Familial Alzheimer's disease from mutations in the *PS1* gene

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Introduction

Alzheimer's disease (AD) is one of the major causes of disability and dependency among older people worldwide [27]. It accounts for 60 to 70% of dementia, which caused 486,000 deaths in 2010 [27, 23]. Familial Alzheimer's disease (FAD) refers to a form of AD which is genetically linked and can be inherited. The only forms of FAD identified are autosomal dominant mutations in one of three genes: amyloid precursor protein (*APP*), presenilin 1 (*PS1*) or presenilin 2 (*PS2*). These forms of autosomal-dominant Alzheimer's disease (ADAD) account for less than 1% of AD cases [2]. However, they have proved very important in understanding the pathophysiology of AD, as these mutations are the only identified deterministic factors for AD [2].

PS1 is found on chromosome 14, particularly on the 14q24 cytogenic location [4]. Great advances in understanding the molecular effects of FAD-linked PS1 mutants (FAD-PS1) have been achieved. The crucial function of PS1 in the γ-secretase protease [34], led to the discovery that all FAD-PS1 appear to act by altering the function of this enzyme, changing the distribution of its products. In particular, the ratio of two forms of amyloid β (Aβ), Aβ42:Aβ40 is increased [6]. This can explain why the mutation is dominant, as even if only some of the γ-secretase have the mutated PS1 in a heterozygote carrier, this ratio will be increased, and only a small increase is required to have pathological effects [20].

Amyloid-β has been linked to AD since the 1980s, when it was found inside amyloid plaques of AD patients [17, 33]. This led to the amyloid cascade hypothesis, which states that deposition of Aβ gives rise to fibrillar amyloid plaques and neurofibrillary tangles, which leads to cell death, inflammation, and eventually dementia.

Although there is no doubt that the presence of plaques and tangles correlates with AD symptoms, the etiology of the disease is still widely debated [17]. However, for the case of ADAD, the fact that Aβ must play a role is strongly supported by the evidence about the function of the mutated genes [26].

Physiology of the APP processing system and related pathways

Amyloid precursor protein (APP) is a type I transmembrane protein which, in neurons, is primarily found in synapses [29]. It has been associated with several functions including cell differentiation, cell adhesion, neurite outgrowth, synapse formation, neuronal migrations, and many signaling pathways including apoptosis [12, 13]. The protein has a large N-terminal domain which interacts with several proteins in the extracellular matrix. The intracellular C-terminal domain interacts with several pathways, and its phosphorylation regulates several processes including its own proteolytic processing [13].

APP is rapidly glycosylated after translation, and then transported through the axon to plasma membranes at synapses [31] (Fig. 1). Once there, its half-life has been measured to be

between 30 minutes and 4 hours. [38, 25, 36, 30]. 30-40% of membrane APP is then cleaved secreting the APPs fragment, while the rest can be degraded or recycled by the endosomal and lysosomal pathways [13, 36, 18].

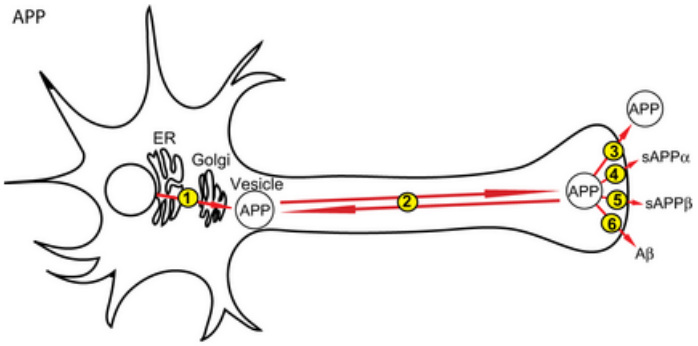

Figure 1: APP axonal transport, from [31]

APP proteolysis can begin with a cleavage in its α or β sites, performed by α and β-secretase, respectively. These are called the non-amyloidogenic and amyloidogenic pathways, respectively. In the case of β cleavage, a C99 fragment remains in the membrane, and is then cleaved by γ-secretase [13]. The N-terminal fragment is secreted outside of the cell, and can be Aβ40, Aβ42, as well as Aβ polypeptides of other lengths. Inside the cell, the C-terminal amyloid intracellular domain (AICD) is also released.

