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Hypothesis

An Alternative View of Familial Alzheimer's Disease Genetics

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Abstract: A probabilistic and parsimony-based view of available genetics data is presented to argue that Hardy and Higgins' amyloid cascade hypothesis is valid but is commonly misinterpreted to support, incorrectly, the primacy of the amyloid beta peptide (A β) in driving Alzheimer's disease pathogenesis. Similar means are used to argue that the pathological effects of familial Alzheimer's disease mutations in the genes *PSEN1* and *PSEN2* are *not* exerted directly via changes in APP cleavage to produce different ratios of A β length. Instead, increase in the activity of the β CTF (C99) fragment of APP is likely the critical pathogenic determinant altered by mutations in the *APP* gene. This model is consistent with the regulation of *APP* mRNA translation via its 5' iron responsive element (IRE). Familial Alzheimer's disease (fAD) mutations in *PSEN1* and *PSEN2* likely affect the stability of presenilin holoprotein and/or γ -secretase multimers with consequences for γ -secretase activity and other important cellular functions. All fAD mutations in *APP*, *PSEN1*, and *PSEN2* likely find unity of pathological mechanism in their actions on endolysosomal acidification and mitochondrial function, with detrimental effects on iron homeostasis and promotion of "pseudo-hypoxia" being of central importance. A β production is enhanced and distorted by oxidative stress and accumulates due to decreased lysosomal function. It may act as a disease-associated molecular pattern (DAMP) enhancing oxidative stress-driven neuroinflammation during the cognitive phase of the disease. We also discuss fascinating, but largely ignored, data on presenilin biology that may be important in understanding presenilins' central role in familial Alzheimer's disease.

Keywords: Alzheimer's Disease; Familial Alzheimer Disease (FAD); presenilins; Amyloid beta Precursor Protein; mutation

1.30. years of the amyloid cascade hypothesis

In 1992 Hardy and Higgins published their famous paper, "*Alzheimer's disease: the amyloid cascade hypothesis*" [9]. The paper captured the excitement of the Alzheimer's disease (AD) research field over the emerging genetic evidence implicating the *AMYLOID BETA A4 PRECURSOR PROTEIN (APP)* gene in the inherited, early onset form of the disease (familial AD, fAD). In the following decades, this paper has been regarded as the keystone supporting the idea that AD pathogenesis is driven by accumulation of the A β peptide, which is derived from APP after this protein is cleaved by β - and γ -secretases (Figure 1). Any theory of AD pathogenesis seeking to challenge the central role of A β in AD pathogenesis must provide a more parsimonious explanation of the genetic data.

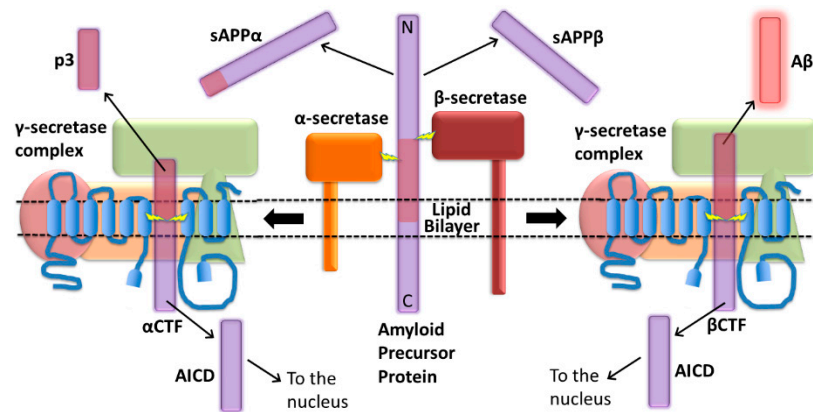


Figure 1. Cleavage of APP by α - or β - then γ -secretases. β -secretase cleavage creates secreted APP β (sAPP β) and β CTF. β CTF is subsequently cleaved by γ -secretase to form A β and the APP intracellular domain (AICD) that can be imported into the nucleus to regulate transcription.

In this paper we will use arguments based in probability and parsimony to assert that the “amyloid cascade hypothesis” as originally presented by Hardy and Higgins is correct but has subsequently been misinterpreted. Similarly, we will also examine the fAD-promoting mutations in the genes *PRESENILIN 1* (*PSEN1*) and *PRESENILIN 2* (*PSEN2*) to argue that their immediate pathogenic effects do not occur via accumulation of A β .

2. Mutations are more likely to destroy function than improve it

The amazing complexity of cellular structures and of living multicellular organisms today only exists because past changes in genetic information (mutations) have, fortuitously, resulted in improved reproductive competitiveness. However, such beneficial mutations are relatively uncommon (improbable). The great majority of mutations result either in no substantial change in cellular function or are detrimental and inhibit reproductive success. The “screwdriver analogy” illustrates this. If a photocopier (a machine for reproducing images on paper) has its screws tightened or loosened at random, most of these changes will likely have little effect, some changes will degrade photocopy quality, while a very few improbable adjustments may actually improve it.

The phenomenon that mutations with no effect are far more likely than mutations with negative effects, that themselves are far more likely than mutations with positive effects, can be referred to as the “mutation effect probability hierarchy” (MEPH). It is well illustrated by examination of mutations in the gene *APP* (Figure 2). The vast majority of genetic variations (mutations) in *APP* in the human population have no known relevance to

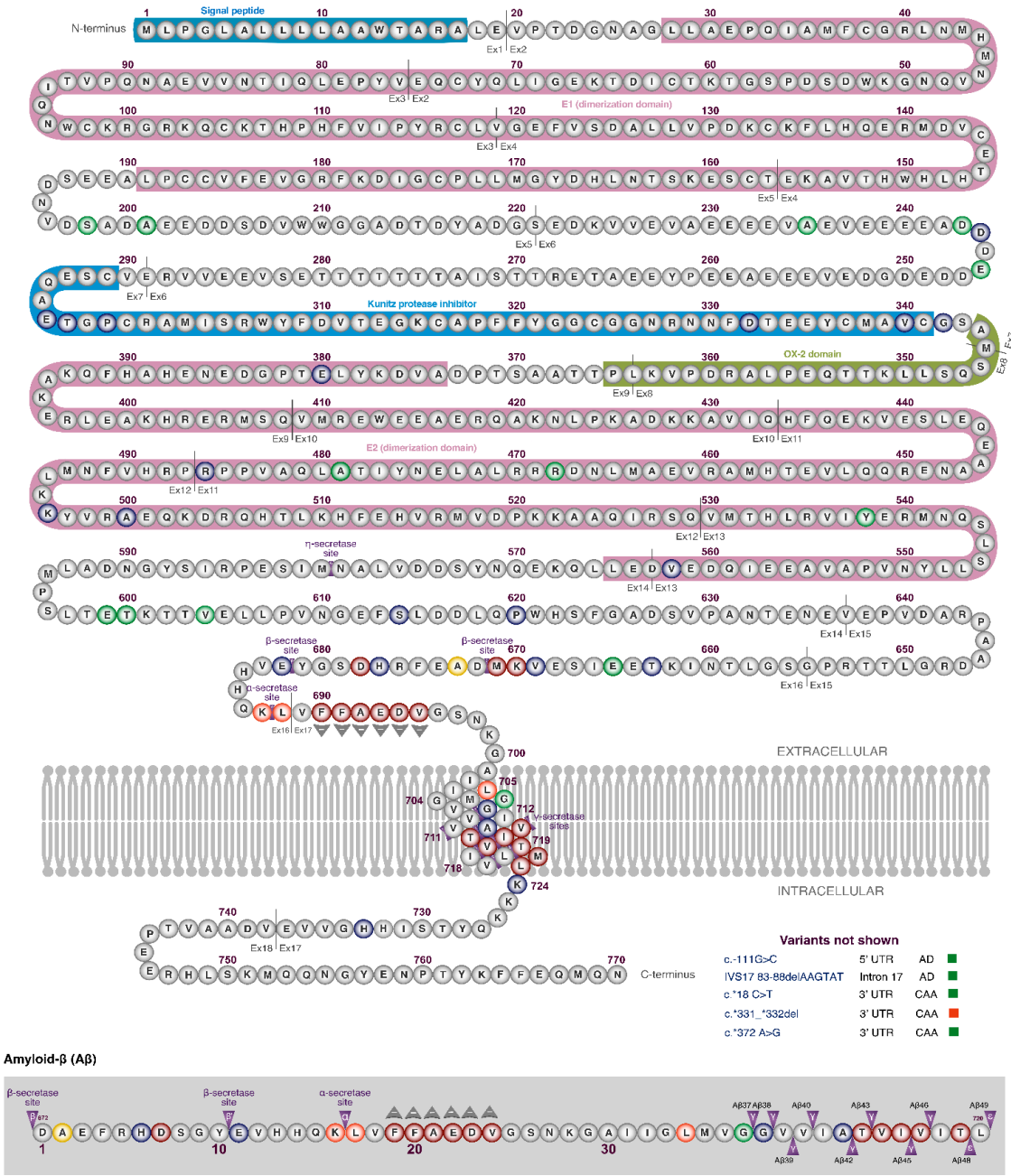


Figure 2. Schematic of the APP protein indicating disease-causing mutations from Alzforum.org (with permission). Refer to Alzforum.org for information on each mutation.

