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Hyperhomocysteinemia and risk of incident cognitive outcomes: an updated dose-response meta-analysis of prospective cohort studies

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HIGHLIGHT:

- Every 5 μmol/L increase in blood homocysteine is linearly associated with a 15% increase in relative risk of Alzheimer-type dementia.

ABSTRACT

Objective: This study aimed to comprehensively assess the dose-response relationship between blood homocysteine levels and risk of all cause, Alzheimer and vascular dementia, as well as cognitive impairment without dementia (CIND).

Method: We searched for all related prospective cohort studies reporting homocysteine as an exposure from patients with cognitive disorders as a result in the PubMed and EMBASE databases up to June 18, 2018. Pooled relative risks (RRs) and corresponding 95% confidence intervals (CIs) were extracted. The dose-response meta-analyses were conducted to assess potential linear and non-linear dose-response relations. Summary RRs and 95% CIs were calculated using a random- or fixed-effects model.

Results: Twenty-eight prospective cohort studies were eligible in this meta-analysis. During average follow-up periods ranging from 2.7 to 35 years there were 2,557 cases (1,035 all-cause dementia, 530 Alzheimer's disease, 92 vascular dementia and > 900 CIND) among 28,257 participants. There was a clear linear dose-response relationship between blood homocysteine concentration and risk of Alzheimer-type dementia (P>0.05 for non-linearity). The pooled RR of Alzheimer-type dementia was 1.15 (95% CI: 1.04 to 1.26; I^2=56.6%, n=5) for every 5 μmol/L increase in blood homocysteine. Sensitivity analysis showed similar results, and there was no clear evidence of publication bias with Begg's and Egger's tests for Alzheimer dementia (P=0.806, 0.084, respectively), strengthening the linear relationship between blood homocysteine levels and risk of Alzheimer dementia. Due to the presence of publication bias and low statistical power, elevated levels of blood homocysteine were not appreciably associated with risk of all-cause, vascular dementia and CIND.

Conclusions: Every 5 μmol/L increase in blood homocysteine is linearly associated with a 15% increase in relative risk of Alzheimer-type dementia. This meta-analysis provides further evidence that a higher concentration of blood homocysteine is associated with a higher risk of Alzheimer-type dementia.

Keywords: homocysteine; Alzheimer's disease; dementia; cognitive impairment; dose-response.

1. Introduction

Age-related cognitive decline or impairment is a major public health problem, affecting about 20% of people aged 70 years and older in the United State (Plassman, et al., 2008). The prevalence of dementia, as a severe cognitive problem, increases with age, so that at the age of 80 years, about one in eight people are affected (Wald, et al., 2011). To search for effective prevention and treatment strategies, it is important to identify the causes of cognitive deterioration (Scarmeas, et al., 2018), and critical to develop an approach to treat or delay cognitive decline (Dolgin, 2016). Furthermore, novel preventive approaches focused on modifying risk factors (Zhou and Haina, 2017) for cognitive disorders are urgently needed to combat this growing epidemic. As a promising molecule for treating or preventing nervous diseases, high blood levels of homocysteine (Hcy) have been closely associated with several diseases that affect the central nervous system, such as epilepsy (Elliott, et al., 2007) and stroke (Lehotsky, et al., 2016).

It has been 20 years since two case-control studies (Clarke, et al., 1998; McCaddon, et al., 1998) found that elevated blood total Hcy levels were associated with Alzheimer's disease (AD). Observational data suggested a link between hyperhomocysteinemia (HHcy) and increased risk of cognitive disorders such as Alzheimer's dementia (Ravaglia, et al., 2005; Zylberstein, et al., 2011), vascular dementia (Miwa, et al., 2016) and cognitive impairment/decline (Haan, et al., 2007; Nurk, et al., 2005). Individual studies, however, have provided conflicting estimates (Ford, et al., 2012; Hooshmand, et al., 2010; Luchsinger, et al., 2004; Miwa, et al., 2016) of the strength of the association between Hcy and a range of cognitive disorders and have not agreed on whether there are relevant associations at all, possibly because of the small sample size examined. Furthermore, the knowledge and understanding of the clinical importance and implications of these associations are limited, and the broad range of literature needs to be reviewed comprehensively to characterize the associations of HHcy with different cognitive outcomes. In addition, previous meta-analyses of case-control and cohort studies on this topic found a significant positive relation between blood Hcy levels and risk of cognitive disorders (Van

Dam and Van Gool, 2009; Wald, et al., 2011). However, no dose-response analyses were conducted, thus questions about the strength and shape of the dose-response relationship between blood Hcy levels and risk of cognitive disorders remain to be addressed.

Given the inconsistency in the literature regarding the role of Hcy in risk of cognitive disorders, we conducted a meta-analysis to review current evidence on the associations of blood Hcy levels with incident risk of all-cause, Alzheimer-type, and vascular dementia, as well as cognitive impairment without dementia (CIND). The present meta-analysis was undertaken to provide an updated, more comprehensive and dose-response review about the relation between blood Hcy and risk of cognitive problems ranging from slight decline to dementia.

2. Methods

2.1. Search strategy

Following the guidelines by the MOOSE (Meta-analysis Of Observational Studies in Epidemiology) statement (Stroup, et al., 2000), we searched the electronic databases (PubMed and EMBASE) from inception to June 18, 2018 using the following terms: homocysteine, hyperhomocysteinemia; blood, plasma, serum, circulat*; dementia, Alzheimer*, cognit*; prospective, cohort, follow up, inciden*, longitudinal, "nested case" (Details of search strategies are shown in Table S1, in Appendix 1). A list of the excluded studies is provided in table S2 in appendix 1. No language restrictions were imposed. Bibliographies of eligible studies and relevant meta-analyses were hand-searched for potential missing studies (Figure 1).

2.2. Study selection

Studies were included if they were prospective cohort or prospective nested case-control studies, investigated an association between blood Hcy levels and cognitive disorders (All-cause dementia, or Alzheimer's disease, or vascular dementia, or cognitive impairment, or cognitive decline or cognitive deficit), classified blood Hcy concentrations into two or more categories, and reported adjusted risk estimates. For the dose–response analysis, the level-specific case numbers and person-years or sufficient data for deriving these numbers were required. The inclusion decisions were made independently by two reviewers (Zhou FT and Chen SR) and any disagreements were resolved by consensus after discussion.

2.3. Data extraction and quality evaluation

For each study included, we extracted the first author's last name, publication year, region (or

country), cohort name, gender distribution (% female), mean age or age range, mean follow-up duration, sample size, number of cases, and person-years stratified by blood Hcy dose, cognitive outcomes, diagnosis criteria of cognitive disorders, sample source, method of measuring blood Hcy concentration, categories of blood Hcy, adjusted covariates, and multivariable-adjusted effects (RR and 95% CI) for each exposure category. The study quality was evaluated with the Newcastle-Ottawa Quality Assessment Scale (NOS), the quality score ranged from 0 to 9. Details of how the criteria were applied are shown in Table S3, in Appendix 1.

2.4. Statistical methods

In this meta-analysis, all associations were estimated as RRs and 95% CIs; HRs were considered equivalent to the RR (Xu, et al., 2015). The ORs were transformed into RRs using the formula RR = OR/[(1-P_0) + (P_0 × OR)] where P_0 is the incidence of the outcome of interest in the non-exposed group. Some studies reported the odds ratio (OR) or hazard ratio (HR) in each category, and the OR (or HR) was considered equivalent to the RR in cohort studies if the value of P_0 was small (Zhang and Yu, 1998). For each of the included studies, we assigned the reported median or mean blood Hcy concentration of each category as the category of blood Hcy concentration. When a study reported only the range of blood Hcy levels for a category, we used the average value of the lower and upper bounds. When the highest category was open-ended, we assigned the lower end value of the category multiplied by 1.5. When the lowest category was open-ended, we set the lower boundary concentration to a fixed value of 3.0 because the lower limit of blood Hcy is normally around 3.0 μmol/L (Zylberstein, et al., 2011). The risk estimate from the most fully adjusted models in the analysis of the pooled RR was used. If the number of cases/non-cases in each category was not available and the authors did not give their reply, a method (Bekkering, et al., 2008) was used to provide approximate data based on the total number and RRs of each category. We excluded the studies without the number of participants and/or cases in the whole cohort, also not providing RRs (ORs) and 95% CI, or without sufficient data to calculate the values required for the dose-response analyses.