γ-secretase has been identified to be a large membrane-bound complex formed by presenilin (PS1 or PS2), anterior pharynx defective-1a (APH-1a) or APH-1b, resenilin enhancer-2 (PEN-2), and nicastrin [34]. See Fig. 2. It acts by first cleaving C99 in the ϵ site, releasing AICD, and then it sequentially cuts the resulting polypeptide in ζ and γ sites. Depending on the result of the ϵ cleavage, the sequence of intermediate polypeptides can primarily be Aβ49>Aβ46>Aβ43>Aβ40 or Aβ48>Aβ45>Aβ42>Aβ38, according to one model [6]. Aβ itself (which is normally mostly in the Aβ40 form) appears to regulate synaptic plasticity and memory formation [28]. Furthermore, Aβ is normally degraded by a set of proteins, predominantly Insulin-degrading enzyme (IDE), and Neprilysin (NEP) [14].

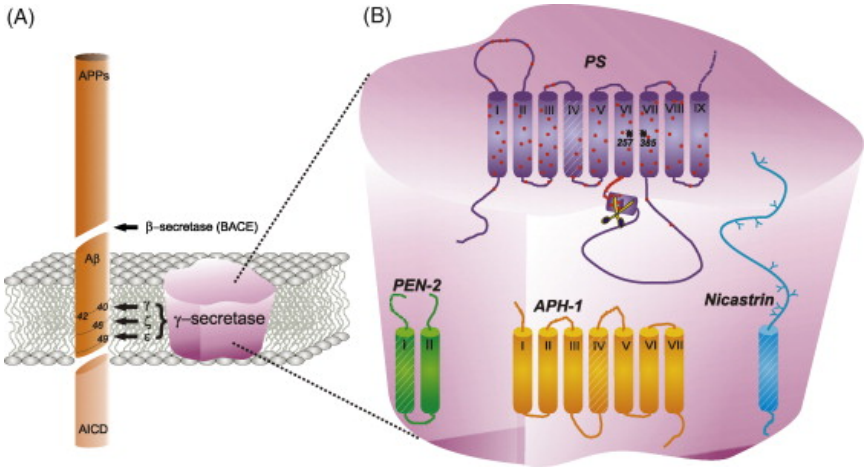

Figure 2: Structure of γ-secretase, from [34]

PS1 is a 7-pass transmembrane protein, which endoproteolyses itself (autoproteolysis) in

order to become active in the γ-secretase complex. This occurs by first binding to the complex; then the loop between the transmembrane domain (TMD) 6 and 7 'dives' into the catalytic cavity, where it is sequentially cleaved in ϵ, ζ, and γ sites, just as for type-1 substrates. This splits PS1 into an N and a C-terminal fragments (NTF and CTF), which remain non-covalently associated. When in γ-secretase, the two fragments are able to laterally diffuse to accommodate type-1 substrates, like APP [10].