AD – this is easily seen by inspecting the data collated in the gnomAD database. When Hardy and Higgins published their hypothesis paper in 1992, it was based on identification of three AD-causative mutations in one codon (V717) of the APP open reading frame and one mutation (E693Q) causing a related disease now known as *cerebral amyloid angiopathy, APP-related, Dutch variant*. Today, we know of at least 27 such deleterious mutations in APP (see Figure 2 and the mutations collated by Alzforum.org). However, only one, uncommon mutation (A673T) found mainly in the small population of Iceland is known to promote mental health by reducing AD risk [10-15].

3. The current “understanding” of fAD genetics

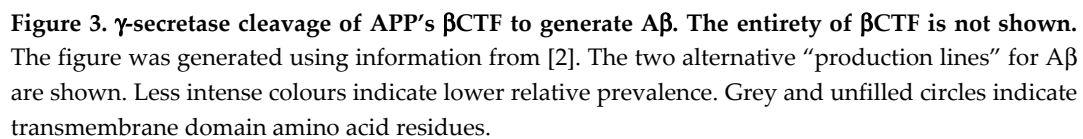
Of the ~20,000 protein-coding genes in humans, only three are known definitively to host mutations causing AD: *APP*, *PSEN1*, and *PSEN2* [16, 17]. A fourth gene, *SORTILIN-RELATED RECEPTOR 1* (*SORL1*) may also cause fAD before the age of 65 years but its relationship to the other fAD genes is relatively poorly understood [17-20].

APP

Decisive for our understanding of how mutation of *APP* drives AD is the fact that simple increased expression of the *APP* coding sequence is sufficient to cause disease. Three doses of *APP*, whether through trisomy of chromosome 21 [21], or duplication of *APP* within chromosome 21 [22], causes early onset of AD. This means that increased expression of the APP protein or one of its fragments is highly likely to be driving the pathogenic changes of AD.

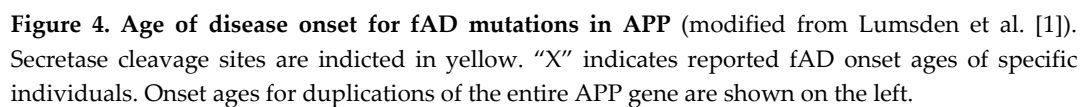
Within the *APP* gene, mutations causing AD are clustered within, and flanking, the sequence coding for A β (Figure 2). This fact is seen as support for the prime role of A β in driving AD pathology. According to the MEPH, we would expect most of these mutations to degrade/inhibit particular APP functions/abilities. Consistent with the MEPH, we find evidence that many of the mutations in the region coding for the γ -secretase cleavage site decrease the ability of the γ -secretase enzyme to cleave APP [23]. Other mutations occur in codons for the α - and β' -cleavage sites of APP respectively, and decrease the proclivity of these sites for cleavage by their relevant enzymes [24, 25], thereby increasing the likelihood of cleavage at the β -site. Interestingly, one unique mutation, the “Swedish mutation” of APP (KM670/671NL) drives development of AD by improving the proclivity of the β -site for cleavage by the β -secretase enzyme BACE1 [26, 27] (while the aforementioned A673T mutation near this site uniquely improves survival by reducing the ability of β -secretase to cleave at the β -site [10]).

The experimental data regarding whether fAD mutations in APP increase or decrease A β production is remarkably variable. The comprehensive analysis published by Xu et al. [23], that saw nearly all fAD mutations of APP decrease γ -secretase cleavage, monitored the γ -secretase cleavage needed to produce A β using cells transfected to express a modified β CTF. The APP intracellular domain (AICD) of the β CTF was fused to a transcription factor to allow detection of cleavage at the ϵ -site (Figure 3) through activation of luciferase expression. By reporting on γ -secretase at the ϵ -site rather than after the subsequent, variable “production line” γ -secretase cleavages (see Figure 3) the rates of γ -secretase cleavage should be consistently comparable between different fAD mutant forms of β CTF. When, instead, levels of the A β product are used to compare γ -secretase cleavage of β CTF mutants in transfected cells, the differing levels of β CTF could have differential effects on lysosomal acidification [28], thereby affecting A β degradation and accumulation. This might partially explain, for example, why Xu et al. observed decreased γ -secretase cleavage of APP due to the fAD mutation D678H whereas Chen et al. [29] saw a relative increase in A β levels. However, Chen et al. also observed that the D678H mutation shifted the initial cleavage of APP from the α -site to the β -site which would increase β CTF levels and also promote A β production. Numerous mutations in the A β region of APP also affect its tendency to aggregate and form fibrils. However, in many cases these mutations are associated with accumulation of A β in brain vasculature in the disease cerebral amyloid angiopathy (CAA) that is distinct from AD e.g. *APP* mutations E693K [30, 31] and L705V [32].



A difficulty with much of the work analysing fAD mutations of *APP*, including their cleavage by γ -secretase, is that much of the research is conducted using transfected cells that significantly over-express *APP*. Also, the fAD genes are very responsive to stress (e.g. [58-60]) and cells in tissue culture (such as the transfected cells used in much fAD gene analysis) are frequently stressed by their unusual environmental conditions [61]. In contrast, fAD mutation-carrying humans are almost always heterozygous, raising the possibility of interactions between mutant and non-mutant forms of *APP* or its fragments. Indeed, dimerization of *APP* is known to affect its γ -secretase cleavage to form $A\beta$ [62-64]. Studies using editing of endogenous *APP* genes in iPSCs to compare heterozygous mutant and non-mutant cells (e.g. [65] below) should produce information that is interpretable with greater certainty (fewer possible variables involved).

In accordance with the “Law of Parsimony” [66] it is highly likely that all the various mutations in APP cause AD through a single mechanism in common. Based on the information above, this is likely to involve an increase in production of the fragment of APP produced by cleavage at the β -site,



Three decades of research focussed on A β has not yet established convincingly the toxic nature of this peptide under normal, physiological conditions, e.g. [67-71]. The apparent recent successes of anti-A β antibody therapies [72] supports a pathological role for this peptide in the clinical phase of the disease, possibly as a disease-associated molecular pattern (DAMP) contributing to the inflammation that is characteristic of AD brains [73, 74]. However, the anti-A β antibody therapies are apparently unable to prevent onset of the dominantly inherited disease [75] and only slow the progression of the sporadic, late onset disease rather than stopping it [72]. This supports that something else drives the pre-clinical phase of AD. In interpreting the results of much A β -based AD research, it is remarkable how seldom the presence and levels of β CTF are assessed. And yet β CTF levels are likely very significantly altered in transgenic mouse models of AD that include APP transgenes (including the models 3xTg, 5xFAD, A7, APP23, APP^{swe}/PSEN1dE9, PDAPP, J20, PDAPP, PS2APP, Tg2576, Tg-ArcSwe, TgCRND8, [76]), while β CTF has been seen to accumulate in AD brains [77-79]. Much of the evidence supporting a role for β CTF in both fAD and sporadic AD was reviewed in a paper by Checler and colleagues [80].

A number of papers below give very strong support for increased β CTF levels as the pathological effect of fAD mutations in *APP*. Note that the first two papers, and our later discussion of the effects of fAD mutations in *PSEN1* and *PSEN2*, link these genes to normal functioning of the cellular endolysosomal system. Many of the late onset AD risk loci discovered by genome-wide

association studies (GWAS) are also important for endolysosomal system function [81, 82]. Indeed, the strongest genetic risk factor for sporadic (non-familial) AD, the $\epsilon 4$ allele of *APOE* (*APOE4*), reduces lysosomal acidification relative to the $\epsilon 3$ allele [83] (in common with *fAD* mutations in *APP* [28] and the presenilins [84]).

Kwart et al. [65] examined the subcellular effects of a variety of *fAD* mutations in *APP* and *PSEN1* after these were edited into a single iPSC cell line (for consistency of genetic background) that was then differentiated into neurons. The mutations consistently caused accumulation of, specifically, APP's β CTF but had inconsistent effects on $A\beta$ levels. Rab5+ early endosome size was correlated with β CTF levels and could also be increased by chemical inhibition of γ -secretase activity (which reduced $A\beta$ to negligible levels).

