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Kepp, Kasper Planeta

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Ten Challenges of the Amyloid Hypothesis of Alzheimer's Disease

Kasper Planeta Kepp*

Technical University of Denmark, DTU Chemistry, DK-2800 Kongens Lyngby, Denmark

*Correspondence: Email: kpj@kemi.dtu.dk Phone: +45 45 25 24 09

Abstract

The inability to effectively halt or cure Alzheimer's Disease (AD), exacerbated by the recent failures of high-profile clinical trials, emphasizes the urgent need to understand the complex biochemistry of this major neurodegenerative disease. In this paper, ten central, current challenges of the major paradigm in the field, the amyloid hypothesis, are sharply formulated. These challenges together show that new approaches are necessary that address data heterogeneity, increase focus on the proteome level, use available human patient data more actively, account for the aging phenotype as a background model of the disease, unify our understanding of the interplay between genetic and non-genetic risk factors, and combine into one framework both the familial and sporadic forms of the disease.

Keywords: Alzheimer's Disease, amyloid hypothesis, challenges, toxicity, aging

Introduction

Alzheimer's Disease (AD) is one of the major health challenges of the 21st century: Based on data from the World Health Organization[1] and Alzheimer International[2], one can estimate that ~30 million people have AD world-wide as of 2016, and prevalence continues to grow. In 2010, a meta-analysis estimated 35.6 million dementia cases[3], corresponding to 21−25 million AD cases (60−70% of dementia cases are classified as AD), implying an annual growth in prevalence of 1½ million. The persistent deterioration associated with the disease is devastating to patients and relatives alike, and the prospected socio-economic burden of AD in an increasingly aged population threatens to undermine future healthcare budgets[4].

In the wake of the early successes of the major mechanistic AD paradigm, the amyloid hypothesis[5][6], the field has experienced clinical disappointments, biochemical confusions, and new arising theories[7][8][9]. Accordingly, our ability to treat the disease remains deeply unsatisfactory, with current treatments only delaying disease progression by months[10][11]. The recent failure of high-profile drug candidates has been a painful wake-up call that has intensified the debate regarding disease mechanisms and treatment strategies[7][8][12][13].

AD is a complex multi-factor disease: It occurs mostly sporadically (>95% of cases) with very limited inheritance, it has a broad clinical spectrum[14], age is the main risk factor[15], and the disease manifests slowly as mild cognitive impairment[14][16][17] and subsequently leads to loss of cognitive capabilities, identity, and activity[18][19]. The complexity is evident from the multiple genetic, life-style, and environmental risk modifiers[20][21].

Genetic variations in the genes coding for the amyloid-β protein precursor (APP)[22] and the two presenilin isoforms PSEN1 and PSEN2[23][24] cause special early-onset familial forms of AD (FAD)[25][26][27]. Thus, APP and PSEN have constituted the main basis for

understanding AD, leading to the amyloid hypothesis discussed in this paper[6][28][29]. In addition, the apolipoprotein E ε4 allele (ApoE4) increases risk by several times for heterozygote carriers and by up to 15-fold for homozygotes[30][31][32]. Many other DNA loci have been identified from genome-wide association studies (GWAS) to possibly confer some risk of AD[33][34]. Some notable ones are *BIN1*, *GAB2*, *GALP*, *ABCA7*, *TNK1*, *TREM2*, *PICALM*, and *CLU*; many of these are involved in membrane transport, endocytosis, immune system, and/or lipid metabolism[25][34][35][36][37].

Among life-style related risk factors associated with dementia (but not yet clearly separated from AD), the most important are smoking[38], alcohol usage[39][40], body mass index[41][42], diabetes[43][44], hypertension[45], physical and mental inactivity[46][47][48], and depression[49][50]. Activity and education[20][51] and particular diets such as anti-oxidant-rich Mediterranean and Indian cuisines[52][53][54] can reduce risk of dementia[55][56][57].

