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Is Hyperhomocysteinemia an Alzheimer's disease (AD) risk factor, an AD marker or neither?

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Abstract

Alzheimer's disease (AD) is the most common form of neurodegenerative disease. The vast majority cases of AD are sporadic, without clear cause, and a combination of environmental and genetic factors have been implicated. The hypothesis that homocysteine (Hcy) is a risk factor for AD was initially prompted by the observation that patients with histologically confirmed AD had higher plasma levels of Hcy, also called hyperhomocysteinemia (HHcy), than age-matched controls. Most evidence accumulated so far implicates HHcy as a risk factor for AD onset, but conflicting results also exist. In this review, we summarize reports on the relationship between HHCy and AD from epidemiological investigations, including observational studies and randomized controlled clinical trials. We also examine recent *in vivo* and *in vitro* studies of potential mechanisms whereby HHcy may influence AD development. Finally, we discuss possible reasons for the existing conflicting data, and provide suggestions for future studies.

Homocysteine and Alzheimer's disease

Alzheimer's disease (AD) is the most common form of dementia and its pathological hallmarks in the brains are neuritic plaques composed mainly by amyloid-β (Aβ) peptides, and neurofibrillary tangles formed by hyperphosphorylated forms of tau protein[1]. Although AD is probably a multifactorial disease and the real cause remains unknown, various hypotheses have been proposed. For example, the amyloid hypothesis suggests that the accumulation of Aβs as the major cause of the disease. Aβs are the 40–42 amino acid peptides cleaved from the amyloid precursor protein (APP) by the subsequential action of the β-secretase-1 (BACE-1) and γ-secretase. By contrast, the tau hypothesis considers abnormally hyperphosphorylated tau as the major culprit of AD[1].

Globally, more than 26 million people have been diagnosed with AD. As the population ages, prevalence of AD keeps rising and is projected to be over 100 million by 2050[2]. More than just a devastating disease for the patients and their families, AD also puts a huge financial burden on the whole society[3]. Altough an effective treatment for AD is unavailable, interventions to control risk factors (e.g. lowering of high blood pressure and high cholesterol levels) can still reduce the number of cases and associated cost. Given the fact that this disease mainly targets people over 65 years old, a small 1 year delay in disease onset would result in 9.2 million fewer cases worldwide by 2050, and save billions in costs

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for the society[2]. An immense effort, therefore, has been spent on identifying risk factors for AD and developing treatments to reduce them.

Hyperhomocysteinemia (HHcy), the abnormal elevation of blood levels of homocysteine (Hcy), has been proposed to be a modifiable risk factor for AD[4]. Hcy is a sulfur-containing, non-protein amino acid produced in the methionine cycle. Its metabolism is at the intersection of two main pathways: remethylation and trans-sulfuration (Figure 1). When the methionine level is low, Hcy is remethylated into methionine; a process which requires vitamin B_{12} and folic acid as cofactors. Methionine is then activated by ATP to form S-adenosyl-methionine (SAM), which serves as the major methyl group donor in the cell. After demethylation, SAM generates S-adenosyl-homocysteine (SAH) and eventually is hydrolyzed back to Hcy for a new cycle. When methionine levels are high, Hcy, through the trans-sulfuration pathway, condenses with serine to form cystathionine, and subsequently cysteine in an irreversible reaction. Therefore, elevated Hcy level, which is associated with low methylation potential, can be reduced by dietary intervention of folic acid and vitamin Bs.

Since the first paper reporting the elevation of Hcy in AD patients in 1990[5], increasing numbers of studies have been conducted to explore the relationship between HHcy and the risk of AD. Evidence from human and animal studies has converged to suggest that moderate elevation of Hcy in aged population is a potential risk factor for AD[6]. With an Hcy level higher than 14 μM, the risk of AD almost doubles in people over 60 years old[7]. However, contradictory evidence also exists, and it is still controversial whether HHcy is an AD risk factor or merely a biomaker[8]. Several potential mechanisms have also been proposed to explain the connections between HHcy and AD, including oxidative stress[9],[10], demethylation[11], cerebrovascular damage[12], endoplasmic reticulum (ER) stress[13], Aβ elevation[11, 14, 15] and tau protein phosphorylation[16].