Jiang et al. [28] examined fibroblasts from trisomy 21 individuals (Down syndrome) who suffer early onset Alzheimer's disease. They showed that the increased dosage of the *APP* gene (present on chromosome 21) led to increased levels of β CTF. This decreased the acidification of lysosomes (and, thereby, the activity of a marker of lysosomal function, the enzyme cathepsin D, CTSD). These phenomena could be modulated by various treatments altering the levels of β CTF in normal and Down syndrome fibroblasts. For example, chemical inhibition of β -secretase cleavage of *APP* (to decrease β CTF levels) decreased lysosomal pH in trisomy 21 syndrome fibroblasts but, interestingly, did not lower lysosomal pH in euploid (normal diploid) fibroblasts.

Montesinos et al. [54] found that APP's β CTF regulates the trafficking of cholesterol from the plasma membrane (PM) to the ER. Increased levels of β CTF inhibits a cell's own cholesterol synthesis and increase the rate of cholesterol trafficking from the PM. The increased ER cholesterol increases formation of the mitochondrial associated membranes (MAM) of the ER through which cells regulate mitochondrial energy production via Ca^{2+} ion release [85]. Increased MAM formation is observed in fibroblasts from both *fAD* and late onset AD patients compared to those from cognitively healthy controls [86].

In previous papers some of these same researchers showed that APP's β CTF and the *PSEN1* and *PSEN2* proteins are all present in the MAM [57, 87] where they regulate sphingolipid and ceramide homeostasis [57].

3. The original amyloid cascade hypothesis included β CTF as a candidate pathogenic agent

The possibility that β CTF is a pathological agent driving AD is actually suggested by Hardy and Higgins in their 1992 paper. (In the following quotation, the authors refer to $A\beta$ as " $A\beta$ P" and I have bolded the text referring to β CTF.)

"Our cascade hypothesis states that $A\beta$ P itself, or APP cleavage products containing

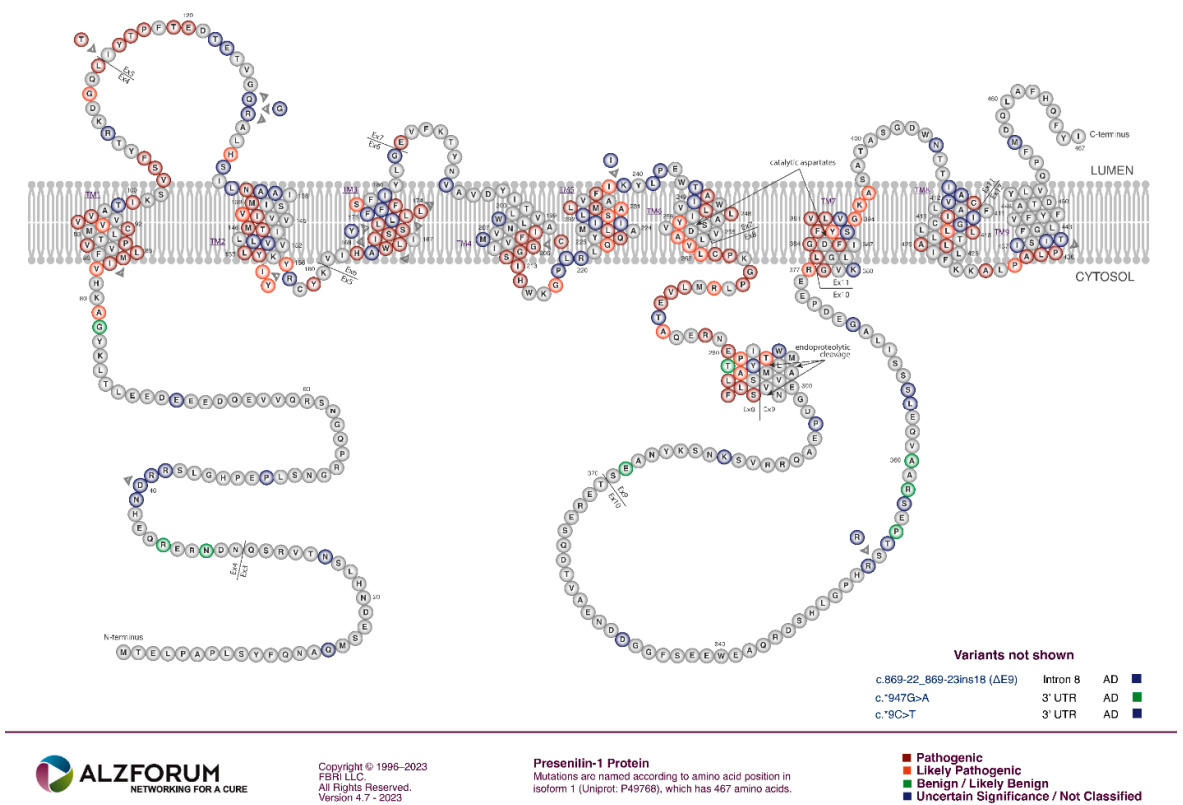
$A\beta$ P, are neurotoxic and lead to neurofibrillary tangle formation and cell death. Thus, two successive events are needed to produce Alzheimer's pathology. First, $A\beta$ P must be generated as an intact entity, either by accumulation of $A\beta$ P or as an $A\beta$ P-containing fragment of APP. Second, this molecule must facilitate or cause neuronal death and neurofibrillary tangle formation. Neve and her colleagues have reported that the $A\beta$ P-containing COOH-terminal fragment is toxic to cultured neurons (18)...

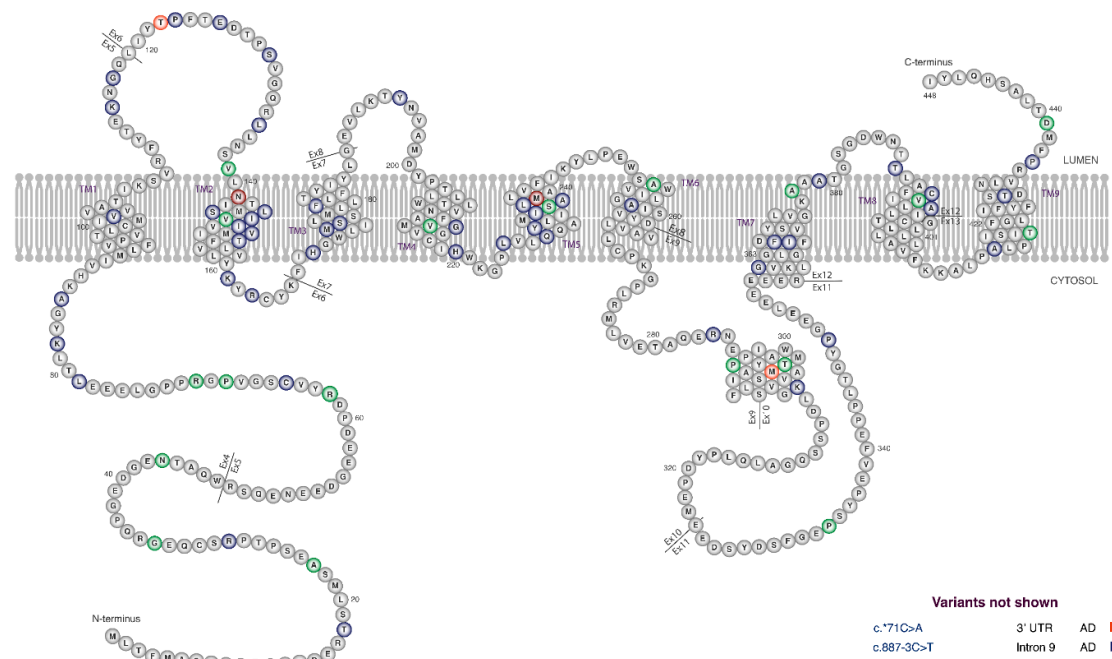
"... The evidence we have described supports the hypothesis that the $A\beta$ P molecule initiates the pathological cascade of Alzheimer's disease. $A\beta$ P-containing COOH-terminal derivatives of APP seem the most likely molecular candidates for initiation of the cascade, with the process presumably taking several decades to produce the full-blown pathology of the disease."

The likelihood that β CTF represents the toxic agent in *APP* mutation-driven AD means that experiments looking at $A\beta$ accumulation in human brains or animal models should also examine β CTF levels to avoid the possibility of inappropriately attributing pathological agency to $A\beta$.

