Correlations of *MTHFR* 677C>T Polymorphism with Cardiovascular Disease in Patients with End-Stage Renal Disease: A Meta-Analysis

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Abstract

Objective: This meta-analysis was conducted to evaluate the correlations of a common polymorphism (677C>T) in the methylenetetrahydrofolate reductase (*MTHFR*) gene with risk of cardiovascular disease (CVD) in patients with end-stage renal disease (ESRD).

Method: The following electronic databases were searched without language restrictions: Web of Science (1945~2013), the Cochrane Library Database (Issue 12, 2013), MEDLINE (1966~2013), EMBASE (1980~2013), CINAHL (1982~2013) and the Chinese Biomedical Database (CBM) (1982~2013). Meta-analysis was performed using STATA statistical software. Odds ratios (ORs) with their 95% confidence intervals (95%CIs) were calculated.

Results: Eight cohort studies met all inclusion criteria and were included in this meta-analysis. A total of 2,292 ESRD patients with CVD were involved in this meta-analysis. Our meta-analysis results revealed that the *MTHFR* 677C>T polymorphism might increase the risk of CVD in ESRD patients (TT vs. CC: OR = 2.75, 95%CI = 1.35~5.59, $P = 0.005$; CT+TT vs. CC: OR = 1.39, 95%CI = 1.09~1.78, $P = 0.008$; TT vs. CC+CT: OR = 2.52, 95%CI = 1.25~5.09, $P = 0.010$; respectively). Further subgroup analysis by ethnicity suggested that the *MTHFR* 677C>T polymorphism was associated with an elevated risk for CVD in ESRD patients among Asians (TT vs. CC: OR = 3.38, 95%CI = 1.11~10.28, $P = 0.032$; CT+TT vs. CC: OR = 1.44, 95%CI = 1.05~1.97, $P = 0.022$; TT vs. CC+CT: OR = 3.15, 95%CI = 1.02~9.72, $P = 0.046$; respectively), but not among Africans or Caucasians (all $P > 0.05$).

Conclusion: Our findings indicate that the *MTHFR* 677C>T polymorphism may be associated with an elevated risk for CVD in ESRD patients, especially among Asians.

Introduction

Hemodialysis (HD) is a common form of dialysis therapy for kidney disease by achieving the extracorporeal removal of waste products such as creatinine, urea and free water from the blood [1]. HD is the most common procedure performed for patients with end-stage renal disease (ESRD), with an estimation of 1.5 million ESRD patients receiving HD worldwide [2]. Despite its important role in treating ESRD, HD also serves as a multiplier for other vascular risk factors, contributing to an increased incidence of cardiovascular disease, such as atrial fibrillation and myocardial ischemia [3,4]. Moreover, it has been reported that mortality caused by cardiovascular disease (CVD) in ESRD patients is 25% higher than that in the general population [5,6]. Therefore, the assessment and prevention of CVD are major considerations for dealing patients with HD [7]. Generally, CVD refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease [8]. Clinically, traditional CVD risk factors include old age, male sex, diabetes mellitus, and systolic hypertension and so on [9]. Over past few decades, studies have demonstrated that the severity and extent of cardiovascular complications in ESRD patients is disproportionate to the number and severity of traditional risk factors [10,11]. In recent years, nontraditional risk factors such as hemoglobin levels, inflammation, oxidative stress, and particularly epigenetic mechanisms of gene regulation have been emphasized as factors that may play an important role in the development of cardiovascular disease in patients with HD [12]. In addition, genetic polymorphisms have received considerable attention as a competitive candidate for the risk of CVD in ESRD patients [13].

