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Development of dementia in mild cognitive impairment (MCI) patients with focus on B-vitamins

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In the memory of
Hedvig Maria

Abstract - English

This thesis focused on the importance of low B-vitamins, hyperhomocysteinemia and the development of dementia and Alzheimer´s disease (AD) in patients with mild cognitive impairment (MCI). The relation between vitamin B_{12}/folate and dementia has been extensively investigated, but the results are inconsistent. The connection of thyroid stimulating hormone (TSH) and dementia is also explored in a lot of studies but neither here is a consensus found. The active fraction of vitamin B_{12}, holo-Transcobalamin (holo-TC), is less studied. The first three studies this thesis were using data from the Geriatric Department of Karolinska University Hospital, Huddinge, Stockholm; the first study also from the Geriatric Department of Falun Hospital. In the fourth study survey data was used from the Kungsholmen Project, a longitudinal population-based study on aging and degenerative disorders in Stockholm.
In *paper I,* we compared the serum levels of vitamin $B_{12,}$ folate and homocysteine in 120 persons with MCI, living either single or in a family setting. We found a higher level of serum homocysteine in men living single compared to men living in a family.
In *paper II,* we investigated the association of homocysteine levels and the development of in 136 MCI patients over a three-year period. The total number of persons who converted to AD was 31%. For the women we found that 45% with Hcy levels >16µmol/L converted to AD compared to 21% with Hcy levels <16µmol/L. Of the men 33% with Hcy levels >20µmol/L, 50% with Hcy levels 20-17µmol/L and none of those with levels<17µmol/L converted.
In *paper III,* we studied predictive factors for the development of AD over a six-year period in 93 subjects with MCI. The percentage of persons converting to AD was 34%. The most important predictive factors were low TSH levels and hyperhomocysteinemia together with low Mini Mental State Examination (MMSE) and age. The importance of Hcy declined with higher age.
In *paper IV,* we investigated the association between total plasma homocysteine (tHcy) and holo-TC and the subsequent development of dementia and AD in 228 persons in a prospective study with a mean follow-up time of 6.7 years. 41% developed dementia of which 31% AD. Subjects with high tHcy (the 4^{th} quartile) had more than twice as high risk of developing AD than persons with low tHcy (the 1^{st} quartile). The 3^{rd} quartile of holo-TC was associated with a reduced risk of AD.

In conclusion the results of our studies suggest that hyperhomocysteinemia may be a risk marker for dementia and AD. This finding can only be confirmed in intervention clinical trials showing a prevention of the development of dementia by lowering of homocysteine. It is not possible to rule out the importance of TSH for the development of dementia from our results, and we found no clear association between dementia and holo-TC.

Abstrakt – Svenska

Denna avhandling har fokuserat på betydelsen av låga B-vitaminnivåer, förhöjt homocystein (Hcy) och utvecklingen av demens och Alzheimer´s sjukdom (AD) hos personer med lätt nedsatt kognitiv svikt (MCI). Kopplingen mellan vitamin B_{12}/folat och demens har blivit föremål för ett intensivt studium men resultaten är hitintills motsägande. Relationen mellan thyroid stimulerande hormon (TSH) och demens har också undersökts i ett stort antal studier men inte heller här har koncensus uppnåtts. Den biologiskt aktiva fraktionen av vitamin B_{12}, holo-Transcobalamin (holo-TC) är mindre studerad. De första tre studierna i denna avhandling har använt data från Geriatriska Kliniken vid Karolinska Universitetssjukhuset, Huddinge, Stockholm, den första studien också från Ger-Rehab. Kliniken, Falu Lasarett. Den fjärde studien har baserats på data från Kungsholmsprojektet, en longitudinell populationsbaserad undersökning av åldrande och degenerativa sjukdomar i Stockholm.
I *studie I* jämförde vi serumnivåer av vitamin $B_{12,}$ folat och homocystein hos 120 personer med MCI, 60 män och 60 kvinnor, varav hälften var ensamboende och hälften levde i familj. Vi fann högre homocysteinnivåer hos ensamboende män än hos de som levde i familj.
I *studie II* undersökte vi sambandet mellan homocysteinnivåer och utvecklingen av AD hos 136 personer med MCI över en treårsperiod. Antalet personer som utvecklade AD var 31 %. Av kvinnorna konverterade 45 % av de med Hcy >16µmol/L till AD jämfört med 21 % av dem med Hcy <16µmol/L. Av männen konverterade 33 % med Hcy >20µmol/L, 50 % med Hcy 20-17µmol/L och ingen av männen med Hcy <17µmol/L.
I *studie III* studerade vi prediktiva faktorer för utveckling av AD hos 93 personer med MCI över en period av sex år. 34 % av personerna utvecklade AD. De viktigaste prediktiva faktorerna var låga TSH- och förhöjda homocysteinnivåer tillsammans med låga Mini Mental State Examination (MMSE) nivåer och hög ålder. Den prediktiva betydelsen av förhöjt Hcy avtog med stigande ålder.
I *studie IV* undersökte vi sambandet mellan total plasma homocystein (tHcy), holo-TC och den efterföljande utvecklingen av demens och AD hos 228 personer i en prospektiv studie med en uppföljningstid av i medeltal 6.7 år. 41% av personerna utvecklade demens, 31% av dessa AD. Personer med höga tHcy (4:de kvartilen) hade mer än dubbelt så hög risk att utveckla AD jämfört med personer med låga tHcy. I den 3:de kvartilen av holo-TC fann vi en reducerad risk för AD.

I sammanfattning visar våra resultat att förhöjda homocysteinnivåer kan vara en riskmarkör för utveckling av demens och AD. Dessa resultatet kan verifieras endast genom kliniska försök som kan visa att utveckling av demens kan förebyggas genom att homocysteinnivåerna sänks. Betydelsen av TSH för demensutveckling kan inte fastställas genom våra resultat och vi fann inget klart samband mellan demens och holo-TC.

List of publications

I **Annerbo S,** Lind B, Lökk J, Wahlund L-O. Vitamin B_{12}, Folate and Homocysteine in Persons with Cognitive Impairment. A comparative study of serum levels among persons living single or in a family. Brain Aging 2003; 3:41-47.

II **Annerbo S,** Wahlund L-O, Lökk J. The relation between Homocysteine Levels and Development of Alzheimer´s disease in Mild Cognitive Impairment Patients. Dementia and Geriatric Cognitive Disorders 2006; 20:209-214.

III **Annerbo S,** Wahlund L-O, Lökk J. The Significance of Thyroid-Stimulating Hormone and Homocysteine in the Development of Alzheimer´s disease in Mild Cognitive Impairment. American Journal of Alzheimer´s Disease & Other Dementias. 2006; 3:182-188.

IV Kivipelto M, **Annerbo S,** Hultdin J, Viitanen M, Fratiglioni L, Lökk J. Homocysteine and Holo-Transcobalamin and the risk of dementia and Alzheimer´s disease: A prospective study. (Manuscript)

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List of abbreviations

AD	Alzheimer´s disease
Aβ	Amyloid β-peptid
APP	Amyloid Precursor Protein
ApoE	Apolipoprotein E
BMI	Body Mass Index
CSF	Cerebrospinal fluid
CDR	Clinical Dementia Rating Scale
CI	Confidence Interval
DLB	Dementia with Lewy Body
DSM	Diagnostic and Statistical Manual of Mental Disorders
EBM	Evidence-based medicine
FAD	Familial Alzheimer´s Disease
FTD	Frontotemporal dementia
Holo-Tc	Holo-Transcobalamin
MMA	Methylmalonate
MCI	Mild Cognitive Impairment
MMSE	Mini Mental State Examination
MTHFR	Methylenetetrahydrofolate reductase
OR	Odds Ratio
PS	Presenilin
SAH	S-Adenosylhomocysteine
SAM	S-Adenosylmethionine
SBU	Swedish Council on Technology Assessment in Health Care
THF	Tetrahydrofolate
TPO-Ab	Thyroid peroxidas antibody
TSH	Thyroid Stimulating Hormone
TRH	Thyroid Releasing Hormone
tHcy	Total Plasma Homocysteine
VaD	Vascular dementia

Introduction

In the past fifty years the average age of the world population has been steadily increasing. In the developed countries the number of persons over 65 years has remarkably increased and the part aged 80+ years is the fastest growing of the elderly population (1). In Sweden, persons aged 80+ comprised approximately 22% of the elderly in 1992. By the year 2025 this group is expected to rise to 32% (2). In connection with the aging of the population the age-related diseases have increased and the focus has been set on the causes of dementia.

Dementia diminishes the individual's ability to live independently and the quality of life. In addition, it imposes a major burden on the health care system. It would therefore be of great benefit if modifiable risk factors for dementia could be identified.

Definition of Dementia

Dementia is a clinical syndrome characterized by impairments in memory and other cognitive functions that are severe enough to affect social, daily life and occupational functioning and represent a significant decline from a previous level of functioning. As dementia is a syndrome with many causes, the diagnoses will be based on fulfillment of a set of criteria. The most commonly used definitions of dementia has been given in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, IV edition (DSM-IV) (3), and World Health Organization's (WHO) International Classification of Diseases, 10th revision (ICD-10) (4).

Mild Cognitive Impairment

Normal aging is connected with decline in many cognitive domains, such as psychomotor speed, executive functioning and episodic memory. Other functions such as semantic memory appear to remain intact (5). Mild Cognitive Impairment *(*MCI) is representing a transitional state between normal aging and mild dementia and longitudinal outcome results indicate that subjects with MCI are likely to develop AD (Alzheimer´s disease) at an accelerated rate (6).

Recommended criteria for MCI include:
1) the person is neither normal nor demented
2) there is evidence of cognitive deterioration shown by either objectively measured decline over time and/or subjective report of decline in conjunction with objective cognitive deficits
3) activities of daily living are preserved and complex instrumental functions are either intact or minimally impaired (7).