The amyloid hypothesis

The Aβ peptides that constitute the plaques characteristically found in patients have historically been a natural starting point for understanding the disease. Aβ is produced from APP found in the membranes of cells and organelles upon protein cleavage by β- and γ-secretases[58][59]. Furthermore, PSEN is the catalytic unit of the γ-secretase complex that degrades (among other substrates such as Notch[60]) APP into Aβ[61]. Thus, the two major genetic risks of FAD, PSEN and APP, suggest a role of Aβ in AD, providing support for the dominating paradigm of AD, the amyloid hypothesis[6][28].

The early version of the amyloid hypothesis, often referred to as the amyloid cascade hypothesis[5][28], asserted that *toxic Aβ-overload* is the cause of AD: It was inspired by the

observed amyloid deposits in AD brains that could suggest an overload mechanism at play[62]. The finding that the plaques consist of Aβ peptides provided support for the amyloid hypothesis[63]. Plaque deposits constitute several years of total production of Aβ, and the steady-state equilibrium between production and clearance of Aβ is maintained at kinetic rates of ~8% per hour[64]. Vascular transport of Aβ across the blood-brain barrier can control Aβ brain levels and is impaired upon aging[65][66][67]; this clearance is reduced in AD[68]. This equilibrium has been thought to be perturbed to gradually increase steady-state levels of toxic Aβ consistent with the buildup of plaques[28]. This version of the hypothesis was a *quantitative gain of toxic function* (or "toxic by degree") mechanism, i.e. *quantitative levels* were seen as a defining culprit of disease, supported by the major plaque deposits.

However, many FAD-causing mutations in PSEN1 do not generally increase Aβ production but actually often lower it[58][69]. Neuro-degeneration and cognitive decline does not correlate with the amount of Aβ plaques[11,12]. Also, 20−40% of cognitively normal people have Aβ plaques in amounts typical for the disease[70]. Thus, total Aβ load, which is dominated by the extracellular plaque pool, relates little to disease progress. This led to modification of the paradigm to imply that not the total Aβ levels, but the ratio between long and short forms of Aβ (mostly $A\beta_{42}/A\beta_{40}$) are molecular determinants of disease[29][71]; $A\beta_{42}$ is well-established to be more toxic than $A\beta_{40}$[6][9], so this argument had support.

Soluble oligomers of Aβ, as first reported by Yankner et al.[72], are more cytotoxic than the fibrils making up the major Aβ plaques[73][74]. The toxicity of oligomers is very dependent on size and conformation[75], and both Aβ-dimers and trimers[76] have been identified as particularly toxic[77]. Yet, the assignment of a single pathogenic form and mechanism of Aβ remains elusive[78][79]. However recently, toxicities of genetic Aβ variants were found to

correlate significantly with conformational features of the peptide variants, with disordered coil structures being more toxic[80]. The toxicity of oligomers depends on structural features as the peptide converts from unstructured monomer to the increasingly β-sheet structured oligomers[81]; these findings suggest that qualitative, rather than quantitative, features of Aβ can in principle cause disease (i.e. "toxic by kind").

The toxic mechanism of $A\beta_{42}$-enriched oligomers supposedly causing AD remains highly debated, and several toxic modes of action have been suggested[82]: They may lead to impairment of long-term potentiation[83], permeabilization of cell membranes[84][85], oxidative stress[86], and calcium dyshomeostasis[87][88]. Exposure of hydrophobic parts of the Aβ variants is likely to cause aggregation and, in various contexts, interactions with membranes and other molecules in the cell to aggravate the toxicity of the peptides[85][89][90]. Aβ has a hydrophilic and a hydrophobic part well-suited for membrane interaction, and multiple studies have documented interaction with membranes[84][85][91] and disruption of prion-protein interaction with NMDA receptors[92] and of the respiratory chains of mitochondria[93][94][95].

