Homocysteine and holotranscobalamin and the risk of Alzheimer disease: A longitudinal study

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ABSTRACT

Objective: To examine the relation between serum levels of homocysteine (tHcy) and holotranscobalamin (holoTC), the active fraction of vitamin B12, and risk of incident Alzheimer disease (AD) in a sample of Finnish community-dwelling elderly.

Methods: A dementia-free sample of 271 subjects aged 65–79 years derived from the Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) study was followed up for 7 years to detect incident AD. The association between serum tHcy and holoTC with AD was analyzed with multiple logistic regression after adjusting for several potential confounders, including common vascular risk factors.

Results: The odds ratios (ORs) (95% confidence interval [CI]) for AD were 1.16 (1.04–1.31) per increase of 1 μmol/L of tHcy at baseline and 0.980 (0.965–0.995) for each increase of 1 pmol/L baseline holoTC. Adjustment for several potential confounders including age, sex, education, *APOE* ε4 allele, body mass index, Mini-Mental State Examination, smoking, stroke, and blood pressure did not alter the associations: ORs (95% CI) for AD became 1.19 (1.01–1.39) for tHcy and 0.977 (0.958–0.997) for holoTC. Adjusting for holoTC attenuated the tHcy–AD link (OR changed from 1.16 to 1.10, 95% CI 0.96–1.25). The holoTC–AD relationship was less influenced by controlling for tHcy (OR changed from 0.980 to 0.984, 95% CI 0.968–1.000). Addition of folate did not change any of the results.

Conclusions: This study suggests that both tHcy and holoTC may be involved in the development of AD. The tHcy–AD link may be partly explained by serum holoTC. The role of holoTC in AD should be further investigated. ***Neurology*® 2010;75:1408–1414**

GLOSSARY

AD = Alzheimer disease; **BMI** = body mass index; **CAIDE** = Cardiovascular Risk Factors, Aging, and Dementia; **CI** = confidence interval; **CV** = coefficient of variation; **DBP** = diastolic blood pressure; ***DSM-IV*** = *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition; **holoTC** = holotranscobalamin; **MMSE** = Mini-Mental State Examination; **OR** = odds ratio; **SAM** = S-adenosylmethionine; **SBP** = systolic blood pressure; **tHcy** = homocysteine.

An association between high serum total homocysteine (tHcy) and cardio/cerebrovascular diseases has long been recognized.[1,2] However, the relation between tHcy, its main determinants (such as vitamin B12 and folate), and Alzheimer disease (AD) risk is still controversial. Cross-sectional studies yielded mixed results.[1] Several longitudinal studies linked elevated tHcy to increased risk of AD, dementia, or cognitive decline,[3-8] while some reported no such associations.[9,10] Low blood levels of vitamin B12 or folate have also been related to the development of AD, dementia, or cognitive impairment, and to increased rate of brain atrophy,[3,4,6,11-15] although the evidence has been inconsistent.[3-6,9,12,14,15]

Holotranscobalamin (holoTC), the biologically active fraction of vitamin B12, may be a more useful marker of B12 status than total serum B12.[16] A decreased concentration of holoTC has been suggested as the first-line test for diagnosing early B12 deficiency.[16] Few longitudinal

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From the Aging Research Center (B.H., A.S., I.K., B.W., M.K.) and KI Alzheimer's Disease Research Center (KI-ADRC) (B.H., B.W., M.K.), Department of Neurobiology, Care Sciences and Society (NVS), Karolinska Institutet, Stockholm, Sweden; National Institute for Health and Welfare (THL) (J.L., S.A., T.L.), Department of Chronic Disease Prevention, Helsinki; and Department of Neurology, University of Eastern Finland, Institute of Clinical Medicine (A.S., M.R., H.S., M.K.) and University Hospital (H.S.), Kuopio, Finland.

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studies have investigated the association between holoTC and risk of AD or cognitive decline.[3,12,15]

Most available studies have so far considered tHcy and its main determinants separately in relation to AD. The aim of the current study is to investigate serum tHcy, holoTC, and folate levels simultaneously, as well as putative interactions between them, in relation to AD risk in a subsample of the population-based Cardiovascular Risk Factors, Aging, and Dementia (CAIDE) study with a 7-year follow-up.