PSEN1 and PSEN2

The majority of mutations causing fAD occur in the gene *PSEN1* both in terms of their relative frequency among fAD mutations in the human population (~63%, [17]) and relative to the number of fAD mutations known in *APP* and *PSEN2* (~80% of the known mutations, [88], Figure 5). Collectively, fAD mutations in *PSEN1* also have the earliest median onset age [89]. Relatively far fewer mutations occur in the gene *PSEN2* and these also have a later median age of onset than even mutations in *APP* [89] (Figure 6). Importantly, both *PSEN1* and *PSEN2* encode proteins with similar structures and functions, implying that disruption of a function they share in common causes AD. Intriguingly, while the large number and wide distribution of fAD-causative mutations in the coding sequences of *PSEN1* and *PSEN2* make it extremely likely that these mutations cause loss of a function(s), two characteristics of these mutations are consistent with action via a gain-of-function mechanism:





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Presenilin-2 Protein
Mutations are named according to amino acid position in isoform 1 (Uniprot: P49810), which has 448 amino acids.

■ Pathogenic
■ Likely Pathogenic
■ Benign / Likely Benign
■ Uncertain Significance / Not Classified

Figure 6. Schematic of the PSEN2 protein indicating disease-causing mutations from Alzforum.org (with permission). Refer to Alzforum.org for information on each mutation.

1. The great majority of fAD mutations in *PSEN1* and *PSEN2* are missense mutations altering the protein coding sequence. Even those mutations causing changes in transcript splicing (e.g. *PSEN1* L113_I114insT [90]) always produce at least one transcript form that preserves the open reading frame to code for a “full-length” protein. We addressed this issue in a previous review [16], describing it as the *reading-frame preservation rule*. Consistent with this (with the exception of one unique and important gain-of-function mutation discussed later [91]), no mutations or genetic variants affecting *PSEN1* or *PSEN2* transcript regulation (without changing protein-coding sequences) have been discovered that increase the risk of AD [92].
2. Loss-of-function mutations that would be expected simply to reduce γ -secretase activity, without otherwise distorting it, do not cause fAD. This is dramatically illustrated by the existence of frameshift mutations in *PSEN1* that truncate the open reading frame and do not cause fAD while causing a completely unrelated disease of the skin, *Acne Inversa, familial 3*, (ACNINV3, also known as hidradenitis suppurativa) [93–95]. Unlike fAD, familial Acne Inversa can also be caused by mutations in genes encoding two other components of the γ -secretase enzyme complex, *NICASTRIN* (*NCSTN*), and *PRESENILIN ENHANCER, GAMMA-SECRETASE SUBUNIT* (*PSENEN*, formerly known as *PEN2*) [93] (See also Figure 7). All these mutations affecting different components of the γ -secretase enzyme complex almost certainly act through reduction of cellular γ -secretase activity but mutations causing fAD *only* occur in *PSEN1* and *PSEN2* and *not* in genes encoding other γ -secretase complex components. Therefore, fAD cannot be due to a simple loss of γ -secretase activity. The *reading frame preservation rule* implies that a gain-of-function mechanism is involved.

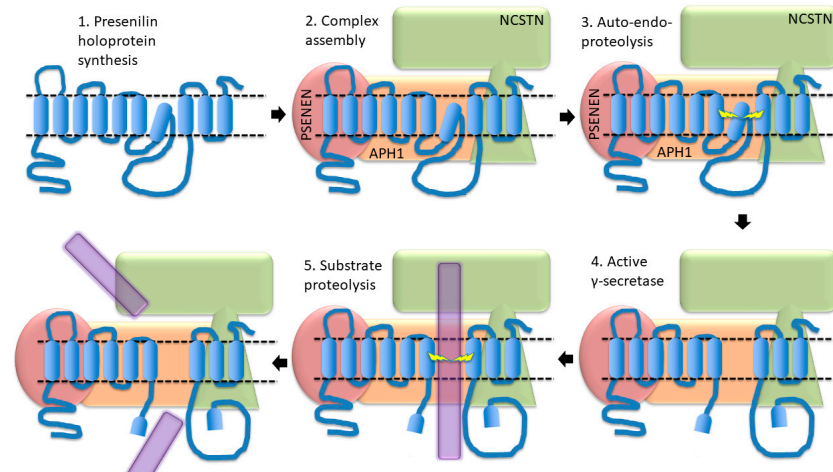


Figure 7. γ -secretase complex assembly, activation, and action. NCSTN, PSENEN (PEN-2), and APH1A/B assemble with the presenilin holoprotein. Auto-endoproteolysis occurs which activates the ability to endoproteolyse other γ -secretase substrates.

The role of both *PSEN1* and *PSEN2* in γ -secretase activity, and the fixation of the AD research field on A β that requires γ -secretase activity for its production from β CTF, has meant that nearly all research on *PSEN1* and *PSEN2* has focussed on γ -secretase. Since fAD mutations in *APP* that affect the protein's γ -secretase cleavage site reduce A β production, while fAD mutations in *PSEN1* and *PSEN2* do not consistently reduce γ -secretase activity (see below), research has focussed on one possible commonality of all these mutations – their effects on A β peptide length ratios no matter what the total level of A β production may be. Some early studies appeared to find correlations between the ages of AD onset caused by different fAD mutations in *PSEN1* and the A β_{42} : A β_{40} ratio caused by these same mutations in transfected cell culture-based assays [96, 97]. However, the comprehensive analysis of 138 *PSEN1* fAD mutations by Sun et al. in 2016 [98] using cell-based and *in vitro* assay systems found these apparent correlations to be statistical artefacts (see Figure 5 in [98]). That work also found that, while the majority of the 138 mutations they analysed decreased γ -secretase activity (as measured by combined A β_{40} and A β_{42} production – a loss of function), ~10% of the fAD mutations increased γ -secretase cleavage of APP (a gain of function, at least in their cell-free assay system that over-expressed mutant *PSEN1* proteins in the absence of wild type *PSEN1*). The increased γ -secretase activity of at least one of these mutations, S365A of *PSEN1*, has been confirmed independently by others [99]. Sun et al. saw that some fAD mutations of *PSEN1* lost all apparent γ -secretase activity while others showed decreased A β_{42} : A β_{40} ratios rather than increased ratios. In summary, Sun et al. saw no consistent changes in γ -secretase activity (when mutant proteins were assayed in homogeneous states) that would support a role for γ -secretase, or for A β , as pathogenic agents promoted by fAD mutations in *PSEN1*.

The idea that A β fragment length is the critical determinant of the AD pathological process was reinvigorated by a study published in 2022 (Petit et al. [2]) analysing a larger number of A β fragments to define a A $\beta_{(37+38+40)}/A\beta_{(42+43)}$ ratio. This ratio reflects more completely the relative activities of the two alternative γ -secretase cleavage pathways of APP that are determined by the initial site of APP γ -secretase cleavage (“ ϵ -cleavage”) after which the γ -secretase enzyme is thought to cleave progressively towards the N-terminal of A β before A β escapes from the lipid bilayer and the enzyme complex (Figures 1 & 3). For the mutants analysed by this study there appears to be a strong correlation between the age of disease onset and the A $\beta_{(37+38+40)}/A\beta_{(42+43)}$ ratio when represented as a percentage of the wild type ratio (see Figure 2 of the Petit et al. paper). In a 2021 paper from some of the same authors, Szaruga et al. [100], changes in A β product ratios are attributed to the tendency of the mutations to destabilise γ -secretase-substrate interactions (as measured by the change in A β fragment production with temperature) and so can be viewed as loss-of-function. Interestingly, neither of these papers analysed any of the 12 *PSEN1* gain-of-function mutations found by Sun et al.

actually to increase total A β production (as measured by combined A β_{40} and A β_{42} production). As might be expected for fAD mutations of APP that decrease cleavage at APP's γ -secretase site, Szaruga et al. found that these mutations also destabilised APP's interaction with the γ -secretase enzyme complex.

A 2023 study (Liu et al. [101]) examined the production of A β fragments with lengths of 37, 38, 39, 40, 42, and 43 aas produced by HEK293 cells lacking endogenous presenilins but transfected to express wild type or one of 130 different fAD mutant forms of *PSEN1* (together with APP's β CTF). It found a best correlation with disease onset age of $r = 0.5807$ ($p < 0.0001$) for the ratio of A β_{37} /A β_{42} . The correlation for the A β_{40} /A β_{42} ratio was also highly significant. Each mutant analysis was, apparently, performed only once and, interestingly, almost a third of these showed higher levels of total A β production (or of A β_{40} plus A β_{42} production as Sun et al. [98] examined) than the wild type controls (see Supplementary Data File 1 for an analysis of the A β production data in Table 1 of Liu et al. [101]).

Presenilin Holoproteins

The presenilin proteins at the catalytic core of the γ -secretase enzyme complex may be auto-activating zymogens. Presenilin proteins are first translated as "holoproteins" that possibly cleave themselves endoproteolytically [102] to form catalytically active heterodimers consisting of the N- and C-terminal fragments (NTF & CTF) within the γ -secretase enzyme complex (Figure 7). Some fAD mutations strongly affect this activating cleavage [103-107]. Petit et al. [2] and Szaruga et al [100] noted that such mutations (e.g. *PSEN1* mutations P433S, R278I and L435F) have milder clinical effects (i.e. later ages of onset) than expected from correlations between other mutants. As described below, the later-than-predicted ages of onset of these mutations strongly inhibiting γ -secretase activity are consistent with an alternative presenilin fAD mutation pathogenic mechanism we shall outline.