Many of the characterized FAD-causing mutations in PSEN1 impair γ-secretase function while increasing the $A\beta_{42}/A\beta_{40}$ ratio[69][96]; some do so while increasing, others while decreasing total Aβ levels which are dominated by the $A\beta_{40}$ isoform[97][98]. Indeed, increased proficiency of so many mutations would be *a priori* unlikely, as proteins are optimized by evolution to perform optimally under the constraints given, and thus most mutations tend to be hypomorphic[99]. PSEN1 mutations also tend to be dominant, i.e. heterozygote carriers are likely to develop AD; this feature is usually interpreted as a gain-of-toxic function because the compensatory presence of the wild type does not prevent disease[69]. Thus, the rationale for inhibiting γ-secretase in the first place, to create phenotypes resembling the FAD-causing

mutants, seems questionable, and indeed, such inhibitors have produced adverse cognitive effects[13][100].

The amyloid hypothesis has recently been reviewed and arguments in its favor have been compiled[101]. The reader may view the present paper as a counter view to the idea that a gain of function of Aβ causes AD and that therapeutic strategies should accordingly focus on Aβ containment.

Ten challenges of the amyloid hypothesis

Among challenges of the amyloid hypothesis, the following ten may be considered noteworthy:

(i) *The "normal plaques" anomaly*: 20−40% of normal elderly have been found by Aizenstein et al. to possess high loads of Aβ plaques[70], and many such people have enough plaques to satisfy common AD diagnosis criteria[102][103]; this anomaly was frequently mentioned[8][104][105]. The amyloid-centric solution to this anomaly is that these normal deposits represent pre-clinical disease states[106]; this hypothesis remains to be tested by carefully monitoring cognitively normal people for emerging clinical indications of AD over several years, correlated against measured plaque load. However, many of these plaques are diffuse and not directly related to pathogenicity[101]; thus the significance of the plaque deposits to disease progress needs to be further addressed.

This challenge also relates to the quality of biomarkers: The Aβ-related biomarkers currently used are $A\beta_{42}$ levels in the cerebrospinal fluid and Positron Emission Tomography (PET) imaging of Aβ plaque deposits[111], which usually accompanies AD diagnosis[18][402]; this method identifies Aβ deposition early in AD pathology, in support of a role of Aβ imbalance in AD[101]. However, plaque deposits are no longer considered pathogenic

themselves[106][107][108][109]. Furthermore, the tendency to form plaque-like aggregates does not correlate with clinical severity of a Aβ variants[9], and plaques are less toxic than various intracellular forms of Aβ[112][113][114][115]. PET does not primarily measure the supposedly pathogenic forms of Aβ[72], since these forms differ substantially in size and structure[75][76][77]. In addition, it is unlikely that the current therapies target the oligomer types Aβ that cause disease[78][79]. Thus, while PET imaging clearly provides important insight into disease features, we need to know much more about the specific molecular forms of Aβ relevant to different stages of the disease[117][118].

(ii) *The Aβ-localization-neurodegeneration anomaly*: Aβ is expressed throughout the brain, but AD initiates in specific parts of the brain, i.e. additional factors contribute to disease, as first observed by Gomez-Isla et al.[107] and later confirmed by Schmitz al.[108]. This point was cited as an anomaly by Bush and Tanzi[109] and has been repeated in later lists of anomalies[8][104][105]. The missing factors explaining why some areas are first hit by AD need to be accounted for.