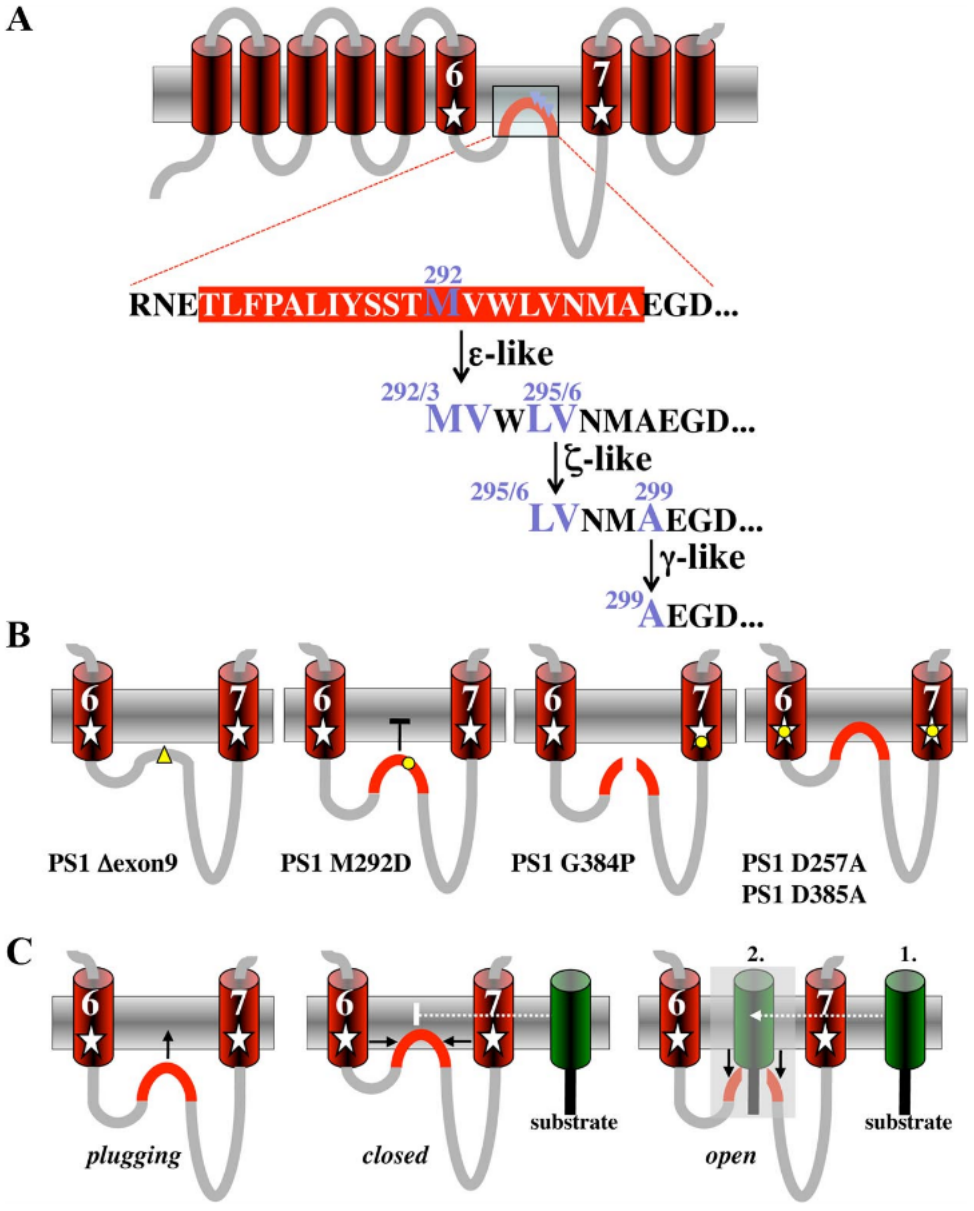

Figure 3: **A** Consecutive clevage locatinos of PS1 in PS1 autoproteolysis. **B** Effect of some FAD-PS1 mutations on its autoproteolysis. See discussion in [10]. **C** Activation of the PS1 active site. PS1 needs to be proteolysed in order to accomodate a type I substrate, in the γ-secretase complex. From [10]

Pathophysiology in APP processing due to FAD-linked PS1 mutants

PS1 mutants found in FAD have been found to affect the active site of PS1 in the γ-secretase complex between TMD 6 and 7 (Fig. 3). A particular mutation, Δexon9 removes the cleavage site domain entirely, and does not allow PS1 endoproteolysis. However, most studied PS1 mutations do allow PS1 endoproteolysis, but change the resulting CTF and NTF [10].