Compared to research on γ -secretase, very little research has examined the biological role of the presenilin holoproteins or their possible role(s) in AD pathogenesis. This is likely because the holoprotein states of presenilins are commonly regarded as short-lived and transitory [108]. However, in the early days of characterisation of presenilin biology, careful analysis by Dewji et al. [109] demonstrated that, when cells are subjected to gentle lysis, most presenilin can subsequently be detected in the holoprotein state. (This suggests the tantalising possibility that presenilin proteins may be involved in responses to mechanical stress in addition to responding to hypoxia/oxidative stress [59]).

The most important paper demonstrating the functional uniqueness of the *PSEN1* holoprotein was published in 2010 by Ralph Nixon and colleagues (Lee et al. [84]). This described an important role for the holoprotein in correct assembly of the vacuolar ATPase (v-ATPase) that pumps H⁺ ions into the endolysosomal system – and particularly the lysosome – for acidification. Here we see a convergence of the activities of the presenilins and the β CTF of APP – all are modulating endolysosomal pH (Figure 8). In fact, Nixon and colleagues have commented (without yet presenting experimental data) that β CTF may bind to v-ATPase to modulate v-ATPase activity [28]. These researchers have,

"...identified a direct physical interaction of lysosomal-associated APP- β CTF with the cytosol-exposed domain of the transmembrane V0a1 subunit of the vATPase. Competition by APP- β CTF with specific V1 subunits for binding to the V0a1 subunit impedes association of the V1 and V0 sectors of the complex....Impaired vATPase assembly ... is rescued by specifically lowering APP- β CTF levels"

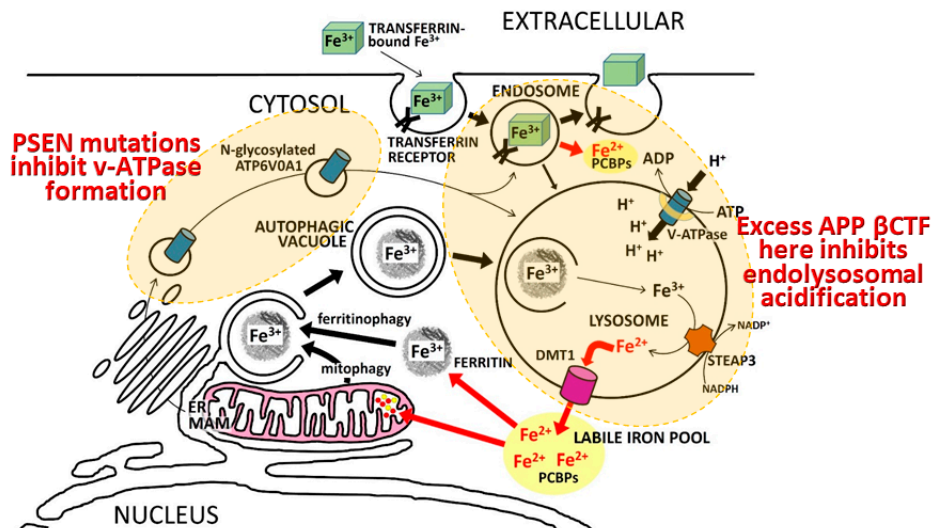


Figure 8. fAD mutations in the presenilins and in APP both affect acidification of the lysosome with consequences for iron importation, the availability of Fe^{2+} ions, and recycling (for Fe^{2+} recovery through autophagy) of mitochondria and of Fe^{3+} stored in ferritin.

Regardless of whether or not βCTF interacts physically with v-ATPase components, the fAD mutant forms of APP, PSEN1, and PSEN2 [110], all affect the acidification of the endolysosomal pathway (as, interestingly, does the late onset AD risk allele APOE4 [83]). Failure to acidify lysosomes sufficiently will inhibit their ability to degrade $\text{A}\beta$ [111], causing $\text{A}\beta$ to accumulate in lysosomes and leading, ultimately, to the formation of neuritic $\text{A}\beta$ plaques [112]. In addition, Kim et al. [113] recently showed evidence that both $\text{A}\beta$ and MAPT (Tau) can bind to v-ATPase components to inhibit their function. Furthermore, Mustaly-Kalimi et al. [114] recently observed decreased v-ATPase expression when cytosolic concentrations of Ca^{2+} ions are raised, while an earlier 2015 paper from Lee et al. [115] found that loss of lysosomal acidification leads to increased levels of cytosolic Ca^{2+} . These observations imply the existence of pathological positive feedback interactions between altered cytosolic Ca^{2+} levels and lysosomal acidification. Overall, the function of v-ATPase has emerged as a hub for the pathological influences of many agents identified as important in the development of AD.

The 2010 Lee et al paper [84] showed evidence that, in the endoplasmic reticulum, PSEN1 holoprotein interacts with the oligosaccharyltransferase complex to promote N-glycosylation of the V0a1 subunit of v-ATPase. This is necessary for correct targeting of the V0a1 subunit to the lysosome for assembly of functional v-ATPase. This PSEN1 holoprotein-dependent function in glycosylation is deficient in mouse cells lacking *Psen1*, but also in human fibroblasts heterozygous for fAD mutations in *PSEN1*. In conclusion, the numerous (and so, presumably, loss-of-function) fAD mutations of *PSEN1* appear to act through a gain-of-function mechanism (see point 2 above) to cause a dominant negative (loss-of-function) effect. How is this possible? We suggest that disruption of a multimeric holoprotein complex provides a parsimonious solution to this quandary.

4. Presenilin holoproteins and/or γ -secretase complexes form multimers

Biochemical evidence supports that the presenilin proteins form multimers [105, 116, 117]. In particular, Heilig et al. [105] and Zhou et al. [99] presented strong evidence from immunoprecipitation that fAD mutant PSEN1 holoproteins can bind (directly or indirectly) to wild type PSEN1 holoproteins. Heilig et al. saw association of differentially-tagged holoproteins in cells co-transfected with expression vectors for these proteins. In contrast, Zhou et al. saw association between pre-purified γ -secretase complexes containing different mutant forms of presenilin. This raises the question of whether the multimeric association involves only the holoproteins or occurs between holoproteins in association with some or all of the other components of γ -secretase complexes. Both Heilig et al. and Zhou et al. reported dominant negative effects of the mutant

proteins on the γ -secretase activity of the wild type (non-mutant) protein. However, under both experimental regimes, the relative reduction in γ -secretase activity was small (~20%) for a 1:1 ratio of mutant to wild type protein resembling a heterozygous situation. This led Zhou et al to conclude,

"To gain a better understanding of the moderate dominant negative effect, the expression level of the WT and mutant PSEN1 alleles, as well as the oligomerization state of γ -secretases, should be carefully examined under in vivo circumstances, especially in patients' brains."

Importantly, Zhou et al. also noted,

"We wish to stress that our experimental data provide no supporting evidence for a potential role of γ -secretase in the development of AD. In fact, a number of the γ -secretase variants with pathogenic PS1 mutations, exemplified by S365A, have WT-level proteolytic activity in terms of A β 42 and A β 40 production (18). The development of AD in the patients with these PS1 mutations cannot be explained by the WT-level proteolytic activity of these γ -secretase variants in vitro. Nonetheless, the dominant negative effect of these PS1 mutations in patients with AD still applies, suggesting such an effect may not be recapitulated by the catalytic function of PS1 in γ -secretase. We speculate that, for the vast majority of patients with AD, the dominant negative effect of PS1 is perhaps effected through other mechanisms that are independent of γ -secretase."

It is also worthwhile noting that a super-resolution microscopy study of presenilin distribution on the surface of cells saw mainly monomers with some possible dimer formation [118]. However, this may not be true of all cellular membranes as the amounts of presenilins/ γ -secretase complexes vary within cells, with particularly high levels being located in the mitochondria associated membranes (MAM) of the endoplasmic reticulum [87].