We first summarized the RRs for the highest versus lowest category of blood Hcy levels in the included studies using the random effects (I^2>50%, the DerSimonian-Laird method) or fixed effects (I^2 ⩽50%, the Mantel-Haenszel method) meta-analysis (high *v* low meta-analysis). For a dose-response meta-analysis, we used the 2-stage generalized least-squares trend estimation method to estimate the study-specific slope lines first and then derive an overall average slope using the method described

by Greenland and Longnecker (Greenland and Longnecker, 1992). We performed a dose–response meta-analysis to examine a potential nonlinear relationship between blood Hcy levels and risk of cognitive disorders by using restricted cubic splines. Restricted cubic spline models with 3 knots were fitted in each study taking into account the covariance among log RR, and the regression coefficients were then combined using multivariate meta-analysis. A test for a non-linear relation was calculated by making the coefficient of the second spline equal to zero, as described previously (Orsini, et al., 2012). When the number of studies reporting a specific outcome was small, we did not carry out a dose-response meta-analysis. To generate a linear dose-response curve, data on the blood Hcy levels, the distribution of cases and person-years, and RRs plus 95% CIs for 3 or more categories were extracted. First, specific linear trends and 95% CIs were estimated from the natural logs of RRs across categories of Hcy by the generalized least-square models method. Then, the estimated linear trends were pooled with fixed- or random-effects meta-analysis, depending on the absence or presence of statistical heterogeneity. If the nonlinearity was not statistically significant, the linear dose-response outcomes were presented in Hcy levels per 3- or 5-unit (μmol/L) increase in the forest plots.

In addition to evaluating the entire cohort, we also performed stratified analyses according to study location, gender distribution (% female), diagnosis criteria, sample source (plasma or serum), method used to quantify blood Hcy levels, mean follow-up duration, study quality score, and adjustment for confounders (age, sex, BMI, APOE-ε4 status, education levels, B vitamins and history of cardiovascular diseases). Study heterogeneity was assessed using the Q-test and I^2 statistic, $p < 0.10$ and I^2>50% indicated evidence of heterogeneity. If the I^2 statistic was 50% or less, a meta-analysis based on a fixed-effect model was conducted, otherwise the random-effects model was used. Sensitivity analyses excluding one study at a time were conducted to explore whether the results were strongly influenced by a specific study. Potential publication bias was assessed by the application of contour-enhanced funnel plots (Peters, et al., 2008), Egger’s linear regression test, and Begg’s rank correlation test. If publication bias was present, we further evaluated the number of missing studies using the trim-fill method and re-calculated the pooled risk estimates with the addition of those missing studies. All statistical analyses were conducted with two-tailed test at the $P < 0.05$ level for statistical significance using STATA v14.0 (Stata Corp, College Station, TX, USA).

3. RESULTS

3.1. Literature search

We identified 410 articles for review of title and abstract. After the initial screening, full text of potentially eligible articles was retrieved for detailed assessment. After full text reviews, 45 articles were excluded (see Fig 1 and Table S2 in appendix 1), and 29 eligible cohort studies from 17 eligible articles were included for meta-analysis, with a total of 28,257 participants and 2,557 patients with cognitive disorders (1,035 cases of all-cause dementia, 530 cases of AD, 92 cases of vascular dementia, and 900 above cases of CIND). A study (Haan, et al., 2007) did not independently calculate the values of the relations of dementia and CIND. We chose not to include (neither in the dementia group nor in the CIND group) it and thus made exclusion decision. All the studies included have been published as full manuscripts and are of high quality (see Table S3, appendix 1). Figure 1 shows a flowchart of the study selection. In addition, the characteristics of the included studies summarize in Table 1, and summary statistics on the exposure and outcome variables are provided in Table 2.

3.2. Study Characteristics

As shown in Tables 1 and 2, there are 10 studies for all-cause dementia, 8 for Alzheimer-type dementia, and 3 for vascular dementia, 8 for CIND. Average follow-up periods ranged from 2.7 to 35 years. Of all the studies included, 8 studies were conducted from the United States, 17 from Europe, 3 from Asia, and 1 from Australia. Most of studies adjusted for age, sex and education levels. Some cohorts also controlled for some conventional risk factors, including body mass index, APOE status, vitamin B status, and cardiovascular disease. One analysis from an article was based on two different nationalities (Yoruba and African Americans), which was considered as two independent studies (Hendrie, et al., 2013).

3.3. Blood Hcy levels and risk of all-cause dementia

Ten cohort studies in nine articles (Ford, et al., 2012; Hendrie, et al., 2013; Kivipelto, et al., 2009; Miwa, et al., 2016; Ravaglia, et al., 2007; Ravaglia, et al., 2005; Seshadri, et al., 2002; Whalley, et al., 2014; Zylberstein, et al., 2011) investigated the association between blood Hcy levels and risk of all-cause dementia, with a total of 11,168 participants and 1,035 incident patients (Tables 1 and 2). The pooled RR for the highest versus lowest Hcy levels was 1.58 (95% CI: 1.33 to 1.87, I^2=17.4%, $P_{heterogeneity}$=0.283) (Fig 2A). Begg's and Egger's tests indicated publication bias (P=0.007, 0.011, respectively; see Table 3). We used the trim and fill method to recalculate the pooled risk estimate, and

the analysis suggested that the imputed risk estimate was 1.471 (95% CI: 1.264 to 1.712), which is slightly decreased in risk but still identical to our original risk estimate.

For dose-response meta-analysis, we excluded two studies (Ravaglia, et al., 2007; Seshadri, et al., 2002) that divided Hcy concentration into only two categories, because this requires for at least three quantitative exposure categories. Therefore, this analysis included eight studies with a total of 9,272 participants and 815 all-cause dementia cases. Using a restricted cubic splines model, we found no evidence of a curvilinear relationship between blood Hcy levels and risk of all-cause dementia (P=0.443 for non-linearity; Fig S6 in appendix 2). For linear dose-response analysis, the summary RR per 3 μmol/L (unit) increase in blood Hcy level was 1.07 (95% CI: 1.03 to 1.12; Fig S2 in appendix 2), with moderate between-study heterogeneity (P=0.034, I^2=53.7%). The summary RR per 5 unit increase was elevated to a height of 1.12 (95% CI: 1.05 to 1.20) with similar heterogeneity (P=0.03, I^2=54.8%; Fig S5 in appendix 2). Begg's and Egger's tests indicated publication bias (P=0.035, 0.012, respectively; see Table 3). The trim and fill method was used to re-calculate our pooled risk estimate. The analysis suggested that the imputed risk estimate was 1.064 (95% CI: 0.993 to 1.142) per 5-unit increase, 1.038 (95% CI: 0.996 to 1.083) per 3-unit increase, with no significance for the adjusted risk estimates.