Mutated FAD-PS1 still form active γ-secretase. However, their altered CTF and NTF change the cataytic properties of the complex. These effects were studied by measuring the kinetic constants of the ϵ and γ cleavages when γ-secretase is processing APP [6]. They measured these on the mutations Y115H, M139V, L166P, I213T, G384A and Δexon9, and found that they had inconsistent effects on the catalytic rate of the ϵ cleavage, meaning that the total number of Aβ produced could either increase or decrease. However, they all impaired γ cleavage, increasing the Aβ42:Aβ38 and Aβ43:Aβ40, as well as the Aβ42:Aβ40 ratio, which has been widely reported to be linked with FAD [6, 32, 19]. Furthermore, their results suggested that an increase in Aβ43 may also be pathologically relevant.

There are proposed alternative hypotheses for the pathological pathways that lead to FAD due to PS1 mutations. These have been weakened by the fact that all tested FAD mutations affect γ-secretase function, as well as the existence of APP mutations that lead to FAD. However, even if not the primary pathologies, some of these mechanisms may act as aggravating factors [6]. Some of these effects are based on the fact that γ-secretase processes several other substrates, like Notch, and so partial loss-of-function of the protease can affect pathways where these molecules are involved. In the case of Notch signaling, however, reduced γ-secretase activity alone did not lead to AD symptoms [6].

Other proposed pathways that may be affected by FAD-PS1 mutations and may contribute to FAD are those were PS1 itself is involved, independently of the γ complex. PS1 appears to be involved in several autophagy trafficking and proteolysis of proteins [1, 37, 22]. Finally, several FAD-PS1 mutations have been shown to alter neuronal Ca^{+2} homeostasis [40, 3, 37].

Amyloid cascade hypothesis and other possible effects leading to AD

As we have discussed, the most likely pathological mechanism to be the primary cause of PS1-linked FAD is the increase of longer amyloid-β relative to shorter ones. In particular, the Aβ42:Aβ40 ratio has been identified as being increased by all the tested FAD-PS1 mutations. However, the way this results in the symptoms of FAD is not yet fully understood [13, 17].

The amyloid cascade hypothesis focuses on the main pathologies observed in AD, amyloid plaques, and neurofibrillary tangles, cell loss, and vascular damage, and suggests that they are ultimately caused by amyloid-β aggregation [11, 26]. One important issue is that many FAD-PS1 mutations don't increase the total concentration of Aβ peptides, nor even that of Aβ42 (which is known to have a higher propensity for aggregation [15]). However, an increase in the Aβ42:Aβ40 ratio that doesn't change the total amount of Aβ, still changes the aggregation kinetics of the peptides leading to stabilization of certain toxic oligomeric aggregates [20].

These intermediate aggregates, or small molecular weight Aβ oligomers, are the subject of one of the major variants of the amyloid cascade hypothesis. While the traditional hypothesis focused on the formation of amyloid plaques, the oligomer hypothesis poses that it is these low-end oligomers that initiate AD pathology, although they may also act in conjunction with amyloid plaques [17, 26]. The current popularity of this hypothesis is based upon a wealth of

evidence. It also helps resolve the paradoxical observation that the amount and location of the amyloid plaques don't correlate well with AD symptoms [17, 26].

Among the many proposed mechanisms, Aβ oligomers binding at synapses have been shown to affect the distribution of critical proteins which causes hyperactivity on certain glutamate receptors. This leads to a Ca^{+2} imbalance and can lead to tau hyperphosphorylation, insulin resistance, oxidative stress, and synapse loss [24], which are major AD neuropathologies. Furthermore, Aβ oligomers have been found to correlate with AD pathologies and to accumulate in the hippocampus, where AD pathologies are most intense at its initial stages. [8].