This review summarizes the studies on the relationship between HHcy and AD, including observational clinical studies and randomized controlled trials. It also presents some of the mechanisms whereby HHcy may influence AD development by considering the latest results from *in vitro* and *in vivo* studies. Finally, several possible explanations for the existing conflicting results are discussed and suggestions for future studies provided. Although Vitamin B deficiency is also reported to be related with cognitive decline and AD, it will not be covered in this paper.

Clinical studies

Most of the direct evidence on the association between HHcy and AD comes from human studies. Regland and colleagues in 1990 first reported elevated Hcy levels in 22 primary degenerative dementia patients[5]. Since then, dozens of studies, both observational and interventional, have been conducted. Here, we summarize the findings from the studies with a minimal sample of 50 subjects.

Observational studies

Observational studies can be further grouped into retrospective or prospective categories.

Retrospective studies—Retrospective studies focus on the comparison between plasma Hcy levels in AD patients and their age-matched healthy controls in an attempt to establish a potential connection. At least 12 studies reported higher Hcy levels in AD patients than in age-matched healthy controls (Table 1). Plasma Hcy levels were ~ 11.5– 16.4 μM in control groups, whereas Hcy levels ranged from 16.3 μM to 23.52 μM in AD patients. By contrast, three other studies reported no difference in blood or cerebral spinal fluid (CSF) Hcy levels

between AD patients and controls[17–19]. Overall, the majority of retrospective studies have observed an increase in Hcy in AD patients. However, they cannot establish whether this increase is the cause or the result of AD. To explore the temporal association between HHcy and AD, prospective studies are required.

Prospective studies—A prospective study generally starts with a group of healthy subjects and follows them for a certain time (ranging from several months to decades), during which the incidence of AD /dementia is recorded, and the corresponding risk ratio calculated. The Hcy base levels from the subgroup of patients who develop AD and the rest of the original group are compared at the end of the study. Due to the age-dependent incidence of AD in normal population, it is quite challenging to get statistically sufficient number of AD-converting cases in most prospective studies. For this reason, in the current paper, we also include prospective studies assessing the relation between Hcy levels and cognitive decline (that does not meet the criteria for AD) in healthy elderly people. As shown in table 2, among 12 of such prospective studies, only 2 did not detect any association between HHcy and AD/cognitive decline. The other 10, with a maximum sample size of 1779 and longest follow-up time of 35 years, all found HHcy was a risk factor for AD or cognitive decline. For instance, one recently published study followed a group of women for 35 years and revealed that midlife high Hcy level is an independent risk factor for the development of late-stage dementia[20]. There are also prospective studies following the disease development in mild cognitive impairment (MCI) and AD patients, in which HHcy predicts the conversion of MCI to AD and further cognitive decline in AD, respectively[21, 22]. Most of these prospective studies reveal that elevation of Hcy levels precedes the cognitive decline or AD development in the elderly, supporting the hypothesis that HHcy is the culprit.

Interventional studies

Despite the strong support offered by observational studies, the cause-effect relationship between HHcy and AD cannot be established without evidence obtained from interventional studies, particularly randomized controlled trials (RCTs). RCTs apply random allocation of different intervention to subjects, thus eliminating the potential selection bias, from either known or unknown prognostic factors. It is considered the gold standard for testing the efficacy of any therapeutic agent. The ideal design of a RCT should have Hcy-reducing treatment(s) or placebo in AD patients appropriately randomized, and should apply measures of cognitive changes after the treatment(s). Compared to observational studies, RCTs are more expensive and complex. Few RCTs have been carried out in AD patients so far, and none recruited AD patients with mild to elevated Hcy levels (Table 3)[23–25]. Among them, only one 6-month-long study found that Hcy-reducing treatment further improves the beneficial effect of cholinesterase inhibitors in AD patients[23]. Other RCTs, despite the observed reduction in the Hcy levels, failed to detect any cognitive benefit in AD patients. In particular, a recent RCT in 340 patients with mild to moderate AD followed for 3 years found that Hcy reducing treatment—high-dose supplement of folate, Vitamin B6 and B12—does not slow down cognitive decline[24].

Potential Mechanisms

The biological association between HHcy and AD revealed in human studies has stimulated a large research effort into the investigation of its underlying mechanism(s). A better understanding of these mechanisms could provide valuable insights for future human studies and ultimately therapeutic opportunities against HHcy-dependent AD development and/or progression.

By using *in vitro* and *in vivo* models diverse possible mechanistic connections between HHcy and AD pathogenesis have been revealed. Below we list some of the most investigated mechanisms.