(iii) *The neglect of normal function*: The normal functions of the central players, Aβ and APP, are not incorporated into the current form of the amyloid hypothesis, although Aβ serves beneficial roles in the normal brain[110][111]. The presence of normal functions of Aβ complicates the idea that Aβ is simply a toxic peptide whose overload triggers AD[112]. Aβ has a therapeutic window, with concentrations below nano-molar (as encountered within cells) being neurotrophic and higher concentrations (as seen in research models of the disease) being toxic[113][114], yet the amyloid hypothesis[101] focuses only on one side of this equation. Normal functions of Aβ are documented in multiple studies: Loss of $A\beta_{40}$ upon secretase inhibition or Aβ antibodies kills cultured neurons[115] and impairs neuronal activity in

mice[110]. Aβ may also protect against metal-induced toxicity[111] and regulate vesicle release in hippocampal synapses[116]. Also, the innate immune system[117][118] and pathogen responses[119] are related to Aβ imbalances, and Aβ can act as a pro-inflammatory agent in such cases[120]. The recently identified roles of infections in elevating AD risk show that inflammatory trauma plays a major role in AD pathology[121][122][123][124]. Clearly, the functions of and responses to Aβ within the neurons are still very far from understood and substantially more complicated than the current overload mechanism implies.

(iv) *The divide between familial and sporadic AD*: The amyloid hypothesis is essentially a FAD hypothesis: It is based on inherited mutations in APP and PSEN and its research models involve such genetic mutations known to overexpress Aβ. This approach is very narrow and certainly does not accurately depict the ~95% sporadic cases caused by risk factors not relating to Aβ, which should be explained. There is thus an urgent need to understand in combination the biochemical causes of the two forms of disease, e.g. by developing sporadic models of AD based on chemical-aging instead of mutations.

v) *Data heterogeneity reduces interpretative value of disease models based on APP variants*: A main basis for the amyloid hypothesis is the overexpression of FAD-related APP mutations in cells and mice. These mutations are located both within (e.g. the Dutch and Italian mutation) and outside (e.g. the Swedish mutation) the Aβ region[9]. The protective A2T mutation[125] has been widely used as a showcase of the amyloid hypothesis[101], and its lower produced Aβ levels fit well to quantitative gain of function as the protective alternative to the Swedish mutation and A2V[126]. The Swedish mutation produces very high Aβ levels and is the most used transgenic mouse model in AD research[127], yet it only models the type of overproduction of Aβ consistent with the now obsolete "cascade" hypothesis.

However, the phenotypes of APP variants are very heterogeneous, a fact sometimes overlooked when focusing on a specific variant[9]. Some of these mutations increase the $A\beta_{42}/A\beta_{40}$ ratio, others lower it. Some are more toxic than wild-type forms, others not significantly so. Some aggregate quickly, others relatively slowly. The EC_{50} values of Aβ variants vary substantially and do not correlate with clinical disease characteristics, and reported aggregation propensities are challenged by measurement uncertainties and differences in lab protocols[9]. This implies that the widely used research models have little, if anything, to do with the gradually disturbed amyloid imbalances of the aging human brain[9]. Even in terms of clinical manifestation, APP variations can give rise to either cerebral amyloid angiopathy (CAA) or classical AD, reflected in differences in the intensity of tangles and plaques[9]. This biochemical and clinical heterogeneity is not accounted for by the amyloid hypothesis, which tends to focus on transgenic models where amyloid levels are uniformly increased, a model that presumes quantitative, rather than qualitative, gain of function.

The trisomy 21 AD-related phenotype is often cited in support of the amyloid hypothesis as a clear-cut case of quantitative gain of function[101]. However, if one looks at the APP phenotypes in total, quantitative gain cannot by itself explain AD as many mutations in both APP and PSEN1 do not increase Aβ levels[9]. Also, A2V, H6R, and D7N variants (using Aβ numbering) lead to $A\beta_{42}/A\beta_{40}$ ratios similar to wild type, but E22G, E22K, and E22Q actually lower the $A\beta_{42}/A\beta_{40}$ ratio[9]. Still, drug development programs are directed towards reducing the amount of Aβ based on a quantitative gain of function mechanism that clearly does not represent the multitude of manifestations of the FAD mutant phenotypes. *The phenotypes of APP variants in their totality strongly suggest that overexpression is a side effect of some APP mutations, but not itself the cause of disease*[9].