The term MCI was used for the first time in the Global Deterioration Scale (GDS 3) and later by the Mayo Clinic group (8, 9). GDS is used to classify subjects from GDS 1 (normal) through GDS 7 (severe stages of dementia). MCI could in this scale correspond to 2 or 3 (7).
Another scale, the Clinical Dementia Rating (CDR), stages subjects along a continuum from normal aging (CDR 0) to severe dementia (CDR 3) where CDR 0, 5 includes subjects with MCI and mild AD (10).

MCI-patients are a heterogeneous group. The occurrence of MCI in the population is not investigated in detail but for people above 65 years the ratio is estimated to be about 15% (11). Several subgroups according to the clinical presentation of the cognitive deficit have been proposed: amnestic MCI, multiple cognitive domain slightly impaired MCI, and single non-memory domain MCI (10). These subtypes of cognitive impairment can be associated with different aetiologies such as neurodegenerative processes, ischemia, metabolic disturbance or trauma (7).

About 10-25% of subjects with MCI progress to dementia per year. As people with amnestic MCI are developing AD in a rate of 10-15% per year it is a strong evidence to suggest that amnestic MCI predominantly has a degenerative aetiology (10). The development from MCI is varied, While some progress to dementia, about 1% per year return to normal Mini Mental State Examination (MMSE: 24-30) and some persons remain stable (6, 12). In a newly published report comparing the rates of conversion to AD between subtypes of dementia in a community-based cohort (n= 581) at age 75 and followed up after 30 months, 48,7% of subjects with amnestic MCI and 26,8% with non amnestic MCI converted to AD. The annual conversion rate for amnestic MCI to AD in the study was approximately 19% and to cognitive health approximately 6% (13).

Conversion to AD is most common in the subjects developing dementia but conversion to other dementias such as Vascular dementia (VaD), frontotemporal dementia (FTD), dementia with Lewy bodies and Parkinson´s disease dementia also occurs (13-15).

As MCI represents a transitional stage between normal aging and dementia, it has been proposed that risk factors for MCI would be the same as for AD i.e. advanced age, low education, APOE ε4 allele, midlife hypertension and hypercholesterolemia (16).

The topic of MCI has generated intense interest as efforts to improve the early detection of AD have increased. AD-based therapeutic strategies could probably be initiated at the MCI stage to delay progression to overt dementia (17).

Alzheimer´s disease

Alzheimer´s disease, the most common type of dementia accounting for 60-70% of cases, is a progressive neurodegenerative disease with certain characteristic pathological and clinical features (18). The etiology is heterogeneous and multifactorial, and the characteristics of AD are a diffuse cerebral atrophy with the presence of extra cellular β-amyloid neuritic plaques, intracellular neurofibrillary tangles, neuronal loss and angiopathy. This process occurs 10-20 years before the symptoms of cognitive decline (19).

The diagnosis is essentially clinical and the criteria for probable AD according to the Diagnostic and Statistical Manual of mental Disorders (DSM-IV) include:
1) dementia established by clinical examination, and documented by a standard test of cognitive function, and confirmed by neurological tests
2) significant deficiencies in two or more areas of cognition
3) progressive deterioration of memory and other cognitive functions
4) no loss of consciousness
5) onset from age 40 to 90, typically after 65
6) no other diseases or disorders that could account for the loss of memory and cognition (20).

AD is a slowly progressive disease and the average duration of manifest AD is between 4 and 20 years (21). The disease most often starts with episodic memory impairment. Multiple cognitive domains deteriorate over time, and the diagnosis AD can be considered when the patient together with memory impairment has at least one other cognitive disturbance such as aphasia (language disturbances), apraxia (impaired ability to carry out motor activities despite the intact motor function), agnosia (failure to recognize or identify object despite the intact sensory function) and disturbance in executive functions severe enough to cause impairment in social or work-related functioning. The definitive diagnosis can only be set by autopsy.

The most common symptoms in AD are short-term memory impairment, aphasia and cognitive decline. Other symptoms, not present in all patients are: aggressiveness, agitations, walking behaviour, insomnia, delusions and hallucinations. As the disease progresses, late symptoms such as autonomic dysfunction, spatial and motor disabilities appear. The premature death is often caused by cardiac infarction, pneumonia or other infections.

Other neurodegenerative dementia diseases

Dementia with Lewy-body (DLB) is characterized by a progressive decline of cognition and is the second most frequent neurodegenerative dementing disorder in the elderly after AD. Clinically, it is associated with fluctuating cognition and hallucinations in association with neurological features of Parkinsonism (22). Especially elderly DLB-patients often show AD pathology.

Frontotemporal degeneration (FTD), accounting for 5% of dementia cases, is a disease affecting subjects younger than 70 years, and starts with a changed personality and progressive language dysfunction. The decline in cognition will be a later symptom (23).

In Parkisons´s disease with dementia the symptoms of cognitive decline will appear more than a year later than the onset of symptoms of Parkinsons´s disease.

Vascular dementia

The second most common type of dementia is vascular dementia (VaD). The diagnosis of VaD needs an abrupt onset and the development of dementia is variable. VaD is related to a variety of pathologic lesions including clinical forms of dementia caused by ischemic, hemorrhagic cerebrovascuar disease or by ischemic-hypoxic brain lesions (24). Among aged persons, both vascular and neurodegenerative changes are present and it is often a challenge to differentiate whether the dementia is due to VaD or AD.

Etiology and risk factors

The etiology of dementia is considered to be a result of an interaction between genetic and environmental factors. A recent review from the Swedish Council on Technology Assessment in Health Care (SBU) on risk factors of dementia and AD included studies on risk factors published between 1987 and 2004 (11).The evidence grading was based on the criteria for internal validity and causality of individual studies, and on the reliability and amount of accumulated evidence. In **table 1** a summary of the main proposed risk factors and protective factors for dementia and Alzheimer´s disease are listed.

Table 1 Proposed risk factors for dementia and Alzheimer´s disease.

Dementia	AD
Risk factors	**Risk factors**
Strong evidence Age	**Strong evidence** Age ApoE ε4 allele
Moderate evidence ApoE ε4 allele Familial aggregation Midlife hypertension Diabetes	**Moderate evidence** Midlife hypertension Familial aggregation
Limited evidence Occupational exposure	**Limited evidence** Midlife hypercholesterolemia Occupational exposure
Insufficient evidence Smoking Late-life hypertension Obesity Cardiovascular disease Hyperhomocysteinemia Inflammatory factors Head trauma Aluminium Dietary factors Folate/B12 insufficiencies Depression Low socioeconomic status	**Insufficient evidence** Smoking Diabetes Late-life hypertension Obesity Cardiovascular disease Hyperhomocysteinemia Inflammatory factors Head trauma Aluminium Dietary factors Folate/B12 insufficiencies Depression Low socioeconomic status
Protective factors	**Protective factors**
Moderate evidence High education Leisure time activities Antihypertensive drugs	**Moderate evidence** High education Leisure time activities
Limited evidence Moderate alcohol drinking	**Limited evidence** Antihypertensive drugs Moderate alcohol drinking
Insufficient evidence Non-steroid anti-inflammatory drugs Statins Social network	**Insufficient evidence** Non-steroid anti-inflammatory drugs Statins Social network

Modified from the SBU-report (11)

The most important risk factor for the development of AD is increasing age. Prevalence as well as incidence increase with advanced age and the occurrence doubles every five years after 65 up to 90 years of age, and then remain stable (18).

Another well known risk factor is the presence of the ε4 allele of apolipoprotein E (ApoE ε4) (25). The human ApoE gene, located on chromosome 19, has tree different alleles: ε4 allele, ε2 allele and ε3 allele of which the ε3 allele is the most common and the ε2 allele the most infrequent. ApoE ε4 is found to decrease the age of onset of AD and intensify all the biochemical disturbances characteristic for the disease as tangle formation, neuronal cell death, oxidative stress, dysfunctions in lipid homeostasis and cholinergic signaling (26).

It has also been reported that ApoE ε4 facilitates the deposition of Amyloid β-peptide (Aβ) in the brain (27) and increases the rate of atrophy in the brain (28). ApoE ε4 has been proposed to alter the effects of various vascular and lifestyle related factors for cognitive functioning and dementia, so that the ApoE ε4 carriers might be more exposed to different adverse environmental factors such as vitamin B12 deficiency and hypertension (29, 30). It has also been suggested that Apo Eε4 may be a predictor for patients with mild cognitive impairment in risk to convert to AD (31).

Familial Alzheimer´s disease (FAD) is a group of genetic forms of AD, caused of specific mutations that are inherited in an autosomal dominant manner. Three different genes have been identified in cases of familial early-onset AD: the amyloid precursor protein (APP) gene on chromosome 21, the presenilin (PS) 1 gene on chromosome 14 and the PS-2 gene on chromosome 1. These mutations account for less than 2% of all cases of AD. One of the mutations, the so-called Swedish mutation APP 670/671, clinically located in the middle of Sweden, has been thoroughly studied (32,33). Another mutation, the so-called Artic mutation, was found in a large family in the Northern part of Sweden (34).

Head trauma has also been suggested to increase the risk of dementia but the evidence is not sufficient (35).

A detailed review is presented below on the role of the risk factors that are central to the present thesis, namely: vitamin B_{12}, holo-transcobalamin, folate, homocysteine and TSH.

Vitamin B_{12} and holo-Transcobalamin

Vitamin B_{12} consists of a group of closely related chemical compounds, which cannot be synthesized in the body and must therefore be taken up through food. The vitamin occurs as methyl-, hydroxo- and deoxyadenosylcobalamin forms in food and is mostly bound to proteins.

Vitamin B_{12} in food is set free to a biologically available form by cooking and also by digestion and intestinal enzymes. Saliva contains a protein, haptocorrin (HC) that binds B_{12}. This complex passes through the ventricle and the vitamin is set free by the action of pancreatic enzyme. Free vitamin then binds to a glycoprotein, intrinsic factor (IF), a transport protein produced by the parietal cells of the ventricle. IF is tightly bound to the vitamin and provides protection against proteolysis. The cobalamin-IF complex is then absorbed via special cubilin receptors in the mucous membrane of the distal ileum.