The importance of PSEN1 and APP's β CTF for the acidification of the endolysosomal pathway, and the involvement of many late onset AD risk loci in endolysosomal function [81], supports that defects in endolysosomal pathway function may be a key, unifying, driver of AD pathology (Figure 8). If all fAD mutations of the presenilins caused loss of γ -secretase activity, then this would also promote accumulation of APP's β CTF to reduce lysosomal acidification and would be consistent with this unifying endolysosomal hypothesis for AD pathogenesis. However, as described above, some fAD mutations appear to increase γ -secretase cleavage of β CTF. There is evidence that correct endolysosomal pH may be important for normal γ -secretase activity [119-122] (although, as discussed by Maesako et al. [122], this conflicts with the supposed pH optimum of 6.3–7.0 for γ -secretase activity established in biochemistry-based assays). Therefore, it is conceivable that the problems with endolysosomal acidification, caused by the effect of any fAD mutation on presenilin holoprotein function, may be sufficient to decrease overall γ -secretase activity in the endolysosomal system, even for those fAD mutations that show increased γ -secretase activity in homogeneous assay systems (i.e. no presence of wild type presenilin). However, a recently discovered mutation in PSEN2 is inconsistent with this idea. Pang et al [91] found that a mutation in the 3' untranslated region (UTR) of PSEN2 transcripts increases the expression of PSEN2, apparently by destroying the binding site of the miR-183-5p microRNA. This mutation was strongly linked to fAD in a Chinese family lacking identifiable fAD mutations in other genes. Since no missense mutations were identifiable in PSEN2, PSEN1 or APP, an implication is that increased γ -secretase activity alone can drive AD pathogenesis.

5. A parsimonious model to account for superficially inconsistent/conflicting presenilin mutation data

Of the ~20,000 protein-coding genes in the human genome, the overwhelming majority of fAD mutations occur in only three – APP, PSEN1, and PSEN2. This restricted number of genes, and the known enzyme/substrate relationship between their protein products, supports that a single mechanism underlies fAD pathogenesis. (Alternatively, protein substrates of γ -secretase are not uncommon – they are encoded by more than 1/200th of the set of all protein-coding genes [33, 34] - so it is not extremely improbable that the fAD-promoting shared activity of mutations in APP, PSEN1, and PSEN2 may be something other than effects on γ -secretase, such as reduced lysosomal

acidification, i.e. while loss of γ -secretase cleavage susceptibility may be important for the action for some fAD mutations in *APP*, changes in γ -secretase activity in heterozygous fAD presenilin mutants need not be related to the pathogenic activity of those presenilin mutations.)

In a 2018 review examining the role of iron in AD [1] we discussed that the disease may have two distinct phases: a preclinical phase of increasing brain hypoxia with increasing reliance on glycolysis, followed by a clinical phase where cognition falters due to “metabolic inversion” of the brain into a hypometabolic state (after breaching a brain metabolic homeostasis capacity threshold - see the “Stress Threshold Change of State” model in Figure 10B). Interestingly, γ -secretase is intimately involved in hypoxia responses. It cleaves p75NTR in a feed-forward mechanism driving stabilisation of the HIF1 α subunit of the transcription factor HIF1, a master regulator of cellular responses to hypoxia [123]. Importantly, γ -secretase activity is also activated by HIF1 α but not via transcriptional regulation. Instead, HIF1 α binds to γ -secretase [124] (possibly directly to presenilin [125]) to increase the pool of active γ -secretase [124]. In experiments using zebrafish heterozygous for a fAD-like mutation of their *PSEN1*-orthologous gene (*psen1*^{Q96_K97del}), under normoxia we saw increased basal expression of genes known to be responsive to hypoxia in the brains of young adult fish that were, nevertheless, still able to upregulate these genes further under acute hypoxia. However, when fish became aged, the response to hypoxia of these hypoxia-responsive genes became inverted – they were downregulated by acute hypoxia. This inverted response occurred at an earlier age in fAD-like mutant fish than in non-mutants, supporting that the fAD-like mutation drives accelerated aging in this aspect of brain metabolic control [126]. The observations above would be consistent with an increase in γ -secretase activity in the brains of young fish and a decrease in γ -secretase activity after an age-dependent “state change” in energy metabolism regulation.

Is there any additional evidence that fAD mutations might drive pathogenesis by first increasing γ -secretase activity before it decreases after a metabolic change of state? When we analysed the transcriptomes of young adult zebrafish brains heterozygous for another fAD-like mutation of the zebrafish *PSEN1*-orthologous gene (*psen1*^{T428del}, equivalent to the T440del mutation of human *PSEN1*), we did see overall increased expression of genes known to be upregulated by Notch signalling that is dependent on γ -secretase activity [127].

The wide distribution and missense nature of fAD mutations in the *PSEN1* and *PSEN2* protein-coding sequences is not consistent with their affecting any particular sub-functional domain in these proteins. However, all such mutations might be expected to affect, to some degree, the conformation of the holoprotein, and that may destabilise holoprotein multimeric complexes. If multimer dissociation is required for conversion of the holoprotein into active γ -secretase then, in heterozygous cells, a fAD-mutant form of holoprotein with decreased intrinsic γ -secretase activity might, nevertheless, promote an overall increase in γ -secretase activity by causing increased activation of non-mutant holoproteins, in addition to displaying its own γ -secretase activity (see Figure 9). Cells heterozygous for fAD mutations encoding proteins with very little intrinsic γ -secretase activity (such as the *PSEN1* P433S, R278I and L435F mutations mentioned above) should show comparatively less total cellular γ -secretase activity than other fAD mutations, and would drive AD pathogenesis more slowly, giving their relatively later ages of disease onset as observed. We suggest, therefore, that a consistent, unifying pathogenic activity of fAD mutations in *PSEN1* and *PSEN2* may be that these all increase γ -secretase activity in heterologous systems co-expressing both fAD mutant and wild type forms of these proteins.

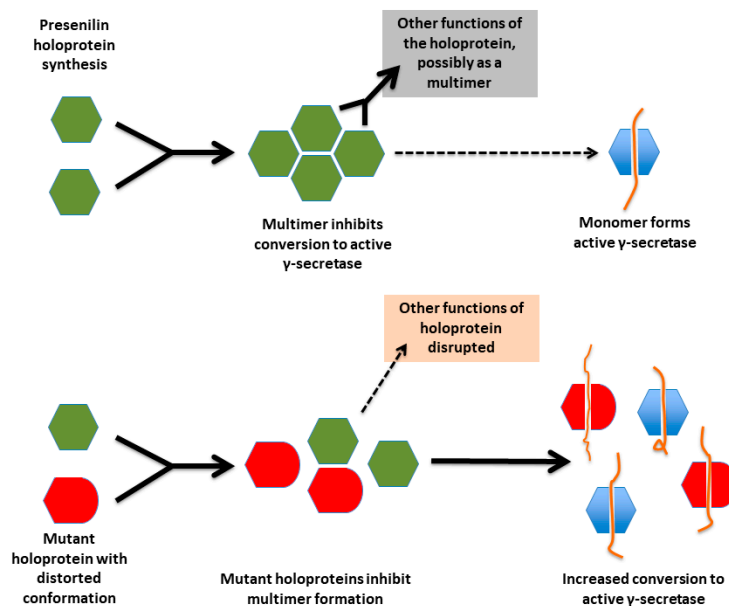


Figure 9. A multimerisation model for how heterozygosity for fAD mutations in the presenilins may increase γ -secretase activity by destabilising multimers of holoproteins or even not-yet-active γ -secretase complexes to give excessive γ -secretase activation.

This hypothesis should be consistent with the mutation fAD onset age/ $A\beta$ fragment ratio correlation of Petit et al [2] described previously since, for those mutations that were examined, the $A\beta$ fragment ratio apparently reflects the degree of destabilisation of the presenilin-substrate interaction (Szaruga et al. [100]) which likely corresponds to the degree of distortion of the presenilin protein within the γ -secretase complex. Any such fAD mutation might be expected to distort the conformation of the holoprotein (or an assembled γ -secretase complex) to a similar relative extent, and this should be reflected in the degree of destabilisation of holoprotein multimeric complexes. We note that, for this holoprotein multimer destabilisation model to function, the mutant and non-mutant forms of presenilin holoprotein must retain some ability to interact/bind each other. The N-terminal sequences of PSEN1 are known to be important for multimer formation [116, 128, 129] and, interestingly, there is no strong evidence for fAD mutations occurring in the PSEN1 coding sequence upstream of codon 79 in the N-terminal region or downstream of codon T440 at the C-terminal end (i.e. conservation of structure in these areas may be critical for the protein interactions needed for fAD mutations elsewhere in the coding sequence to exert a pathogenic effect – see Figure 5).

6. Additional evidence consistent with increased γ -secretase activity due to fAD mutations

There are a number of curious observations that appear consistent with the idea that fAD mutations of the presenilin genes may increase γ -secretase levels. These involve the gene *PSEN2*.

