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Hypothesis

# Alzheimer's Amyloidopathy: An Alternative Aspect

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**Abstract**. The 'amyloid hypothesis' dominates Alzheimer's disease (AD) research but has failed to deliver effective therapies. Amyloid precursor protein (*APP*) and presenilin-1 (*PSEN1*) genetic mutations are undoubtedly pathogenic, albeit by unclear mechanisms. Conversely, high dose B-vitamins convincingly slow brain atrophy in a pre-stage state of sporadic AD. Here we suggest a link between sporadic and genetic AD: 1) Increased serum homocysteine, a marker of B-vitamin deficiencies, is a significant risk factor for sporadic AD. It also correlates with elevated levels of antichymotrypsin, a serine protease inhibitor. 2) Family members with codon 717 *APP* mutations and dementia have low serum vitamin $B_{12}$ values. Overexpression of the *APP* domain coding for a Kunitz type serine protease inhibitor might explain this. 3) *PSEN1* mutations disrupt lysosomal function due to reduced proteolytic activity. They also trap cobalamin ($B_{12}$) within lysosomes, leading to intracellular deficiency of the vitamin. In summary, *APP* and *PSEN1* mutations both confer a risk for reduced protease activity and $B_{12}$ bio-availability. Comparably, sporadic AD features a constellation of increased protease inhibition and B-vitamin deficiencies, the central part of which is believed to be $B_{12}$. These concordant observations in three disparate AD etiologies suggest a common neuropathogenic pathway. This hypothesis is evaluable in laboratory and clinical trials.

Keywords: Alzheimer's disease, amyloid, proteolysis, vitamin $B_{12}$ deficiency

## INTRODUCTION

In 1991, amyloid precursor protein (*APP*) gene mutations were found in families with early-onset Alzheimer's disease (AD). Three different mutations at codon 717 implied pathogenicity; amyloid became a prime focus of AD research. Amyloid-β protein precursor (AβPP) is a transmembrane protein cleaved by two proteases (β- and γ-secretase) into amyloid-β (Aβ). This is regarded as a central pathogenic event in AD [1]. According to the 'amyloid hypothesis' Aβ is neurotoxic, its deposition leading to amyloid plaques, neuronal death, and ultimately dementia. This idea was strengthened by finding γ-secretase (presenilin) mutations in other families with early-onset AD, and from the utility of cerebrospinal fluid Aβ levels as a marker of AD diagnosis. In a whirl of optimism, the hypothesis prompted development of anti-amyloid drugs to treat *all* patients with AD, regardless of age or the presence of such mutations.

Sadly, more than 25 years later, this hypothesis has not delivered effective therapies. Perhaps its focus was misguided [2, 3]? Aβ itself is barely toxic [4]; even its capacity to induce oxidative stress remains controversial [5]. Importantly, AβPP has trophic

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functions and is involved in neuronal survival, neurite outgrowth, and neurorepair in the brain [6]. We suggest that Aβ deposition does not drive the pathogenic process but is a consequence of the disease state. *APP* and *presenilin* mutations are undoubtedly pathogenic but the resultant increased Aβ deposition is likely a relatively innocent bystander—the mutations leading to AD by other mechanisms.

## VITAMIN $B_{12}$ AND FAMILIAL AD

A serendipitous finding was made in one of the families with an *APP* gene mutation; it was associated with lower serum vitamin $B_{12}$ values [7]. We suggested this might result from systemic protease inhibition [8]. An insert in the *APP* sequence has striking homology to the Kunitz family of serine protease inhibitors. These are expressed in most tissues and inhibit the activity of proteases such as trypsin and chymotrypsin. If overexpressed, they could interfere with protein-bound transfer of vitamin $B_{12}$ posing a risk for its deficiency.

Presenilin-1 (PSEN1) is required for lysosomal proteolysis, and lysosomal function is disrupted by AD-related *PSEN1* mutations [9]. Lysosomes are a key component for endocytosis of protein-bound molecules; in defective proteolysis proteins accumulate detrimentally in lysosomes. This was recently explored by an Australian group [10]. Firstly, *in vitro* neuroblastoma cells were transfected with the 'Swedish' *APP* mutation and then treated with a protease inhibitor. Secondly, wild type mice were compared with transgenic (APPxPSEN1) mice. Radio-labelled cobalamin ($B_{12}$) was used to study lysosomal function. Both experiments showed that cobalamin became trapped within lysosomes, i.e., intracellular cobalamin availability is reduced. This is analogous to the cobalamin F group of hereditary $B_{12}$ deficiency (see Fig. 1). In infancy this leads to failure to thrive and mild to severe developmental delay, although there is a good response to $B_{12}$ supplementation if started at an early stage [11].