Methylene tetrahydrofolate reductase (MTHFR), encoded by the *MTHFR* gene, is a key enzyme in folate metabolism which plays a critical role in the synthesis and methylation of DNA encoding for an enzyme responsible for re-methylation of homocysteine (Hcy) to methionine [14–16]. Actually, Hazra et al. confirmed that the functional polymorphism in MTHFR
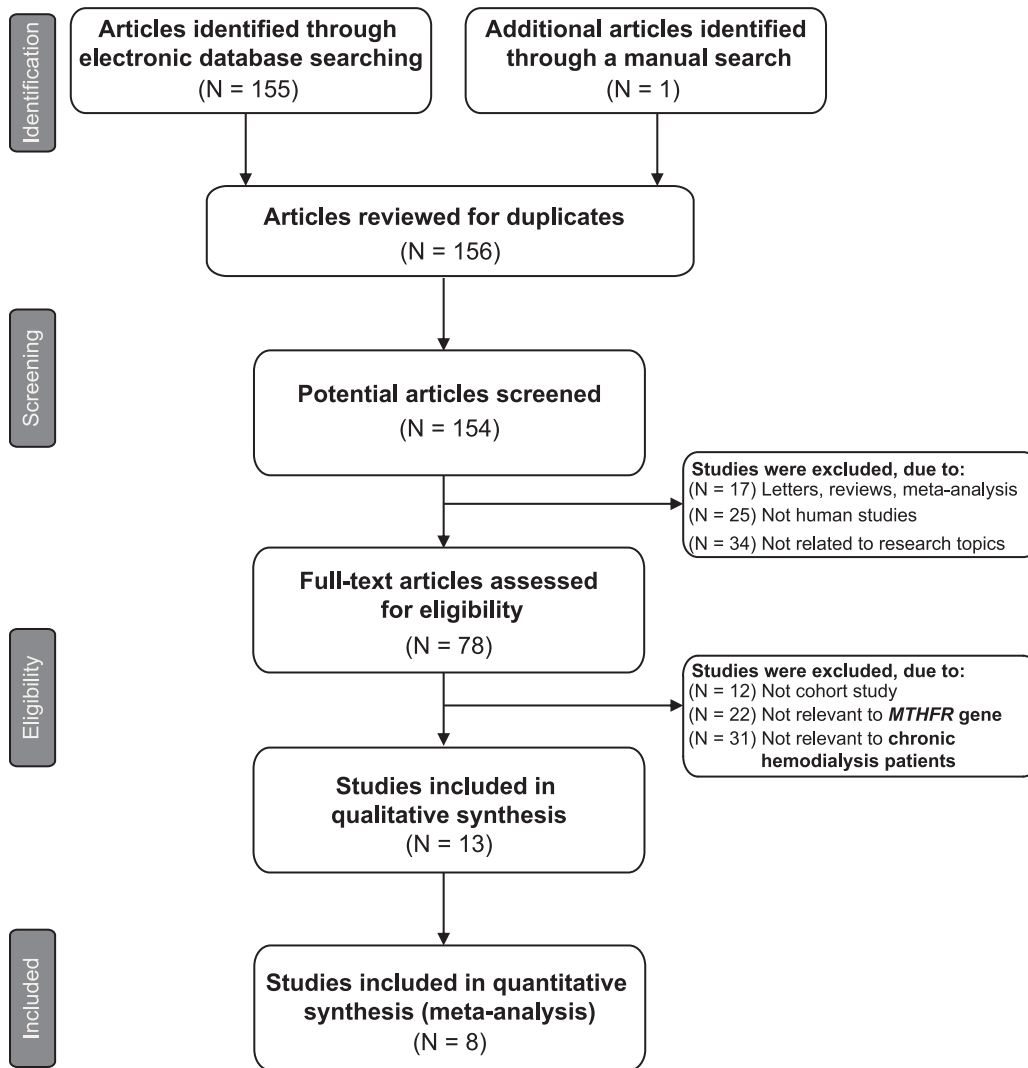
Figure 1. Flow chart shows study selection procedure. Eight cohort studies were included in this meta-analysis.
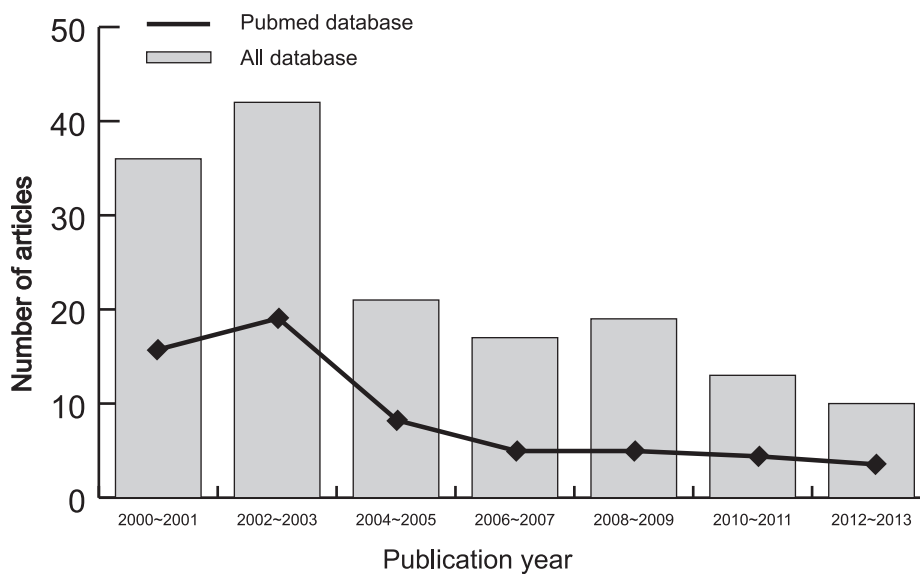doi:10.1371/journal.pone.0102323.g001

Figure 2. The distribution of the number of topic-related literatures in the electronic database over the last decade.
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Table 1. Baseline characteristics and methodological qualities of all included studies.

First author	Year	Country	Ethnicity	Case number	Gender (M/F)	Age (years)	Genotyping method	Genotype frequency			HWE test (P value)	NOS score
								CC	CT	TT		
Lee et al [21]	2011	Korea	Asians	152	83/69	56.8±13.6	PCR-RFLP	44	72	36	0.538	7
Ibrahim et al [30]	2009	Egypt	Africans	50	29/21	41.6±11.8	Direct sequencing	30	16	4	0.385	6
Aucella et al [28]	2005	Italy	Caucasians	461	217/244	58.8±15.6	PCR-RFLP	134	220	86	0.801	8
Fukasawa et al [23]	2003	Japan	Asians	337	196/141	61.5±13.7	Direct sequencing	111	158	68	0.390	8
Morimoto et al [32]	2002	Japan	Asians	168	87/81	60.7±13.1	PCR-RFLP	78	67	23	0.167	7
Haviv et al [29]	2002	USA	Caucasians	120	72/48	48.0±13.0	PCR-RFLP	40	60	19	0.656	7
Wrone et al [24]	2001	USA	Caucasians	459	230/226	-	Direct sequencing	216	184	59	0.049	7
Kimura et al [31]	2000	Japan	Asians	545	319/226	58.7±12.5	PCR-RFLP	199	251	95	0.303	8

Legend: M – male; F – female; SNP - single nucleotide polymorphism; HWE - Hardy-Weinberg equilibrium; NOS - Newcastle-Ottawa Scale; PCR-RFLP - polymerase chain reaction-restriction fragment length polymorphism.
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Ala222Val (also reported as MTHFR C677T), rs1801133, on chromosome 1 was associated with plasma homocysteine in a healthy population [17]. The human MTHFR gene is located on the short arm of chromosome 1 (1p36.3), is comprised of 11 exons and 10 introns, and is 19.3 kb in length [17]. Currently, multiple researchers have demonstrated that genetic variations in the MTHFR gene might alter its production and function, thereby affecting individual prevalence rates of some diseases and even cancers [18,19]. More specifically, multiple polymorphic sites such as 677C>T (rs1801133) and 1298A>C (rs1801131) within the MTHFR promoter region have been reported in previous studies, of which 677C>T has been suggested to be implicated in the susceptibility to CVD in ESRD patients [20,21]. It should be noted that plasma Hcy levels have been found to be an important risk factor for the development of CVD in ESRD patients [22]. However, polymorphism at nucleotide 677C>T may reduce the activation and thermo-stability of MTHFR, and subsequently inhibit the generation of 5-Methylenetetrahydrofolate and inter-fere with the normal metabolism and re-methylation of Hcy, thereby resulting in the accumulation of Hcy and an elevated plasma Hcy concentration in individuals; however, contradictory results have also been reported [23,24]. Given the conflicting evidence on this issue, we conducted a meta-analysis of all available cohort studies to determine whether MTHFR genetic polymorphisms contribute to the pathogenesis of CVD in ESRD patients.