3.4. Blood Hcy levels and risk of Alzheimer-type dementia

The association between blood Hcy levels and risk of Alzheimer-type dementia was investigated in eight studies (Hooshmand, et al., 2010; Kivipelto, et al., 2009; Luchsinger, et al., 2004; Miwa, et al., 2016; Ravaglia, et al., 2007; Ravaglia, et al., 2005; Seshadri, et al., 2002; Zylberstein, et al., 2011) with a total of 5777 participants and 530 patients with Alzheimer's disease (Tables 1 and 2). As shown in Table 3, the pooled RR of Alzheimer-type dementia for the highest versus lowest category of blood Hcy was 1.74 (95% CI 1.32 to 2.29), with significant heterogeneity (I^2=58.5%, P=0.018) (Fig 2B). Begg's test indicated no publication bias (P=0.063), but Egger's test indicated publication bias (P=0.004; Table 3). We used the trim and fill method to re-calculate the pooled risk estimate. The analysis suggested that the imputed risk estimate was 1.372 (95% CI: 1.033 to 1.822), which is less than our original risk estimate, but its significance remains clear.

For dose-response meta-analysis, three studies (Hooshmand, et al., 2010; Ravaglia, et al., 2007; Seshadri, et al., 2002) were excluded due to only two categories in Hcy levels. Therefore, in this analysis five studies were included with a total of 3,610 participants and 362 Alzheimer-type dementia cases. Similarly, we observed that there was no significant non-linear relationship between blood Hcy

levels and risk of Alzheimer-type dementia (P=0.586 for non-linearity; Fig 3B). For linear dose–response analysis, the summary RR per 3-unit increase in Hcy was 1.09 (95% CI: 1.02-1.15; Fig S3 in appendix 2) with moderate between-study heterogeneity (P=0.056, I^2=56.6%). The summary RR per 5 μmol/L increase, pooled RR was elevated to 1.15 (95% CI: 1.04 to 1.26) with similar heterogeneity (P=0.058, I^2=56.6%; Fig 3A). Begg's and Egger's tests indicated no publication bias (P=0.806, 0.084, respectively; Table 3). Figure 3 shows the results of non-linear dose-response meta-analysis, and every 3 or 5 μmol/L increases in blood Hcy was estimated to be associated with a 9% or 15% higher risk of Alzheimer-type dementia, respectively (Table S4 in appendix 1).

3.5. Blood Hcy levels and risk of vascular dementia

For the association between blood Hcy levels and incident risk of vascular dementia were investigated in three studies (Miwa, et al., 2016; Ravaglia, et al., 2007; Zylberstein, et al., 2011) with a total of 2706 participants and 92 patients with vascular dementia (Tables 1, 2 and 3). As shown in Table 3, the pooled RR of vascular dementia for the highest versus lowest category of blood Hcy levels was 1.78 (95% CI 0.58 to 5.42; Fig S1 in appendix 2), with significant heterogeneity (I^2=70.6%, P=0.033). Although Begg's and Egger's tests indicated no publication bias (P=1, 0.377, respectively), the statistical power was too low to draw definitive conclusions regarding the association. For dose-response meta-analysis, one study (Ravaglia, et al., 2007) was excluded due to only two categories in Hcy levels. Therefore, this analysis included two studies with a total of 1,902 participants and 58 vascular dementia cases. Because the number of the studies of the association between Hcy levels and risk of CIND was small, then we did not perform a nonlinear dose-response analysis (there was no significance for linear dose-response analysis; Table 3).

3.6. Blood Hcy levels and risk of cognitive impairment without dementia (CIND)

For the association between blood Hcy levels and incident risk of CIND, there were eight studies in six articles (Dufouil, et al., 2003; Kado, et al., 2005; Kalmijn, et al., 1999; Mendonca, et al., 2017; Nurk, et al., 2005; Reitz, et al., 2009) included, with a total of 8,606 participants and 900 and more patients with CIND (see Tables 1, 2 and 3, a case number not reported in one study [31] included). As shown in Table 3, the pooled RR for the highest versus lowest category was 1.34 (95% CI 1.02 to 1.74), with small heterogeneity (I^2=53.7%, P=0.033 for heterogeneity; Fig 3). There was no evidence of publication bias with Begg's and Egger's tests for CIND (P=0.174, 0.097, respectively). For dose-response meta-analysis, one study (Kado, et al., 2005) not reporting the number of cases could not

be included in the dose-response analysis. Therefore, this analysis included seven studies with a total of 8,226 participants and 900 cases. Using a restricted cubic splines model, it was shown that there was no significant non-linear relationship between blood Hcy levels and risk of CIND (P=0.0974 for non-linearity; Fig S8, in appendix 2). For linear dose–response analysis, the summary RR for a 3-unit increase in Hcy was 1.04 (95% CI: 1.00 to 1.08; Fig S4 in appendix 2), with moderate between-study heterogeneity (I^2=53.7%, P=0.033). For a 5-unit increase, the pooled RR was 1.06 (95% CI: 0.99 to 1.13; I^2=62%, P=0.015; Fig S7, in appendix 2). Begg's and Egger's tests indicated publication bias (both of P values: 0.034). We used the trim-fill method to re-calculate the pooled risk estimate. The analysis suggested, however, that the imputed risk estimate was 1.007 (95% CI: 0.960 to 1. 056) for every 3-unit increase, 1.012 (95% CI: 0.935 to 1.095) for every 5-unit increase, with no significance for the adjusted risk estimates (see Table 3).

3.7. Study quality, subgroup analyses, and sensitivity analyses

Assessment of study quality yielded an average score of 7 (9 representing the highest quality), and 13 studies had a score of ≧7 (Table S3 in appendix 1). Mean (median) study quality scores were 7.4 for all-cause dementia, 7.1 for Alzheimer-type dementia, 7.3 for vascular dementia, 6.6 for CIND.

To evaluate the robustness of the risk estimates, several stratified analyses were done based on study location, gender distribution (% female), mean follow-up duration, diagnosis criteria, quantification method of Hcy, study quality and adjustment for critical confounders. Table 4 shows the different subgroup analyses. The positive associations between blood Hcy levels and risk of all-cause and Alzheimer-type dementia persisted in most of subgroup analyses. For all-cause dementia, most of the subgroups (mean follow-up duration, sample source, quantification method, NOS score) follow the overall trend and show statistically significant increases. Subgroup analyses showed no significant associations for studies reporting participants from beyond Europe, following the diagnosis criteria of ICD-10, and with small number of female participants (50% below) (Table 3), as well as with no adjustment for sex and education levels. We noted that in other subsets associations were detected. For Alzheimer-type dementia, there were significant associations in most of the subgroup analyses, with exception of the serum subgroup, the subgroup with a low NOS score, and the subgroup including the studies (Table 2). For CIND, most of the subgroups demonstrated no significant relationship. Sensitivity analyses demonstrated that the estimates were not substantially altered for all-cause dementia, Alzheimer-type dementia and CIND (Figs S16-S24 in appendix 2).

4. DISCUSSION

To the best of our knowledge, the present meta-analysis is the largest and most comprehensive evaluation of the dose-response relationships between Hcy levels and risks of incident cognitive disorders in the general population. There were positive associations between Hcy levels and risks of these disorders, including all-cause and Alzheimer-type dementia, and cognitive impairment without dementia, suggesting increases of 58%, 74%, and 34%, respectively. Nevertheless, there was a non-significant association for vascular dementia. Particularly importantly, there was a linear, dose-dependent relationship between Hcy levels (per 5 or 3 μmol/L increase) and risk of incident Alzheimer-type dementia (a 15% or 9% increase in risk, respectively). The findings from the current meta-analysis of prospective cohort studies support the notion that an increased level of blood Hcy appears to play a causal role in the development of AD, but neither in the other dementia nor in cognitive impairment.