In summary, *APP* and *PSEN1* mutations confer a risk for $B_{12}$ deficiency, presumably due to reduced proteolytic activity.

Of course, the vast majority of AD patients have neither *APP* nor *PSEN1* mutations; the disease occurs sporadically, with a relatively late age of onset. Elevated antichymotrypsin and homocysteine blood levels are consistently reported in sporadic AD. In a meta-analysis of 13,000 AD patients and a similar number of healthy controls, antichymotrypsin and homocysteine were significantly elevated [12]. However, these have never been considered to be directly related.

## HOMOCYSTEINE AND SPORADIC AD

Reports of low vitamin $B_{12}$ and folate blood levels in AD prompted investigation of the utility of serum homocysteine as a marker of such deficiencies in dementia. Several long-term prospective studies in healthy populations yielded startling results: elevated homocysteine predicts dementia up to several decades before its onset [13–15]. 'Treating' AD with B vitamins became an interesting option, but a demanding challenge. Established AD is too advanced to achieve evident effects, and it is difficult to evaluate how such treatment modifies brain function in a clinical trial. Erroneous methodology can give false negative outcomes [16, 17].

The Oxford-based OPTIMA group formulated a careful design to overcome such pitfalls. They focused on patients with mild cognitive impairment (MCI), regarded as a 'pre-stage' state with a highly increased risk for developing AD. They also used magnetic resonance imaging as a highly sensitive and accurate tool to detect 'brain atrophy rate changes' as a primary outcome measure.

In 2010, OPTIMA reported on their 24-month randomized double-blind controlled clinical trial in which MCI patients ($n = 168$; age > 70 years) were given either placebo or vitamins $B_{12}$, $B_6$ and folic acid—all necessary for optimal homocysteine metabolism [18]. As part of normal aging, our brains lose volume of around 0.5% annually [19]. In the OPTIMA cohort, brain volume of the placebo group diminished by 1.08% per year, compared with 0.76% for the treatment group ($p < 0.001$); i.e., brain volume reduction was 30% slower in treated patients. In other words, MCI patients treated with B vitamins significantly slowed their rate of brain volume loss. Global cognition, episodic and semantic memory also improved in treated patients with supra-median homocysteine levels [20]. Directed acyclic graph analysis showed that vitamin $B_{12}$ was the main driver in the protective effect of B vitamins in slowing both brain atrophy and cognitive decline in this study [21].

We believe this trial is 'epoch-making' in AD prevention strategies. Effective homocysteine metabolism is crucial for all cells (Fig. 1). B vitamin adequacy supports a chain of vital reactions: methylfolate is de-methylated to support DNA synthesis (its