METHODS

Study population. The present study included a subsample of 271 subjects, derived from the dementia-free cohort participating in 1998 in the first reexamination of the CAIDE study in Finland. The CAIDE study has been described in detail elsewhere.[17] Briefly, CAIDE participants were examined at midlife within the framework of the North Karelia project and the FINMONICA study in 1972, 1977, 1982, or 1987. Individuals still alive, aged 65–79 years at the end of 1997, and living in the areas of Kuopio and Joensuu, were the target for the 1998 reexamination. A second reexamination of the same cohort was conducted in 2005–2006.

The 271 subjects included in the present study were selected based on availability of serum samples from 1998 for tHcy, holoTC, and folate measurements. The mean (SD) follow-up duration of the CAIDE subsample from 1998 reexamination (baseline for this study) was 7.4 (0.3) years. There was no clinically significant difference between the CAIDE subsample and the entire dementia-free CAIDE cohort.

Reexamination in 1998. This survey comprised a self-administered questionnaire on sociodemographic characteristics, health-related behaviors, and medical history, including cerebrovascular, cardiovascular, and renal conditions. Nurses especially trained for the survey checked the questionnaire to ensure that they were fully completed. Height, weight, and blood pressure were measured. Body mass index (BMI) was calculated as weight (in kilograms) divided by height squared (in meters). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured from the right arm of the subject after sitting for 5 minutes.

Second reexamination. In the 2005–2006 reexamination, the survey methods were similar to those applied in the 1998 reexamination. Cognitive impairment and dementia were identified in 3 phases: screening phase, clinical phase, and differential diagnosis phase. In the screening phase, subjects who scored ≤24 in the Mini-Mental State Examination (MMSE),[18] had a decline of ≥3 points in MMSE since the 1998 reexamination, or had a delayed recall in Consortium to Establish a Registry for Alzheimer's Disease word list[19] of <70%, or for whom there was serious informant concern regarding the participant's cognition, were referred for thorough neurologic, cardiovascular, and detailed neuropsychologic examinations (the clinical phase). A review board consisting of the study physician, the study neuropsychologist, and a senior neurologist ascertained the primary diagnosis based on all available information. Subjects with possible dementia were invited to the differential diagnosis phase, which included brain imaging, CSF analysis, EKG, and blood tests. All data accumulated from the screening and clinical phases were carefully reanalyzed by the review board before establishing the final diagnosis. Dementia was diagnosed according to *DSM-IV* criteria,[20] and AD was diagnosed according to the US National Institute of Neurologic and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association criteria.[21]

Standard protocol approvals, registrations, and patient consents. The CAIDE study was approved by the local ethics committee (University of Kuopio and Kuopio University Hospital, Kuopio, Finland), and written informed consent was obtained from all participants.

Biochemical analyses. Venous blood samples were taken at the 1998 reexamination and serum specimens were stored at or below −20°C until analysis at the National Institute for Health and Welfare. Serum tHcy was determined by chemiluminescent microparticle immunoassay and serum folate was determined by chemiluminescent microparticle folate binding protein assay by Architect *i* System (Abbott Laboratories, Abbott Park, IL). The interassay coefficients of variation (CV) of homocysteine were 5.9% and 5.4% at the levels of 6.6 μM/L and 11 μM/L and for folate 13% and 11% at the levels of 7.5 and 31 nM/L. Holotranscobalamin was measured by microparticle enzyme immunoassay by AxSym System (Active-B12 [holotranscobalamin], Axis-Shield, Dundee, UK, Abbott Laboratories). At the levels of 48 and 97 pM/L, the interassay CV were 7.1% and 8.0%.

Blood leukocyte samples were analyzed to determine *APOE* genotype in 1998. To extract DNA, a standard phenol-chloroform technique was used; *APOE* genotypes were analyzed by PCR and *Hha*I digestion.[22] Participants were classified as positive for the *APOE* ε4 allele genotype if they had 1 or 2 ε4 alleles.

Statistical analyses. Differences between the AD and no dementia groups were assessed using binary logistic regression, with diagnosis as the dependent variable, and results are presented as mean (SD) for continuous or number (%) for categorical variables. Homocysteine and holoTC serum concentrations were further categorized by using the corresponding median values: low tHcy was defined as concentrations ≤12.3 μmol/L and low holoTC was defined as concentrations ≤83.3 pmol/L and individuals were compared according to median defined tHcy or holoTC categories.

