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Presenilins function in ER calcium leak and Alzheimer's disease pathogenesis

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Abstract

Alzheimer disease (AD) is the most common neurodegenerative disorder worldwide and is at present, incurable. The accumulation of toxic amyloid-beta (A β) peptide aggregates in AD brain are thought to trigger the extensive synaptic loss and neurodegeneration linked to cognitive decline, an idea that underlies the amyloid hypothesis of AD etiology in both the familial (FAD) and sporadic forms of the disease. Genetic mutations causing FAD also result in the dysregulation of neuronal calcium (Ca²⁺) handling and may contribute to AD pathogenesis, an idea termed the calcium hypothesis of AD. Mutations in presenilin proteins account for majority of FAD cases. Presenilins function as catalytic subunit of γ -secretase involved in generation of A β peptide. Recently, we discovered that presenilins function as low-conductance, passive ER Ca²⁺ leak channels, independent of γ -secretase activity. We further discovered that many FAD mutations in presenilins results in loss of ER Ca²⁺ leak function activity and Ca²⁺ overload in the ER. These results provided potential explanation for abnormal Ca²⁺ signaling observed in FAD cells with mutations in presenilins. The implications of these findings for understanding AD pathogenesis are discussed in this article.

Alzheimer's disease

Alzheimer's disease (AD) is the most common type of age-related dementia. It is a worldwide epidemic which currently affects approximately 27 million people and because there is no known cure or therapies that significantly slow disease progression, this number is projected to quadruple by 2050 (Brookmeyer et al., 2007). Clinical characteristics of AD include a decline in memory, particularly in short-term and working memory, apathy, depression, impaired judgement, disorientation, confusion, changes in behaviour and difficulty speaking, swallowing and walking. The key pathological hallmarks of AD are extensive neurodegeneration of the median temporal lobe (the hippocampus in particular), parietal lobe, select regions of the frontal cortex and cingulate gyrus (Giannakopoulos et al., 2009; Wenk, 2003); the presence of extracellular senile plaques composed of dense-core deposits of amyloid β peptide (A β), dystrophic neuritis and activate microglia (Hardy, 2009; Hardy and Selkoe, 2002); and intracellular neurofibrillary tangles (NFTs) comprised of hyper-phosphorylated microtubule-associated protein tau (MAPT) (Small and Duff, 2008). The majority of AD cases are sporadic (SAD) and characterized by the late age of disease

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onset (>60 years of age). A small percentage (1–2%) of all AD cases are characterized by an earlier onset (<60 years) and genetic inheritance, known as familial AD (FAD). Mutations in the genes encoding presenilin-1 (*PSEN1*), presenilin-2 (*PSEN2*) and amyloid precursor protein (*APP*) account for more than 50% of autosomal-dominant FAD cases (Bergmans and De Strooper, 2010; Bertram and Tanzi, 2008; Hardy, 2009; Hardy and Selkoe, 2002). Mutations in the *MAPT* gene do not lead to AD but instead result in a variant of frontotemporal dementia (FTDP17) (Cruts and Van Broeckhoven, 2008; Hutton et al., 1998; Small and Duff, 2008).