Oxidative stress

As a thiol group, Hcy can undergo auto-oxidation to generate hydrogen peroxide and other reactive oxygen species, leading to oxidative stress[26]. It can also promote oxidative stress by reducing activity of antioxidants such as glutathione, probably through increasing SAH level[27]. Hcy binding to proteins has also been recently reported to be able to induce oxidative stress under physiological conditions[28]. Experiments in cell lines, primary neuronal culture and rodent hippocampus slices, have all shown that high amounts of Hcy induce oxidative stress[10, 29]. Hcy can also augment the toxicity of Aβ and metal ions by exacerbating their pro-oxidant activity[9, 30], whereas antioxidants such as vitamin E can rescue these Hcy-induced deficits[9].

Considering that the central nervous system (CNS) is extremely vulnerable to oxidative imbalance, owing to its high oxygen consumption, high iron and lipid concentration, and the relatively low activity of antioxidant defense, the oxidative stress induced by high concentration of Hcy is likely to impair important neural functions. This impairment probably leads to cognitive problems in AD.

Demethylation

The S-adenosyl-homocystein (SAH) hydrolase reaction, which forms Hcy from SAH, is reversible, with the equilibrium actually favoring the condensation of Hcy and adenosine to form SAH[31] (Figure 1). For this reason, HHcy is usually associated with elevation of intracellular SAH, a competitive universal inhibitor for methyltransferase, and depletion of S-adenosyl-methionine (SAM), the major methyl group donor, resulting in an overall decrease of cellular methylation[32]. HHcy-dependent demethylation of DNA/histone or proteins can modulate both gene expression and enzyme activities involved in AD pathogenesis. In both cell culture and AD transgenic mouse models, HHcy demethylates the promoters of BACE-1 and Presenilin 1(a major component of the γ-secretase complex), resulting in an increase in their protein levels and Aβ formation[11, 33–35]. At the protein level, demethylation of protein phosphatase 2A (PP2A) can alter its substrate specificity and enzyme activity[36]. PP2A is a major brain Ser/Thr phosphatase and mediates tau protein phosphorylation[37]. Its expression levels have been found significantly reduced in brains of patients with AD[38]. Although kinases such as glycogen synthase kinase 3β (GSK3β) and cyclin-dependent kinase 5 (CDK5) are also involved in tau phosphorylation, previous study suggested that the decrease of PP2A activity, rather than the increase of these kinases activity, is crucial for tau hyperphosphorylation associated with neurofibrillary tangles formation[39]. Interestingly, *in vitro* and *in vivo* data also show that high doses of Hcy or SAH, through PP2A demethylation, increases phosphorylation of tau and APP, alters APP processing and promotes Aβ production[16, 40].

Cerebrovascular damage

For more than four decades, HHcy has also been recognized as a risk factor for cardiovascular disease (CVD)[41]. Its deleterious effects on the vascular system of the brain could also partially explain the connection between HHcy and AD. For example, HHcy-induced cerebrovascular impairments correlate with the cognitive deficits in mice[12].

Furthermore, HHcy impairs the integrity of blood-brain barrier (BBB), both in AD patients and AD transgenic mice[42, 43], and causes leakage of detrimental molecules into the tightly-controlled CNS environment, leading to cell damage and cognitive decline[44]. HHcy-

dependent BBB alteration[45] could be also responsible for abnormal GABA signaling[45], with subsequent damage to endothelial cells[46] and astrocytes[47]. Interestingly, a high-folate diet treatment in MCI patients with HHcy is effective in restoring the BBB integrity, providing further support for this mechanism[48]. However, whether HHcy can directly cause vascular damage is still under debate, and conflicting data also exist, showing that HHcy is only associated with AD and not with vascular dementia[49]. In summary, the contribution of HHcy-dependent vascular damage to AD pathogenesis is still very much controversial.

Excitatory damage

Hcy and its acidic derivatives such as homocysteic acid are analogs of glutamate and N-methyl-D-aspartate (NMDA), and act as agonists on the NMDA receptors[50]. These derivatives are also metabolic glutamate receptor agonists[51], and activators of their downstream signaling pathways, which can result in brain excitotoxicity. Thus, *in vitro* studies have provided evidence that glutamate receptor antagonists (including MK801and MSOP) could attenuate the damage to neurons that are exposed to high concentrations of Hcy[52].