Methods

Publication search

The following electronic databases were searched without language restrictions: Web of Science (1945~2013), the Cochrane Library Database (Issue 12, 2013), MEDLINE (1966~2013), EMBASE (1980~2013), CINAHL (1982~2013) and the Chinese Biomedical Database (CBM) (1982~2013). We used the following keywords and MeSH terms in conjunction with a highly sensitive search strategy: ["SNP" or "mutation" or "genetic polymor-phism" or "variation" or "polymorphism" or "single nucleotide polymorphism" or "variant"] and ["cardiovascular disease" or "CVD" or "cardiovascular risk" or "Hemodialysis" or "HD"] and ["methylenetetrahydrofolate reductase" or "MTHFR" or "methylene tetrahydrofolate reductase" or "5,10-methylenetetra-hydrofolate reductase"]. We also conducted a manual search to find other potential articles based on references identified in retrieved articles.

Inclusion and exclusion criteria

The following criteria were used to determine the eligibility of included studies: (1) the study design must be a clinical cohort study; (2) the study must relate to the relationships of MTHFR 677C>T polymorphisms with the risk of CVD in ESRD patients; (3) all patients met the diagnostic criteria for HD; (4) the study must provide sufficient information about the frequencies of the MTHFR 677C>T polymorphism. If a study could not meet the inclusion criteria, it was excluded. The most recent or the largest sample size publication was included when the authors published several studies using the same subjects. The supporting PRISMA checklist is available as Checklist S1.

Data extraction

Data were systematically extracted by two authors from each included study using a standardized form. The form used for data extraction documented the most relevant items including publi-cation year, language of publication, the first author's surname,
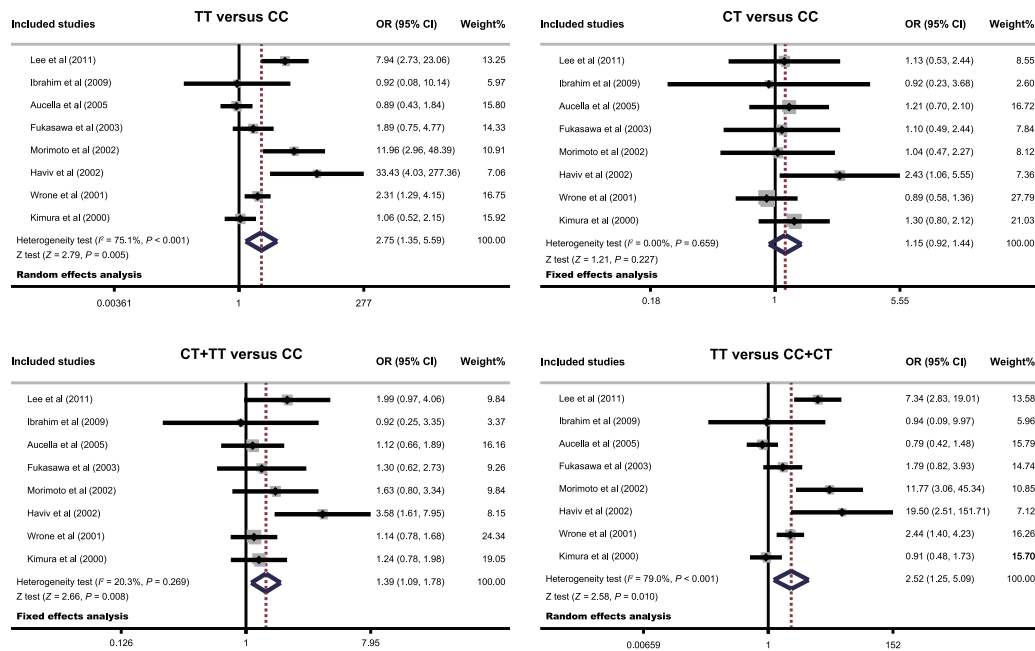
Figure 3. Forest plots for the relationships between the *MTHFR* 677C>T polymorphism and the risk of cardiovascular disease in chronic hemodialysis patients.
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geographical location, sample size, design of study, the source of the subjects, source of samples, allele frequencies, genotyping method of SNP, evidence of HWE in healthy controls, etc.

Methodological assessment

Methodological quality was independently assessed by two observers according to the Newcastle-Ottawa Scale (NOS) criteria [25]. The NOS criteria includes three aspects: (1) subject selection: 0~4 points; (2) comparability of subject: 0~2 points; (3) clinical outcome: 0~3 points. NOS scores range from 0 to 9 with a score ≥7 indicating good quality. The supporting NOS score criteria are available in Supplement S1.

Statistical analysis

Meta-analysis was performed with the use of the STATA statistical software (Version 12.0, Stata Corporation, College Station, TX, USA). Odds ratios (ORs) with their 95% confidence intervals (95%CIs) were calculated as estimates of relative risk for CVD in ESRD patients. The Z test was used to estimate the statistical significance of pooled ORs. Heterogeneity among studies was estimated by the Cochran's Q-statistic and I^2 tests [26]. If a Q-test shows a $P<0.05$ or $I^2>50\%$, indicating significant heterogeneity, the random-effects model was conducted; otherwise the fixed-effects model was used. We also explored reasons for heterogeneity using meta-regression and subgroup analyses. In order to evaluate the influence of single studies on the overall estimate, a sensitivity analysis was performed. Funnel plots and Egger's linear regression test were applied to investigate publication bias [27].

