S, Arulselvi S, Parveen S and Mukhopadhyay AK. MTHFR (677 and 1298) and IL-6-174 G/C genes in pathogenesis of Alzheimer's and vascular dementia and their epistatic interaction. Neurobiol Aging 2012; 33: 1003, e1001-1008.
[31] Pandey P, Pradhan S, Modi DR and Mittal B. MTHFR and ACE gene polymorphisms and risk of vascular and degenerative dementias in the elderly. Brain Cogn 2009; 71: 295-299.

[32] Yoo JH, Choi GD and Kang SS. Pathogenicity of thermolabile methylene tetra hydro folate reductase for vascular dementia. Arterioscler Thromb Vasc Biol 2000; 20: 1921-1925.
[33] Bottiglieri T, Parnetti L, Arning E, Ortiz T, Amici S, Lanari A and Gallai V. Plasma total homocysteine levels and the C677T mutation in the methylene tetra hydro folate reductase (MTHFR) gene: a study in an Italian population with dementia. Mech Ageing Dev 2001; 122: 2013-2023.
[34] Wu DB, Wang LN, Ye L, Liu JW and Wang L. Analysis of MTHFR C677T polymorphism in patients with SAD and VD. Chin J Neuromed 2006; 5: 775-780.
[35] Sun YN, Zhang BS and Cheng Y. Correlation of methylene tetra hydro folate reductase 677C/T polymorphism and plasma homocysteine level with Alzheimer's disease and vascular dementia. Chin J Geriatr 2014; 33: 948-951.
[36] Olsson Y, Brun A and Englund E. Fundamental pathological lesions in vascular dementia. Acta Neurol Scand Suppl 1996; 168: 31-38.
[37] Fewell Z, Davey Smith G and Sterne JA. The impact of residual and unmeasured confounding in epidemiologic studies: a simulation study. Am J Epidemiol 2007; 166: 646-655.
[38] Smith GD and Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003; 32: 1-22.
[39] Mokry LE, Ahmad O, Forgetta V, Thanassoulis G and Richards JB. Mendelian randomisation applied to drug development in cardiovascular disease: a review. J Med Genet 2015; 52: 71-79.