Aβ elevation and tau phosphorylation

Recent studies provide evidence that HHcy could directly affect Aβ and tau metabolism. Direct injection of Hcy into rat brain increases Aβ level and tau phosphorylation[53, 54]. Both diet- and genetic-induced HHcy result in an elevation of Aβ levels and deposition in the brain of transgenic mouse models of AD-like amyloidosis[14, 15, 55]. GSK3 phosphorylation levels were found reduced in AD transgenic mice with HHcy, suggesting that this signaling pathway may be involved in HHcy-induced Aβ elevation, as GSK3 activation (low phosphorylation levels) can modulate the Aβ production through the γ-secretase pathway[14, 56]. Moreover, HHcy is found to bind to Aβ40 and favor its β sheet structure formation both *in vitro* and *in vivo*, thus facilitating its deposition[57]. Hcy thiolactone, an intramolecular thioester of Hcy, may also react with Aβ and cause its precipitation[58]. Another derivative of Hcy, homocysteic acid, increases intracellular Aβ42 *in vitro*[59], and intracranially injected antibody against this metabolite to 3xTg mice results in Aβ reduction and cognitive preservation[60].

ER stress

The ER is the principal site for protein synthesis and maturation in the cell. Its physiological functions include regulation of protein production, folding, modification and targeting. ER stress can be induced when some proteins become unfolded or misfolded and tend to accumulate and/or aggregate. If not resolved, this phenomenon slows down protein synthesis, activates protein degradation pathways and eventually triggers apoptosis[61]. HHcy can induce ER stress by disrupting disulphide bonds and causing misfolding of proteins traversing the ER. *In vitro* Hcy-induced ER stress can trigger neuronal apoptosis, and synergize the toxic effect of Aβ[62].

Several ER stress response proteins, including GRP78, GRP94, X-box-binding protein 1 and HHcy-induced ER response protein (Herp), can also be increased by HHcy[63–65]. The Herp protein has been shown *in vitro* to increase Aβ level through the γ-secretase pathway[13]. Even though its levels are elevated in an AD transgenic mouse overexpressing human APP Swedish mutation, i.e. Tg2576[66], with diet-induced HHcy[67], they are unaltered in a genetic HHcy model mouse, i.e., $CBS^{+/-}$. By contrast, incubation of cells with high Hcy concentration induces an increase in Aβ formation, but not significant changes in Herp protein or mRNA levels[67].

Other mechanisms

Although less investigated, many other mechanisms have been proposed to explain the association between HHcy and AD. For example, DNA repair mechanisms could be impaired by HHcy and lead to apoptosis and hypersensitivity to other types of damages[68, 69]. Higher Hcy levels are associated with hippocampal or cortical atrophy in healthy elderly adults[70, 71] and white matter changes in AD patients[72]. Other mechanisms that have also been suggested include inhibition of adult neurogenesis[73], immune activation[74], and blockage of the nitric oxide (NO) signaling pathway[75].

Reconciling the differential findings on HHcy and AD

So far, we have summarized the clinical data regarding the relationship between HHcy and AD and introduced some of the potential mechanisms accounting for it. The controversial data from the human studies are hard to interpret. Even though most observational studies suggest that HHcy is a risk factor for AD, RCTs conducted so far cannot demonstrate a beneficial effect of HHcy reducing treatment in AD patients. As the RCT design is still relatively new in the neurodegenerative disease field and most of these trials show negative results, it could suggest that there might be some intrinsic methodological problems[3]. We believe that more careful consideration and better designed studies are required before jumping to the conclusion that there is no cause-effect relationship between HHcy and AD based on the failure of these trials. Here we will discuss several possibilities to explain the discrepancy in the existing data and provide some suggestions for future research.

Recruitment of AD patients with actual HHcy

One common aspect of the published RCTs is that they recruited subjects with Hcy levels in the normal range for the particular age of the population, rather than subjects with actual HHcy. Based on observational studies, only mildly elevated Hcy level (>14μM), rather than normal Hcy (<14μM) per se, is a risk factor for AD. Thus, providing folate and B vitamins to patients with Hcy within the normal range level is very unlikely to result in any benefit. By contrast, the same treatment in AD patients with mild/high HHcy may provide significant and meaningful benefits.