Vitamin B_{12} is then transported in blood by transcobalamin II, which comprises approximately 20-30% of B_{12} in plasma with a short half-life in blood, and by haptocorrins. Only transcobalamin II bound $B_{12,}$ holo-Transcobalamin, is taken up by receptors of the cells. In lysosomes, $B_{12,}$ is released from transcobalamin II by proteolysis. Without transcobalamin II, vitamin B_{12} cannot gain entry into the cell. Haptocorrin bound B_{12} has a long half-life and must be recirculated by the liver through the bile for new uptake in the distal ileum (36).

Cobalamin is co-enzyme in three different biochemical processes and in two different forms. In the synthesis of methionine from homocysteine (Hcy) methylcobalamin is the co-enzyme and in the conversion of methylmalonate (MMA) to succinate and of leucine to β-leucine, adenosylcobalamin is the co-enzyme.

The most common causes of cobalamin deficiency include too low content of IF and intestinal diseases affecting the ileum. Due to the difficulties in accurately measuring the levels of B_{12} in the body, interest has focused on metabolic processes where cobalamin is active so as to find suitable markers of B_{12} deficiency. The metabolites methylmalonate and homocysteine have been used as suitable and sensitive, albeit non-specific markers of B_{12}.

Folate

Folate is found in foods of both plant and animal sources, such as leafy green vegetables, beans, whole grains, potatoes, mushrooms, liver and beef. Folic acid occurs naturally in food as a mixture of mono-and polyglutamate forms. The pure synthetic form is used in fortification of food stuff.

Folates in the food, mostly polyglutamates, are hydrolyzed to monoglutamates, including 5-methyltetrahydrofolate, and absorbed in the proximal jejunum. This occurs with the help of proteins and releasing enzymes in the mucosa of the duodenum and jejunum. In the cells, folate is reduced to tetrahydrofolate and storages in form of polyglutamates.

Folate in blood is contained in erythrocytes (red blood cells, RBC-folate) and plasma. RBC-folate levels indicate a person's folate consumption during recent months, while plasma or serum folate levels reflect the few days prior to blood sampling.

Disturbed transport and metabolism may cause both vitamin B_{12} and folate deficiency in the elderly (37, 38). As the diet in Sweden is low in folate, fortification of grain has been discussed. The daily need of folate is 300 µg/day for adults and 400 µg/day for women of childbearing age with tissue supplies lasting for 3 – 4 months. The fact that high levels of folate may mask a deficiency of vitamin B_{12} it has been an argument against fortification of food in our country. Another argument against folate fortification has been the reported risk of twin birth with increased folate intake (39).

Low levels of B_{12} in combination with folate deficiency have been shown to cause cognitive disturbances in elderly persons (40). Folate deficiency without low cobalamin levels may also cause neurological damage (41). A cohort study of elderly Latinos (n=1798) reported an association between low red blood cells folate and development of dementia despite folic acid fortification (42).

In a selected sample of non-demented Swedish elderly participants, low serum folate (<10nmol/l) and vitamin B_{12} (<150pmol/l) were predictive of AD at a three-year of follow-up (43). The sample however was small (370 subjects), and a clear association was only detected when both vitamins were taken into account. On the other hand other cross-sectional studies have failed to find an association (44, 45).

Homocysteine

Homocysteine is a non-essential sulphur-containing amino acid derived from the metabolism of methionine, an essential amino acid derived from dietary proteins. The metabolism of Hcy comprises a methionine cycle and a linked folate cycle (Figure 1).

The enzymes methionine adenosyltransferase (MAT) and adenosine triphosphate (ATP) mediates the formation of methionine to S-adenosylmethionine (SAM), which is the most important methyl donor in the organism and the only methyl donor in CNS. SAM methylates DNA, proteins, phospholipids, neurotransmitters, myelin sheaths and is also a necessary intermediate in synthesis of the polyamines spermine and spermidine. SAM is demethylated to S-adenosyl-homocysteine (SAH) which is hydrolysed to homocysteine in a reversible reaction. Methionine synthase (MS) mediates the remethylation of homocysteine to mehionine, in a reaction where methylcobalamin (vitamin B_{12}) is a cofactor and 5-methyltetrahydrofolate the methyl donor. MS is present in all mammalian cells (46).

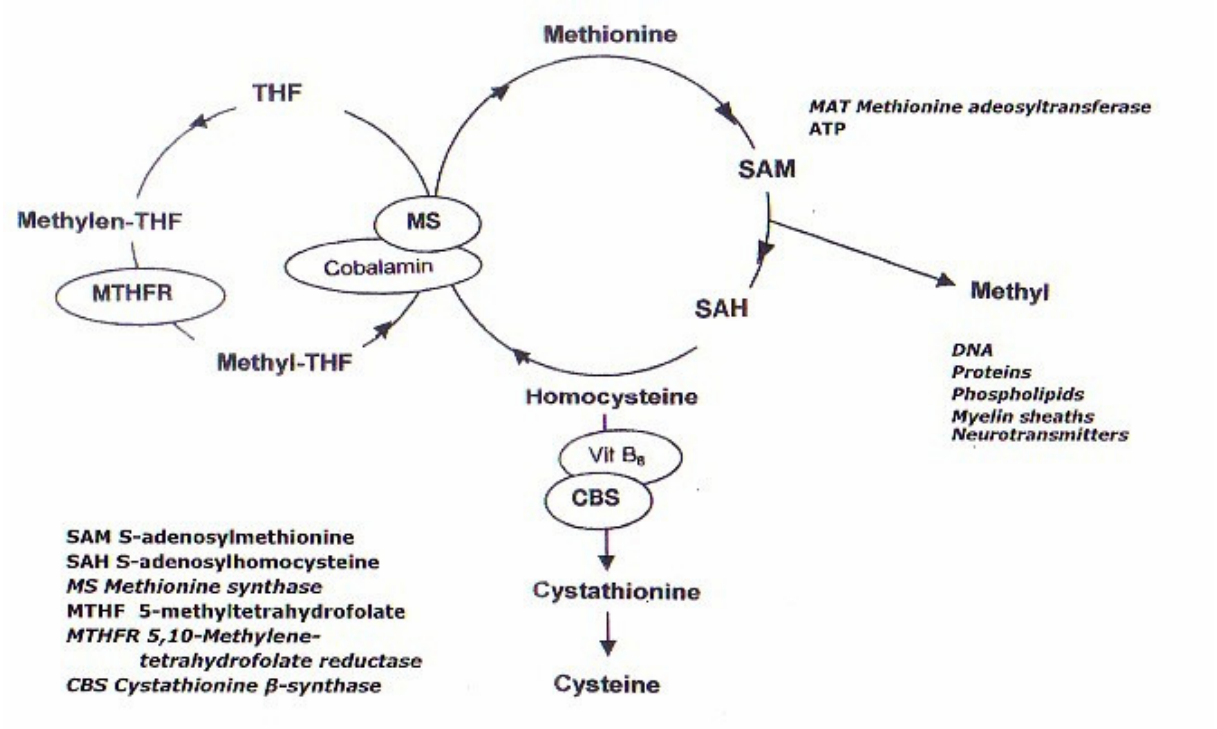


Figure 1. Homocysteine metabolism. The methionine cycle at the right and the folate cycle at the left.

If there is a functional lack of vitamin B_{12} and/or folate, the remethylation will be impaired and homocysteine accumulates. Decreased remethylation of homocysteine may impair the methylation reactions required for normal brain function.
The methyl donor 5-methyltetrahydrofolate is formed from 5, 10-methyltetrahydrofolate in the folate cycle by the enzyme methylenetetrahydrofolate reductase (MTHFR).

Except nutrient related disturbances in the two pathways of Hcy, remetylation to methionine or transsulfuration to form cystathionine, hyperhomocysteinemia can also be caused by genetic mutations. A C>T single nucleotide polymorphism in MTHFR (MTHFR C677) makes the enzyme thermolabile. The T-allele reduces the enzyme activity by approximately two thirds in TT-homozygotes (47). MTHFR enzyme has a key role in the folate cycle for routing 5, 10-MTHFR to either DNA-synthesis or 5-MTHFR, the folate form needed for re-methylation of Hcy to methionine.

The effect of the T-allele on folate and Hcy status is damaging, but emerges to depend on the levels of folate and vitamin B_{12} (48). A recent survey suggested that a synergistic effect of ApoE ε4 and MTHFR C677 related to an insufficiency in the metabolism of folate and vitamin B_{12} could be a risk factor for development of AD (49).

Homocysteine in plasma is mostly bound to plasma proteins, particularly albumin (50). A small amount is unbound and contains a very reactive sulphydryl group that may have toxic effects, for example by producing oxygen radicals (51). A recent study found a significant elevation of plasma concentrations of Hcy in patients with Parkinson's disease (PD) who were taking levodopa. (52).This could possibly be due to consumption of methylgroups when forming dopamine from levodopa in the dopamine depleted Parkinson patients. Clinically it has recently been reported that elevated Hcy levels in Parkinson patients could be a predictive factor for hip fractures (53).
The positive association between Hcy and amyloid β-protein levels in this study increase the possibility that these circulating factors could interact to effect AD risk and cognition in PD (54).