The associations between tHcy, holoTC, folate (as continuous variables), and subsequent AD development were examined using multiple logistic regression analyses. We present the results as odds ratios (ORs) with 95% confidence intervals (CI). Analyses were adjusted for baseline age, sex, years of full-time education, and follow-up time (model 1), and then additionally for other potential confounding or mediating factors, including *APOE* ε4 status, baseline BMI, SBP, DBP, MMSE score, history of stroke, and smoking (model 2). All variables were entered as continuous into the models except sex, *APOE* ε4, history of stroke, and smoking, which were dichotomized. As creatinine values were not available, additional analyses were adjusted for presence of renal conditions (yes/no) during the study. No participants used B-vitamin or other vitamin supplementations. No mandatory folic acid fortification is performed in Finland.

We also ran additional analyses to investigate the effects of holoTC or folate on the relation between tHcy and AD, as well as the effects of tHcy or folate on the relation between holoTC and AD. Interaction terms were entered in the models in order to investigate possible interactions between tHcy, holoTC, and folate in relation to AD risk, as well as tHcy–*APOE*, tHcy–sex,

Table 1 Baseline characteristics of the study population[a]

Characteristics	No dementia (n = 254)	AD (n = 17)	p Value
Age, y	70.5 (3.5)	73.4 (4.3)	0.003
Sex, women	155 (61.0)	13 (76.5)	0.213
Education, y	9.1 (3.2)	9.4 (4.9)	0.73
MMSE	26.3 (2.0)	25.8 (2.1)	0.24
BMI, kg/m^2	28.0 (4.0)	25.3 (3.4)	0.005
Systolic blood pressure, mm Hg	153.3 (21.5)	142.2 (22.9)	0.042
Diastolic blood pressure, mm Hg	82.8 (10.0)	77.2 (9.7)	0.03
APOE ϵ4 allele	82 (32.3)	10 (58.8)	0.032
History of stroke	15 (5.9)	1 (5.9)	0.997
Ever smoked	90 (35.4)	4 (23.5)	0.324
tHcy, μmol/L	12.6 (3.1)	14.9 (5.9)	0.01
HoloTC, pmol/L	93.3 (51.6)	61.6 (27.4)	0.01
Folate, nmol/L	7.1 (3.9)	7.9 (3.4)	0.39
Duration of follow-up, y	7.4 (0.3)	7.5 (0.4)	0.142

Abbreviations: AD = Alzheimer disease; BMI = body mass index; holoTC = holotranscobalamin; MMSE = Mini-Mental State Examination; tHcy = total homocysteine.
[a] Values are mean (SD) or n (%).

tHcy–age, holoTC–*APOE*, and holoTC–sex, holoTC–age interactions. The level of significance was <0.05 in all analyses. PASW for windows (17th ed.; Chicago, IL) was used for all statistical analyses.

RESULTS The baseline sociodemographic and clinical characteristics of the 271 participants are presented in table 1. After 7.4 years of follow-up, 17 (6.2%) subjects were diagnosed with AD and 254 individuals represented the control group. The mean (SD) age of subjects was 70.7 (3.6) years and 62% were female. As expected, people who developed AD were older at baseline and had lower BMI, lower BP, and higher frequency of *APOE* ϵ4 allele. They also had higher tHcy and lower holoTC levels compared to subjects without dementia.

Comparing participants based on median tHcy values (12.3 μmol/L) revealed that those with higher tHcy were older (71.2 [3.9] vs 70.2 [3.4] years, $p = 0.027$), less likely to be female (46.6% vs 73.5%, $p < 0.001$), and had lower holoTC (72.4 [36.6] vs 105.5 [55.8] pmol/L, $p < 0.001$) and folate (5.8 [2.9] vs 8.2 [4.2] nmol/L, $p < 0.001$) levels. No significant difference was observed for other clinical characteristics. However, when the cutoff was set at the 80th percentile of tHcy (15 μmol/L), more people with AD tended to belong to the high homocysteine group (12% vs 5%, $p = 0.064$).

Individuals were further compared according to median holoTC values (83.3 pmol/L); those with higher holoTC were younger (70.0 [3.3] vs 71.4 [3.9] years, $p = 0.002$) and had lower tHcy (11.6 [3.0] vs 13.8 [3.5] μmol/L, $p < 0.001$) and higher folate (7.7 [3.8] vs 6.7 [3.9] nmol/L, $p = 0.047$) values. In addition, more people with AD tended to belong to the low holoTC group (9% vs 4%, $p = 0.077$).

tHcy and risk of developing AD. The OR of AD for each increase of 1 μmol/L in baseline serum tHcy values was 1.16 (95% CI 1.04–1.31). This association remained after adjusting for age, sex, education, and follow-up time (model 1). Furthermore, adjusting for *APOE* ϵ4, BMI, MMSE, SBP, DBP, history of stroke, and smoking did not influence the tHcy–AD relationship (table 2). When analyses were stratified according to median holoTC, the association between tHcy and AD remained only in individuals who had holoTC below median values (OR = 1.19 [1.04–1.37], $p = 0.014$).