Presenilins and Familial Alzheimer's disease

The majority of FAD is caused by missense mutations in the *PSEN1* and *PSEN2* genes, which display 80% sequence homology. Since the discovery of their potential involvement in AD pathogenesis over 16 years ago (Sherrington et al., 1995), 182 mutations in *PSEN1* and 14 in *PSEN2* have been identified to be causative in FAD (www.molgen.ua.ac.be/ADMutations) (Cruts; Cruts and Van Broeckhoven, 1998). Presenilins (PSs) belong to the family of aspartic proteases and they are involved in Regulated Intramembrane Proteolysis (RIP), a mechanism that is used to cleave peptide bonds within the lipid bilayer (Brown et al., 2000; Urban et al., 2002) (reviewed in (Hass et al., 2009)). Presenilins are 50 kDa holoproteins that contain 9 transmembrane (TM) domains (Laudon et al., 2005; Spasic et al., 2006) and they reside primarily in the endoplasmic reticulum (ER) membrane (Annaert et al., 1999) with the amino-terminal oriented towards the cytosol and the carboxy-terminal toward the ER lumen (Fig 1). The PSs aggregate with nicastrin, anterior pharynx defective 1 (Aph-1) and presenilins enhancer 2 (Pen-2) subunits to produce the multimeric γ -secretase complex, which is transported to the cell surface and endosomal structures to cleave type I transmembrane proteins such as Notch and the amyloid precursor protein (APP). Following assembly, PS1 and PS2 holoproteins undergo endoproteolysis in the cytosolic loop between TM6 and TM7, resulting in the generation of a 35 kDa amino-terminal fragment (PS-NTF) and an 18–20 kDa carboxy-terminal fragment (PS-CTF), which remain associated with each other in the “mature” γ -secretase complex (De Strooper et al., 1997; Edbauer et al., 2003; Kimberly et al., 2003; Lazarov et al., 2006; Takasugi et al., 2003; Thinakaran et al., 1996; Wolfe et al., 1999; Yu et al., 1998). The cleavage of APP sequentially by β -secretase then γ -secretase results in the generation and release of A β , the principal constituent of the amyloid β in the brains of AD patients (De Strooper et al., 1998; Wolfe et al., 1999). Consistent with the role of PSs as the catalytic subunits of γ -secretase (Bergmans and De Strooper, 2010; De Strooper et al., 1998; Wolfe et al., 1999), PS-FAD mutations result in a shift in the proteolysis of APP such that there is an increase in the production the hydrophobic, aggregation-prone 1–42 A β fragment, increasing A β 42/40 ratio and contributing to A β plaque formation (De Strooper et al., 1998; Scheuner et al., 1996; Wolfe et al., 1999). In addition, loss of PSs or mutation of the catalytic aspartates D257 or D385 in TM6 and TM7, respectively, affect APP processing (Figure 1) (Wolfe et al., 1999).

Presenilins and Intracellular Calcium Signaling

The identification of the biological functions of PS has been a global effort since the discovery that they were involved in AD pathogenesis. It appears that PSs have a wide range of functions beyond RIP and γ -secretase, including β -catenin regulation, protein trafficking, apoptosis in both mammals and plants (reviewed in (Hass et al., 2009)). Interestingly, many PS-FAD mutations result in deranged neuronal calcium (Ca²⁺) signaling (reviewed in (Bezprozvanny and Mattson, 2008; Demuro et al., 2010; Stutzmann, 2007; Supnet and Bezprozvanny, 2010a)), which suggests a role for PS in the regulation of intracellular Ca²⁺ signaling. The connection between PS-FAD mutations and abnormal Ca²⁺ signaling has

been known approximately 2 decades (Ito et al., 1994), however, the molecular mechanism(s) behind this observation are still controversial (Bezprozvanny and Mattson, 2008; Demuro et al., 2010; Stutzmann, 2007; Supnet and Bezprozvanny, 2010a).

A consistent observation is that PS-FAD mutations predominantly result in the exaggerated release of Ca^{2+} from overloaded ER stores. Skin fibroblasts from human patients that harbour a mutation in PS1-A246E showed exaggerated Ca^{2+} release from IP_3 -gated stores compared to controls after treatment with bombesin and bradykinin (Ito et al., 1994). Alterations in Ca^{2+} signalling were detected before the development of overt clinical symptoms and such changes were not present in cells from subjects that failed to develop AD (Etcheberrigaray et al., 1998). These initial results were recapitulated experimentally in various model systems expressing FAD-related mutations in PS and the data suggested that in addition to contributing to altered γ -secretase function, PS mutations had a significant impact on Ca^{2+} signaling in AD models. These early observations supported the “ Ca^{2+} hypothesis of AD” which states that deranged Ca^{2+} signaling plays an important role in AD pathogenesis (Bezprozvanny and Mattson, 2008; Demuro et al., 2010; Khachaturian, 1989; Stutzmann, 2007; Supnet and Bezprozvanny, 2010a, b).