Hcy can also be toxic to neurons and can enhance their vulnerability to being damaged by Aβ (55). Deposition of Aβ in the brain increases as a part of normal aging but it seems that Hcy accelerates dementia by stimulating Aβ deposition in the brain (56). The mechanisms by which homocysteinemia create vascular damage is probably through endothelial injury caused by oxidative stress (57, 58). (**Figure 2)**

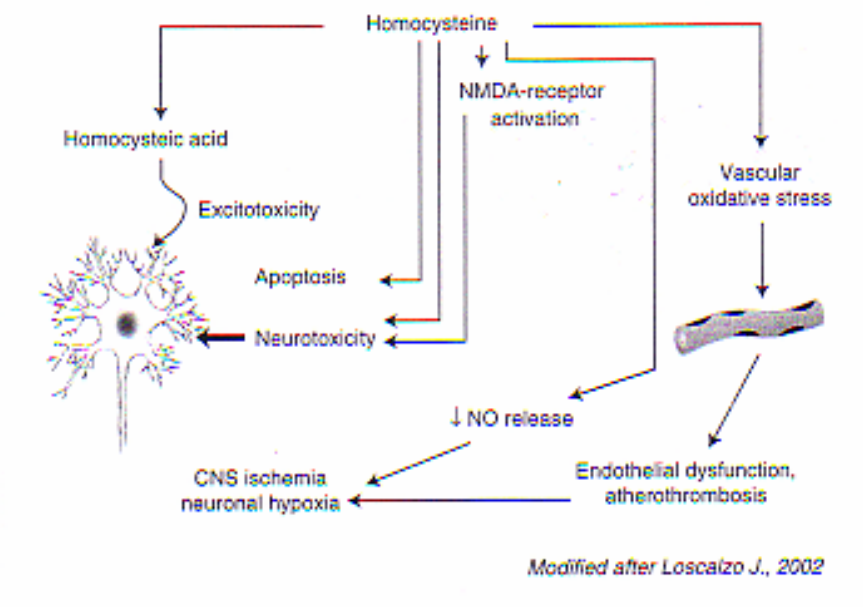


Figure 2. How homocysteine exerts neurological damage.Modified from Loscalco (58). (NMDA = N-methyl-D-aspartrate; CNS = central nervous system)

In spite of the numerous surveys suggesting hyperhomocysteinemia being a risk factor for AD the evidence is still insufficient. The Swedish Council on Technology Assessment in Health Care (SBU) has in a recent report evaluated the possible association between homocysteine and dementia/AD in all studies on this topic between 1985 and 2004. The result was that only one positive and one negative survey of medium/high quality together with three positive and two negative studies of medium/low quality fulfilled strict evidence-based medicine (EBM) criteria (11). However, a newly published population based study (n=18.000), the Hordaland Homocysteine Study, reported that increased Hcy levels were associated with cognitive deficit whereas low Hcy levels were associated with better physical and mental health (59).

The investigations of hyperhomocysteinemia mostly have focused on development of dementia, but only a few are concerned with specific cognitive abilities during normal aging. In a current study of cognitively normal subjects increased plasma Hcy levels were correlated to poorer performance at a specific measure of language abilities (60).
It is of importance to notice hyperhomocysteinemia also is dependent of an interaction of both inherited and acquired factors as well as lifestyle. **(Figure 3)** (61).

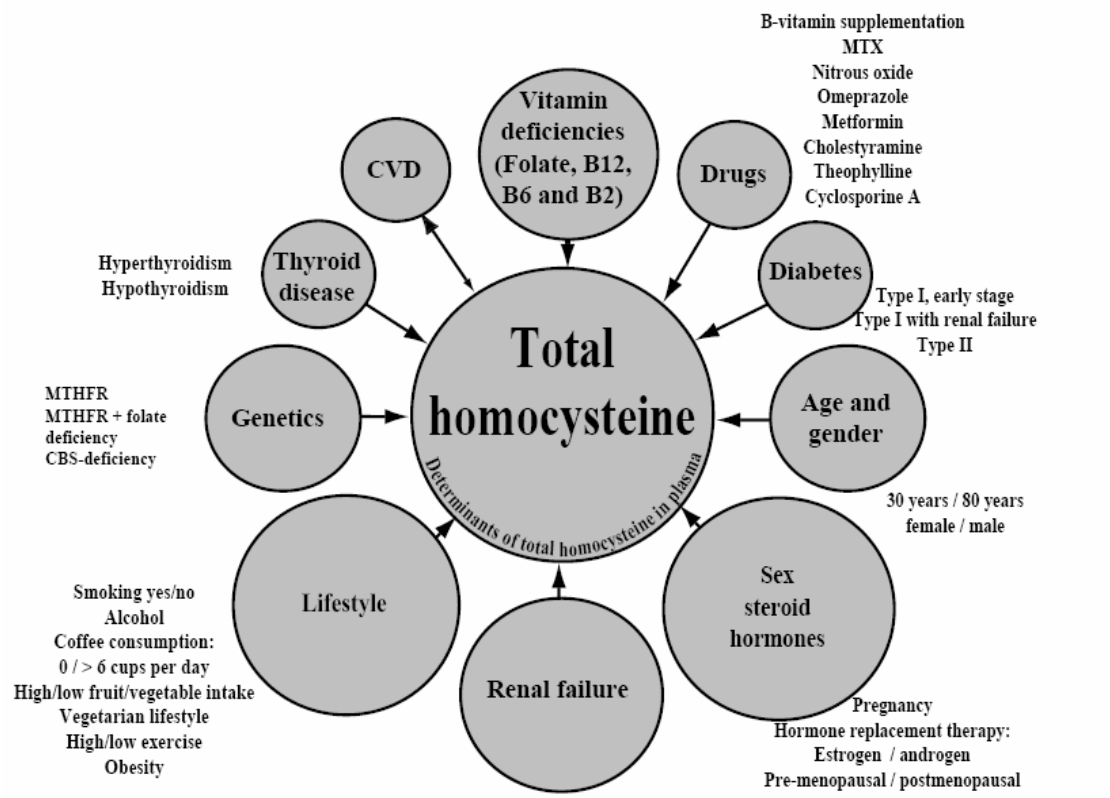


Figure 3 – Interaction between physiological and patophysiological factors that influence vitamin status and the homocysteine metabolism. *Modified after Schneede J. Seminars in thrombosis and hemostasis. 2000* (61).

Methylmalonate (MMA)

Branched amino acids are important precursors for neurotransmitter synthesis in the brain. The brain tends to decrease in size and weight with age, and the synthesis of neurotransmitters may be reduced. Methylmalonate is a dicarboxylic acid related to the metabolism of branched amino acids such as valine, isoleucine and methionine and fatty acids.

Proprionyl CoA and methylmalonyl CoA are intermediates in the breakdown of the amino acids. Proprionyl CoA is carboxylated at the expence of an ATP in a reaction catalyzed by proprionyl-CoA carboxylase to D-methylmalonyl-CoA.

The D-methylmalonyl-CoA is racemized to L-methylmalonyl-CoA, the substrate for the methylmalonyl CoA-mutase that converts it into succinyl-CoA with 5´-Deoxyadenosyl-cobalamin as co-enzyme. In cobalamin deficiency the methylmalonyl CoA is converted to MMA and results in an intracellular increase in MMA followed by increased levels in the serum. The restricted catabolism of MMA disturbs the synthesis of fatty acids and pathologic fatty acids can result in a decrease of myelin production and brain injury (62).

Analysis of p-MMA has been suggested of being a more specific and sensitive marker for vitamin B_{12} deficiency than p-Hcy (63). A newly reported survey showed that subjects with higher serum concentrations of MMA had higher concentrations of Hcy in cerebrospinal fluid (CSB), suggesting that low vitamin B_{12} status (indicated by raised serum concentration of MMA) can partly cause neurological damage by increasing Hcy in the brain. As vitamin B_{12} has a limited role in determining of MMA in cerebrospinal fluid (CSF), elevated MMA in CSF could be related to increased substrate rather than to decreased B_{12} status or methyl malonyl mutase activity (64).

Thyroid-stimulating hormone

Thyroid-stimulating hormone (TSH) targets the thyroid gland and triggers the release of thyroid hormones. TSH is released in response to thyroid hormone-releasing hormone (TRH) from the hypothalamus. As circulating concentrations of thyroid hormone raise, the rates of TRH and TSH production decline.

A low TSH-level is found in persons with hyperthyroidism and can also be a result of thyroxin substitution which leads to suppressed TSH in hypothyroids. Some decrease in TSH secretion in older subjects has been supported by the observation that among patients with primary hypothyreoidism, serum TSH concentrations are lower in older patients as compared with younger ones (65).

This might be associated with a decrease in TRH secretion. One explanation for the decrease in TRH and TSH secretion in older subjects could be their lesser need for thyroxin (T_4) secretion. Hence, TSH secretion most likely decreases slightly with age, although serum TSH concentrations generally remain within the reference range for younger subjects (66).

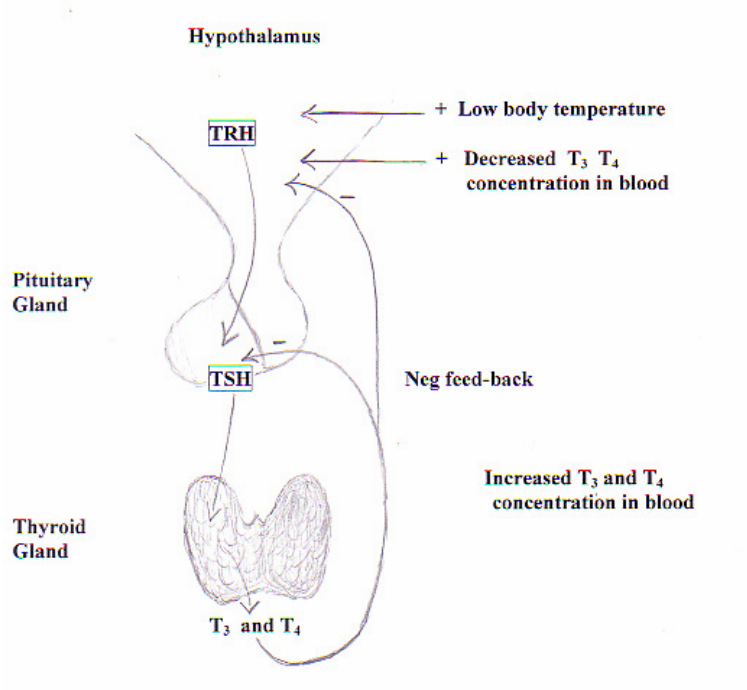


Figure 4 – Regulation of thyroid hormones.