4.1. Exploration of heterogeneity and publication bias

In the current meta-analysis of Alzheimer-type dementia, there was no between-study heterogeneity in the subgroups of HPLC and IMx assay, indicating that quantification method of Hcy contributed to most of the observed heterogeneity. In addition, this heterogeneity might partly be explained by the fact whether the included studies adjusted for B vitamins. Sensitivity analyses showed that exclusion of any single study did not substantially alter the primary overall RRs, which further confirmed in the direction and magnitude of the findings in the present study. There were no missing studies imputed in regions of the contour enhanced funnel plots. In the linear dose-response analysis for Alzheimer-type dementia, Egger's and Begg's tests suggested no evidence of publication bias ($p>0.05$).

4.2. Comparisons with other studies

The first meta-analysis on this topic was published in 2009 (Van Dam and Van Gool, 2009), and included only 3 cohort studies. Two years later, Wald et al. (Wald, et al., 2011) reported the relationship between serum Hcy and dementia risk. Although this meta-analysis conducted by Wald has included 8 cohort studies with 8669 participants, it should be noticed that it was obviously different with the current study. First, the authors included a study (Haan, et al., 2007) in which the cases were mixed and not the dementia-only patients (excluded by us, as above described). Thus, this probably affected, to a

certain degree, the precision of the pooled results. Secondly, the authors did not distinguish Alzheimer disease from other dementia, and included the data containing the number of all-cause dementia cases. Our meta-analysis of cohort studies for AD aimed to explore the relationship of Alzheimer with Hcy, and included simply AD cases meeting the either NINCDS or DSM criteria. In addition, sensitivity analysis and publication bias test were not performed in this meta-analysis, which potentially decreased the stability of the pooled results (Wald, et al., 2011). A report (Beydoun, et al., 2014) included 5 studies in AD patients and performed a pooled analysis only in high versus low categories of blood Hcy levels. Another meta-analysis study (Nie, et al., 2014) reported the association of HHcy with risk of cognitive decline. In fact, however, the cases from the study contained both dementia and cognitive decline. In other words, the authors defined abnormal cognitive function as cognitive decline, and included dementia and cognitive decline no dementia.

More comprehensively, our current meta-analysis examined the relationships between blood Hcy levels and risks of cognitive disorders including all-cause dementia, Alzheimer-type dementia, CIND, as well as vascular dementia, providing greater statistical power and more precise estimates because of pooling of multiple studies. It was also worth noting that the current dose-response analysis was performed in a broad range of, simple but not composite, cognitive outcomes, from cognitive decline no dementia to dementia. Importantly, there was a clear linear, but no curvilinear dose-response relationship between blood Hcy levels and risk of incident Alzheimer-type dementia. Our results support the notion that HHcy is a linear risk factor for AD.

4.3. Implications

Our finding has important implications for prevention and treatment of AD. It was reported that the overall pooled prevalence of HHcy in China was 27.5%, particularly in northern populations, the inlanders, males and the elderly (Yang, et al., 2014). The results of the current study imply that the increased incidence of AD might be attributed to the rapid elevation in blood Hcy. Clinical trial studies found that lowering Hcy levels with folic acid and B-vitamins could interfere with cognitive decline and AD (Cacciapuoti, 2013; Chen, et al., 2016; Rommer, et al., 2016). A report showed that B-vitamin supplementation could slow the atrophy of specific brain regions associated with AD process (Douaud, et al., 2013). Overall, high levels of Hcy can be reduced or even reversed through changes in nutrition, and efforts toward early detection of HHcy in conjunction with implementation of lifestyle changes to improve Hcy metabolism may represent a viable strategy to reduce the risk of incident Alzheimer-type

dementia, which is strongly supported by our results.

4.4. Strengths and limitations

Several strengths of the current study include the comprehensive analyses of blood Hcy in relation to a range of mild-to-severe cognitive impairment risks; linear and non-linear dose-response analyses; the detailed subgroups, sensitivity and influence analyses; a large number of cases and participants; a complete quality assessment, and large populations. This meta-analysis was based on some prospective cohort studies from various populations. The sample size was large and the follow-up period was long enough. Compared with previous meta-analyses on this topic, the current dose-response meta-analysis covered broader classifications of cognitive outcomes. The estimates from the fully adjusted models for each study were used in our analyses to reduce the potential of confounding. This can help to quantify the associations and test the shape of these possible associations.

Despite these strengths, our study also has some limitations. Firstly, as a meta-analysis of observational studies, there was the possibility of remaining residual confounding due either to known but unmeasured or imperfectly measured risk factors, or to factors that are not yet known to influence cognitive function risk. Secondly, most of the studies included have found that raised Hcy remained associated with cognitive impairment even after adjusting for B vitamins (Kivipelto, et al., 2009; Ravaglia, et al., 2005; Smith and Refsum, 2016; Whalley, et al., 2014; Zylberstein, et al., 2011), suggesting being independent of B vitamins for the association of cognitive impairment risk with blood Hcy levels. During follow-up, however, participants in the included studies might take in B vitamins, by means of either direct use or alterations induced by diet, resulting in changes in blood Hcy levels. In this sense, it might not be well-controlled for the potential confounder (B vitamins). Thirdly, differential adjustment for confounders across different studies could potentially influence our study findings. However, this was not observed in pooled analyses using HR associated with models with versus without adjustment for risk factors. Fourthly, our meta-analysis was conducted with summary statistics, rather than individual data which allow more precise delineation of the dose-response relation and further control of potential residual confounding. Lastly, as a meta-analysis of published literature, publication bias may have affected our findings. There was no evidence of publication bias in the analyses for Alzheimer-type dementia, but there was some indication of missing negative studies.

4.5. Conclusions

Our dose-response meta-analysis shows that every 5 μmol/L increase in blood Hcy is linearly

associated with a 15% increased risk for Alzheimer-type dementia. Hyperhomocysteinemia is also a risk factor for cognitive disorders, including not only dementia (non-Alzheimer type) but also cognitive impairment/decline. More prospective cohort studies, with large numbers of participants, especially those from developing countries, are needed to provide a more precise assessment of the effects of blood Hcy on non-Alzheimer type dementia and cognitive impairment.

Conflict of interest

The authors declare no conflict of interest.

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Table and Figure Legend

Figure 1. Screening and selection process of studies investigating effects of blood Hcy concentration on risk of cognitive diseases.