HoloTC and risk of developing AD. The association between holoTC and AD risk is shown in table 2. Serum holoTC values were related to decreased risk of AD; OR was 0.980 (95% CI 0.965–0.995) for each increase of 1 pmol/L in baseline serum holoTC. This association remained even after adjusting for all study covariates. Additional controlling for presence of renal conditions did not alter the results (OR [95% CI] were 1.19 [1.01–1.39] for tHcy and 0.977 [0.957–0.997] for holoTC). No significant relation between folate and AD was detected (table 2).

Combined effect of tHcy, holoTC, and folate. The relation between tHcy and risk of AD was slightly attenuated by adjusting for holoTC: OR for tHcy changed from 1.16 to 1.10 (95% CI 0.96–1.25) (table 3). The association between holoTC and AD risk was less influenced by controlling for tHcy: OR for holoTC changed from 0.980 to 0.984 (95% CI 0.968–1.000). Adding folate to the models did not change the association between tHcy and AD (OR [95% CI] 1.17 [1.04–1.31]) or holoTC and AD (OR [95% CI] 0.981 [0.966–0.996]) appreciably (table 3). No evidence of interaction was detected between tHcy, holoTC, or folate in relation to AD risk when interaction terms were entered into the models. In addition, no significant interactions

Table 2 Odds ratios (95% confidence intervals) examining the association of serum homocysteine, holotranscobalamin, and folate with incident Alzheimer disease

Variable	Model 1[a]	Model 2[b]
Homocysteine	1.18 (1.02-1.36)	1.19 (1.01-1.39)
Holotranscobalamin	0.980 (0.963-0.997)	0.977 (0.958-0.997)
Folate	1.03 (0.91-1.16)	1.01 (0.87-1.18)

[a] Model 1: adjusted for age, sex, education, and duration of follow-up.
[b] Model 2: additionally adjusted for *APOE* ϵ4 allele, body mass index, Mini-Mental State Examination, systolic blood pressure, diastolic blood pressure, smoking, and history of stroke.

Table 3 Odds ratios (95% confidence intervals) examining the combined association of serum homocysteine, holotranscobalamin, and folate with incident Alzheimer disease

Independent variable	Crude model	Adjusted for			
		Homocysteine	Holotranscobalamin	Folate	Both
Homocysteine	1.16 (1.04–1.31)	—	1.10 (0.96–1.25)	1.17 (1.04–1.31)	1.10 (0.97–1.26)
Holotranscobalamin	0.980 (0.965–0.995)	0.984 (0.968–1.000)	—	0.981 (0.966–0.996)	0.985 (0.970–1.001)
Folate	1.05 (0.94–1.17)	1.07 (0.96–1.19)	1.06 (0.95–1.18)	—	1.06 (0.95–1.18)

were found between tHcy and *APOE*, tHcy and sex, tHcy and age, holoTC and *APOE*, and holoTC and sex in relation to the risk of AD (results not shown). However, a significant interaction between holoTC and age was observed; the protective effect of holoTC became more pronounced with increasing age (adjusted OR for the interaction 0.994, 95% CI 0.989–0.998).

Stratified analyses by *APOE* ϵ4 status or by sex did not reveal any significant differences between *APOE* ϵ4 carriers and noncarriers or between men and women with respect to the association of tHcy or holoTC with AD risk (results not shown).

DISCUSSION Our results indicate that elevated serum tHcy concentrations measured 7.4 years earlier is associated with an increased risk of developing AD. The observed association appeared to be independent of age, sex, education, *APOE* ϵ4 genotype, renal conditions, and other potential confounders including common vascular risk factors. In addition, higher holoTC values were independently related to reduced AD risk. The protective effect of holoTC was more pronounced with increasing age.