A naturally occurring transcriptional isoform of *PSEN2* exists named PS2V [130]. This name is derived from the fact that PS2V transcripts exclude sequences from what was once denoted as exon 5 (i.e. “V”) of *PSEN2* (but is now denoted as exon 6). PS2V levels are increased in AD brains due to an oxidative stress-sensing and -regulatory mechanism involving binding of HMGA1 protein to *PSEN2* transcripts during splicing [130, 131]. Interestingly, the importance of this regulatory mechanism is demonstrated by its conservation during approximately half a billion years of vertebrate evolution – except, oddly, in mice and rats [132]. PS2V mRNAs encode a truncated form of *PSEN2* protein that cannot have any γ -secretase activity itself, but that nevertheless increases γ -secretase activity in cells [133]. This increase appears to be caused by suppression of the unfolded protein response – presumably in the ER [133-135]. (This illustrates once again the involvement of the presenilins in cellular stress responses). The existence of PS2V is relevant to early onset AD due to the existence of the fAD mutation K115Efs of *PSEN2* [48]. This deletion of two nucleotides truncates

the *PSEN2* open reading frame so that it would encode a protein similar to that of the truncated PS2V isoform. Like PS2V, the K115Efs mutant protein can also increase γ -secretase activity [60]. Note however, that, while the K115Efs mutation was initially thought to be a possible exception to the reading frame preservation rule of presenilin fAD mutations, Braggin et al. [136] reported that this mutation also induces forms of alternative splicing that generate mRNAs with restored open reading frames but with significant coding changes. Therefore, currently, we cannot resolve whether the K115Efs mutation causes fAD through increased γ -secretase activity due to constitutive PS2V-like activity, or by encoding distorted full-length holoproteins, or both.

Another curious experimental result suggests that the most intensively investigated fAD mutation of *PSEN2*, N141I, shows greater than normal γ -secretase activity. Mastrangelo et al. [137] attempted to rescue embryo development in mice lacking *Psen1* activity by inserting transgenes expressing either wild type human *PSEN2* or N141I mutant *PSEN2*. Only the N141I mutant form could compensate for the absence of *Psen1* activity and so allow embryo survival. Whether this superior ability of the N141I mutant is due to overall increased γ -secretase activity, or increased γ -secretase activity in a particular area of cells (e.g. due to fAD mutations changing presenilin proteins' intracellular distributions as described by [138]), or some other mechanism is not known.

Finally, most invertebrates have only one presenilin gene but a duplication event early in the evolution of the vertebrates gave rise to *PSEN1* and *PSEN2*. As these genes have similar structures (and, therefore, similar functions) it is unsurprising that they are both loci for fAD mutations. However, *PSEN2* hosts only ~10% as many fAD mutations as *PSEN1*, despite that these two genes represent almost identically sized "targets" for mutagenesis. Also, fAD mutations of *PSEN2* tend to show less severity (later age of onset) than those of *PSEN1* [89]. Keeping in mind that ~10% of the fAD mutations in *PSEN1* apparently increase the intrinsic γ -secretase activity of the mutant protein [98], one possibility is that only mutations in *PSEN2* that increase the intrinsic γ -secretase activity of its encoded protein are sufficiently severe to cause disease. (Alternatively, since only *PSEN2* protein appears to bind directly to MFN2 protein to promote MAM formation, and since the N141I fAD mutant form of *PSEN2* was shown to bind MFN2 more avidly than wild type *PSEN2* [139], it may be that, in contrast to fAD mutations of *PSEN1*, mutations of *PSEN2* must affect both endolysosomal acidification and MAM formation if they are to cause AD – possibly a less likely event than fAD mutation of *PSEN1* affecting endolysosomal acidification alone.)

7. Notes on the importance of iron and hypoxia in fAD pathogenesis

Our transcriptome analyses of young adult brains from zebrafish heterozygous for fAD-like mutations in the *psen1*, *psen2*, or *sorl1* genes identified oxidative phosphorylation in mitochondria as the cellular process disrupted in common between these brains. Some fAD mutations may alter mitochondrial activity by affecting MAM formation or function [139]. However, Yambire et al. [140] demonstrated that disturbance of lysosomal acidification has profound effects on mitochondrial activity by affecting the availability of ferrous iron ions (Fe^{2+}) in cells required by mitochondria to form the iron-sulphur clusters in their electron transport chain (ETC) that performs oxidative phosphorylation. When lysosomal acidification is reduced, (i.e. pH is increased), lysosomes may accumulate poorly soluble ferric iron (Fe^{3+}) since the conversion of Fe^{3+} to the more soluble Fe^{2+} form (that can be exported for uptake by mitochondria) is inhibited. Interestingly, neuritic A β plaques are complexed with iron [141] and, since Lee et al. [112] have explained how the plaques arise through accumulation of A β in distorted lysosomes, it is now clear how this association between A β and iron may occur.

Most ATP is produced through oxidative phosphorylation. Production of ATP is the most essential cellular function since most other cellular activities are dependent on the energy it provides. For example, ATP is consumed by v-ATPase to drive lysosomal acidification. However, an additional, underappreciated, but essential role of ATP is as a "hydrotrope" inhibiting aggregation of the proteins in cells that are, collectively, close to solution saturation concentration in the cytosol [142]. The generalised aggregation of proteins observed as cells age [143] probably reflects falling

ATP levels together with molecular crosslinking due to rising oxidative stress (particularly if the proteins are associated with iron [141]).

Disturbance of mitochondria's ETC, whether through deficiency of either oxygen or Fe^{2+} , commonly generates reactive oxygen species causing oxidative stress [144]. The co-dependence of cells on oxygen and Fe^{2+} for ETC function is so tight that transcriptional responses to deficiencies of oxygen and Fe^{2+} are both regulated by the HIF1 transcription factor [145]. In fact, the effects of Fe^{2+} deficiency are sometimes described as "pseudo-hypoxia". Hypoxia is thought to play a major role in the development of AD [146, 147]. The oxidative stress caused by hypoxia greatly increases the activity of the molecular machinery producing A β [59, 148-150] that may then be protective through its activity as an antioxidant [151-155].

It is clear that the fAD mutations in *APP*, *PSEN1*, and *PSEN2* likely all generate pseudo-hypoxic effects by inhibiting correct lysosomal acidification, thereby limiting cellular Fe^{2+} maintenance and disturbing oxidative phosphorylation (Figure 10A). In this sense, the fAD mutations accelerate the increase in hypoxia and oxidative stress that brains normally experience during aging [156] due to the gradual degradation of vascular function [157-159]. At some point, the brain "shifts" into a hypometabolic state, possibly as a final effort to avoid the rising ravages of oxidative stress that also drive neuroinflammation [160] (Figure 10B). There are many observations consistent with an overarching role for hypoxia in AD, such as the increased risk of AD due to sleep apnoea [161], decreased bloodflow in AD brains [162], lower levels of HIF1 α in postmortem AD brains [163] and data from model organisms studied by us [126] and others (e.g. Qian et al.'s [164] demonstration that cholinergic basal forebrain neurons – that express p75NTR [165] and are selectively lost in AD [166] – are particularly sensitive to degeneration in their mouse model of sleep apnoea, and that this is mediated by Hif1 α). An additional intriguing connection between AD and hypoxia that we have not seen emphasised elsewhere relates to the "Two-Hit" hypothesis of Alzheimer's disease described in 2004 by Mark Smith and colleagues [50, 167]. They postulated that, "...although either oxidative stress or abnormalities in mitotic signalling [aka cell cycle events, CCEs] can independently serve as initiators, both processes are necessary to propagate disease pathogenesis...and [are] invariant features of disease." CCEs are seen when neurons inappropriately enter the cell cycle but do not undergo cell death. Both oxidative stress and CCEs result from hypoxia [168] with sufficient levels of HIF1 α being essential for preventing inappropriate entry of cells into the cell cycle during hypoxia [169].