Recent studies have also shown that reducing HHcy is beneficial for cognition in animal and human trials using subjects with elevated Hcy levels. Two months of Hcy-reducing treatment in Tg2576 mice with HHcy decreases brain amyloid levels and improve their cognitive impairments[76]. In humans, the FACIT study, a folate acid treatment in 818 seniors between 50–70 years old with HHcy, showed benefit in cognition after 3 years of treatment[77]. In another study, 5 year folate and vitamin B supplement in older women (n=5540) with CVD showed significant cognitive improvement only in subjects with low folate/vitamin B levels at baseline before treatment[78]. These studies further support the idea that subjects with circulating Hcy levels above 14μM are the ones who best benefit from Hcy-lowering strategies. Another study using folate treatment in subjects over 65 years old with HHcy failed to detect cognitive improvement. However, compared to the studies just mentioned, it had a smaller sample size (n= 246) and shorter treatment period (2 years)[79].

Taken together, these data suggest that future prevention studies with Hcy reducing strategies should recruit subjects with actual HHcy and treat them for an appropriate length of time.

Appropriate cognitive tests

The average Hcy reducing treatment period of most of the published studies is relatively short compared to the years or decades of accumulated HHcy-dependent deleterious effects

on cognition. Given this and the fact that the treatment generally occurs in patients at their late stage of life, the beneficial effect of treatment is very likely to be subtle and probably needs appropriate and very sensitive cognitive assessments to detect it. Global cognitive measures such as MMSE are unlikely to be sensitive enough for small and domain-specific changes after Hcy-reducing treatment[3]. The FACIT study, which detected cognitive benefit after a 3-year folate diet treatment in healthy people, would have missed the changes in memory and information processing speed in the treated group, if they had only implemented MMSE rather than domain-specific tests such as the sensorimotor speed test[77]. Appropriate cognitive tests, particularly tests focusing on memory and information processing speed, should be included in future HHcy-lowering study in AD patients.

Population difference

Another possible explanation for the discrepancy between the observational studies and RCT is that these studies usually include different patient populations. It takes considerable effort to be part of a RCT, because it requires initial screening, blood sample collection, taking daily medication and participating in different kinds of cognitive tests during the follow-up period. Thus, the subject group is more limited to certain type of patients who are easier to recruit for long-term treatment. These subjects are usually more educated, more concerned about their own health and more likely to take care of themselves. By contrast, observational studies require less effort (if any) from the patients. Therefore, samples collected by observational study are very likely from a population which is larger and more diverse than the one who participates in a RCT. Hcy-reducing treatment(s) may yield better results if a larger and more diverse population is recruited in the trials.

Influence of other factors: lessons from cardiovascular studies

Because HHcy was originally recognized as a risk factor for CVD, many more studies has been conducted in this area than in the AD field. Thus, some lessons learned from those studies could be useful to better understand the connection between HHcy and AD. One thing particularly intriguing is that, similar to what we described in AD, only observational studies, but not RCTs, provide evidence supporting HHcy as a risk factor for CVD. Most folate and vitamin B_{12} treatment in CVD patients failed to show any improvement[80]. Are there some common factors that could explain the failure of Hcy-reducing treatment(s) both in CVD and AD?

Interestingly, the AtheroGene study found that the pathogenic effect of HHcy is significantly influenced by the overall cardiovascular redox state[81]. This study followed 643 subjects with coronary artery disease for 7.1 years and found that both HHcy and GPx-1, one of the most important antioxidant enzymes, were the strongest predictors for future cardiovascular events. Further analysis revealed that in subjects with high GPx-1 activity, HHcy did not predict future cardiovascular events. In the subjects with low GPx-1 activity, plasma Hcy level above the median, however, increased the cardiovascular risk 3.2 fold. This finding suggests that other associated factors, such as oxidative stress, could influence the effect of HHcy on CVD. This would partly explain the lack of benefit secondary to Hcy reduction alone in CVD. It should be interesting to see if a similar effect can also be found in AD patients.

Hcy, folate or vitamin B

Although many studies have examined the connection between Hcy and AD, there is hardly any clinical study that can clearly distinguish a difference in the effect of Hcy, folate or vitamin B12 on cognition and/or AD pathogenesis. None of the current trials can tell if the observed cognitive decline is mediated by HHcy or vitamin Bs. This is probably due to the complexity of Hcy metabolism which closely connects Hcy with folate and B vitamins.

Given that almost all the Hcy reducing treatment in RCTs use diet supplement of folate or vitamin B12 or both, the failure of these RCT on slowing AD development cannot rule out the potential of HHcy-reducing treatment using alternative non-vitamin approaches. These non-vitamin-related means, such as the use of N-acetylcysteine, are also imperative to determine the actual effect of HHcy in AD development[82]. Thus, as shown by several studies, long-term folate treatment could induce adverse effects through mechanisms such as NO signaling inhibition[24, 83]. These adverse effects may offset the benefits of HHcy reducing treatment in AD and provide an important rationale to test a non-vitamin reducing therapeutic approach.