Results

Eligible studies

Initially, the highly sensitive search strategy identified 156 articles. After screening the titles and abstracts of all retrieved articles, 78 articles were excluded. Then full texts were reviewed

and 65 articles were further excluded. Another 5 studies were also excluded due to lack of data integrity (Figure 1). Finally, 8 cohort studies were included in this meta-analysis [21,23,24,28–32]. Publication years of the eligible studies ranged from 2000 to 2011. Figure 2 shows the distribution of the number of topic-related literatures in electronic databases over the last decade. A total of 2,292 ESRD patients with CVD were involved in this meta-analysis. Four studies were conducted among Asians, three studies among Caucasians, and one study among Africans. The polymerase chain reaction-amplified genes with restriction endonucleases (PCR-RFLP) was performed in 5 studies, while 3 studies used the direct DNA sequencing method. The genotype frequencies of controls were all in HWE (all $P>0.05$). The NOS scores of all included studies were ≥6. The main characteristics of all eligible studies are listed in Table 1.

Quantitative data synthesis

Meta-analysis findings on the relationship between the *MTHFR* 677C>T polymorphism and susceptibility to CVD in ESRD patients is shown in Table 2. The random-effects model was conducted due to significant heterogeneity between studies. Our meta-analysis results revealed that the *MTHFR* 677C>T polymorphism might increase the risk of CVD in ESRD patients (TT vs. CC: OR = 2.75, 95%CI = 1.35~5.59, $P = 0.005$; CT+TT vs. CC: OR = 1.39, 95%CI = 1.09~1.78, $P = 0.008$; TT vs. CC+CT: OR = 2.52, 95%CI = 1.25~5.09, $P = 0.010$; respectively) (Figure 3).

Subgroup and meta-regression analysis were conducted based on ethnicity to investigate potential sources of heterogeneity. Our results suggested that the *MTHFR* 677C>T polymorphism was associated with the incidence of CVD in ESRD patients among Asians (TT vs. CC: OR = 3.38, 95%CI = 1.11~10.28, $P = 0.032$; CT+TT vs. CC: OR = 1.44, 95%CI = 1.05~1.97, $P = 0.022$; TT vs. CC+CT: OR = 3.15, 95%CI = 1.02~9.72, $P = 0.046$; respectively), but not among Africans or Caucasians (all $P>0.05$). Further subgroup analyses based on genotyping method suggested

Table 2. Meta-analysis of the relationships between the MTHFR 677 C>T polymorphism and the risk of cardiovascular disease in patients with end-stage renal disease.

	Sample size	TT vs. CC			CT vs. CC			CT+TT vs. CC			TT vs. CC+CT		
		OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Overall		2.75	1.35–5.59	0.005	1.15	0.92–1.44	0.227	1.39	1.09–1.78	0.008	2.52	1.25–5.09	0.010
Ethnicity													
Asians (n=4)	1202	3.38	1.11–10.28	0.032	1.18	0.85–1.65	0.327	1.44	1.05–1.97	0.022	3.15	1.02–9.72	0.046
Africans (n=1)	50	0.92	0.08–10.14	0.943	0.92	0.23–3.68	0.902	0.92	0.25–3.35	0.895	0.94	0.09–9.97	0.962
Caucasians (n=3)	1040	2.72	0.76–9.78	0.125	1.25	0.75–2.07	0.389	1.52	0.84–2.73	0.165	2.35	0.69–7.96	0.171
Genotyping method													
PCR-RFLP (n=5)	1446	4.00	1.19–13.53	0.025	1.31	0.98–1.74	0.065	1.61	1.11–2.32	0.012	3.42	1.04–11.23	0.043
Direct sequencing (n=3)	846	2.11	1.30–3.42	0.003	0.93	0.65–1.34	0.695	1.16	0.83–1.61	0.394	2.14	1.37–3.33	0.001

Legend: OR - odds ratio; 95%CI - 95% confidence interval; PCR-RFLP - polymerase chain reaction-restriction fragment length polymorphism.
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that the MTHFR 677C>T polymorphism was closely linked to an increased risk of CVD in the majority of subgroups (as shown in Table 2).

Meta-regression analysis confirmed that none of the considered factors was the main source of heterogeneity (as shown in Table 3). Sensitivity analysis suggested that no single study could influence the overall pooled estimates (Figure 4). We found no evidence of obvious asymmetry in the funnel plots (Figure 5). Egger's test also did not display strong statistical evidence for publication bias (all $P>0.05$).

Discussion

MTHFR can catalyze a rate-limiting reaction in the transformation of methionine into Hcy and back exclusively through the transfer of the methyl group to and from methyl-tetrahydrofolate, separately [33–35]. It is well documented that an elevated level of the sulphur amino-acid Hcy in plasma is an independent risk factor for atherosclerosis, coronary heart disease, stroke and other CVDs [36,37]. Meanwhile, the increased fasting level of total Hcy was found to establish an independent contribution to the excess incidence of fatal and non-fatal cardiovascular outcomes [38]. Indeed, the formation of hyperhomocysteinemia (HHcy) was closely associated with folic acid, Vitamin B_6 and B_{12} supplementation [39], but also correlated with sulfur amino acid metabolism [40]. As for MTHFR, since the methyl groups for Hcy mediated DNA-methylation to methionine are related to the provision of 5-methylenetetrahydrofolate, which can be irreversibly transferred from 5 to 10-methylenetetrahydrofolate through MTHFR catalysis, the MTHFR gene probably plays a crucial role in the re-methylation of Hcy [41]. On the other hand, it has been estimated by statistical data in clinical studies that HD patients suffering from mild to moderate HHcy account for a great proportion of the high-risk population for CVD [42]. Therefore, it was hypothesized that common polymorphisms in the MTHFR gene could be functional and associated with the development and progression of CVD in ESRD patients. Recently, the usage of human MTHFR gene mutations in predicting the increased Hcy levels and susceptibility to CVD has been widely adopted [13,34]. In particular, a relatively common functional polymorphism in MTHFR rs1801133 (677C>T) in exon 4 may influence the plasma Hcy in ESRD patients. Abundant evidence has supported the view that a half reduction in enzymatic capability might lead to a mild Hcy, as well as total elevation of plasma Hcy concentrations in individuals, both of which might contribute to the genetic predisposition to CVD [30,43].