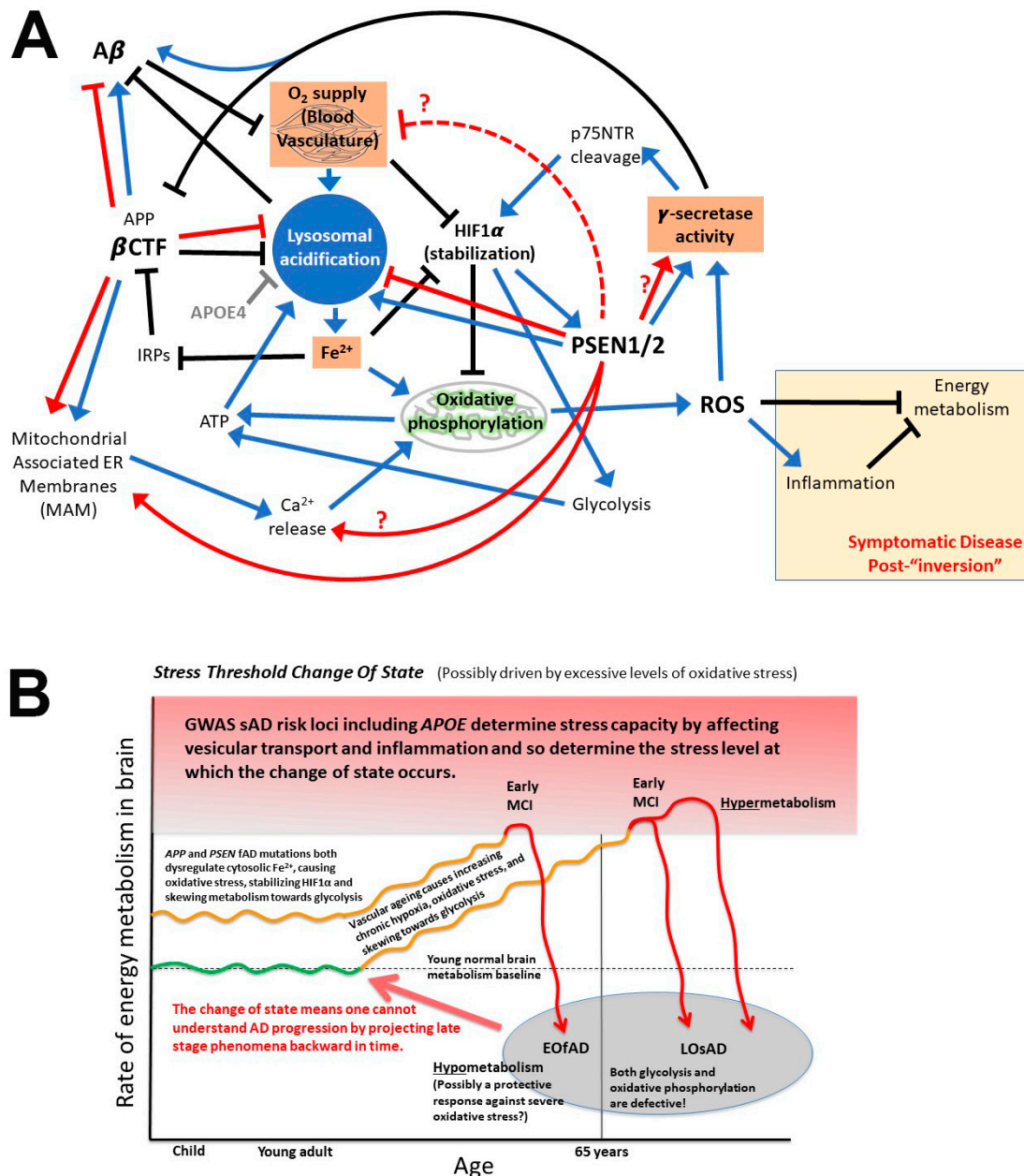


Figure 10. Factors influencing development of Alzheimer's disease. **A**, Many interconnected cellular factors place brains under stress before AD cognitive change become evident. Blue arrows indicate stimulation, black bars indicate inhibition, red arrows are the effects of fAD mutations in *APP* or *PSEN1/2*. Red dashed inhibition line refers to reported non-cell autonomous deleterious effects on vasculature of transgenic human *PSEN1* fAD mutant genes when expressed solely in mouse neurons [3]. fAD mutations in *APP* may increase or decrease $A\beta$ production. Components important in hypoxia/pseudo-hypoxia are indicated by brown boxes. **B**, Stress threshold change of state hypothesis of AD reprinted from [1]. If fAD mutations of the *PSEN1/2* genes drive increased γ -secretase activity this might also drive early raised hypoxia responses. "Inversions" of gene [4, 5], protein [6], and biomarker [7] expression and in other phenomena [8] have previously been observed between pre- and post-clinical disease states.

8. What is APP's role in iron homeostasis?

The Iron Regulatory Proteins, IRP1 and IRP2, act to maintain cellular iron homeostasis by binding to Iron Responsive Elements (IREs) in the transcripts of genes to regulate their translation and/or stability (reviewed by [170]). The importance of iron homeostasis to cell function and survival

is indicated by the fact that more than 10% of protein-coding genes may include IREs in their transcripts [171]. Considerable research has focussed on the existence of an IRE in the 5' untranslated region (UTR) of *APP* mRNA that inhibits translation of APP when cells are deficient in Fe^{2+} . A number of papers have suggested that APP acts to stabilise the cellular iron exporting protein ferroportin while others show data in conflict with this (discussed by us previously in [171]). The ability of β -secretase inhibitors to enhance ferroportin stability is regarded as evidence of a role for APP in stabilisation of ferroportin [172]. However, this stabilisation by β -secretase inhibition would also be expected if the common partner of ferroportin, the ferroxidase hephaestin, was a β -secretase substrate. There is evidence that this is the case [173].

The fact that excessive expression of APP can cause fAD supports that iron-dependent regulation of APP mRNA translation may be a significant influence in fAD pathogenesis. We note that the IRE-dependent regulation of APP mRNA translation is consistent with a role for APP's β CTF in modulating pH in the endolysosomal system. When cells are replete in Fe^{2+} , β CTF levels would be higher since APP translation would not be repressed by the binding of Iron Responsive Proteins (IRPs) to APP's 5' IRE. The consequent increase in endolysosomal pH would reduce importation of Fe^{3+} and its conversion to Fe^{2+} .

Iron is seen to accumulate in AD brains leading to the common speculation that this excessive iron load drives oxidative stress via the Fenton reaction [174, 175]. However, the mechanism described in this paper, whereby insufficient endolysosomal acidification contributes to fAD, supports an alternative idea – AD brains suffer from a functional ferrous iron deficiency in a background of ferric iron accumulation. This is supported by transcriptome analysis of postmortem AD brains indicating increased expression of iron importation protein TFRC [171], the transcripts of which are stabilised during Fe^{2+} deficiency through binding of IRPs to multiple IREs in their 3' UTR [176, 177].

9. Predictions arising from these proposed mechanisms

If fAD mutations in APP exert their effect by increasing β CTF levels, then we expect that ongoing research from the AD research community will continue to find a strong association between these levels and disease pathologies – when researchers take the care to examine β CTF levels in addition to their usual examination of $\text{A}\beta$ levels.

If fAD mutations in *APP*, *PSEN1*, and *PSEN2* all cause AD through their action on endolysosomal system acidification, then a prediction of this model would be existence of an inverse correlation between mutations' effects on lysosomal pH and age of disease onset. The most severe mutations with the earliest ages of disease onset should show the relatively highest lysosomal pH (when comparing heterozygous mutations under uniform conditions in cellular systems with otherwise identical genetic backgrounds). Unfortunately, it is currently challenging to make precise measurements of lysosomal pH.

Testing of the hypothesis that all fAD mutations in the presenilins lead to increased γ -secretase activity could be performed by restoring the wild type sequence in iPSCs derived from an individual heterozygous for a fAD mutation in *PSEN1* or *PSEN2*. Comparison of the γ -secretase activity between the mutant and the wild type cells (possessing otherwise identical genetic backgrounds) could then be performed after their differentiation to neurons or other cell types.

To test the idea that brains "invert" into a hypometabolic state upon exceeding a particular oxidative stress threshold, a model organism such as the zebrafish could be exposed to varying levels of reactive oxygen species (e.g. by placement under varying degrees of hypoxia) and then metabolic states assessed via metabolomics and transcriptomics strategies.

10. Conclusions

The most parsimonious explanation for the many types of fAD mutation affecting *APP* is that these increase the expression of APP's β CTF. This alters cellular cholesterol distribution and reduces lysosomal acidification (likely affecting Fe^{2+} ion homeostasis thus driving pseudo-hypoxia) to impact mitochondrial function. The identification of excessive β CTF as the pathogenic agent promoted by

fAD mutations in *APP* is consistent with Hardy and Higgins' amyloid cascade hypothesis as originally published.

A unifying mechanism for fAD promotion by mutations in *PSEN1* and *PSEN2* is that these drive pseudo-hypoxia by reducing lysosomal acidification thereby disrupting Fe^{2+} ion homeostasis. The possibility exists that these mutations also promote a pseudo-hypoxic state by increasing γ -secretase activity. The formation of multimeric complexes by presenilin holoproteins and/or γ -secretase complexes may be fundamental to understanding how mutations in the presenilin genes cause fAD. Nevertheless, this phenomenon remains almost completely uninvestigated.

Hypoxia and pseudo-hypoxia cause increased production of $\text{A}\beta$ as brains age. Failure of lysosomal acidification results in accumulation of undegraded $\text{A}\beta$, likely with co-accumulation of Fe^{3+} , in the lysosomes of neurons. Upon neuronal death these are observed as neuritic plaques.

Supplementary Files: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Supplementary Data File 1: Data from Liu et al 2022 [101], "TABLE 1 $\text{A}\beta$ s secretion from PS dKO293 cells co-transfected with WT or mutant PSEN1 and APP-C99 (pg/mL)".

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