In spite of the known effects of clinical thyroid disorders on cognitive function, little is known about the relationship between thyroid hormone levels and cognitive performance among older persons with levels of thyroid hormone within the normal reference range. A lot of studies have investigated the association between dementia, particularly AD, and thyroid function, but the findings are inconsistent. Studies are reporting either no significant association (67-70) or an association between hypothyroidism and AD (71, 72).

One survey suggested subclinical hyperthyroidism to be related to a higher risk of AD, in the presence of serum antibodies to thyroid peroxidase (TPO-Ab), with a more than threefold increased risk of dementia and AD at follow-up, after an average of two years, in subjects with reduced TSH levels at baseline (73).

However, individuals whose TSH levels were slightly reduced within the normal range had an increased risk for AD (74).
Subclinical thyroid diseases, with variations in thyroid function within the normal range and, elevated or suppressed TSH levels with normal levels of triijodothyronine (T_3) and T_4, have often been connected with cognitive dysfunction (75,76). Moreover, association between hyperhomocysteinemia and decreased TSH has also been reported in some studies (74, 77).

There is an open question whether thyroid dysfunction results from or contributes to AD pathology. The hippocampus and nucleus of amygdala in the medial temporal lobe have a high density of thyroid hormone receptors and patients with mild AD have more atrophy in those parts of the brain compared to healthy elderly (78), though a recent study could not find an association between TSH levels and hippocampal or amygdalal atrophy (70).

Anyway, there are a number of possible explanations for the finding of lowered TSH levels in AD. A decrease of the thyrotrophic-releasing hormone (TRH) can enhance the phosphorylation of tau-protein and other proteins that are potentially involved in the pathogenesis of AD (76). TRH increases the acetylcholine synthesis and release in rats, indicating that reduction of TRH may cause acetylcholine decrease, an important factor in the development of AD (79). It is also possible to think of an additive effect on the combination of low TRH and low folate as the neuron myelination is dependent of the folate levels.

As well subclinical as clinical thyroid diseases are connected with cardio-vascular disease and vascular risk factors (80-82), and with accumulating epidemiologic evidence that vascular risk factors increase the risk of AD, one probable explanation for the connection between thyroid function and AD might involve a mediating role of those factors (74).

Aims

The general aim of this doctoral thesis is to examine whether hyperhomocysteinemia will affect the risk of dementia and AD.

The specific aims of the four studies are:

* To compare levels of vitamin B_{12}**,** folate and homocysteine in subjects with MCI living either single or in a family setting.

* To investigate over a period of three years the connection of hyperhomocysteinemia and the development of dementia in subjects with MCI.

* To investigate the predictive importance of vitamin B_{12}**,** folate, homocysteine, MMSE, BMI, standard laboratory parameters as hemoglobin, glucose, cholesterol and TSH for the development of Alzheimer´s disease in persons with a MCI-diagnosis over a six year period.

* To investigate the predictive importance of homocysteine and holo-Transcobalamin for the development of dementia and Alzheimer´s disease.

Subjects and methods

Study population

Studies I – III had a clinical based setting with subjects from the Geriatric Department, Huddinge University Hospital and for Study I also from the Department of Geriatrics-Rehabilitation, Falun Hospital.

Study IV was performed as an epidemiological study with data derived from the Kungsholmen Project, a longitudinal population-based study on aging and dementia that began collecting data in 1987.

Study I

In the first study we examined the association between vitamin B_{12}**,** folate, homocysteine and MMSE score in persons with MCI. The subjects were recruited from the Department of Geriatrics-Rehabilitation, Falun Hospital, and the Geriatric Department, Huddinge University Hospital. The survey comprised 120 persons, 60 men and 60 women. In each group half of the subjects lived in a family and the other half lived single.

Information on age, sex, weight, diagnosis, form of habitation, vitamin B_{12}**,** folate, creatinine and MMSE score was recorded.
Six persons who were not able to do the MMSE- test were excluded before the statistical analyses. We compared the mean levels of the parameters between the groups of different habitation and the correlation between Hcy and other parameters. Student´s *t*-test was used for differences between groups and Pearson´s correlation coefficient (r) was used for studying the correlation of variables. All statistics were performed in the Excel program of Microsoft Software and the level of significance was set at < 0.05 in all analyses.

Study II

In the second study we investigated the importance of homocysteine levels on the subsequent development of Alzheimer´s disease for a follow-up time of three years in MCI patients recruited from the memory investigation unit of the Geriatric Department, Huddinge University Hospital. Hcy was analyzed in 136 persons, 68 men and 68 women, at the first visit to the Department.

Information on age, sex, vitamin B_{12}**,** folate, hemoglobin, creatinine, thyroid profile and MMSE score was recorded. Substitution of vitamin B_{12} and folate before and after baseline blood sampling was also documented. 19 men and 18 women were treated with vitamin B_{12} and/or folate and excluded from the study, so also 3 men with a diagnosis of commotio cerebri in their anamnesis, by reason of the homocysteine-lowering effect of the vitamins and the suspected connection of commotio cerebri and development of dementia. **Figure 5**.

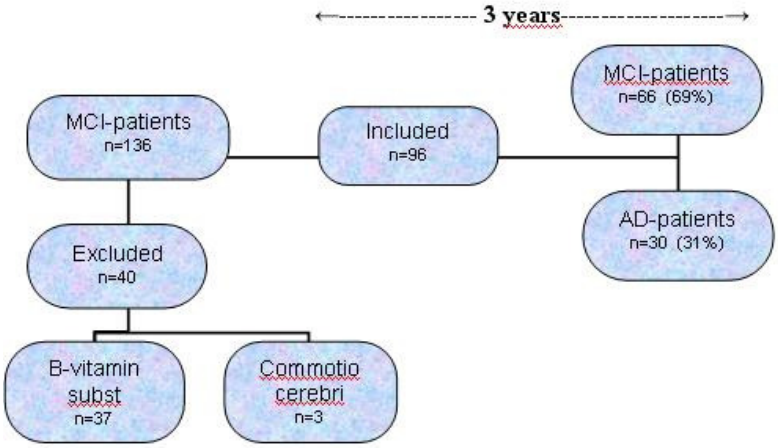


Figure 5. Study design.

Plasma Hcy was sampled in a non-fasting situation at baseline and stored below -20°C. Homocysteine levels were analyzed with liquid chromatography and mass spectrometry, LC-MS/MS**.** The laboratories' reference limits are set to 5-20 µmol/L for men and to 5-16 µmol/L for women. For the male subjects we used three cut-off points for Hcy: >20µmol/L, 20-17µmol/L and <17µmol/L, and for the female two cut-off points: >16µmol/L and <16µmol/L.

Differences between the subjects who developed dementia and those who did not were evaluated by using x^2 (chi-square) analyses and Student´s *t*-tests in the Excel program of Microsoft Software. Correlations for male and female between Hcy and other parameters were performed by Pearson´s correlation test.

Study III

The third study included 93 persons, 45 men and 48 women recruited from the memory unit of the Geriatric Department, Huddinge University Hospital with a follow-up time for six year. The clinical investigation of the subjects included physical and psychiatric evaluation, blood, urine and CSF (Cerebrospinal fluid) -analysis, cranial MRI (Magnetic Resonance Imaging) or CT (Computer tomography), cranial SPECT (Single Photon Emission Computed Tomography) and qEEG (quantitative EEG). A neuropsychological test (including MMSE) was made by an experienced psychologist according to a fixed protocol.

In the present study we included all patients with a MCI diagnosis consecutively during a six months period. The survey focused on the predictive value of vitamin B_{12}**,** folate, homocysteine, Thyroid-Stimulating Hormone, standard laboratory parameters and concomitant diseases for development of Alzheimer´s disease in MCI patients.
The diagnosis of AD was assessed according to the criteria of DSM-IV and ICD-10. The MCI diagnosis was assessed according to the revised Petersen criteria (7).

Associations between potential risk factors and the outcome variable "dementia" were assessed using the independent-samples *t*-test (two-tailed) and Mann-Whitney U test for the continuous variables and for categorical variables, x^2 (chi-square) analyses. A stepwise forward logistic regression analysis was performed to determine the most important predictive factors.

Study IV

The Kungsholmen Project (KP) is a longitudinal population-based study on aging and dementia started in 1987. The initial population included all registered inhabitants (n=2368) who were living in the Kungsholmen parish of Stockholm and were aged 75 years (82). Until now, five follow-ups at approximately 3-year intervals were completed before the data collection was terminated in the year 2000. At baseline and each follow-up, physicians clinically examined all subjects, trained personnel administered psychological tests, and nurses collected data on family and personal history, according to a standard protocol. Dementia and type of dementia were defined according to the DSM-III-R and DSM-IV (83).

The setting of this study was a nested case control and the sample was randomly selected from the subjects participating in the KP and who had stored blood samples available. The controls were age (±2 years) and gender matched with the cases. Blood samples were available for 228 subjects who were non-demented at the time of the sample collection. A total of 128 subjects was from the study baseline and followed for 9 years, and 100 from the first follow-up and followed for 6 years to identify incident dementia. No significant differences were found regarding age, gender and education compared to the cases and controls in the complete cohort and those included to this study (128 vs. study baseline participants, and 100 vs. the 1st follow-up participants).

Biochemical analysis

Total fasting homocysteine (tHcy) concentrations in plasma were analyzed with a fluorescence polarization immunoassay on an IMx® unit (Abbott Laboratories, IL, USA). At mean levels of 12.8 and 25.7 µmol/L, intra-assay coefficients of variation (CV) were 1.3% and 2.1%, respectively. Inter-assay CV:s were 1.4% and 1.0%, respectively.