We undertook the present meta-analysis to elucidate the association of genetic mutations in the MTHFR gene with the incidence of CVD in ESRD patients. Our meta-analysis results showed that the MTHFR 677C>T polymorphism was associated with an increased risk of CVD in ESRD patients, indicating that variants in the MTHFR gene may play a pivotal role in the pathology of CVD in ESRD patients. Although the mechanism by which MTHFR genetic polymorphisms lead to the development of CVD in ESRD patients still remains partially understood, a possible explanation is that the MTHFR 677C>T polymorphism may lead to a mild decrease in MTHFR activity which plays a crucial role in the remethylation of Hcy to methionine, resulting in elevated total plasma Hcy levels which may contribute independently to the excess incidence of fatal and nonfatal CVD. Haviv YS et al. have suggested a major role for plasma Hcy level as a determinant of CVD in ESRD patients, whereby the MTHFR variants and low serum folate are important determinants of plasma Hcy level and therefore may also have an indirect impact
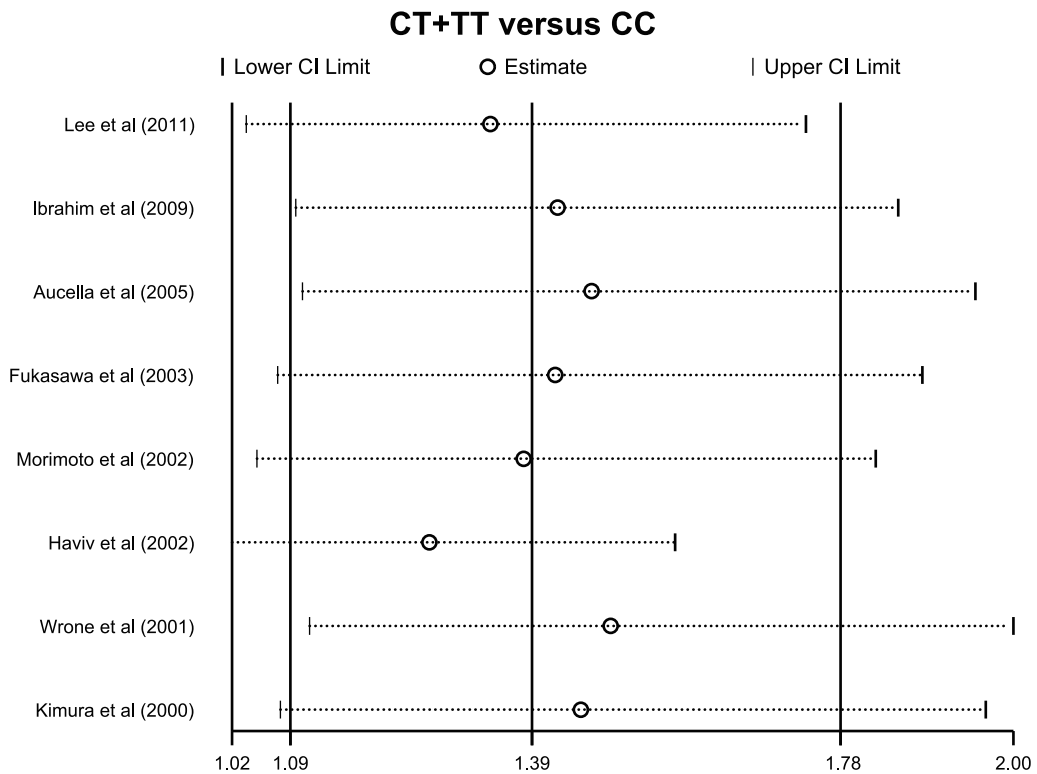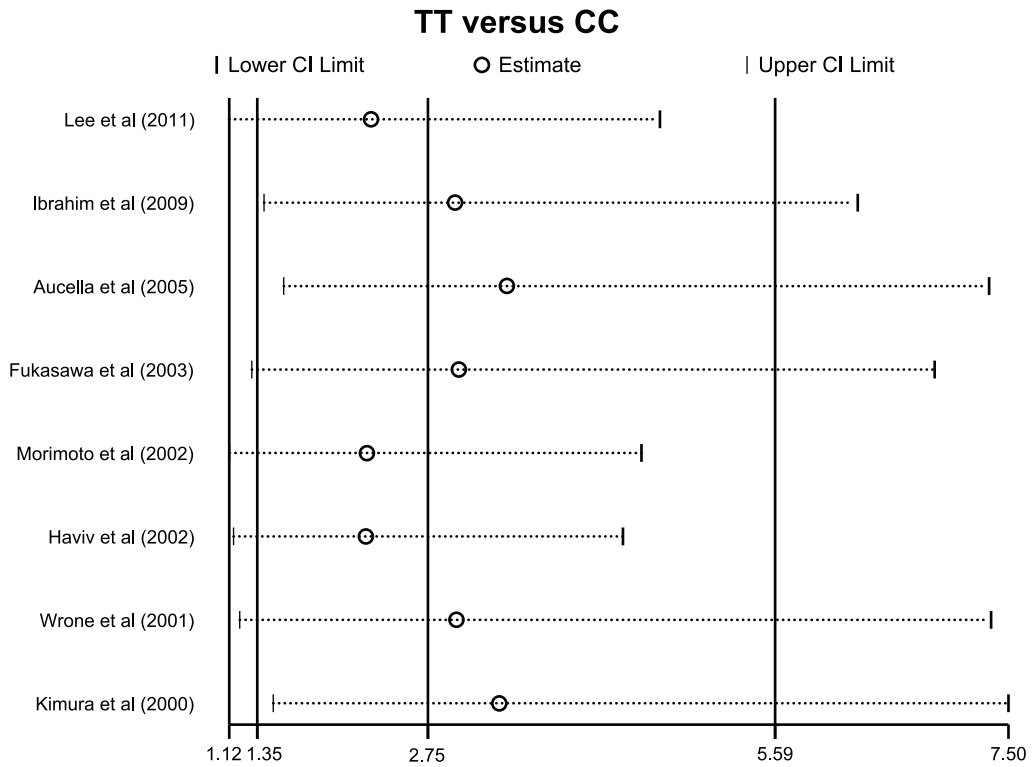
Figure 4. Sensitivity analysis of the summary odds ratio coefficients on the relationships between the *MTHFR* **677C>T polymorphism and the risk of cardiovascular disease in chronic hemodialysis patients.**
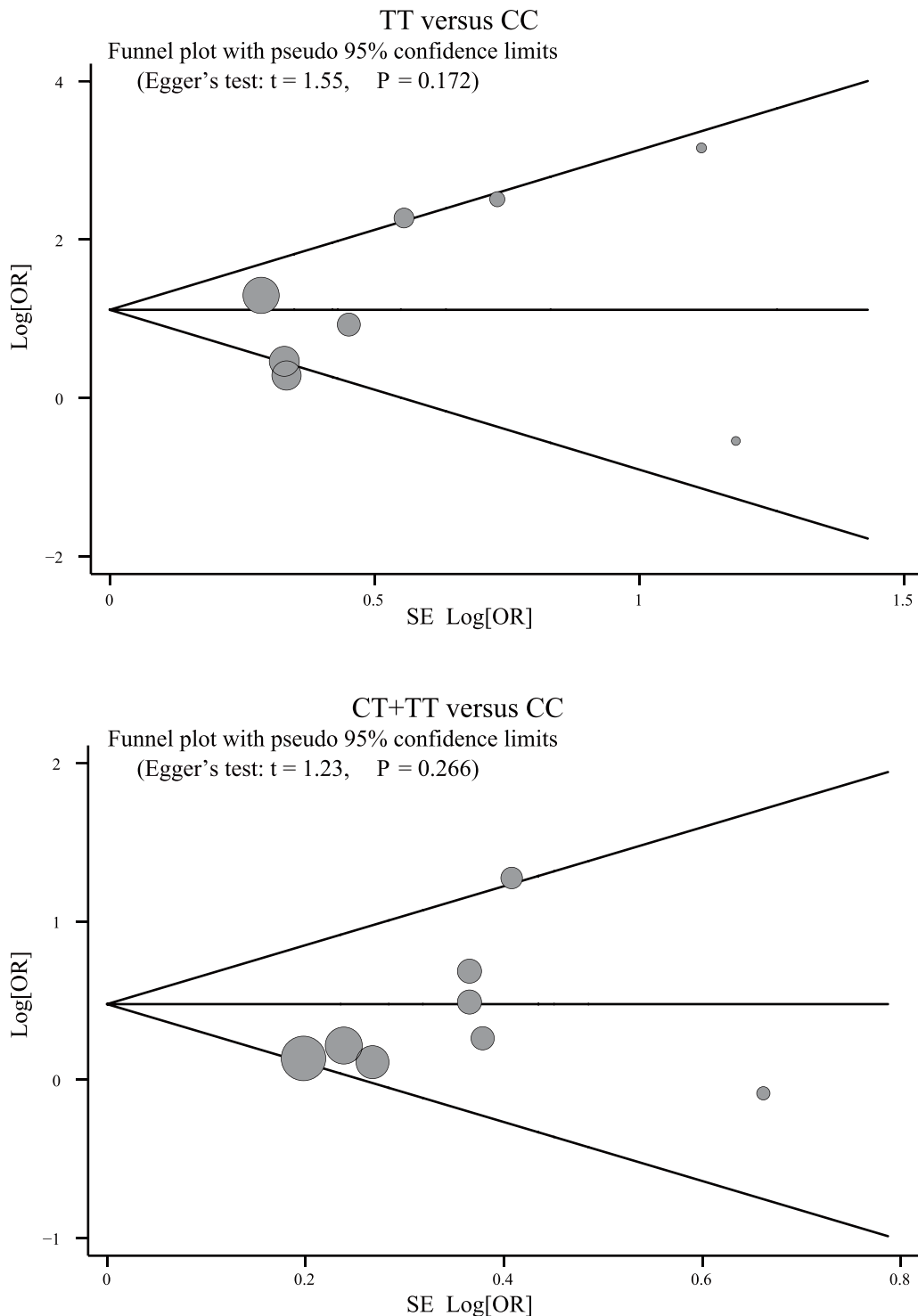
Figure 5. Funnel plot of publication biases on the relationships between the *MTHFR* 677C>T polymorphism and the risk of cardiovascular disease in chronic hemodialysis patients.
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on the cardiovascular status [29]. Since heterogeneity obviously existed in our meta-analysis, we performed stratified analyses based on ethnicity and genotyping method. Subgroup analysis by ethnicity indicated that there were marked associations between the *MTHFR* 677C>T polymorphism and an increased risk of CVD in ESRD patients among Asians. In short, our findings were in accordance with previous studies that found that the *MTHFR* 677C>T polymorphism may alter the risk of developing CVD in ESRD patients, suggesting that these polymorphisms may be useful as biomarkers for the early diagnosis of CVD in ESRD patients.