Tau hyperphosphorilation is particularly well studied, as it leads to neurofibrillary tangles, which have been found in brain regions showing AD symptoms [26]. Tau normally stabilizes microtubule assemblies [16], but when it is hyperphosophorilated, it promotes microtubule dissasembly [39]. This in turn leads to dysfunctional axonal transport in neurons. The hyperphosphorilated tau also aggregate into neurofibrillary tangles in the neurons, causing further pathology [26]. See Fig. 4.

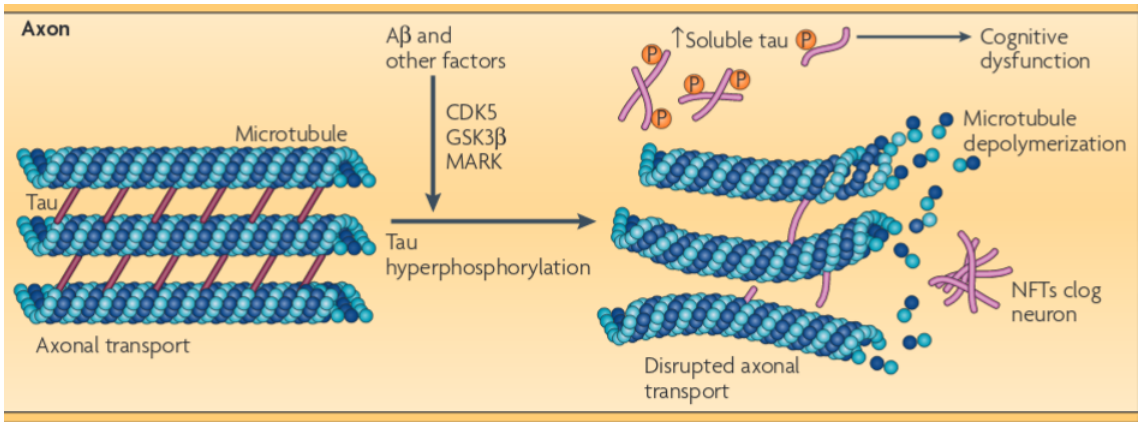

Figure 4: Hyperphosphorilation of Tau is mediated by several Aβ-dependent factors, like the CDK5 GSK3β and MARK kinases. Adapted from [21]

Other proposed hypotheses, like the mitochondrial cascade hypothesis, the dual pathway hypothesis, and the cell cycle re-entry hypothesis may be relevant for sporadic AD, but not for FAD. On the other hand the metabolism hypothesis and the vascular hypothesis are related to Aβ aggregation and could play a role in FAD [17].

As I have mentioned, the early stages of AD affect the hippocampus most strongly. The hippocampus is involved in the formation of new memories, so synaptic malfunction and cell loss in this region impairs this process, a condition called anterograde amnesia, which is characteristic of early stage AD. At later stages AD symptoms, spread to other parts of the brain, radially outwards, causing increasingly severe symptoms, altering personality and mood, and basic cognitive functions [5]. Death is usually caused indirectly, for instance by pneumonia [35].

Conclusion

The role of Aβ in the pathology of familial Alzheimer's disease has been established, but the way it causes the symptoms of AD is not yet fully understood. Although there is considerable evidence for the amyloid cascade and oligomer hypotheses, as well as many proposed mechanisms to cause AD pathology, it seems that an integrative systems biology approach may be necessary to fully understand this complex disease [5]. AD is an insidious disease that starts decades

before cognitive symptoms are observed. Understanding these early stages is important for clinical treatment and diagnoses [7]. These early stages can also give insight into the etiology of the disease.

It is currently proposed that the the early stages of AD are characterized by an stress-response interaction between the impaired pathways (particularly, the APP processing pathway), and homeostatic networks (predominantly, proteostasis networks). It is severe or accumulated stress on these networks that eventually causes system failure and leads to apoptotic cell death [5, 9]. The need to sustained accumulative stress can also be the reason for the association between AD and age.

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