Holo-Transcobalamin was analyzed with a radioimmunoassay, HoloTC RIA (Axis-Shield, Dundee, Scotland). At mean levels 34.5 and 98.8 pmol/L intra-assay total CV:s were 6.6% and 6.0%, respectively. Inter-assay CV:s were 6.6% and 9.5%, respectively.

These analyses were performed at the department of Medical biosciences, clinical chemistry, Umea University Hospital, Umea, Sweden.
All other biochemical analyses were performed by routine methods at the laboratory of clinical chemistry, Karolinska Institute, Stockholm, Sweden.
APOE genotypes were grouped as having any ε 4 allele vs no ε 4 allele.

Statistical analyses

Differences among demented and non-demented persons were analysed using Mann-Whitney U test and χ^2 –test as appropriate. The associations between Hcy and holo-TC and the subsequent development of dementia and AD were analysed using Cox regression analyses. Differences in follow-up time were taken into account in the analyses. The analyses were adjusted first for age, sex and education (model 1) and then additionally for other potential confounding factors (model 2). Finally, we carried out analyses adjusting for holo- TC concerning homocysteine- dementia association and for homocysteine levels concerning holo-TC- dementia association (model 3).
Homocysteine and holo-TC were first analysed as continuous variables and then classified as quartiles. The level of significance was < 0.05 in all analyses. The analyses were conducted using the SPSS for Windows, v.12.0.1 (SPSS Inc., Chicago, IL).

Ethical permission

Study I –IV was approved by the Ethical Committee of the Karolinska Institute, Stockholm and Study I also by the Ethical Committee of Dalarna, Falun.

Results and discussion

Paper I

Vitamin B12, Folate and Homocysteine in persons with Cognitive Impairment. A comparative study of serum levels among persons living single or in a family.

Both vitamin $B_{12,}$ and folate deficiency have been shown to be common in elderly people in many studies (38, 85, 86). There is also a great volume of surveys reporting an association between vitamin B_{12} /folate deficiency and elevated Hcy levels and cognitive decline (87). Results are often presented from cross-sectional, cohort and case-control studies.

In this study the aspect was social-medical, and we compared the levels of vitamin $B_{12,}$ folate and Hcy in men (n=60, mean age 71) and women (n=60, mean age 73) with MCI living either in a family setting or single. There were no significant differences between the groups concerning mean levels of MMSE, vitamin B_{12} and folate. For vitamin B_{12} the mean values were in the lower part of the reference region in all groups.

Inadequate nutrition with low content of B_{12}-vitamin and folate can be one explanation, so also a reduced intake of food. Another source of deficiency is the food processing. Microwave heating of food may destroy 30-40% of the vitamin B_{12} content in food (88). Natural folates are destroyed faster in microwave heating than in conventional heating to the same temperature. In conventional cooking about 50% of natural folates is destroyed (89). It is possible that single living men more often use microwave heating of food as men in this generation were not particularly active in cooking. To choose pre-cooked food, which has to be heated in microwave oven, reduces folate, cobalamin and vitamin B_6 (89, 90).

The main finding in the study was a significant ($p < 0.01$) difference in Hcy levels found between men in families (15,5µmol/l) and men living single (21,9 µmol/l).
The mean values for Hcy were above the upper reference for all groups, except for men living in families.

Limitations of the study must be addressed as elevated Hcy levels can be a result of several lifestyle factors. The most important are smoking, high consumption of alcohol, high coffee consumption and low physical activity (61). As information of the person's height were not available it was impossible to determine BMI and get a more adequate picture of the nutritional status. Nor was it possible to figure out the anamnestic food factors as the study was retrospective. It has been estimated that food intake decreases by about 30% in persons at the age of 80 years (91).

Swedish nutrition studies of representative elderly groups have shown that food habits on average were good, but that the variance was big. Parts of the population were apparently already in the beginning of their 70s at risk of malnutrition. Underfeeding, however, seems to be very unusual among elderly people in Sweden and is almost always related to cancer or dementia. (92). A higher nutrition intake, on the other hand, is common but too much fat in the food can occur at the same time as malnutrition concerning essential nutrients such as proteins, minerals and vitamins.

As the absorption of vitamin B_{12} is depending of the function of the ventricle it would have been preferable to have the possibility to examine the role of the ventricle. The prevalence of atrophic gastritis is increasing with age (93-95). Reduced acid production in chronic atrophic gastritis plays an important role in the malabsorbtion of vitamin B_{12} as the cleavage of the vitamin from proteins decreases with lack of gastric acid.

Another draw-back of the study was the fact that the subjects were not followed up. The patients would have been followed over time to make it clear if the differences in homocysteine levels were related to the development of dementia.

Age-related impairment of renal function may reduce the plasma clearance of Hcy. In this study, however, the creatinine levels were within the reference region for all groups.
Hcy was, as expected, negatively correlated to folate and vitamin-B_{12} in all groups.
The levels of vitamin B_{12} and folate are dependent of a combination of both inner and outer factors in elderly persons (96). The measuring of Hcy is one way to diagnose the deficiency states of the vitamins, however non-specially, as Hcy correlates negatively with those vitamin levels.

The results of this survey proposed that cognitively impaired men living single showed a greater risk of developing deficiency of vitamin B_{12} and folate, and thereby raised Hcy levels, than men living in a family setting. Hcy levels can be lowered with B_{12} and folate; however, it is not obvious if the lowering also is linked to clinical effects in the individual patients (43, 97, 98). Although a recent article with 818 healthy participants showed improvement in certain domains of cognitive function that tended to decline with age after a three year folic acid supplementation (99).

There are also small studies on cognitively impaired patients supplemented with vitamin B_{12} and folate suggesting an improvement in cognition in those with elevated Hcy and having mild-moderate dementia (97). The best results in treatment of a vitamin B_{12} and folate deficiency have been reported when the supplementation is started within six months from the detection of cognitive decline (100, 101). Early diagnoses of deficiency of these vitamins are of great importance.

Paper II

The relation between homocysteine levels and development of Alzheimer´s disease in Mild Cognitive Impairment (MCI) patients

The aim of this 3-year follow-up study was to analyze the connection between levels of homocysteine in the blood from patients with MCI and the development of AD. The connection between hyperhomocysteinemia and AD has been mostly investigated in cross-sectional clinical settings where elevated tHcy levels have been reported in subjects with AD (102-104). Two population-based prospective studies and a case controlled survey also reported that high tHcy was associated with an increased risk of dementia and AD (105-107). However, this finding has not been confirmed in other studies (108-111).

Totally 96 patients with diagnosed MCI were included in the survey. The total number of persons who converted to AD was 30 (31%). One subject developed FTD (Fronto-Temporal Dementia) and one dementia NUD (Non Ultra Descriptus). The 37 patients treated with vitamin B_{12} and/or folate, and initially excluded from the study, did not develop dementia.

Numerous longitudinal studies on MCI-patients are dealing with the development to dementia in general and Alzheimer's disease in particular. In this survey the conversion rate to AD was 31% within 3 years and the annual conversion rate approximately 10%. This finding is in line with the results of some other studies.

The observed conversion rate among studies differs between 19% development of AD after 3 years of follow-up to 48% after 4 years (112, 113).Those differences could probably be a result of the different criteria applied, different settings of the studies, and different length of the follow-up period.

There was a significant difference in the mean level of Hcy between the men who converted to AD and those who did not. For the women there were significant differences between the groups regarding the mean levels of age, MMSE and vitamin B_{12} at baseline.
Of the males 26% developed AD within the 3 year period. When grouping the men, 33% of the men with Hcy-levels >20 μmol/l converted to Alzheimer's disease, 50% with Hcy-levels 17-20 μmol/l while none of the men with Hcy-levels <17umol/l converted.

This result contrasts to other studies showing an increasing trend in conversion to AD with higher Hcy levels (105). Explanations for the conflicting results could be a possible self-administrated vitamin supplementation, or a non-documented treatment of B-vitamins before the baseline sampling.

Of the 50 female patients, 36% converted to AD. 45% of subjects with Hcy-levels >16 umol/l within the three-year period, compared to 21% of those with Hcy-levels <16 μmol/l. There was a statistically significant difference between the groups ($p<0.05$). This finding is in line with the increasing tendency in conversion to AD with higher levels of Hcy (105). As expected we found negative correlations between Hcy/folate and Hcy/B_{12}, in some of the groups significant.

The small sample size was a general drawback of the study while the strengths included the prospective design and the relatively long follow-up period.

When using the cut-off points for Hcy we did not find a positive correlation between elevated homocysteine levels and development of AD. Looking at the whole group of men and women who converted to AD the findings were in line with a recently published study on non-demented patients followed for 8 years (105). In that survey the RR (relative risk) of developing AD was 1, 8 (CI 1,3-2,5) per SD increase of Hcy at baseline. Additionally, there are also some cross-sectional studies which have shown a significant increase of Hcy levels in AD patients compared to controls (45, 113).

A significant difference between vitamin B_{12} levels was found for the women who developed AD and those who did not. The deficiency of vitamin B_{12} has long been suggested to be associated with cognitive impairment in the elderly (40, 103, 107, 114). However, other studies have failed to find such an association (44, 45, 115).

There is a common preliminary conclusion that raised Hcy levels are inversely correlated with cognition scores and may mediate an increased risk of developing dementia and AD (1, 34-36). The source of hyperhomocysteinemia most often is a result of deficiency of vitamin B_{12} and folate.

The findings in this survey support that low/normal homocysteine levels may have a possible protective effect on dementia conversion in MCI patients. Such a relation has also been reported in a recent survey, the Hordaland Homocysteine study, where low Hcy levels were associated with better physical and mental health compared to subjects with hyperhomocysteinemia (59).