The SAH / SAM hypothesis

A novel and emerging hypothesis is that SAH is potentially the pathologic mediator of HHcy-dependent effect on AD pathogenesis. It postulates that HHcy is basically a marker of an altered cellular methylation potential, which by affecting the transcription of genes in neuronal cells, modulates molecular events of functional importance in the neurobiology of AD. In particular, HHcy results in high intracellular SAH levels. Because SAH is a competitive inhibitor with SAM, elevated SAH will result in reduced levels of methyltransferase activity, which will then cause reduced levels of methylation of DNA, histone and transcription factors ultimately altering gene expression. Importantly, because SAH and SAM are not readily diffusible through mammalian membranes, they are not at equilibrium between cells and plasma. To remove excessive intracellular SAH, the cell must first convert it to Hcy, which can then be secreted. This hypothesis could also provide a biochemically explanation for the negative results of the vitamin lowering Hcy strategies. Thus, despite the fact that folic acid reduces plasma Hcy levels, it does not influence SAH intracellular concentration and actually, by facilitating methionine synthesis indirectly even increases SAH production, which further aggravates hypomethylation. If this hypothesis holds true, the real target in a condition of HHcy should be SAH and not Hcy itself. However, at the moment, there is no available therapeutic strategy which would specifically target intracellular SAH. Studies targeting SAH by gene therapy approach in animal models of HHcy and AD are warranted.

Animal studies

Recent animal studies, especially the ones using AD-like transgenic mouse models, provide further insights into the potential mechanisms by which HHcy might influence AD development. The availability of genetically induced HHcy has been used to test the hypothesis of its influence on the AD-like phenotype of these mouse models. Crossing heterozygous cystathionine-beta-synthase mutant mice, which spontaneously develop HHcy, with a transgenic mouse model AD-like amyloidosis resulted in higher brain Aβ levels[15]. In that study the author reported no apparent changes in APP metabolism. Other studies have implemented a diet-induced HHcy models approach to test the same hypothesis. However, caution needs to be taken before comparing the results from different HHcy inducing approaches (e.g. high methionine diet vs. low folate diet), as these different approaches could trigger different downstream mechanisms. Moreover, data accumulated so far indicate that the diet-induced HHcy is also affected by the strain difference. As shown in Table 4, although 6 weeks of a low-folate diet increases plasma Hcy to over 300 μM in the TgCRND8 mice at the age of 3 months[11], 7 months feeding of a similar diet can only elevate plasma Hcy to 35μM in Tg2576 mice[55]. The difference in the transgene between the Tg2576 and TgCRND8 mice (APP Swedish mutation vs. APP Swedish and Indiana mutation) as well as their genetic background (C57BL6 vs. Hybrid C3H/He-C57BL/6) could provide some explanations for the discrepancy in the obtained Hcy levels by dietary intervention. Interestingly the C57BL6 mice per se also show small elevation in plasma Hcy after low-folate diet feeding[12]. By contrast, studies in human and animal models have also

shown that extreme HHcy (Hcy levels over 100 μM) as caused by rare genetic mutations or severe nutrition deficits is not associated with significant elevation of $A\beta^{7, 84}$. Therefore, since extreme HHcy rarely occurs in the normal aging population it is unlikely that is involved in AD pathogenesis.

Concluding remarks

In its sporadic form, AD is a chronic neurodegenerative disease resulting from a complex interplay between exogenous and endogenous factors. Although our knowledge about risk factors is still incomplete, consistent evidence suggests that there are some modifiable factors which play a role in AD pathogenesis. In its original description, HHcy was considered an AD risk factor, but later on it has become evident that it could only be just a marker of the disease. Some recent negative RTCs do not support either definition, and have even seriously questioned the validity of the therapeutic approaches to lower Hcy in AD. However, based on the discussion provided in this article, we believe that the evidence in support of this recent hypothesis should be considered inconclusive and cannot be used to deny a role for HHcy in AD.