Table 3. Univariate and multivariate meta-regression analyses of potential source of heterogeneity.

Heterogeneity factors	Coefficient	SE	Z	P	95%CI	
					LL	UL
Publication year						
Univariate	0.028	0.122	0.23	0.820	−0.212	0.267
Multivariate	−0.078	0.067	−1.17	0.241	−0.209	0.053
Ethnicity						
Univariate	−0.439	0.697	−0.63	0.528	−1.806	0.927
Multivariate	−0.132	0.339	−0.39	0.697	−0.796	0.532
Genotyping method						
Univariate	−0.769	0.922	−0.83	0.405	−2.577	1.039
Multivariate	0.562	0.372	1.51	0.131	−0.167	1.291

Legend: SE - standard error; 95%CI - 95% confidence interval; UL - upper limit; LL - lower limit.
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As the first meta-analysis focused on the relationships between the *MTHFR* 677C>T polymorphism and the risk of CVD in ESRD patients, our study has some limitations. First, our results lacked sufficient statistical power to assess correlations between the *MTHFR* 677C>T polymorphism and susceptibility to CVD in ESRD patients due to a relatively small sample size. Second, as a retrospective study, our meta-analysis may have been influenced by subject selection bias, thereby affecting the reliability of our results. Third, our meta-analysis failed to obtain original data from the included studies, which may have limited further evaluation of the potential roles of the *MTHFR* 677C>T polymorphism in the development and progression of CVD in ESRD patients. Importantly, the inclusion criteria of cases and controls were not well defined in all included studies, which might also have influenced our results.