Many studies have since revealed that neurons from mice harbouring FAD-PS mutations display numerous changes to proteins involved in Ca^{2+} regulation, specifically affecting protein expression and function. Early reports revealed that cortical neurons from PS1-M146V mice showed increased cytosolic Ca^{2+} compared to wild type (WT) after treatment with caffeine, suggesting a role for the ryanodine receptors (RyanRs) in the altered Ca^{2+} response (Chan et al., 2000). Furthermore, RyanR protein levels and channel function are increased in mouse models containing PS mutations PS1-M146V and PS2-N141I (Chan et al., 2000; Lee et al., 2006; Smith et al., 2005). The PS1-M146V mutation augmented Ca^{2+} release from IP_3 - and caffeine- gated stores in hippocampal and cortical neurons in slice preparations of young 3XTg-AD mice, prior to extensive plaque pathology (Chakroborty et al., 2009; Goussakov et al., 2010; Stutzmann et al., 2006). Clinical mutations of PS2 can also enhance Ca^{2+} release from IP_3 R-gated ER stores (Leissring et al., 1999). Presenilin 1 mutations and genetic knockout attenuate capacitative Ca^{2+} entry (CCE), a refilling mechanism for depleted ER Ca^{2+} stores (Akbari et al., 2004; Giacomello et al., 2005; Herms et al., 2003; Leissring et al., 2000; Yoo et al., 2000; Zatti, 2004; Zhang et al., 2010). The gating of IP_3 R can be modulated by PS1-M146L and several other PS1-FAD mutants (Cheung et al., 2010; Cheung et al., 2008). *Xenopus laevis* oocytes expressing PS1-M146V have increased sarco-/endoplasmic reticulum Ca^{2+} ATPase (SERCA) activity compared to those with WT PS1 (Green et al., 2008), a mechanism that could contribute to the overfilling of ER Ca^{2+} store. Taken together, PSs have profound effects on the activity and/or expression of many proteins involved in intracellular Ca^{2+} signalling. Over time such changes to Ca^{2+} dynamics are thought to lead to changes in synaptic stability and function, cell energetics and ultimately neurodegeneration.

Presenilins function in ER Ca^{2+} leak

Recently, we discovered that PSs function as low-conductance, passive ER Ca^{2+} leak channels, independent of γ -secretase activity (Tu et al., 2006). We found that many FAD mutations in PS1 disrupts this Ca^{2+} leak function (Nelson et al., 2010; Nelson et al., 2007; Tu et al., 2006), resulting in overloaded ER Ca^{2+} stores and exaggerated ER Ca^{2+} release in double PS knock-out fibroblasts (Nelson et al., 2010; Nelson et al., 2007; Tu et al., 2006), cultured hippocampal neurons from PS double-knockout mice and PS1-M146V mutant neurons (Zhang et al., 2010) and lymphoblasts from FAD patients (Nelson et al., 2010). These observations suggest that PS1 plays a pivotal role in deranged Ca^{2+} in AD and they also provide further support for the contribution of PS1 to Ca^{2+} homeostasis in neurons.