In summary, the hypothesis that HHcy is a risk factor for AD onset is still very much alive and viable, but a great deal of work needs to be done. This will involve both basic science research as well as clinical studies. In particular, we lack good animal models of HHcy which more closely reflect the human condition, and clinical trials with better study design are urgently needed. If, in the near future, these two issues are addressed, then we will be able to establish or deny a mechanistic link between AD and HHcy in a conclusive manner.

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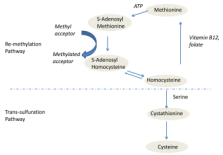

Figure.

Homocysteine metabolism is at the intersection of two main pathways: remethylation and trans-sulfuration. When the methionine level is low, homocysteine is remethylated into methionine; a process which requires vitamin B_{12} and folic acid as cofactors. Methionine then forms S-adenosyl-methionine (SAM), which serves as the major methyl group donor in the cell. After demethylation, SAM generates S-adenosyl-homocysteine (SAH) and eventually is0 hydrolyzed back to homocysteine for a new cycle. When methionine levels are high, homocysteine, through the trans-sulfuration pathway, condenses with serine to form cystathionine, and subsequently cysteine in an irreversible reaction.

Table 1

Retrospective study of HHcy and AD/Cognitive decline

	Study	Average age of participants	Sample size	Hcy level
Hcy level elevated in AD	Joosten, Lesaffre et al. 1997[85]	80 years	AD n=52 Hospitalized non-dementia n=50 Healthy control at home n=49	AD: 18.3 μM Hospitalized: 14.2μM At home Control:12.3 μM
	Clarke, Smith et al. 1998[86]	73 years	Histological AD n=76 Clinical AD n=164 Health control n=108	Histological AD: 16.3 μM Clinical AD: 15.3 μM Control: 13.2 μM
	McCaddon, Davies et al. 1998[87]	79 years	AD: n = 30 Control: n = 30	AD: 21.9 μM Control: 12.2 μM
	Selley, Close et al. 2002[88]	77.5 years	AD: 27 Control: 25	AD: 21.05 μM Control: 16.01 μM
	Nagga, Rajani et al. 2003[89]	N/A	AD: n = 56 VD: n = 50 Control: n = 101	Plasma Hcy is significantly increased in dementia group
	Selley 2003[90]	75 years	AD: n=25 Control: n=25	AD: 23.52 μM Control: 19.04 μM
	Gallucci Zanardo et al. 2004[91]	77 years	AD: n=137 VD: n = 40 Control: n = 42	AD: 21.4 μM VD: 24.4 μM Control: 15.5 μM
	Genedani, Rasio et al. 2004[92]	75 years	AD: n = 22 PD: n = 29 Control: n = 57	AD: 22.5 μM PD: 18 μM Control: 15.5 μM
	Mizrahi, Bowirrat et al. 2004 [93]	78 years	AD: n=75 Control: n = 155	AD: 20.6 μM Control: 16.4 μM
	Quadri, Fragiacomo et al. 2004 [94]	77 years	AD: n = 74 Control: n =55	AD: 16.8 μM Control: 14.6 μM
	Guidi, Galimberti et al. 2005[95]	72 years	AD: n =97 Control: n = 23	AD: 19.0 μM Control: 13.0 μM
	Linnebank, Popp et al. 2010 [96]	73 years	AD: n = 60 Control: n = 60	Higher plasma Hcy is associated with AD
	Trojanowski, Vandeerstichele et al. 2010[97]	75 years	AD: n = 200, MCI: n = 400 Control: n = 200	Both AD and MCI have significantly higher plasma Hcy than control
No difference detected	Mizrahi, Jacobsen et al. 2003[17]	N/A	AD: n = 60 Control: n = 60	AD: 12.3 μM Control: 11.5 μM ns
	Ariogul, Cankurtaran al. 2005[18]	72 years	AD: n = 121 Non dementia patients: n = 795	AD: 17 μM Non dementia patients: 16.4 μM
	Serot Barbe et al. 2005 [19]	75 years	AD: n = 38 Control: n =22	AD: 115 μM Control: 125 μM