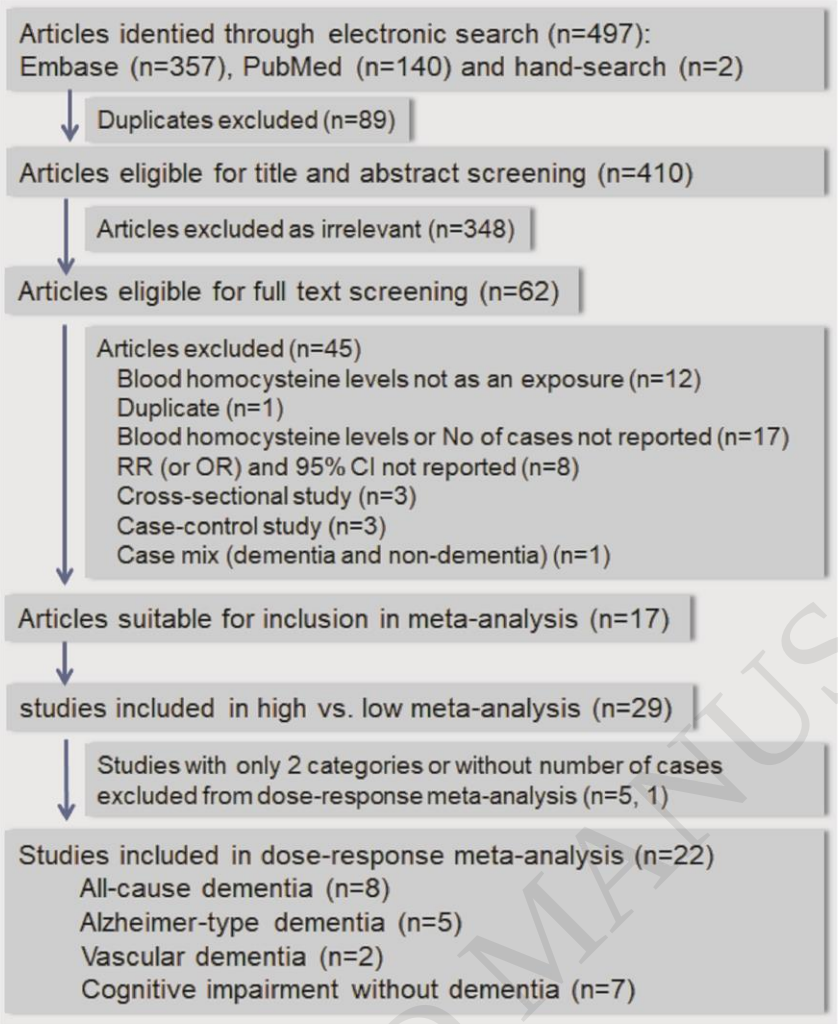


Figure 2. Summary relative risk of all-cause (A, weights from fixed effects analysis) and Alzheimer-type dementia (B, weights from random effects analysis), as well as CIND (C, weights from fixed effects analysis), highest vs lowest blood Hcy category. *indicates mean value.

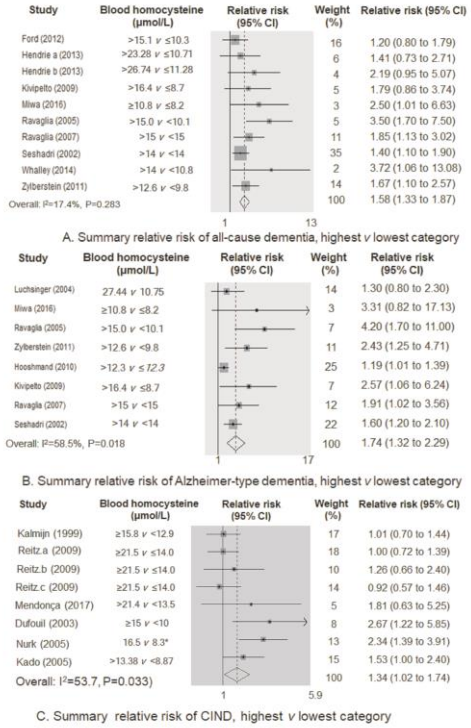
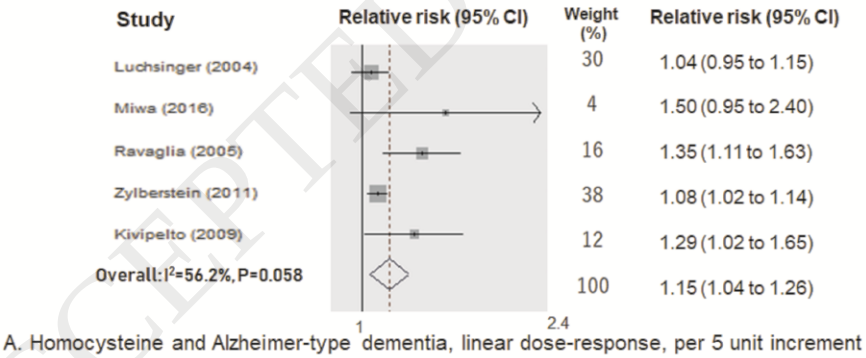
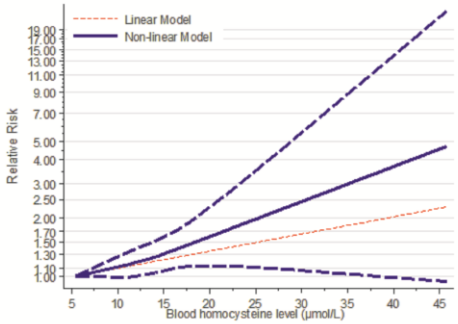


Figure 3. Blood Hcy and risk of Alzheimer-type dementia, linear and nonlinear dose-response analysis. A, linear dose-response analysis (per 5 μmol/L increase); B, non-linear dose-response analysis. The solid line represents the best fitting cubic spline model. The area between two dash lines represents the 95% CI.



A. Homocysteine and Alzheimer-type dementia, linear dose-response, per 5 unit increment



B. Homocysteine and Alzheimer-type dementia, non-linear dose-response

Table 1. The characteristics of the identified prospective studies of blood Hcy levels and risk of cognitive disorders.

Table 1. Characteristics of the identified prospective studies of blood Hcy levels and risk of cognitive disorders										
Author; year; country	**Cohort name**	**baseline age (mean±SD or range)**	**Female (%)**	**No of participants**	**Mean follow-up time**	**Diagnosis criteria**	**Adjusted confounders**	**Sample source**	**Quantification method of Hcy**	**Disease type**
Ford, 2012; Australia	Health in Men Study cohort	67.4±4.7 (62.7-72.1)	0	2959	5.8	ICD-10	Age, history of ischaemic heart disease and of stroke	Plasma	HPLC	All-cause dementia
Hendrie, 2013[a];USA	Indianapolis-Ibadan dementia project (African Americans)	76.75±5.08	70.4	912	4.7	DSM-III-R/ICD-10	Age, education, APOE-Ɛ4, smoking, time of enrollment	Plasma	NR	
Hendrie, 2013[b]; USA	Indianapolis-Ibadan dementia project (Yoruba)	75.88±4.87	64.1	819	5.1	DSM-III-R/ICD-10	Age, education, APOE-Ɛ4, smoking, time of enrollment	Plasma	NR	
Kivipelto, 2009; Sweden	Kungsholmen Project	81±4.6	74.6	213	6.7	DSM-III-R	Age, sex, education, BMI, albumin, Hb, creatinine, APOE-Ɛ4, MMSE score, holo-TC, B12 and folate	Plasma	IMx assay	
Miwa, K, 2016; Japan	Japanese cohort of participants with	67.2±8.4	41	643	7.3	DSM-III-R	Age, sex, education, APOE-Ɛ4, BMI, MMSE, hypertension,	Plasma	HPLC	

	vascular risk factors						previous cerebrovascular events, eGFR and MRI-findings			
Ravaglia, 2005; Italian	CSBA	73.6±6.3	53.2	816	3.8	DSM-IV	Age, sex, education, APOE-Ɛ4, serum creatinine, serum folate, serum Vit B12, history of stroke	Plasma	IMx assay	
Ravaglia, 2007; Italian	CSBA	73.6±6.3	53.1	804	3.7	DSM-IV	Age, gender, education, APOE-Ɛ4, history of cardiovascular disease, history of stroke, physical activity, BMI, serum creatinine, serum folate, serum Vitamin B12	Plasma	IMx assay	
Seshadri, 2002; UK	Framingham Study cohort	76	61.1	1092	8	NINCDS	Age, sex, APOE-Ɛ4, educational status, history of stroke, smoking status, alcohol intake, diabetes mellitus, BMI, SDP	Plasma	HPLC	
Whalley, 2014; UK	Aberdeen 1921 Birth Cohort study	78 (77-79)	44.3	173	5	ICD-10	Sex, IQ, education, deprivation, plasma vita B12, folate, heart diseases, hypertension and plasma micronutrients	Plasma	HPLC	
Zylberstein, 2011; Sweden	Prospective Population Study of Women in Gothenburg	46.8	100	1368	35	DSM-III-R	Age, education, BMI, cholesterol, triglycerides, SBP/DBP, smoking, creatinine, vit B12	Serum	IMx assay	