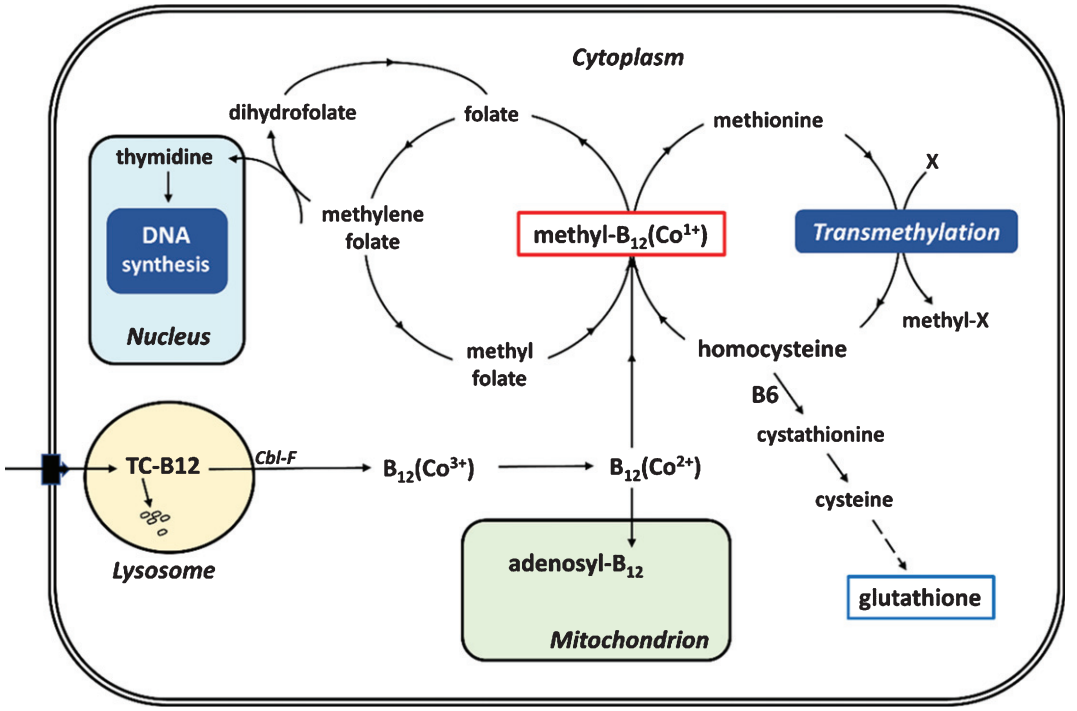


Fig. 1. Simplified scheme of entry and utilization of vitamin $B_{12}$ by mammalian cells. TC is transcobalamin degraded in the lysosome; Cbl-F is the site causing the Cobalamin F group of hereditary $B_{12}$ deficiency; ($Co^{1+}$) indicates valency of the cobalt atom in the cobalamin/$B_{12}$ molecule; X is substrate transmethylated by a methyltransferase.

methyl group is transferred to $B_{12}$); methyl-$B_{12}$ remethylates homocysteine to methionine which, in its activated form S-adenosyl-methionine, is the transmethylating agent for hundreds of different methyl acceptors. Moreover, vitamin $B_6$ enables a proportion of homocysteine to be metabolized to cysteine, a precursor of the key cellular antioxidant glutathione.

If *any* of these reactions are compromised, cells undergo cytostatic involution: in the brain, an increasing number of neurons collapse [22]. Dementia is the ultimate consequence. Essentially, this is why insufficiency of these particular B-vitamins is more closely related to AD brain cell death than is amyloid deposition.

A recent meta-analysis included almost 22,000 cognitively healthy elderly individuals treated with B vitamins, including $B_{12}$, for up to 7 years. It was concluded that although B vitamins did not improve *global* cognitive function, they did improve specific subdomains (information processing, sensorimotor speed and short term memory) when administered for over 3 years. However, no clear conclusion can yet be drawn as to whether B vitamins can 'treat' AD if administered sufficiently early [16].

Other important questions remain. How is increased homocysteine, and thus the need for B-vitamins, explicable in relation to sporadic AD? And how does protease inhibition *exactly* link familial and sporadic AD? Perhaps oxidative stress or elevated serine protease inhibition, such as by antichymotrypsin, or both, are key pieces of the missing picture in sporadic AD?

## ANTICHYMOTRYPSIN AND SPORADIC AD

Antichymotrypsin is an acute-phase protein induced during inflammation. Of hepatic origin, it inhibits serine proteases and protects tissues from excess proteolytic damage. High levels occur in a substantial proportion of patients with sporadic AD, albeit with unexplained consequences. The largest study ($n = 359$) found that antichymotrypsin increases in parallel with disease progression [23].

Antichymotrypsin is expressed in astrocytes, and found in amyloid plaques, tightly bound to Aβ [24]. Interestingly, a secreted form of Kunitz type serine protease inhibitor, nexin-II, is a potent

Table 1
Binding proteins and their degradation as a multistep process necessary for delivery of vitamin $B_{12}$ to its ultimate target cells

| Compartments | Processing events |
|---|---|
| In the mouth and stomach | $B_{12}$ is released from food and bound by **Haptocorrin**, a salivary glycoprotein with high affinity and broad specificity |
| The upper intestinal tract of duodenum and jejunum | Pancreatic proteases degrade **Haptocorrin** and release $B_{12}$, which is subsequently bound to **Intrinsic factor**, a second glycoprotein with high specificity |
| In the enterocytes of ileum intestine mucosa | **Intrinsic factor** is degraded in the lysosome, and $B_{12}$ is released into the blood stream |
| In the blood stream | $B_{12}$ is associated with two separate carriers in the blood: **Haptocorrin** for passive storage in the circulating blood, and **Transcobalamin** as an active carrier that binds $B_{12}$ avidly and mediates its transport into target cells |
| In the target cell | **Transcobalamin** is degraded in the lysosome, and $B_{12}$ is released to be further processed intracellularly (see Fig. 1) |

antichymotrypsin showing identity to AβPP [25]. The exact origin of antichymotrypsin in plaques has, to our knowledge, not been determined [26].