In conclusion, our findings indicate that the *MTHFR* 677C>T polymorphism may contribute to increased CVD risk in ESRD patients. Thus, the *MTHFR* 677C>T polymorphism may be a useful and promising biomarker for the early detection and prognosis of CVD in ESRD patients. However, due to the limitations mentioned above, more research studies with larger sample sizes are still needed to provide a more representative statistical analysis.

Supporting Information

Checklist S1 The PRISMA 2009 checklist.
(DOC)

Supplement S1 The Newcastle-Ottawa Scale for assessing methodological quality.
(DOC)

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Author Contributions

Conceived and designed the experiments: XHG. Performed the experiments: XHG HYZ. Analyzed the data: XHG YW. Contributed reagents/materials/analysis tools: GYZ YW. Wrote the paper: XHG.

References

1. Mehrotra R, Chiu YW, Kalantar-Zadeh K, Bargman J, Vonesh E (2011) Similar outcomes with hemodialysis and peritoneal dialysis in patients with end-stage renal disease. Arch Intern Med, 171(2): 110–118.
2. Palacio S, Gonzales NR, Sangha NS, Birnbaum LA, Hart RG (2011) Thrombolysis for acute stroke in hemodialysis: international survey of expert opinion. Clin J Am Soc Nephrol, 6(5): 1089–1093.
3. Winkelmayer WC, Patrick AR, Liu J, Brookhart MA, Setoguchi S (2011) The increasing prevalence of atrial fibrillation among hemodialysis patients. J Am Soc Nephrol, 22(2): 349–357.
4. McIntyre CW (2010) Haemodialysis-induced myocardial stunning in chronic kidney disease - a new aspect of cardiovascular disease. Blood Purif, 29(2): 105–110.
5. Weiner DE, Scott TM, Giang LM, Agganis BT, Sorensen EP, et al (2011) Cardiovascular disease and cognitive function in maintenance hemodialysis patients. Am J Kidney Dis, 58(5): 773–781.
6. Matias PJ, Jorge C, Ferreira C, Borges M, Aires I, et al (2010) Cholecalciferol supplementation in hemodialysis patients: effects on mineral metabolism, inflammation, and cardiac dimension parameters. Clin J Am Soc Nephrol, 5(5): 905–911.
7. Shiotsu Y, Mori Y, Nishimura M, Sakoda C, Tokoro T, et al (2011) Plasma S100A12 level is associated with cardiovascular disease in hemodialysis patients. Clin J Am Soc Nephrol, 6(4): 718–723.
8. Anderson JL, May HT, Horne BD, Bair TL, Hall NL, et al (2010) Relation of vitamin D deficiency to cardiovascular risk factors, disease status, and incident events in a general healthcare population. Am J Cardiol, 106(7): 963–968.
9. Stenvinkel P, Carrero JJ, Axelsson J, Lindholm B, Heimburger O, et al (2008) Emerging biomarkers for evaluating cardiovascular risk in the chronic kidney disease patient: how do new pieces fit into the uremic puzzle? Clin J Am Soc Nephrol, 3(2): 505–521.
10. Kendrick J, Chonchol MB (2008) Nontraditional risk factors for cardiovascular disease in patients with chronic kidney disease. Nat Clin Pract Nephrol, 4(12): 672–681.
11. Chonchol M, Kendrick J, Targher G (2011) Extra-skeletal effects of vitamin D deficiency in chronic kidney disease. Ann Med, 43(4): 273–282.
12. Cozzolino M, Biondi ML, Galassi A, Cusi D, Brancaccio D, et al (2010) Vascular calcification and cardiovascular outcome in dialysis patients: the role of gene polymorphisms. Blood Purif, 29(4): 347–351.
13. Fung MM, Salem RM, Lipkowitz MS, Bhatnagar V, Pandey B, et al (2012) Methylenetetrahydrofolate reductase (MTHFR) polymorphism A1298C (Glu429Ala) predicts decline in renal function over time in the African-American Study of Kidney Disease and Hypertension (AASK) Trial and Veterans Affairs Hypertension Cohort (VAHC). Nephrol Dial Transplant, 27(1): 197–205.
14. Spellicy CJ, Northrup H, Fletcher JM, Cirino PT, Dennis M, et al (2012) Folate metabolism gene 5,10-methylenetetrahydrofolate reductase (MTHFR) is associated with ADHD in myelomeningocele patients. PLoS One, 7(12): e51330.
15. Garner JL, Niles KM, McGraw S, Yeh JR, Cushnie DW, et al (2013) Stability of DNA methylation patterns in mouse spermatogonia under conditions of MTHFR deficiency and methionine supplementation. Biol Reprod, 89(5): 125.
16. Field MS, Shields KS, Abarinov EV, Malysheva OV, Allen RH, et al (2013) Reduced MTHFD1 activity in male mice perturbs folate- and choline-

dependent one-carbon metabolism as well as transsulfuration. J Nutr, 143(1): 41–45.

17. Hazra A, Kraft P, Lazarus R, Chen C, Chanock SJ, et al (2009) Genome-wide significant predictors of metabolites in the one-carbon metabolism pathway. Hum Mol Genet, 18(23): 4677–4687.

18. Abbas S, Raza ST, Ahmed F, Ahmad A, Rizvi S, et al (2013) Association of Genetic polymorphism of PPARgamma-2, ACE, MTHFR, FABP-2 and FTO genes in risk prediction of type 2 diabetes mellitus. J Biomed Sci, 20(1): 80.