Luchsinger, 2004; USA	Washington Heights-Inwood Columbia Aging Project	76.2±5.7	70.7	679	4.72	NINCDS	Age, sex, education, APOE-Ɛ4, stroke	Plasma	HPLC	Alzheimer-type dementia
Miwa, 2016; Japan	Japanese cohort of participants with vascular risk factors	67.2±8.4	41	643	7.3	DSM-IV	Age, sex, education, APOE-Ɛ4, BMI, MMSE	Plasma	HPLC	
Ravaglia, 2005; Italian	CSBA	73.6±6.3	53.2	816	3.8	NINCDS-ADRDA	Age, sex, education, APOE-Ɛ4, serum creatinine, serum folate, serum vitamin B-12, history of stroke	Plasma	IMx assay	
Zylberstein, 2011; Sweden	Prospective Population Study of Women in Gothenburg	46.8	100	1368	35	DSM-III-R	Age, education, BMI, cholesterol, triglycerides, SBP/DBP, smoking, creatinine and vita B12, without cerebrovascular disease	Serum	IMX assay	
Hooshmand, 2010; Sweden/Finland	CAIDE study	70.7±3.6	62	271	7	NINCDSADRDA	Age, sex, education, and duration of follow-up, APOE-Ɛ4, BMI, MMSE, SBP/DBP, smoking, history of stroke.	Serum	Chemi-luminescent microparticle immunoassay	
Kivipelto, 2009; Sweden	Kungsholmen Project	81±4.6	74.6	213	6.7	DSM-III-R	Age, sex, education, BMI, albumin, haemoglobin, creatinine, APOE-Ɛ4, MMSE, holo-TC, B12, folate	Plasma	IMx assay	

Ravaglia, 2007; Italian	CSBA	73.6±6.3	53.1	804	3.7	NINCDSADRDA	Age, sex, education, APOE-Ɛ4, history of cardiovascular disease, history of stroke, physical activity, BMI, serum creatinine, serum folate, serum Vita B12	Plasma	IMx assay	
Seshadri, 2002; USA	Framingham Study cohort	76	61.1	1092	8	NINCDS	Age, sex, APOE-Ɛ4, education, history of stroke, smoking status, alcohol intake, diabetes mellitus, BMI, SDP	Plasma	HPLC	
Miwa, 2016; Japan	Japanese cohort of participants with vascular risk factors	67.2±8.4	41	643	7.3	DSM-IV	Age, sex, education level, APOE-Ɛ4, BMI, MMSE	Plasma	HPLC	Vascular dementia
Zylberstein, 2011; Sweden	The Prospective Population Study of Women in Gothenburg	46.8	100	1368	35	DSM-III-R	Age, education, BMI, cholesterol, triglycerides, SBP/DBP, smoking, creatinine, Vit B12	Serum	IMx assay	
Ravaglia, 2007; Italian	CSBA	73.6±6.3	53.1	804	3.7	NINCDS-AIREN	Age, sex, education, APOE-Ɛ4, history of cardiovascular disease, history of stroke, physical activity, BMI, serum creatinine, serum folate, serum Vit B12	Plasma	IMx assay	
Kalmijn, 1999; Netherlands	Prospective Rotterdam Study	67.7±7.1	60	819	2.7	MMSE; the MMSE score of >1 point /year	Age, sex, education, and baseline MMSE	Serum	HPLC	Cognitive impairment /decline

Reitz, 2009; USA	all-cause MCI	77.4±5.8	70.4	516	5.2	DSM-IV	Age, gender, ethnic group, APOE-Ɛ4	Plasma	HPLC	
Reitz, 2009; USA	amnestic MCI	77.4±5.8	70.4	432	5.2	DSM-IV	Age, gender, ethnic group, APOE-Ɛ4	Plasma	HPLC	
Reitz, 2009; USA	non-amnestic MCI	77.4±5.8	70.4	467	5.2	DSM-IV	Age, gender, ethnic group, APOE-Ɛ4	Plasma	HPLC	
Mendonça, 2017; UK	Newcastle 85+ Study	>85	61	763	5	sMMSE	Alcohol intake, smoking status, APOE-Ɛ4 (rs429358/rs7412), sex, education, BMI, depression, hypertension, diabetes type 1/2, history of cardiovascular diseases, physical activity.	Plasma	IMx assay	
Nurk, 2005; Norway	Hordaland Homocysteine Study	66	0	2189	6	ICD-9	Sex, APOE-Ɛ4, education, history of cardiovascular disease and hypertension, depression score	Plasma	HPLC	
Kado, 2005; USA	MacArthur Studies of Successful Aging	74±2.7	58	370	7	Five standardized cognitive performance tests	Age, sex, and baseline cognitive function	Plasma	HPLC	
Dufouil, 2003; France	Epidemiology of Vascular Ageing (EVA) study	67±3	58.6	1107	2	MMSE	Age, gender, education level, baseline cognition, BMI, alcohol consumption, smoking status, hypertension, hypercholesterolemia,	Plasma	HPLC	

							glycemic status, history of vascular disease, folate and vitamin B12 concentrations			

NR=not reported; HPLC=high-performance liquid chromatography assay; ICD-9/10=the International International Classification of Diseases, Ninth/Tenth Revision; DSM-III=the Diagnostic and Statistical Manual of Mental Disorders 3rd edition; NINCDSADRDA=National Institute of Neurologic and Cognitive Disorders and Stroke-AD and Related Disorders Association criteria; NINCDS-AIREN=National Institute of Neurological and Communicative Disease and Stroke-Alzheimer's Disease and Related Disorders Association; BMI=body mass index; APOE -Ɛ4=apolipoprotein E-Ɛ4; MMSE=Mini-mental State Examination; MCI=mild cognitive impairment; CSBA=Conselice Study of Brain Aging; SBP=systolic blood pressure; DBP=diastolic blood pressure.

Table 2. Relative risk for cognitive disorders in studies included in systematic review and dose-response meta-analysis on blood Hcy concentration (μmol/L) and risk of cognitive disorders.

Table 2. Risk relative for cognitive disorders in studies included in systematic review and dose-response meta-analysis on blood homocysteine concentration (μmol/L) and risk of cognitive disorders.

Author; year	Sample size	Case No	Person-years	Category	Hcy exposure	Mean	Multivariable-adjusted RR (95%CI)	Disease type
Ford, 2012	1033	43	5991.4	4	≤10.3	6.87	1 (ref)	All-cause dementia
	995	48	5771		NR	12.6	0.88 (0.57-1.36)	
	988	65	5730.4		NR	13.9	1.06 (0.70-1.61)	
	981	74	5689.8		>15.1	22.65	1.2 (0.8-1.79)	
Hendrie, 2013[a]	283	19	1330.1	4	4.44-10.71	7.58	1 (ref)	
	284	22	1334.8		10.72-16.99	13.86	1.16 (0.58-2.28)	
	284	32	1334.8		17.0-23.27	20.14	1.78 (0.94-3.38)	
	284	28	1334.8		23.28-29.52	26.4	1.41 (0.73-2.71)	