Our novel proposal that PSs function as Ca^{2+} or ion conductance channels has been challenged (Cheung et al., 2010; Cheung et al., 2008). In particular, it has been argued that PSs lack an ion conductance pore and therefore would not be able to function as ion channels (Cheung et al., 2010; Cheung et al., 2008). The crystal structure of PS has not been elucidated and the presence of an ion conductance pore cannot be confirmed or refuted based on structural analysis studies. However, recent biochemical studies of PS1 by two independent groups (Sato et al., 2006; Sato et al., 2008; Tolia et al., 2006; Tolia et al., 2008) have revealed the presence of a water-filled cavity within the hydrophobic membrane, which would allow for the hydrolysis required for proteolytic cleavage by γ -secretase. These groups utilized cysteine-scanning mutagenesis (SCAM), a technique that was developed to identify residues which line a pore of a channel (Akabas et al., 1994; Akabas et al., 1992). In studies of PS1 it was determined that TM7 domain containing the catalytic aspartate residue D385 is facing a water-filled cavity in the lipid bilayer. In contrast, TM6 domain containing D257 catalytic residue was not water accessible. It was reasoned that significant translocation of TM6 is required to support proteolytic cleavage of the γ -secretase substrates within the membrane (Sato et al., 2006; Sato et al., 2008; Tolia et al., 2006; Tolia et al., 2008) (Fig 1B). Again using SCAM, the investigators were also able to demonstrate the involvement of TM domain 9 in forming the hydrophilic pore (Sato et al., 2008; Tolia et al., 2008). Being water-accessible and highly flexible, TM domain 9 is potentially involved in the translocation of substrates from the initial binding site to the catalytic site of PS1 (Tolia et al., 2008). Recent NMR studies of PS1-CTF in micelles revealed unusual features of TM domains 7 and 9, where TM domain 7 was discovered to be a putative half-membrane-spanning helix and TM domain 9 was a kinked helix (Sobhanifar et al., 2010). In addition, they discovered a higher rate of hydrogen-deuterium exchange in these regions, implying either higher exposure to the aqueous environment and/or increased dynamics (Sobhanifar et al., 2010). These data further confirm the flexibility of TM domain 9 and the involvement of TM domain 7 and 9 in forming a water-filled catalytic core.

Could this hydrophilic pore (Fig 1B) also serve as an ion conductance channel? Recently, in order to answer this question and to map the ion conductance pore in PS1, we systematically evaluated ER Ca^{2+} leak activity supported by a series of cysteine point mutants in TM6, TM7 and TM9 of mPS1 expressed in PS double knockout mouse embryonic fibroblasts (Nelson et al., 2011). Using Ca^{2+} imaging and stable cell rescue lines, our results indicated that TM7 and TM9, but not TM6, play an important role in forming the ion/ Ca^{2+} conductance pore of PS1 (Nelson et al., 2011). These results are consistent with previous SCAM and NMR analysis of PS1 (Sato et al., 2006; Sato et al., 2008; Sobhanifar et al., 2010; Tolia et al., 2006; Tolia et al., 2008) and provide further support to our hypothesis that the hydrophilic catalytic cavity of presenilins also constitutes the Ca^{2+} conductance pore. However, it is important to remember that Ca^{2+} channel function is supported by the holoprotein of PS1 in the ER (Tu et al., 2006) and γ -secretase function is supported by cleaved PS1 in the plasma membrane (De Strooper, 2003). Therefore, these 2 functions are mutually exclusive and never performed by the same PS1 molecule simultaneously (Fig 1B).

What about the location and composition of the ion/ Ca^{2+} conductance channel? Interestingly, it was discovered that PS1 forms homodimers in intact mammalian cells in fluorescent lifetime imaging microscopy using differentially labeled PS1 constructs experiments (Herl et al., 2006). It has been reported that PS1 dimers affect γ -secretase activity and function (Cervantes et al., 2001; Cervantes et al., 2004; Hebert et al., 2003). However, the involvement of PS1 dimers and how the configuration relates to the ion conductance function of PS1 is not known. It was revealed that PS1 holoproteins with D257A mutation were also able to form dimers, suggesting that cleavage within the loop was not necessary for dimerization (Herl et al., 2006). These data align with our finding that residues in TM domain 6, which contains D257, did not affect the Ca^{2+} channel function of