These results are in line with previous prospective studies on late-life tHcy levels and risk of AD. Data from the Framingham study (follow-up over 8 years),[5] the Conselice Study of Brain Aging in Italy (follow-up 4 years),[4] and the Kungsholmen Project in Sweden (follow-up 6.7 years)[3,23] indicated elevated tHcy as a risk factor for AD. Furthermore, high midlife tHcy levels increased the risk of late-life AD 35 years later in the Prospective Population Study of Women in Gothenburg.[8] In addition, the Sacramento Area Latino Study on Aging (follow-up 4.5 years) reported that elevated tHcy associated with low B12 status had the strongest association with combined incidences of dementia and cognitive impairment without dementia. In contrast, in the WHICAP project no significant association between tHcy and AD was detected after adjustments.[9] Possible explanations for this difference are the relatively short follow-up period (4.7 years) and the rather homogenously high tHcy concentrations in this sample (which did not permit enough variability to detect an association).[4,9]

The association between holoTC and AD has previously been less investigated. One case-control study reported lower holoTC values in patients with AD.[24] Another study which additionally examined TC776C>G polymorphism (a genetic determinant of holoTC that has been suggested to influence AD risk in some studies[25]) found no difference in holoTC values between patients with AD and controls, although genotype influenced the age at disease onset.[26] In addition, results from the Kungsholmen Project showed that moderate (third quartile) but not high (fourth quartile) holoTC levels are associated with reduced AD risk at follow-up.[3]

The exact mechanisms behind the observed associations remain to be determined, but certain hypotheses can be considered. The effects of holoTC or B12 on AD risk may be partly mediated by tHcy, since tHcy concentration is dependent on vitamin B12 status.[1,2] High tHcy levels have been related to endothelial dysfunction, impaired nitric oxide activity, atherosclerosis,[27,28] and subsequent increase in the risk of various cardiovascular and cerebrovascular events which may increase the risk of dementia and AD.[29-32] Experimental studies have shown that elevated homocysteine may potentiate β-amyloid peptide generation[33] and its neurotoxicity,[34] or may cause DNA damage[35] and impair DNA repair in neurons.[36] In addition, homocysteine may convert to homocysteic acid, a highly potent neurotoxic metabolite and an *N*-methyl-D-aspartate receptor agonist,[37] which may further promote β-amyloid peptide generation in the brain.[37]

Alternatively, the effects of holoTC or B12 may be mediated through its impact on S-adenosylmethionine (SAM) concentrations. SAM is the primary methyl donor in many biochemical reactions involved in normal brain functions, including the production of cell-membrane phospholipids, myelin, monoaminergic neurotransmitters, and nucleic acids.[15,38] Vitamin B12 is needed for remethylation of homocysteine to methionine and subsequent formation of SAM. Deficiency of SAM may be linked to white matter damage and brain

atrophy,[14] factors associated with cognitive decline and dementia.[38] One recent study reported an association between B12 status and WML severity. However, no relation between B12 indicators (including holoTC) and cognition was detected. The authors concluded that the influence of B12 on WML may be too small to result in effects on cognition. The lack of association with cognition may be due to differences in B12 status, other population characteristics, and in approach (i.e., focus on elderly without dementia, unspecified dementia incidence and type, WML–cognition link not investigated in the study).[15]

Little is currently known about the interactions between tHcy and holoTC in relation to AD risk. In the present study, interaction terms were not significant. However, the tHcy–AD association was attenuated by adjusting for holoTC. This could be due to the small sample size, but it may also point to an important effect of holoTC on tHcy, suggesting that holoTC itself may explain the tHcy–AD relationship. Interestingly, the holoTC-AD link was less influenced by adjusting for tHcy. This supports the hypothesis that factors other than tHcy may also explain the association between holoTC and incidence of AD.

Folate was not related to AD risk in our study, nor were there any significant interactions between folate, tHcy, and holoTC in relation to AD. Low folate levels have been indicated as a risk factor for AD in some (but not all) studies.[1] Differences in results could be due to population differences. However, similar to our results, no association between folate and AD or dementia was found in the Kungsholmen Project, suggesting that tHcy or holoTC may be a better and earlier marker for AD.[3]

The strengths of this study are the population-based design, follow-up period of at least 7 years, and evaluation of both late-life tHcy and holoTC in relation to incident AD. Compared to vitamin B12 values, holoTC levels may represent a more sensitive assay of B12 status.[16,24] Also, a large number of potential confounding factors were taken into account. The 271 subjects are from a well-characterized longitudinal study (CAIDE) specifically designed to investigate risk factors for dementia and AD. The long follow-up period, the comprehensive evaluation and diagnostic protocol at each examination and recruitment of dementia-free subjects at baseline, and adjusting the analyses for baseline MMSE make our findings less prone to the influence of reverse causality (i.e., effects of preclinical dementia on tHcy or holoTC).