Hendrie, 2013b	259	10	1320.9	4	3.56-11.28	7.42	1 (ref)	
	259	13	1320.9		11.29-19	15.15	1.27 (0.52-3.09)	
	259	14	1320.9		19.01-26.73	22.87	1.39 (0.57-3.38)	
	259	22	1320.9		26.74-34.46	30.6	2.19 (0.95-5.07)	
Kivipelto, 2009	53	19	355.1	4	5-8.7	6.85	1 (ref)	
	53	14	355.1		8.8-12.5	10.65	0.83 (0.39-1.77)	
	53	15	355.1		12.6-16.3	14.45	1.12 (0.51-2.42)	
	54	35	361.8		16.4-20	18.2	1.79 (0.86-3.74)	
Miwa, 2016	214	11	1562.2	3	≤8.2	5.47	1 (ref)	
	214	9	1562.2		8.3-10.7	9.5	0.78 (0.27-1.94)	
	215	27	1569.5		≥10.8	16.2	2.5 (1.01-6.63)	
Ravaglia, 2005	211	13	801.8	4	<10.1	6.73	1 (ref)	
	204	23	775.2		10.1-12.5	11.3	1.7 (0.6-3.5)	
	184	21	699.2		12.6-15.0	13.8	2.1 (0.9-4.1)	
	217	55	824.6		>15.0	22.5	3.5 (1.7-7.5)	
Ravaglia, 2007	590	56		2	<15		1 (ref)	
	214	53			>15		1.85 (1.13-3.02)	
Seshadri, 2002	321	55		2	<14		1 (ref)	
	771	56			>14		1.4 (1.1-1.9)	
Whalley, 2014	46	4	230	3	<10.8	7.2	1 (ref)	
	49	11	245		10.8-14	12.4	2.63 (0.65–10.59)	
	78	17	390		>14	21	3.72 (1.06–13.08)	
Zylberstein, 2011	254	39	8890	3	3.1-9.8	6.45	1 (ref)	
	564	49	19740		9.8-12.6	11.2	1.3 (0.84-2.0)	
	441	63	15435		12.6-78.9	45.75	1.67 (1.10-2.57)	

Luchsinger, 2004	177	26	835.44	4		10.75	1 (ref)	Alzheimer-type dementia
	184	29	868.48			14.09	1.10 (0.7-2.0)	
	164	24	774.08			17.52	1 (0.6-1.8)	
	154	30	726.88			27.44	1.3 (0.8-2.3)	
Miwa, 2016	214	1	1562.2	3	≤8.2	5.47	1 (ref)	
	214	1	1562.2		8.3-10.7	9.5	0.71 (0.09-4.49)	
	215	22	1569.5		≥10.8	16.2	3.31 (0.82-17.13)	
Ravaglia, 2005	211	8	801.8	4	<10.1	6.73	1 (ref)	
	204	17	775.2		10.1-12.5	11.3	2.5 (0.93-6.0)	
	184	14	699.2		12.6-15.0	13.8	2.5 (0.93-6.1)	
	217	31	824.6		>15.0	22.5	4.2 (1.7-11.0)	
Zylberstein, 2011	254	12	8890	3	3.1-9.8	6.45	1 (ref)	
	564	40	19740		9.8-12.6	11.2	1.54 (0.78-3.05)	
	441	46	15435		12.6-78.9	45.75	2.43 (1.25-4.71)	
Hooshmand, 2010	271	17		2	≤12.3		1 (ref)	
					>12.3		1.19 (1.01-1.39)	
Kivipelto, 2009	53	13	355.1	4	5-8.7	6.85	1 (ref)	
	53	8	355.1		8.8-12.5	10.65	0.71 (0.26-1.91)	
	53	12	355.1		12.6-16.3	14.45	1.45 (0.57-3.69)	
	54	28	361.8		16.4-20	18.2	2.57 (1.06-6.24)	
Ravaglia, 2007	590	38		2	<15		1 (ref)	
	214	30			>15		1.91 (1.02-3.56)	
Seshadri, 2002	321	27		2	<14		1 (ref)	
	771	56			>14		1.6 (1.2-2.1)	
Miwa, 2016	214	3	1562.2	3	≤8.2	5.47	1 (ref)	Vascular

	214	4	1562.2		8.3-10.7	9.5	1.29 (0.15-6.48)	dementia
	215	14	1569.5		≥10.8	16.2	6.29 (1.10-18.42)	
Zylberstein, 2011	254	9	8890	3	3.1-9.8	6.45	1 (ref)	
	564	15	19740		9.8-12.6	11.2	0.76 (0.35-1.64)	
	441	13	15435		12.6-78.9	45.75	0.70 (0.28-1.72)	
Ravaglia, 2007	590	15	2183	2	<15		1 (ref)	
	214	19	791.8		>15		1.76 (0.70-4.23)	
Kalmijn, 1999	248	49	669.6	3	<12.9	8.6	1 (ref)	CIND
	262	72	707.4		12.9-15.8	14.35	1.04 (0.73-1.49)	
	309	106	834.3		>15.8	23.7	1.01 (0.70-1.44)	
Reitz, 2009a	172	44	894.4	3	7.5-14.0	10.75	1 (ref)	
	173	49	899.6		14.1-21.4	17.75	1 (0.73-1.36)	
	171	39	889.2		21.5-29.6	25.55	1 (0.72-1.39)	
Reitz, 2009b	143	15	743.6	3	7.5-14.0	10.75	1 (ref)	
	140	17	728		14.1-21.4	17.75	1.09 (0.58-2.06)	
	149	17	774.8		21.5-29.6	25.55	1.26 (0.66-2.40)	
Reitz, 2009c	157	29	816.4	3	7.5-14.0	10.75	1 (ref)	
	156	32	811.2		14.1-21.4	17.75	1.08 (0.60-1.95)	
	154	22	800.8		21.5-29.6	25.55	0.92 (0.57-1.46)	
Mendonça, 2017	189	17	945	4	<13.5	9	1 (ref)	
	188	15	940		13.5-16.7	15.1	1.71 (0.68-4.30)	
	200	12	1000		16.7-21.4	19.05	0.97 (0.37-2.56)	
	186	13	930		>21.4	32.1	1.81 (0.63-5.25)	
Kado, 2005	370	NR		2	<8.87		1 (ref)	
					13.38-40		1.53 (1.0-2.4)	

Nurk, 2005	452	33	2712	5		8.3	1 (ref)	
	440	37	2640			10	1.05 (0.58-1.89)	
	472	50	2832			11.5	1.70 (1.01-2.88)	
	386	51	2316			13.3	1.66 (0.95-2.91)	
	432	63	2592			16.5	2.34 (1.39-3.91)	
Dufouil, 2003	1131	30	2262	4	<10	6.5	1 (ref)	
	702	29	1404		10-11.9	10.95	1.57 (0.73-3.38)	
	694	24	1388		12-14.9	13.45	1.29 (0.55-3.02)	
	520	35	1040		⩾15	22.5	2.67 (1.22-5.85)	

Hendrie, H. C. 2013[a]: cohorts of elderly African Americans; Hendrie, H. C. 2013[b]: cohorts of elderly Yoruba; Reitz, C. 2009[a]: cases were all-cause mild cognitive impairment (MCI); Reitz, C. 2009[b]: cases were amnestic MCI; Reitz, C. 2009[c]: cases were non-amnestic MCI; NR=not reported; CIND=cognitive impairment without dementia.

Table 3. Meta-analysis of blood Hcy levels and risk of cognitive disorders.