PS1 and could support a possible homodimer configuration of PS1 for the ion channel (Nelson et al., 2011). Further experiments utilizing mutations of other TM domains that could be involved in the formation of the ion/ Ca^{2+} conductance pore of PSs are required. For example, recent application of SCAM approach demonstrated that TM1 of mPS1 is water exposed and facing the catalytic pore of γ -secretase (Takagi et al., 2010). Recent reports suggest that there are distinct forms of γ -secretase, which opens up the possibility for alternative functions or composition of the complex and perhaps PS1. It has recently been demonstrated that there may be two physically different forms of γ -secretase-associated PS1, one that is relatively proteinase K-sensitive and one that is significantly more resistant (Nakazawa et al., 2006). In agreement with our studies, these results demonstrate the existence of a stable subset of PS1 which could serve as the ion/ Ca^{2+} conductance pore. Moreover, it was discovered that γ -secretase of hematopoietic origin has an atypical subunit composition (PS1-NTF:PS1-CTF ratio) with significantly altered subunit stoichiometry compared to epithelial γ -secretase (Placanica et al., 2010). These atypical γ -secretases display a unique activity profile, having reduced activity for APP and Notch (Placanica et al., 2010), illustrating a potentially unique subset of PS1 with alternative functions outside of APP processing. However, for definitive information regarding the location and composition of the PS1 ion/ Ca^{2+} conductance pore, the crystal structure of PS must be elucidated.

ER Ca^{2+} leak and variant AD

Clinical studies indicated that many patients with FAD mutations in PS1 display significant phenotypic heterogeneity, including variable age of onset, penetrance, myoclonus and seizures, Parkinsonism, apraxia/ataxia, FTD and psychiatric symptoms (Larner and Doran, 2006; Ryan and Rossor, 2010; Shepherd et al., 2009). There are also variations in PS1-FAD neuropathology. For instance, greater NFT formation, altered amyloid β plaque composition, hippocampal sclerosis, the appearance of Pick bodies and neuropathological involvement of the basal ganglia and/or brainstem (Larner and Doran, 2006; Ryan and Rossor, 2010; Shepherd et al., 2009). An intriguing subset of PS1-FAD patients manifest symptoms of spastic paraparesis (SP, known as variant AD), or progressive spasticity of the lower limbs (Houlden et al., 2000; Larner and Doran, 2006; Ryan and Rossor, 2010; Shepherd et al., 2009). The linkage of SP to PS1-FAD mutations, R278T and M233T, was first reported in 1997 (Kwok et al., 1997). The PS1 deletion of exon 9 (ΔE9) was associated with SP in a Finnish family (Crook et al., 1998). However, not all patients carrying PS1- ΔE9 within the same family will present with SP (Verkkoniemi et al., 2001), which suggest the involvement of other modifiers and environmental factors. Upon pathological examination of patients harbouring PS1-FAD mutations associated with SP, their brains usually contain abundant large, non-cored amyloid- β plaques, named cotton wool plaques (CWP) composed primarily of A β 42 peptide and lacking surrounding neuritic dystrophy and glial activation (Bergmans and De Strooper, 2010; Karlstrom et al., 2008; Larner and Doran, 2006; Tabira et al., 2002). Patients also display corticospinal tract degeneration at the level of the medulla and the spinal cord (Verkkoniemi et al., 2001). It is believed that the accumulation of CWP plaques in the basal ganglia, brainstem and spinal cord is responsible for the Parkinsonism and spastic phenotypes clinically observed in these patients. Currently, the reasons for these unique clinical and pathological phenotypes observed in these PS1-FAD pedigrees are not understood (Bergmans and De Strooper, 2010; Karlstrom et al., 2008; Larner and Doran, 2006; Ryan and Rossor, 2010; Shepherd et al., 2009; Tabira et al., 2002).

Could this variant AD somehow be explained by the altered ER Ca^{2+} signaling caused by PS1-FAD mutations? Over a series of experiments (Nelson et al., 2010; Nelson et al., 2007; Tu et al., 2006), we have tested a total of 23 FAD mutations in PS1, 1 FAD mutation in PS2 and 3 FTD implicated PS1 mutations. We concluded that 14 FAD mutations abolished ER