Table 2

Prospective study of HHcy and AD/Cognitive decline

	Study	Average age of participants	Sample size	Duration	Results
HHcy as predictor for cognitive decline/AD	McCaddon, Hudson et al. 2001 [98]	> 60 years	n= 32	5 years	Baseline Hcy level predict cognitive decline
	Dufouil, Alperovitch et al. 2003 [99]	67 years	n= 1241	4 year	Cognitive decline 2.8 fold higher in people with Hcy >15 μM
	Seshadri, Beiser et al. 2002[7]	76.6 years	n= 1092	8 years	Plasma Hcy > 14 μM nearly double the AD risk
	Ravaglia, Forti et al. 2005 [100]	74 years	N= 816	4 years	HHcy associated with AD risk
	Tucker, Qiao et al. 2005 [101]	67 years	n= 321	3 years	Hcy level predict the cognitive decline
	Annerbo, Wahlund et al. 2006[21]	> 60 year	n= 93 MCI patients	6 years	Hcy level predict the AD development
	Haan Miller et al. 2006 [102]	> 60 years	n= 1779	4.5 years	High hcy level is associated with dementia
	Annerbo, Kivipelto et al. 2009 [103]	> 75 years	n= 200	6.7 year	High Hcy level is related with high AD risk
	Zylberstein, Lissner et al. 2011[20]	38–60 years	n= 1368	35 years	Baseline Hcy level in midlife can predict the AD risk in later stage
	Oulhaj, Refsum et al.[22]	> 50 years	n= 97 AD patients	1.5–9.5 years	Raised hcy level predict the cognitive decline in Ad patients
HHCy is not predictor of cognitive decline/ AD	Kalmijn, Launer et al. 1999 [101]	>55 years	n= 702	2.7 years	No association between HHcy and AD
	Luchsinger, Tang et al. 2004 [102]	>60 years	N= 679	5 years	HHcy is not associated with memory score

Table 3

Randomized Controlled Trials on HHcy and AD

Treatment effect	Study	Age	Sample size	Subjects	Duration	Medication per day	Results
Yes	Connelly, Prentice et al. 2008[23]	76.2 years	N =57	AD without HHcy	6 months	Folate: 1mg	Hcy reducing treatment improves the effect of anti-cholinesterase drugs on cognitive decline
No	Sun, Lu et al. 2007[25]	75.3 years	N = 89	Mild AD patient without HHcy	26 weeks	Folate: 1mg Vitamin B_{12}: 0.5mg	Although Hcy levels decrease after treatment, no difference found in cognition
	Aisen, Schneider et al. 2008 [24]	76 years	N =340	Mild to moderate AD without HHcy	3 years	Folate: 5 mg Vitamin B_6: 25mg Vitamin B_{12}: 1mg	No sign of slowing AD development after treatment

Table 4

Diet-induced HHcy studies in AD transgenic mouse models

Mouse strain	Diet	Starting age	Duration	Hcy level	Effect	study
APP_{695} Line C3-3	No folate 4.5g/kg D,L-homocysteine	7 months old	3 months	Increased from 3 μm to 27 μm	Hippocampus neuronal death No change in Aβ levels	Kruman, Kumaravel et al. 2002 [69]
Tg2576	No folate No methionine 4.5g/kg D,L-homocysteine	16–18 months old	6 months	Increased from 100 μm to 200 μm	Impaired water maze performance No change in Thioflavin-S-staining of amyloid aggregate	Bernardo McCord et al. 2007 [106]
	Methionine 7.7g/kg	8 months old	7 months	Increased from 6 μm to 35 μm	Increase Aβ levels and deposition GSK3 signaling pathway altered Impaired cognitive tests	Zhuo, Portugal et al. 2010 [14]
	No folate, vitamin B_6 or B_{12}	8 months old	7 months	Increased from 6 μm to 28 μm	Increase Aβ level and deposition	Zhuo and Pratico 2010 [55]
	No folate, vitamin B_6 or B_{12}; Methionine 7.7g/kg	8 months old	7 months	Increased from 6 μm to over 150 μm	No change in Aβ levels	Zhuo and Pratico 2010 [84]
	Methionine 7.7g/kg	9 months old	5 months	Increased from 3 μm to 15 μm	2 months normal diet feeding restores the Hcy levels to normal, and slow Aβ deposition	Zhuo and Pratico 2010 [76]
TgCRND8	No folate, vitamin B_6 or B_{12}	15 days	45 days or 60 days	Increased from 0.2 μm to over 200 μm (45 days) or over 400 μm (60 days)	Increase Aβ level and deposition Increase PS1 and BACE1 mRNA and protein level expression	Fuso, Nicolia et al. 2008 [11]
					PSNE1 gene is regulated by HHcy	Fuso, Nicolia et al. 2009 [107]
					SAM treatment restores the methylation and oxidative system damaged by HHcy	Cavallaro, Fuso et al. 2010 [108]