The main limitations of our study include the relatively small sample size, and availability of tHcy or holoTC measurements at only one time point, which may underestimate their associations with the disease.[2,39] tHcy was measured in serum.[2] Since creatinine values were not available, history of renal conditions was considered in the analyses. Selective survival may also have contributed to underestimation of the relation between tHcy and AD risk, because elevated tHcy has been related to increased mortality in previous studies.[2] Larger studies are needed to investigate possible differences between *APOE* ϵ4 carriers and noncarriers or between men and women regarding the effects of tHcy or holoTC on incident AD. Although holoTC may be an earlier and more sensitive marker of B12 deficiency,[16,24] the best indicator or combination of indicators of B12 status (i.e., B12, methyl malonic acid) in relation to AD risk remains to be determined.

Our results indicate the involvement of both serum tHcy and holoTC in the development of AD. This emphasizes the need for further studies on the role of sensitive markers of B12 status in identifying individuals who are at increased risk of AD. High Hcy and low levels of vitamin B12 are surprisingly common conditions in the elderly, both in developed and developing countries.[1,40] However, few randomized controlled trials have so far investigated the usefulness of vitamin B12 supplements in preventing cognitive impairment or dementia, with mixed results.[1,38] Limitations of statistical power, study duration, and choice of target population make such studies difficult to interpret. Supplementation may be most effective in prevention during a critical time window, and larger and better planned randomized controlled trials are necessary to formulate efficient treatment guidelines (dose, treatment start and duration, target population).

AUTHOR CONTRIBUTIONS

Statistical analysis was conducted by Dr. Babak Hooshmand, Dr. Ingemar Kåreholt, and Dr. Alina Solomon.

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DISCLOSURE

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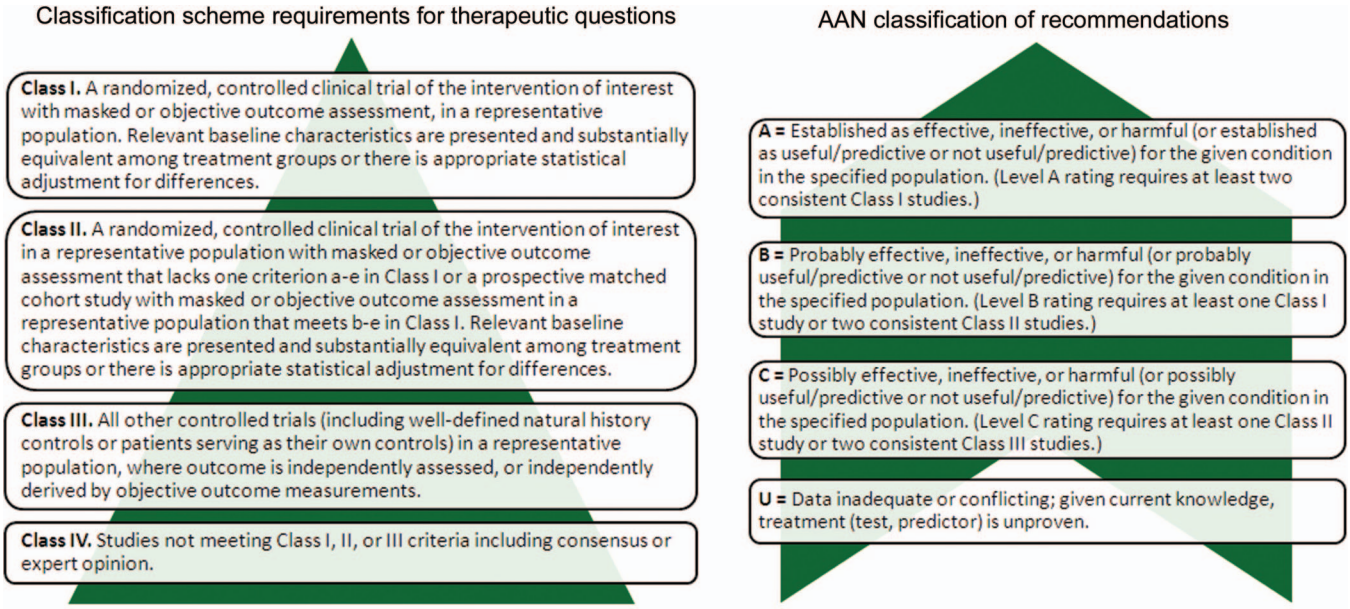

Homocysteine and holotranscobalamin and the risk of Alzheimer disease: A longitudinal study

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