vi) *Toxicity does not reflect pathogenicity*: The amyloid hypothesis lends support from specific Aβ toxicities measured in cultured cells. Many reported Aβ cytotoxicities[128] and aggregation tendencies[129] occurred at micro-molar concentrations, representative of ~1 year of total brain production, administered locally and instantaneously at 1000-fold higher than biological concentrations[130][131]; yet the true human disease is age-dependent and only manifest very gradually. Arguably, many amyloid toxicity studies simply prove the principle of Paracelsus that the dose makes the poison and are hardly informative. Some toxic modes associated with physiologically relevant concentrations have been reported[76], and more research in this direction seems required. Yet, quantitative measures of clinical severity (age of onset, survival times) of genetic Aβ variants do not correlate with measured toxicities[9]. Thus, any relation between toxicity in cells and mice and the real human aging brain disease remains speculative and potentially explains why such research models have not produced successful clinical treatments.

(vii) *The absence of genetic risk factors relating to Aβ turnover*: Many more mutations in PSEN1 than in APP cause AD, although APP contains the final Aβ product; this anomaly was first emphasized by Shen and Kelleher[132]. Also, the shortage of β- and α-secretase mutations and mutations in zinc peptidases such as insulin degrading enzyme involved in amyloid degradation undermine the concept that APP cleavage and Aβ production is central to disease. The FAD-related mutations, and in particular the *absent* FAD-related mutations, argue for a secondary role of APP processing (and hence, amyloid buildup) relative to other functions.

(viii) *The curious nature of the $A\beta_{42}/A\beta_{40}$ ratio:* Many FAD mutations tend to lower the levels of both amyloid isoforms, and the amyloid hypothesis relies on the $A\beta_{42}/A\beta_{40}$ ratio to argue why PSEN mutations cause AD[13][133], yet the curious nature of only this ratio (but not

total levels of $A\beta_{42}$) being pathogenic remains to be explained, i.e. how does this produce gradual buildup of toxic oligomers emphasized by the amyloid hypothesis[133]. This ratio has recently been directly correlated to clinical severity, although this does not necessarily imply causation[97]. It could be that reduced enzymatic function causes disease and that higher ratios is a side consequence[132][134].

Some possible solutions to this anomaly can be suggested: A *competitive seeding* that depends on *relative* amounts of isoforms rather than *total* $A\beta_{42}$ levels (which tend to also decrease in PSEN1 phenotypes) could mean that local surplus of longer isoforms seed degradation-resistant oligomers that enable a gradual buildup of oligomers. Also, one could imagine that hetero-oligomers enriched in longer isoforms may be less prone to degradation, thus causing a gradual buildup of pathogenic oligomer pools. Such mechanisms could demystify the $A\beta_{42}/A\beta_{40}$ ratio as a culprit of disease and provide it with a mechanistic basis.

(ix) *The aging effect*: The amyloid hypothesis does not explain the main risk factor, age, i.e. why Aβ gradually accumulates but then transits into gradual cognitive impairment and AD, as emphasized by the two-hit hypothesis[135]. The aging human proteome undergoes remarkably systematic changes with a general down-regulation of genes involved in synaptic function, including calcium homeostasis and vesicular transport, whereas genes involved in stress response, inflammation, lipid metabolism are generally up-regulated[15]. These features should be incorporated into models of AD to capture the aging effect, yet the amyloid hypothesis has little direct coupling to the aging phenotype. At the same time, the other important histopathological features of AD that relate more directly to chemical aging, e.g. metabolic deficiencies, metal ion imbalances, and oxidative stress, are not well accounted for by the amyloid hypothesis[104], and these manifestations need also to be explained in relation to amyloid imbalance.