Some authors have suggested, on the basis of cross-sectional investigations, that increased Hcy levels are not a causative factor in dementia and AD, but only a marker for concomitant vascular disease, independent of cognitive status (110, 116). Argument against this interpretation has been reported in other cross-sectional studies (117), but only intervention trials can give definitive confirmation of a causal relationship between hyperhomocysteinemia and AD. In some small surveys and in individual patients Hcy lowering intervention with vitamin B_{12}/folate have been successful in reversing dementia, thus arguing in favour of such a relationship (97, 118, 119).

Paper III

The significance of TSH and homocysteine in development of Alzheimer´s disease in MCI: A six year follow-up study.

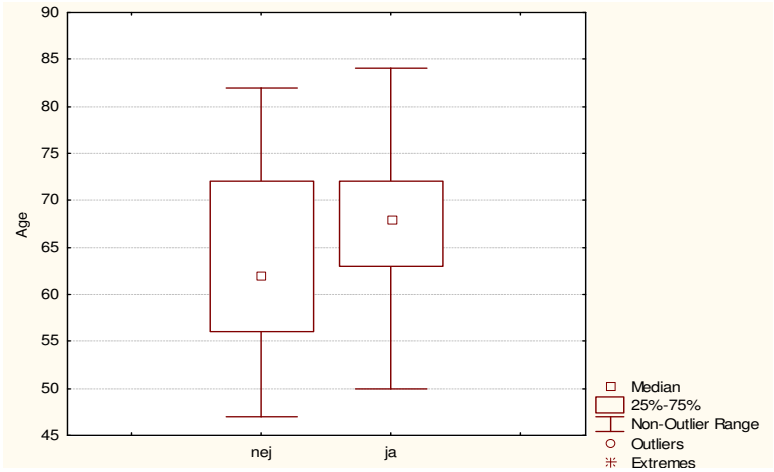
In this survey we investigated the predictive importance of Hcy, vitamin B_{12}, folate, standard laboratory parameters and concomitant diseases for development of AD in MCI patients. The development of dementia was followed for 6 years in 93 subjects with MCI diagnosis. The mean age for men (n=45) was 64,7(±9,2 SD) and for women (n=48) 65,4(±8,9 SD) years.

The conversion rate to AD was 34% within 5 years, the annual rate approximately 7%. This result is in line with result from other studies (10, 112, 113). The 61 persons who did not convert to dementia became the control (NC) group, 32 men and 29 women.

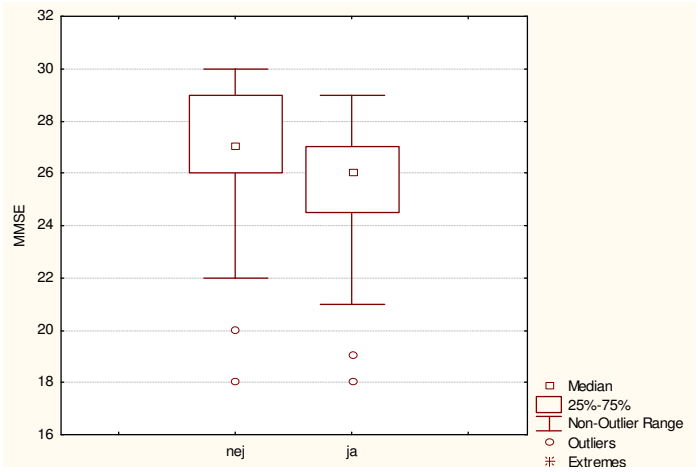
Table 2. Differences in some baseline characteristics between Nonconverting (NC) patients and patients converting to Alzheimer´s disease.

	n	NC	N	C	p
Age, years	61	63,6±9.6	32	67.7 ± 7.2	0.0249
Homocysteine µmol/L	59	16.8 ± 4.0	32	18.4 ± 3.2	0.0342
TSH mU/L	49	1.8 ± 1.4	27	1.3±1.0	0.0394
MMSE	61	27.0 ±2.5	32	25.3 ±2.6	0.0029

Figure 6-9. Distribution of the significant variables age, MMSE, Hcy and TSH.



Dementia



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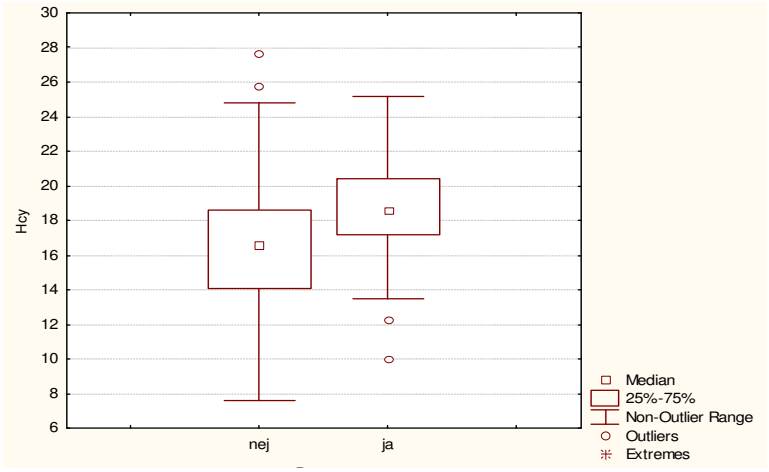
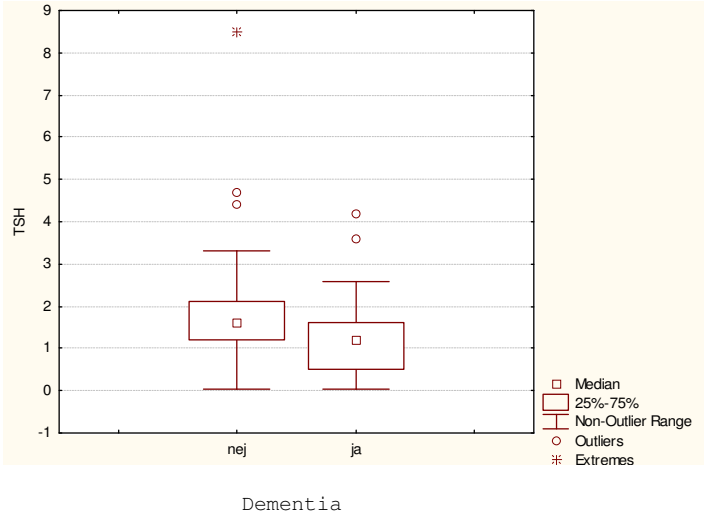


Dementia



In order to determine independent predictors for dementia, a forward stepwise logistic regression was performed. Hcy was not significant between the groups in the main effects model. After adjustment for age the significance of TSH and MMSE remained.

Table 3. Odds Ratio (OR) and 95% confidence intervals for risk of Alzheimer´s disease

	Age	MMSE	TSH
Unadjusted OR	1.084	0777	0.287
(95% CI)	(1.014-1.160)	(0.636-0.948)	(0.088-0.931)
Adjusted OR[1]	1.054	0.774	0.322
(95% CI)	(1.002-1.109)	(0.646-0.927)	(0.107-0.971)

[1]Adjusted for age

Table 4. Odds Ratio (OR) and 95% confidence intervals for risk of Alzheimer´s disease

	Hcy 60 years	Hcy 65 years
OR[1]	1.287	1.087
(95% CI)	(1.031-1.608)	(0.985-1.368)

[1] Interaction Hcy/age

When we added Hcy and the interaction Hcy*age to the main effects model, this new model provided a significant improvement over the main effects model (p<0, 05). This indicated that the most important variables for the development of AD in the survey, except age, were MMSE, TSH and Hcy at lower ages.

The connection between hyperhomocysteinemia and development of AD has been reported in several studies (102-105). In this survey we found that the importance of raised Hcy levels was decreasing in elderly subjects. As AD is a slowly progressive disease where both genetic and environmental factors can be included, it is possible that other causes may be of more importance in older subjects. It may be of interest to investigate the importance of midlife hyperhomocysteinemia in comparison with other midlife risk factors for AD such as hypercholesterolemia and hypertension (120-123).

Decreased levels of TSH are found in subjects with hyperthyroidism and also in those with supplemented hypothyroidism. Numerous studies have explored the connection between thyroid function and dementia, with varied results (67-72). A random sample, aged 49-71, from the Maastricht Aging Study, could not find any cross-sectional associations involving cognitive performance and TSH (124), whereas a recent study from the Kungsholmen Project reported that a decline in verbal fluency and visuospatial abilities was associated with a simultaneous decline in TSH (125).

A limitation of the study, except for the small study sample, was the fact that we did not have the opportunity to compare TSH levels from different samples. It would also have been of interest to determine the levels of T_4 and T_3, but the variables were only recorded in a few subjects. A recent study showed that high levels of plasma fT_4, the biologically active form of T_4, were significantly negatively correlated to MMSE scores, suggesting that high fT_4 could worsen MCI (126). Neither was TPO-Ab (Thyroid peroxidase antibodies) available, which could have contributed to evaluation of the thyroid status.

There were no significant differences of the standard laboratory parameters, concomitant diseases or the use of thyroxin substitution between the groups.
The findings of hyperhomocysteinemia in connection with low TSH levels as predictors for the development of AD in our survey needs to be further investigated in well planned studies with large sample size in order to clarify the importance of these variables.

Paper IV

Homocysteine and Holo-Transcobalamin in relation to the development of Alzheimer´s disease

In this prospective survey we investigated the importance of tHcy and holo-TC for the development of dementia and AD. The association of hyperhomocysteinemia and dementia has been thoroughly investigated (102-111), whereas only a few reports have shown the impact of low holo-TC, the biological active fraction of vitamin B_{12} (127,128).

The study comprised 228 subjects, 41% developed dementia of which 31% AD, and 135 controls were matched. 75% of the subjects were females and the follow-up time was 3-9 years (mean 6.7). The mean age at baseline was 82 (±4.5) for the demented and 79.7 (±4.0) for the control group ($p<0.001$). The groups also differed in MMSE 26.3 (±1.9) and 27.7 (±2.0) ($p<0.001$), respectively, as well as in ApoE4 allele - 46.2% and 35.6% ($p<0.003$), respectively, which was expected.