Elevated plasma antichymotrypsin in sporadic AD might reflect its reactivity towards chronic low-grade inflammation. Increased homocysteine levels, in turn, may either arise from an independent oxidative stress reaction caused by such inflammation, or from antichymotrypsin disrupting cleavage of bonds between $B_{12}$ and its several carrier proteins. Other than as a transient phenomenon following injection, $B_{12}$ is not found in the 'free' state. Like all co-enzymes, it is attached to protein in its natural form. Released from food by saliva, $B_{12}$ and other corrinoids can be considered as 'rough diamonds' to be carefully sorted, selected and finally delivered as 'crown jewels' for co-enzyme functions in their target cells (see Fig. 1). This complex process includes at least 14 proteins, functioning as carriers, receptors or enzymes, for which separate genes or complementation groups have been identified in patients with inherited $B_{12}$ disorders [27, 28].

Importantly, this intricate $B_{12}$ trafficking is a multistep process in terms of repeated protein binding and proteolysis (Table 1). For each repetition, $B_{12}$ is preferentially selected and attached to yet another binder protein and then carried to a location where the binder is degraded and $B_{12}$ is released. For a minireview, see Gherasim et al. [29].

Our hypothesis is based on the concept that impaired proteolysis in *any* of the compartments in Table 1 predisposes to the development of $B_{12}$ deficiency. Some proof already exists. For example, trafficking events in the upper gastrointestinal tract are defective in pancreatic insufficiency, since three pancreatic proteases (trypsin, chymotrypsin, and elastase) are required to partially degrade haptocorrin and release $B_{12}$ [30, 31].

Elevated antichymotrypsin is therefore a candidate for causing $B_{12}$ deficiency due to insufficient proteolysis and aberrant protein binding at the blood and/or the target cell/lysosomal level, and its potential to interfere with carriage and delivery of $B_{12}$ into the brain requires further investigation.

## CONCLUSION

AβPP is important in maintaining the aging brain. Genetic mutations in *APP* and *PSEN1* lead to pre-senile AD. However, solely focusing on 'anti-amyloid therapy' might be counter-productive in discovering a panacea for dementia.

Conversely $B_{12}$ deficiency, as part of a broader scheme of deficiencies relating to homocysteine and folate metabolism, is a feature of both genetic and sporadic AD; it perhaps reflects reduced proteolytic activity.

Our hypothesis makes testable predictions and suggestions for future research. Additional confirmatory studies of the effects of *APP* and *PSEN1* mutations on $B_{12}$ intracellular processing would be helpful. It would also be valuable to assess the vitamin $B_{12}$ and homocysteine status of a large cohort of patients with familial AD, and to conduct clinical trials of B vitamins on such patients.

It is worth noting additional 'downstream' effects attributable to this hypothesis and the resulting elevated homocysteine levels. There is clinical and experimental evidence linking hyperhomocysteinemia with tau hyperphosphorylation [32–34]. Tau hyperphosphorylation and its subsequent aggregation results in the other key pathological feature of AD,

neurofibrillary tangles. Elevated homocysteine might also contribute to protein aggregation and subsequent neurodegeneration. This can occur by a mechanism involving protein N-homocysteinylation of lysine residues by homocysteine thiolactone [35–37] or, more speculatively, by undergoing self-fibril formation to induce seeding of other protein aggregates, including Aβ polypeptide itself [38].

Regardless of such possible underlying pathogenic mechanisms, it is reassuring to know that high dose B-vitamin supplements convincingly reduce brain atrophy and cognitive decline in a pre-stage state of sporadic AD. This is an excellent starting-point for finding the best preventive means of this devastating disorder.

## DISCLOSURE STATEMENT

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/18-1007r1).

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