19. Zhang J, Qiu LX, Wang ZH, Wu XH, Liu XJ, et al (2010) MTHFR C677T polymorphism associated with breast cancer susceptibility: a meta-analysis involving 15,260 cases and 20,411 controls. Breast Cancer Res Treat, 123(2): 549–555.

20. Perna AF, Lanza D, Sepe I, Conzo G, Altucci L, et al (2013) Altered folate receptor 2 expression in uraemic patients on haemodialysis: implications for folate resistance. Nephrol Dial Transplant, 28(5): 1214–1224.

21. Lee SY, Kim HY, Park KM, Hong SG, Kim HJ, et al (2011) MTHFR C677T polymorphism as a risk factor for vascular calcification in chronic hemodialysis patients. J Korean Med Sci, 26(3): 461–465.

22. Heinz J, Kropf S, Luley C, Dierkes J (2009) Homocysteine as a risk factor for cardiovascular disease in patients treated by dialysis: a meta-analysis. Am J Kidney Dis, 54(3): 478–489.

23. Fukasawa M, Matsushita K, Kamiyama M, Mikami Y, Araki I, et al (2003) The methylenetetrahydrofolate reductase C677T point mutation is a risk factor for vascular access thrombosis in hemodialysis patients. Am J Kidney Dis, 41(3): 637–642.

24. Wrone EM, Zehnder JL, Hornberger JM, McCann LM, Coplon NS, et al (2001) An MTHFR variant, homocysteine, and cardiovascular comorbidity in renal disease. Kidney Int, 60(3): 1106–1113.

25. Stang A (2010) Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol, 25(9): 603–605.

26. Zintzaras E, Ioannidis JP (2005) HEGESMA: genome search meta-analysis and heterogeneity testing. Bioinformatics, 21(18): 3672–3673.

27. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L (2006) Comparison of two methods to detect publication bias in meta-analysis. JAMA, 295(6): 676–680.

28. Aucella F, Margaglione M, Grandone E, Vigilante M, Gatta G, et al (2005) The C677T methylenetetrahydrofolate reductase gene mutation does not influence cardiovascular risk in the dialysis population: results of a multicentre prospective study. Nephrol Dial Transplant, 20(2): 382–386.

29. Haviv YS, Shpichinetsky V, Goldschmidt N, Atta IA, Ben-Yehuda A, et al (2002) The common mutations C677T and A1298C in the human methylene-tetrahydrofolate reductase gene are associated with hyperhomocysteinemia and cardiovascular disease in hemodialysis patients. Nephron, 92(1): 120–126.

30. Ibrahim S, El Dessokiy O (2009) Prevalence of methylenetetrahydrofolate gene (MTHFR) C677T polymorphism among chronic hemodialysis patients and its association with cardiovascular disease: a cross-sectional analysis. Clin Exp Nephrol, 13(5): 501–507.

31. Kimura H, Gejyo F, Suzuki S, Miyazaki R (2000) The C677T methylene-trahydrofolate reductase gene mutation in hemodialysis patients. J Am Soc Nephrol, 11(5): 885–893.

32. Morimoto K, Haneda T, Okamoto K, Ishida H, Kikuchi K (2002) Methylenetetrahydrofolate reductase gene polymorphism, hyperhomocysteine-mia, and cardiovascular diseases in chronic hemodialysis patients [PMID: 11744804]. Nephron, 90(1): 43–50.

33. Yang Q, Bailey L, Clarke R, Flanders WD, Liu T, et al (2012) Prospective study of methylenetetrahydrofolate reductase (MTHFR) variant C677T and risk of all-cause and cardiovascular disease mortality among 6000 US adults. Am J Clin Nutr, 95(5): 1245–1253.

34. Clarke R, Bennett DA, Parish S, Verhoef P, Dotsch-Klerk M, et al (2012) Homocysteine and coronary heart disease: meta-analysis of MTHFR case-control studies, avoiding publication bias. PLoS Med, 9(2): e1001177.

35. Pereira TV, Rudnicki M, Pereira AC, Pombo-de-Oliveira MS, Franco RF (2006) 5,10-Methylenetetrahydrofolate reductase polymorphisms and acute lymphoblastic leukemia risk: a meta-analysis. Cancer Epidemiol Biomarkers Prev, 15(10): 1956–1963.

36. Yun KU, Ryu CS, Oh JM, Kim CH, Lee KS, et al (2013) Plasma homocysteine level and hepatic sulfur amino acid metabolism in mice fed a high-fat diet. Eur J Nutr, 52(1): 127–134.

37. Benight NM, Stoll B, Chacko S, da Silva VR, Marini JC, et al (2011) B-vitamin deficiency is protective against DSS-induced colitis in mice. Am J Physiol Gastrointest Liver Physiol, 301(2): G249–259.

38. Di Minno MN, Tremoli E, Coppola A, Lupoli R, Di Minno G (2010) Homocysteine and arterial thrombosis: Challenge and opportunity. Thromb Haemost, 103(5): 942–961.

39. Stanger O, Fowler B, Piertzik K, Huemer M, Haschke-Becher E, et al (2009) Homocysteine, folate and vitamin B12 in neuropsychiatric diseases: review and treatment recommendations. Expert Rev Neurother, 9(9): 1393–1412.

40. Stipanuk MH, Ueki I (2011) Dealing with methionine/homocysteine sulfur: cysteine metabolism to taurine and inorganic sulfur. J Inherit Metab Dis, 34(1): 17–32.

41. Safarinejad MR, Shafiei N, Safarinejad S (2011) Genetic susceptibility of methylenetetrahydrofolate reductase (MTHFR) gene C677T, A1298C, and G1793A polymorphisms with risk for bladder transitional cell carcinoma in men. Med Oncol, 28 Suppl 1: S398–412.

42. Shemin D, Bostom AG, Selhub J (2001) Treatment of hyperhomocysteinemia in end-stage renal disease. Am J Kidney Dis, 38(4 Suppl 1): S91–94.

43. Rubino E, Ferrero M, Rainero I, Binello E, Vaula G, et al (2009) Association of the C677T polymorphism in the MTHFR gene with migraine: a meta-analysis. Cephalalgia, 29(8): 818–825.