Ca²⁺ leak function of PS (Fig 2A). These conclusions are based on the lack of channel activity in planar bilayers with recombinant PSs and by failure of these mutants to rescue Ca²⁺ signaling defects in PS DKO MEF cells. In contrast, another 10 FAD mutations in PS1 appeared to be functional and were able to rescue ER Ca²⁺ leak defects in PS DKO fibroblasts (Fig 2A). The PS1-ΔE9 FAD mutant acted as a “gain of function” (GOF) mutant based on bilayer experiments (Tu et al., 2006). Other functional PS1 mutants were able to rescue Ca²⁺ signaling defects in PS DKO MEF cells but were not tested in bilayers. These mutants may correspond to a GOF, normal function, or partial loss of function mutations and were classified as “functional”. All 3 PS1 mutants implicated in FTD (L113P, G183V, and Rins352) were functional in ER Ca²⁺ leak assay (Fig 2A). The Rins352 mutation is probably not pathogenic as the same patient also has a mutation in progranulin, which is most likely responsible for the disease (Cruts and Van Broeckhoven, 1998). The pathogenic status of the L113P and G183V mutations remains to be elucidated. The “loss of function” (LOF) mutations are spread out through the sequence of PS1 (within exons 5, 6, 7, 8, 11, and 12 of PSEN1 gene) and are mostly localized to the TM domains (Fig 2A). Most “functional” PS1 FAD mutations are concentrated in the initial portion of a large cytosolic loop between TM6 and TM7 (Fig 2A), the region encoded by exons 8 and 9 of the *PSEN1* gene.

Is there any correlation between ER Ca²⁺ leak phenotype of PS1-FAD mutants and clinical phenotypes of FAD patients with the same mutations? In our analysis we discovered that PS1-ΔE9 and most other PS1-FAD mutants that were “functional” for Ca²⁺ leak (L85P, P264L, R269G, ΔE8, E280G, T291P, N405S, and C410Y) segregated with the CWP pathology and/or SP clinical phenotype. Remarkably, all “functional” mutations are associated with CWP/SP phenotype and none of the “functional” mutations led to typical dense-core plaque (DCP) AD pathology. Based on these results we concluded that the disruption of both ER Ca²⁺ leak and γ-secretase function of PS1 causes pathological changes in the brains of PS1-FAD patients. To represent these ideas quantitatively, we plotted the increase in Aβ_{42/40} ratio against the increase in ER Ca²⁺ levels for each of the PS1-FAD mutants (Fig. 2B). The fold increase values for Aβ_{42/40} ratios (Fig. 2B) for each of the PS1 FAD mutants were extracted from <http://www.molgen.ua.ac.be/ADMutations> (Cruts; Cruts and Van Broeckhoven, 1998). The change in ER Ca²⁺ levels was plotted based on our studies of ER Ca²⁺ leak activity. We made an assumption that for LOF mutants the ER Ca²⁺ levels were increased 2 fold, for “functional” mutants the ER Ca²⁺ levels were unchanged, and for the GOF mutant PS1-ΔE9 the ER Ca²⁺ levels were reduced by 10%. The Aβ_{42/40} ratios and ER Ca²⁺ levels were normalized to the corresponding values for the wild-type (WT, open triangle). The 3 PS1-FAD mutants with confirmed DCP pathology (M139V, M146L, A246E) (Ataka et al., 2004; Halliday et al., 2005; Le et al., 2001) were plotted as solid circles, the 8 PS1-FAD mutants with confirmed CWP pathology (L166P, P436Q, P264L, ΔE8, E280G, ΔE9, T291P, C410Y) (Karlstrom et al., 2008; Larner and Doran, 2006; Tabira et al., 2002) were plotted as open circles, and the 2 PS1-FAD mutants with unknown pathology (G384A, L85P) were plotted as diamonds. For the remaining 9 PS1-FAD mutants (G217D, K239E, V261F, E273A, L420R, A426P, A431E, R269G, and N405S) Aβ_{42/40} values were not available and these mutants could not yet be plotted. The late onset, partially penetrant PS1-A79V mutation and PS2-N141I mutation were not plotted.