(x) *Clinical performance*: The ultimate test of any disease theory, the development of medicine from the principles of the ruling paradigm, has not yet been successful[7][12]; the absence of any Aβ-centric drug on the market or indeed any successful phase 3 trial has led to calls for modification of the amyloid hypothesis[11]. The most promising current drug candidates are antibodies such as solanezumab that target various Aβ forms[136]. Solanezumab did not improve cognitive function in the two major phase 3 trials[137], but if one analyses the combined data there is a positive effect on cognition that should be explored further[138][139]. Aducanumab has also shown several promising data and is currently a promising candidate[140][141]. However, another antibody, Bapineuzumab, has been found not to improve cognition[142] and produce adverse effects[143] even though it does lower Aβ levels[144]. Clearly, we need to understand better these different outcomes, specifically how the various antibodies bind and modify the conformations and properties of Aβ, as the various conformations of Aβ that are targeted affect pathology differently[7][80].

In many of these cases, the clinical human data were substantially less encouraging than the mouse and cell data used to research new treatments[13][100], a troubling finding that is however consistent with the poor correlations between human and cell and mouse data from meta-analysis[9]. This problem emphasizes two major challenges in current AD research; the need for accurate models of the conformational epitopes targeted by therapies and the need for preclinical disease models that more accurately reflect the aging human brain.

Concluding remarks and perspectives

The ten challenges above emphasize that new approaches are required if the amyloid paradigm is to be retained. While the heterogeneity in clinical data is due to risk modifiers and thus a

problem to *any* paradigm of AD, heterogeneity in Aβ-specific data is due to different lab protocols and to the special chemical features of this enigmatic peptide: Efforts are ongoing to produce consistent, stable, and reproducible monomeric and oligomeric Aβ samples to remove some of the protocol-based heterogeneity[145][146]. Even beyond sample management, the structural variability of these highly disordered peptides[147] renders observed properties such as toxicity very conformation-dependent, and, since conformation relates to chemical environment, observed properties are highly sensitive to concentration, pH, ionic strength, co-solvents, and the time scale of the experiment[90][148].

Considering the major structural variability of Aβ, the "physiologically relevant" Aβ structures can be sought by correlating specific structures directly to clinical and biochemical data: This provides statistically significant relationships between fundamental chemical properties of Aβ variations and their clinical and biochemical phenotypes[80][90][149]: Remarkably, hydrophobic exposure in disordered structures correlates with the diagnosis age of patients carrying a specific variant, whereas other structures do not. This suggests that these disordered conformations of Aβ are the physiologically relevant ones[149]. Also, the differences in experimental Aβ toxicities can be explained by distinct structural features, notably the amount of hydrophobic exposure seen in the average structural ensemble of each peptide variant, providing statistically significant correlations to EC_{50} data[80][90]. Thus, Aβ aggregation and cell toxicity is caused by hydrophobic exposure in specific disordered amyloid states that could be targeted by molecular intervention, e.g. antibodies[150][151]. However, in the light of the poor correlation between toxicity assays and clinical disease features[9], the question still remains whether this oligomerization-driven cell toxicity has anything to do with AD.

To move forward on these various challenges, we must i) solve the data heterogeneity issue of peptide preparations and measurements; ii) actively use available human patient data

that tell us about the real aging human disease, considering the challenges of mouse models[127]; iii) unite sporadic and familial disease forms as we move beyond the FAD-mutation-based research models and towards chemical-aging models that account for the aging phenotype as emphasized e.g. by the two-hit hyopothesis[135]; iv) think effectively at the proteome rather than single-gene level; v) account for the normal functions of APP and Aβ, as their absence within the current paradigm is conspicuous; the elaborate splicing of APP clearly occurs in the neurons for a reason; and vi) consider all manifestations of disease, including oxidative stress and metal ion imbalances, mitochondrial disease, immune system responses, and metabolic deficiencies.

The amyloid paradigm may have been an excellent starting point, but it is, as argued above, very far from the full solution.

Conflict of interest statement

The author reports no conflicts of interest.

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