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 (\textit{APP}) and presenilin-1 (\textit{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 \textit{APP} mutations and dementia have low serum vitamin B\textsubscript{12} values. Overexpression of the \textit{APP} domain coding for a Kunitz type serine protease inhibitor might explain this. 3) \textit{PSEN1} mutations disrupt lysosomal function due to reduced proteolytic activity. They also trap cobalamin (B\textsubscript{12}) within lysosomes, leading to intracellular deficiency of the vitamin. In summary, \textit{APP} and \textit{PSEN1} mutations both confer a risk for reduced protease activity and B\textsubscript{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\textsubscript{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\textsubscript{12} deficiency

INTRODUCTION

In 1991, amyloid precursor protein (\textit{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-\beta protein precursor (\textit{A\betaPP}) is a transmembrane protein cleaved by two proteases (\textit{\beta}- and \textit{\gamma}-secretase) into amyloid-\beta (\textit{A\beta}). This is regarded as a central pathogenic event in AD [1]. According to the ‘amyloid hypothesis’ \textit{A\beta} is neurotoxic, its deposition leading to amyloid plaques, neuronal death, and ultimately dementia. This idea was strengthened by finding \textit{\gamma}-secretase (presenilin) mutations in other families with early-onset AD, and from the utility of cerebrospinal fluid \textit{A\beta} 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]? \textit{A\beta} itself is barely toxic [4]; even its capacity to induce oxidative stress remains controversial [5]. Importantly, \textit{A\betaPP} has trophic
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 B₁₂ 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 B₁₂ 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 B₁₂ 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 (B₁₂) 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 B₁₂ 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 B₁₂ supplementation if started at an early stage [11].

In summary, APP and PSEN1 mutations confer a risk for B₁₂ 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 B₁₂ 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 B₁₂, B₆ 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 B₁₂ 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
methyl group is transferred to \( B_{12} \); methyl-\( B_{12} \) re-
methyllates homocysteine to methionine which, in its
activated form \( S \)-adenosyl-methionine, is the trans-
methyllating agent for hundreds of different methyl
acceptors. Moreover, vitamin \( B_6 \) enables a propor-
tion 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 increas-
ing number of neurons collapse [22]. Dementia is the
ultimate consequence. Essentially, this is why insuffi-
ciency of these particular B-vitamins is more closely
related to AD brain cell death than amyloid depo-
sition.

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 antichymo-
trypsin, 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 \( A\beta \)[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<sub>12</sub> to its ultimate target cells

| Compartments                                      | Processing events                                                                 |
|---------------------------------------------------|-----------------------------------------------------------------------------------|
| In the mouth and stomach                          | B<sub>12</sub> 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<sub>12</sub>, 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<sub>12</sub> is released into the blood stream |
| In the blood stream                               | B<sub>12</sub> 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<sub>12</sub> avidly and mediates its transport into target cells |
| In the target cell                                 | Transcobalamin is degraded in the lysosome, and B<sub>12</sub> 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<sub>12</sub> and its several carrier proteins. Other than as a transient phenomenon following injection, B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> disorders [27, 28].

Importantly, this intricate B<sub>12</sub> trafficking is a multistep process in terms of repeated protein binding and proteolysis (Table 1). For each repetition, B<sub>12</sub> is preferentially selected and attached to yet another binder protein and then carried to a location where the binder is degraded and B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> [30, 31].

Elevated antichymotrypsin is therefore a candidate for causing B<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> 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<sub>12</sub> intracellular processing would be helpful. It would also be valuable to assess the vitamin B<sub>12</sub> 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,
neurofibriillary tangles. Elevated homocysteine might also contribute to protein aggregation and subsequent neurodegeneration. This can occur by a mechanism involving protein N-homocysteinilation 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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