The resulting diagram can be divided into 4 quadrants (Fig. 2B): quadrant I (high ER Ca²⁺, small increase in Aβ_{42/40}), quadrant II (high ER Ca²⁺, large increase in Aβ_{42/40}), quadrant III (low ER Ca²⁺, small increase in Aβ_{42/40}), quadrant IV (low ER Ca²⁺, large increase in Aβ_{42/40}). Interestingly, all 3 mutations with confirmed DCP are located in quadrant I, and all mutations with confirmed CWP are located in quadrants II, III, or IV (Fig. 2B). Given these results, we propose that both ER Ca²⁺ dyshomeostasis and increases in Aβ_{42/40} ratios

can play a role in development of AD pathology. If the pathology is driven by both ER Ca^{2+} overload and an increase in $\text{A}\beta_{42/40}$ ratios, then the formation of DCP is favoured (quadrant I). However, if the pathology is primarily driven by an increase in the $\text{A}\beta_{42/40}$ ratio, then the formation of $\text{A}\beta_{42}$ -enriched CWP plaques is strongly favored. We propose that this situation can occur when ER Ca^{2+} levels are not affected (quadrant III) or when ER Ca^{2+} levels are affected but an increase in $\text{A}\beta_{42/40}$ ratios is large (quadrant II). The extreme case of this situation is quadrant IV, which corresponds to both low ER Ca^{2+} levels and large increase in $\text{A}\beta_{42/40}$ ratios (Fig. 2B). Indeed, the only mutation located in quadrant IV (PS1- ΔE9) results in the most robust and penetrant CWP/SP clinical phenotype (Brooks et al., 2003; Jacquemont et al., 2002).

The proposed classification has a predictive power. For example, we predict that mutations G384A (located in quadrant II) and L85P (located in quadrant III) should also be linked with CWP pathology. It will be of interest to test this prediction experimentally. Although the pathological evaluation of these mutants has not yet been reported, the SP clinical phenotype has been previously described for PS1-L85P patients (Takao et al., 2002), consistent with our predictions. The proposed hypothesis provides a novel insight into the relative contributions of Ca^{2+} -driven and amyloid-driven pathological pathways in AD patients. Therefore, if pathology is primarily driven by an increase in $\text{A}\beta_{42/40}$ ratio, then formation of $\text{A}\beta_{42}$ -enriched CWP plaques is favored. It has been previously proposed that only mutations that cause large increases in $\text{A}\beta_{42/40}$ ratios, such as PS1- ΔE9 and PS1-436Q, can lead to CWP plaques (Houlden et al., 2000). Our proposal can be considered as expansion of the same idea which now adds “ Ca^{2+} dimension” to the “amyloid dimension”. In agreement with the earlier proposal (Houlden et al., 2000), we argue that PS1-FAD mutations linked with large increases in $\text{A}\beta_{42/40}$ ratio should yield CWP phenotype independently from effects of the same mutation on ER Ca^{2+} leak (quadrants II and IV on Fig. 2B). However, even PS1 mutations that have relatively small effects on $\text{A}\beta_{42/40}$ ratios would yield CWP phenotype if ER Ca^{2+} leak is not effected (quadrant III on Fig. 2B).

On another hand, if pathology is driven by both Ca^{2+} dyshomeostasis and relatively modest increases in the $\text{A}\beta_{42/40}$ ratio, then typical DCP or “diffuse” plaques are favored (quadrant I on Fig. 2B). It is possible that the relative contribution of these pathways may vary in different members of the same pedigree, resulting in a variable presentation of CWP/SP phenotype in most cases (Karlstrom et al., 2008; Lerner and Doran, 2006; Tabira et al., 2002). In agreement with this idea, the most robust CWP/SP phenotype was reported for PS1 ΔE9 mutant (Brooks et al., 2003; Crook et al., 1998), which has the largest effect on γ -secretase activity and acts as a GOF mutant for ER Ca^{2+} leak channels (quadrant IV, Fig. 2B). Our hypothesis indeed predicts that the mutant with these properties should shift the balance as far as possible towards pure amyloid-driven pathology and formation of CWP plaques.