Table 3 Meta-analysis of blood Hcy levels and risk of cognitive disorders

Comparison	No of studies	Cases/participants	Pooled RR (95% CI)	Heterogeneity (I^2), P value	P values for Begg's and Egger's tests	Adjusted RR (95% CI) via the trim-and-fill method
All-cause dementia						
Highest *v* lowest	10	1,035/11,168	1.58 (1.33 to 1.87)	17.4, 0.263	0.007, 0.011	1.471 (1.264 to 1.712)
per 3-unit increment	8	926/10,364	1.07 (1.03 to 1.12)	53.7, 0.034	0.035, 0.012	1.038 (0.996 to 1.083)*
per 5-unit increment	8	926/10,364	1.12 (1.05 to 1.20)	53.7, 0.035	0.035, 0.012	1.064 (0.993 to 1.142)*

Alzheimer-type dementia						
Highest *v* lowest	8	530/5,777	1.74 (1.32 to 2.29)	58.8, 0.018	0.063, 0.004	1.372 (1.033 to 1.822)
per 3-unit increment	5	362/3,610	1.09 (1.02 to 1.15)	56.6, 0.056	0.806, 0.081	
per 5-unit increment	5	362/3,610	1.15 (1.04 to 1.26)	56.6, 0.058	0.806, 0.084	
Vascular dementia						
Highest *v* lowest	3	92/2,706	1.78 (0.58 to 5.42)	70.6, 0.033	1, 0.377	
per 3-unit increment	2	58/1,902	1.18 (0.79 to 1.76)	90.1, 0.001		
per 5-unit increment	2	58/1,902	1.32 (0.68 to 2.58)	90.1, 0.001		
CIND						
Highest *v* lowest	8	900*/8,606	1.34 (1.02 to 1.74)	53.7, 0.033	0.174, 0.097	
per 3-unit increment	7	900/8,226	1.04 (1.00 to 1.08)	62.0, 0.015	0.034, 0.034	1.007 (0.960 to 1.056)*
per 5-unit increment	7	900/8,226	1.06 (0.99 to 1.13)	62.0, 0.015	0.035, 0.034	1.012 (0.935 to 1.095)*
RR=relative risk; CI=confidence interval; Hcy=homocysteine; CIND=cognitive impairment without dementia * indicates that p-value had a significant level (P<0.05) after the trim-and-fill correction.						

Table 4. Stratified meta-analyses of Hcy levels and risk of cognitive impairment.

Table 4. Stratified meta-Analyses of blood homocysteine levels and risk of cognitive disorders

Category	All-cause dementia			Alzheimer-type dementia			CIND		
	n	RR (95% CI)	I^2, %	n	RR (95% CI)	I^2, %	n	RR (95% CI)	I^2, %

Location									
Europe	6	1.67 (1.38 to 2.07)	30.4	5	2.04 (1.25 to 3.34)*	72	4	1.73 (1.00 to 2.99)*	68.9
America	2	1.67 (0.99 to 2.79)	0	2	1.53 (1.19 to 1.96)	0	4	1.12 (0.89 to 1.41)	6.4
Australia-Asia	2	1.34 (0.93 to 1.95)	49.4	1	3.31 (0.82 to 17.13)	0			
Mean follow-up duration									
Below 5 years	3	1.97 (1.39 to 2.79)	40.6	4	1.60 (1.05 to 2.45)*	65.1	2	1.54 (0.60 to 3.96)*	79.5
5 years or above	7	1.52 (1.26 to 1.82)	0	4	1.79 (1.40 to 2.28)	0	6	1.32 (0.98 to 1.78)*	50.8
Female (%)									
50 or above	7	1.64 (1.37 to 1.97)	4	7	1.7 (1.28 to 2.25)	61.6	7	1.15 (0.97 to 1.38)	31.1
50 below	3	1.87 (0.93 to 3.74)*	53.4	1	3.31 (0.82 to 17.13)	0	1	2.34 (1.39 to 3.91)	0
Diagnosis criteria									
ICD	2	1.80 (0.62 to 5.19)*	64.6	0			1	2.34 (1.39 to 3.91)	0
DSM-III-R/IV	7	1.90 (1.50 to 2.40)	0	3	2.56 (1.55 to 4.22)	0	3	1.01 (0.79 to 1.30)	0
NINCDS-AIREN/ADRDA	1	1.40 (1.10 to 1.90)	0	5	1.54 (1.15 to 2.06)*	62.5			
MMSE							3	1.53 (0.79 to 2.95)*	61.9
other							1	1.53 (1.00 to 2.40)	0
Sample source									
plasma	9	1.59 (1.34 to 1.90)	26.3	6	1.73 (1.40 to 2.15)	22.5	7	1.42 (1.05 to 1.94)*	55.9
serum	1	1.67 (1.10 to 2.57)	0	2	1.58 (0.80 to 3.12)*	76.2	1	1.01 (0.70 to 1.44)	0
Quantification method of Hcy									
HPLC	4	1.42 (1.14 to 1.77)	30.7	3	1.56 (1.22 to 1.99)	0	7	1.32 (1.00 to 1.75)*	59.4
IMx assay	4	1.92 (1.46 to 2.53)	0	4	2.46 (1.70 to 3.56)	0	1	1.69 (0.63 to 5.25)	0

Adjustment for confounding factors										
Age										
	Yes	9	1.58 (1.34 to 1.86)	12.5	8	1.74 (1.32 to 2.29)*	58.5	6	1.14 (0.95 to 1.37)	39
	No	1	3.72 (1.06 to 13.08)	0	0			2	2.20 (1.38 to 3.50)	0
Sex										
	Yes	6	1.60 (1.35 to 1.89)	31.5	8	1.74 (1.32 to 2.29)*	58.5	8	1.34 (1.02 to 1.74)*	53.7
	No	4	1.67 (0.99 to 2.79)	0	0					
BMI										
	Yes	4	1.81 (1.37 to 2.39)	0		2.56 (1.55 to 4.22)	0	2	1.27 (1.21 to 4.27)	0
	No	6	1.51 (1.24 to 1.84)	46		1.54 (1.15 to 2.06)*	62.5	6	1.23 (0.95 to 1.61)*	54.1
APOE										
	Yes	6	1.64 (1.34 to 2.01)	23.7	7	1.65 (1.24 to 2.19)*	57.3	5	1.29 (0.89 to 1.85)*	56.7
	No	4	1.54 (1.17 to 2.01)	28.3	1	2.43 (1.25 to 4.71)	0	3	1.46 (0.90 to 2.36)*	64.6
Education										
	Yes	9	1.70 (1.42 to 2.02)	6.1	8	1.74 (1.32 to 2.29)*	58.5	4	1.73 (1.00 to 2.99)*	68.6
	No	1	1.20 (0.80 to 1.79)	0				4	1.12 (0.90 to 1.39)	6.4
B vitamins										
	Yes	5	1.98 (1.52 to 2.59)	0	4	2.46 (1.70 to 3.56)	0	1	2.67 (1.22 to 5.85)	0
	No	5	1.42 (1.16 to 1.74)	0	4	1.29 (1.13 to 1.47)	36.6	7	1.20 (1.01 to 1.42)	46.9
History of cardiovascular diseases										
	Yes	6	1.78 (1.29 to 2.45)*	50.2	6	1.64 (1.23 to 2.18)*	63.5	3	2.31 (1.55 to 3.45)	0
	No	4	1.69 (1.25 to 2.28)	0	2	2.74 (1.27 to 5.89)	0	5	1.09 (0.90 to 1.31)	0
NOS score										
Less than 7 stars					1	1.30 (0.80 to 2.30)	0	3	1.65 (1.19 to 2.27)	36.5
7 stars		6	1.56 (1.28 to 1.90)	0	5	1.64 (1.19 to 2.27)*	60.8	4	1.17 (0.91 to 1.51)	0

8 stars	4	2.01 (1.20 to 3.38)*	63	2	2.44 (1.45 to 4.10)	47.1	1	2.80 (1.20 to 6.20)	

RR=relative risk; CI=confidence interval; HPLC=high-performance liquid chromatography assay; ICD=the International International Classification of Diseases; DSM-III=the Diagnostic and Statistical Manual of Mental Disorders 3rd edition; NINCDSADRDA=National Institute of Neurologic and Cognitive Disorders and Stroke-AD and Related Disorders Association criteria; NINCDS-AIREN=National Institute of Neurological and Communicative Disease and Stroke-Alzheimer's Disease and Related Disorders Association; BMI=body mass index; APOE= apolipoprotein E; MMSE=Mini-mental State Examination;NOS=Newcastle-Ottawa Scale. *indicates the pooled result using a random effects model.