The tHcy levels were 21.3 µmol/L (±13.9) for the demented compared to 16.7 µmol/L (±6.4) for the controls. For holo-TC there were no significant differences in levels between the groups 142,5pmol/l (±188) and 115,9 pmol/L (±119), respectively.

Distribution of tHcy in demented and controls is presented in **Figure 10** and for holo-TC in **Figure 11**.

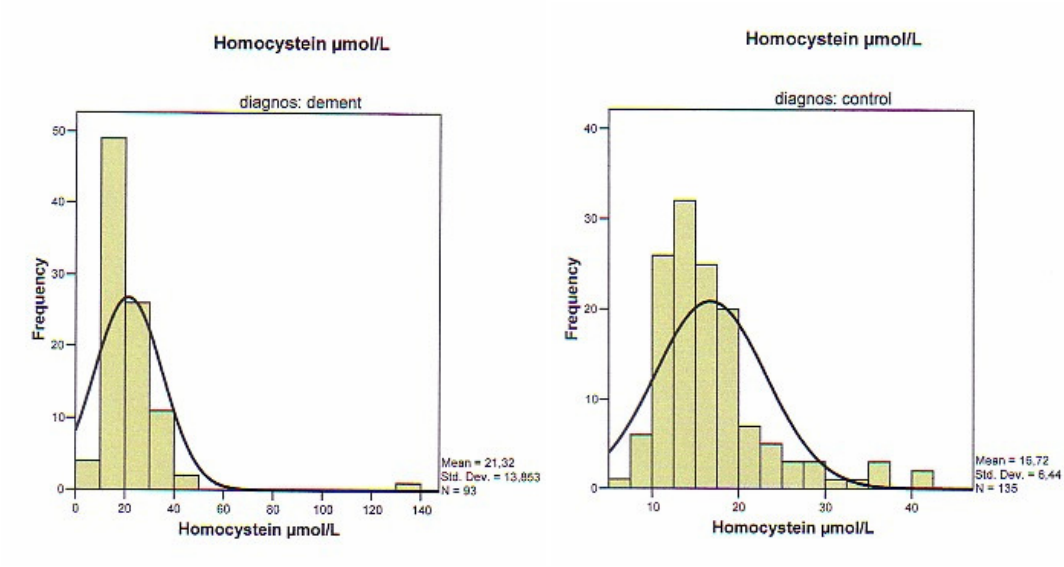


Figure 10. Histogram of plasma total homocysteine concentrations for the demented and control group.

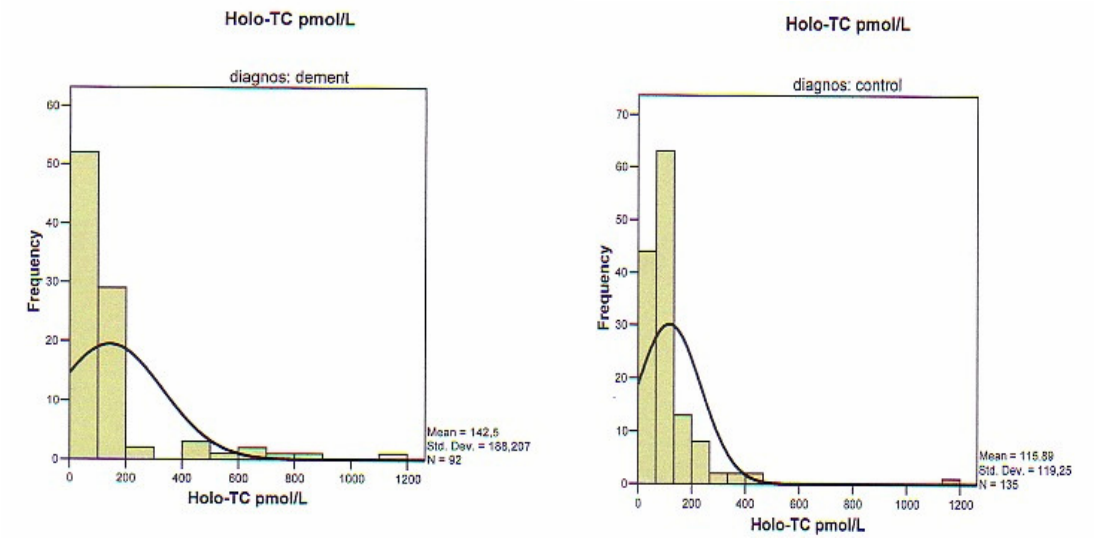


Figure 11. Histogram of holo-Transcobalamin concentrations in the demented and control group.

tHcy was positively correlated with age in both demented and control group (r=0.256, p<0.02) and (r=0.26, p<0.01), negatively correlated with MMSE (r=-0.071, p<0.501) and (r=-0.248, p<0.004), B12 (r=-0.320, p=0.002) and (r=-0.227, p<0.008), and Holo-TC (r=-0.013, p=0.90) and (r=-0.163, p<0.060), respectively.

Hcy as a continuous variable was related with an increased risk of dementia and AD, **Table 5**.

Table 5. Continuous variable. Relative Risk (RR) and 95% confidence intervals for risk of dementia and AD.

	Model 1	Model 2
Homocysteine/dementia	1.015	1.037
	(1.003-1.027)	(1.007-1.069)
Homocysteine/AD		1.05
		(1.02-1.09)

Multiple logistic regression analyses were used to create the models.
Model 1 was adjusted for age, sex and education
Model 2 was additionally adjusted for BMI, creatinine, albumin, hemoglobin, Apo-E and MMSE

Classification of tHcy as quartiles showed a more than twofold risk of dementia and AD in the highest quartile. The result remained after adjustment for confounding factors.

Holo-TC as a continuous variable was not associated with an increased risk of dementia. We found a reduced risk of dementia and AD in the 3rd quartile of holo-TC, and this association remained for AD even after all the adjustments.

In the current study we found that hyperhomocysteinemia increased the risk of developing dementia and AD among 228 subjects in the Kungsholmen Project, aged 75-92 years. In the 4th quartile of plasma homocysteine the risk of dementia and AD was doubled and remained after adjustment for potential confounding factors, such as age, sex, education, BMI, MMSE, Apo-ε4 allele, creatinine, haemoglobin, albumin and glucose. We found no clear association for holo-TC, but the 3rd quartile showed a slightly reduced risk of AD.

The findings are close to two earlier prospective studies, the Framingham study (105) and a study from Bologna, Italy (106). The Framingham survey, comprising 111 demented subjects, of those 83 with AD, found a nearly doubled risk of AD in subjects with tHcy levels >14µmol/L, and the Italian study including 112 demented subjects, 70 with AD, a risk increase was seen in tHcy levels >15µmol/L. A recent cohort study reported an association between hyperhomocysteinemia and development of dementia and CIND (Cognitive Impairment Non Demented). The risk was reduced in subjects with higher vitamin B_{12} levels (129). The Washington Heights-Inwood Columbia Ageing Project (WHICAP) comprising 109 subjects who developed AD reported no association with hyperhomocysteinemia (109).

The connection between low levels of holo-TC and development of dementia/AD has been reported only in a very few surveys from later years. One cross-sectional study from the Medical Research Council´s (MRC) Cognitive Function and Ageing Study (CFAS) cohort with 84 non-demented subjects aged 69+ years reported an association between low holo-TC and lower cognitive functions, supporting our findings (127). Another study from the Oxford Project to Investigate Memory and Ageing (OPTIMA) cohort, 51 patients with confirmed AD and 65 controls showed an association between low holo-TC and AD in subjects with hyperhomocysteinemia (128).

A limitation of this study was the rather small sample size; however, earlier prospective studies have presented surveys of the same sample size as the current study. The long follow-up period and the presentation of many potential confounding factors in relation to our data may strengthen the study.

The most common source of moderate to severe fasting tHcy are deficiencies of vitamin B_{12} and folate but an influence of several inherited and acquired factors as well as lifestyle factors are also involved (61). The great interest in the investigation of homocysteine as a risk factor for dementia and AD is the possibility to reduce tHcy levels with B_{12} and folate medications.

Until now a successful effect of treatment has been reported only in a few studies (97, 99) and in a recent survey from the WHICAP group, where a lower incidence of AD was reported for the highest quartile of folate intake (129). However, some clinical treatment trials have failed to prove an improvement of cognitive failure or dementia (130 -132), though, the treatment period in those studies was rather short.

Our findings in this prospective nested case-control study were an association between hyperhomocysteinemia and the development of dementia and AD, but no clear association for holo-TC. It is of importance to find markers or instruments for patients prone to develop AD that makes it possible to start an intervention at an early stage of AD as the brain changes start to develop decades before the clinical picture of dementia is overt.

Further investigations

The definitive proof of homocysteine´s role in cognitive decline is dependent of clinical trials to establish whether lowering of the homocysteine concentration with B vitamin treatment will prevent a proportion of the elderly from developing dementia or reversing already established cognitive impairment. The most interesting group to study would be persons with mild cognitive impairment, as the findings have shown that an intervention in this group should be more agreeable and beneficial than treating a non-symptomatic population with elevated homocysteine levels (133). If lowering homocysteine could stop only 10% of subjects with mild cognitive impairment from developing AD, several hundred thousand persons worldwide would benefit every year (134).

To further study the impact of thyroid disease, hypo- as well as hyperfunction, together with thyroid medication is another important research track to follow with regard to our findings. As the proportion of elderly in the population increases, the incidence of dementia increases and will become an enormous public health problem. In a resent report the total societal worldwide cost of dementia, on the basis of a dementia population of 29.3 million persons was estimated to be 315.4 billion dollar in year 2005 (135). Research into the field of dementia is of great importance and has the potential to affect the lives of patients, relatives and caregivers as well as to have an impact on the whole health care system.

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