Neuronal Ca^{2+} signalling and sporadic AD

What are the implications of these findings for sporadic AD? The age-related changes in neuronal Ca^{2+} signalling has been well established (Foster, 2007; Gant et al., 2006; Toescu and Verkhratsky, 2007). It has been shown that Ca^{2+} release from intracellular stores is increased in the aging neurons, similar to neurons that express PS1-FAD mutants with loss of ER Ca^{2+} leak function. Thus, it is possible that overloaded ER Ca^{2+} stores result in “accelerated aging” phenotype (Bezprozvanny, 2009). Other features of aging neurons include depolarized mitochondria, reduced cytosolic Ca^{2+} buffering capacity, and activation of calcineurin and calpains. All these changes indicate that aging neurons experience significant dysregulation of intracellular Ca^{2+} handling. Consistent with importance of Ca^{2+} signalling in sporadic AD, most sporadic AD patients present dense core plaque phenotype.

However, some of sporadic AD patients also present CWP pathology (Le et al., 2001), suggesting that CWP are not specific for early onset FAD resulting from PS1 mutations. The evidence suggests that there is significant and complex interplay between the “amyloid hypothesis” and the “Ca²⁺ dysregulation hypothesis” of AD pathogenesis, and it appears that PSs are master-regulators of these processes. It is possible that the relative contribution of “Ca²⁺-driven” and “amyloid-driven” pathogenic processes may vary between different brain regions, resulting in different abundance of CWP and DCP plaques in different areas of the brain for SAD and FAD patients. Future studies will be needed to test the proposed hypothesis and to relate our findings to understanding the pathogenesis of familial and sporadic AD.

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Figure 1. ER Ca^{2+} leak and γ -secretase function of PS1

A. The domain structure of PS1. TM domains 1 – 9 are indicated. The yellow stars indicate the catalytic aspartates D257 and D385. Based on biochemical (Laudon et al., 2005; Spasic et al., 2006) and structural (Sobhanifar et al., 2010) studies.

B. ER Ca^{2+} channel (top) and γ -secretase (bottom) conformations of hydrophilic pore in PS1. Adapted from (Nelson et al., 2011). Proposed arrangement of TM6, TM7, TM8 and TM9 is indicated. Amino acids are represented by a single letter code. Light blue residues are exposed to water in SCAM experiments and dispensable for ER Ca^{2+} leak activity. Dark blue residues are exposed to water and essential for ER Ca^{2+} leak activity. Stars indicate the D257 and D385. The model is based on SCAM analysis (Sato et al., 2006; Sato et al., 2008; Tolia et al., 2006; Tolia et al., 2008) and results of ER Ca^{2+} leak assays (Nelson et al., 2011).

Figure 2. Variant AD and ER Ca²⁺ leak function of PS1

A. Schematic representation of PS1-FAD mutations analyzed in ER Ca²⁺ leak function experiments. Adopted from (Nelson et al., 2010). The nine transmembrane domains (TM1-TM9) of presenilins, the γ-secretase catalytic aspartate residues (D257 and D385, yellow) and the site of the endoproteolytic cleavage of presenilins are shown. The effects of FAD mutations on ER Ca²⁺ leak activity of presenilins is color coded – red color is used for LOF mutations, blue color is used for “functional” mutations. The positions of 3 PS1 FTD-implicated mutants are also shown (light blue color).

B. Effects of PS1-FAD mutations on ER Ca²⁺ levels and Aβ42/40 ratios. Adopted from (Nelson et al., 2010). For each PS1-FAD mutant the Aβ42/40 ratios and ER Ca²⁺ levels were normalized to the corresponding values for wild-type (WT) (open triangle). The PS1-FAD mutants with confirmed DCP pathology (M139V, M146L, A246E) are plotted as solid circles. The PS1-FAD mutants with confirmed CWP pathology (L166P, P436Q, P264L, ΔE8, E280G, ΔE9, T291P, C410Y) are plotted as open circles. The PS1-FAD mutants with unknown pathology (G384A, L85P) are plotted as open diamonds. The diagram is divided into 4 quadrants (I, II, III, IV) as shown and explained in the text.