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## Lysosome and Calcium Dysregulation in Alzheimer's Disease – Partners in Crime

MaryKate McBrayer\* and Ralph A. Nixon\*<sup>†,‡</sup>

\*Center for Dementia Research, Nathan S. Kline Institute, 140 Old Orangeburg Road, Orangeburg NY 10962

<sup>†</sup>Department of Psychiatry, New York University Langone Medical Center, 550 1<sup>st</sup> Avenue, New York NY 10016

<sup>‡</sup>Department of Cell Biology, New York University Langone Medical Center, 550 1<sup>st</sup> Avenue, New York NY 10016

### Abstract

Early onset familial Alzheimer's disease (FAD) is caused by mutations of Presenilin 1, Presenilin 2, and amyloid precursor protein. Beyond the effects of PS1 mutations on proteolytic functions of the gamma-secretase complex, mutant or deficient PS1 disrupts lysosomal function and calcium homeostasis, both of which are considered strong pathogenic factors in FAD. Loss of PS1 function compromises assembly and proton-pumping activity of the vacuolar-ATPase on lysosomes, leading to defective lysosomal acidification and marked impairment of autophagy. Additional dysregulation of cellular calcium by mutant PS1 in FAD has been ascribed to altered ion channels in the endoplasmic reticulum; however, rich stores of calcium in lysosomes are also abnormally released in PS1-deficient cells secondary to the lysosomal acidification defect. The resultant rise in cytosolic calcium activates calcium-dependent enzymes, contributing substantially to calpain over-activation that is a final common pathway leading to neurofibrillary degeneration in all forms of AD. Here we discuss the close inter-relationships among deficits of lysosomal function, autophagy, and calcium homeostasis as a pathogenic process in PS1-related FAD and their relevance to sporadic AD.

### Keywords

Alzheimer's disease; lysosomes; calcium regulation; calpains

### Introduction

Autosomal dominant mutations of Presenilin 1 (PS1), Presenilin 2 (PS2), and amyloid precursor protein (APP) cause an early-onset form of Alzheimer's disease (EOAD). Although EOAD accounts for fewer than 5% of all AD cases, investigations of the three responsible genes have thus far provided many of the available clues to suspected pathogenic mechanisms in AD. Mutations of PS1, which are responsible for the vast majority of early-onset AD cases, can accelerate disease onset to ages as early as the late 20's. In most cases of EOAD, the defining lesions of AD – neurofibrillary tangles (NFT) and neuritic plaques-- as well as characteristic autophagic –lysosomal pathology, resemble the features of later onset “sporadic” AD (sAD), although these abnormalities are usually

more severe. Notable clinical and neuropathological heterogeneity, however, is sometimes seen among families carrying one of the >120 known PS1 mutations [1, 2] [3].

Presenilin 1 (PS1), a ubiquitous protein with 9 transmembrane domains, exists as a 65Kda holoprotein in the endoplasmic reticulum (ER). The molecule is cleaved by a furin-like “presenilinase” to generate a two-chain form [4, 5], which constitutes the catalytic subunit of the gamma ( $\gamma$ )-secretase enzyme complex composed of four additional subunits. Delivered from the ER to diverse vesicular destinations in the cell, gamma-secretase mediates the intramembranous cleavage of well over 25 different substrates, which are mainly type I membrane proteins [6, 7], including most notably APP. The gamma-secretase generates a neurotoxic amyloid- $\beta$  peptide ( $A\beta$ ) from a membrane bound carboxyl-terminal fragment of APP generated by  $\beta$ -APP cleaving enzyme (BACE-1)[8]. The pathogenic effects of PS1 mutations in AD are commonly ascribed to a small increase in the generation of a toxic 42 amino acid peptide ( $A\beta_{42}$ ) relative to a less toxic 40 amino acid form ( $A\beta_{40}$ ) [7, 9, 10]. Recent findings, however, indicate that AD-causing PS1 mutations often confer loss of function of the secretase, so that even if the  $A\beta_{42}/A\beta_{40}$  ratio is modestly higher, as is often, though not invariably the case [11], the absolute levels of these peptides may be markedly lowered [12, 13] and not always a higher  $A\beta_{42}$  to  $A\beta_{40}$  ratio [11]. Thus, additional or alternative explanations for pathogenicity of PS1 mutations have been sought [14][15, 16].

PS1 may contribute to AD pathogenesis through loss of its other functions [17] while serving as a component of gamma-secretase, which may include cell adhesion, neurite outgrowth, and synaptic plasticity [18, 19] or alternatively, by acting via its secretase-independent roles as PS1 holoprotein, which include lysosomal acidification essential for autophagic protein degradation [20], wnt signaling [21], and cellular calcium regulation [22–24]. In this brief review, we will focus specifically on the multiple roles of the PS1 holoprotein and how loss of its function in the ER may link ER and lysosome pathogenic mechanisms in EOAD which accelerate neurodegeneration.

### **Mutations or deletion of PS1 cause autophagy defects by disrupting v-ATPase assembly and lysosome acidification**

Autophagy is a lysosomal degradative pathway for recycling diverse cellular constituents [25, 26], particularly under conditions of metabolic stress (Figure 1). Essential for survival of neurons, autophagy is solely responsible for the cellular turnover of damaged or obsolete organelles and is vital to eliminating misfolded and aggregated proteins, which are poorly degraded by the ubiquitin-proteasome system. Autophagy has been reported to be altered in different neurological disorders, and is considered to be a pathogenic factor in several of these diseases, particularly AD and Parkinson’s disease [27]. In AD, disruption of autophagy results in a particularly profuse accumulation of autophagic vacuoles within grossly swollen dystrophic neurites of affected neurons [28] which stems from defects in the lysosomal clearance of autophagy substrates by lysosomes [29, 30]. Growing evidence has linked lysosome system failure during autophagy to multiple pathological outcomes in AD, including accelerated amyloidogenesis, neuritic dystrophy, apoptosis, and possibly, tauopathy [31–34].

Mutations of PS1 considerably exacerbate autophagy pathology in EOAD and mouse models of AD [35] and similar pathology can be induced in neurons and non-neuronal cells by deleting PS1 [20, 36, 37]. Exploring the basis for these effects, Lee and colleagues [20] demonstrated that PS1 holoprotein serves as a chaperone in the ER for the vATPase V0a1 subunit, a 6-pass transmembrane protein constituent of the proton pump responsible for full acidification of lysosomes. Binding to the PS1 holoprotein, as this subunit is being translated in the ER, stabilizes the correct folded state and facilitates its glycosylation, believed to be

necessary for transit from the ER [20, 38–40]. In cells lacking PS1, the V0a1 subunit of the v-ATPase is poorly glycosylated and unstable, which prevents adequate delivery to lysosomes and assembly of the multi-subunit v-ATPase, leading to greatly diminished levels and activity of the proton pump [41] resulting in failure to fully acidify lysosomes, which is a requirement for lysosomal protease activation and autophagy [20, 30]. Similar lysosomal acidification defects have now been observed in PS1 KO blastocysts, PS1 KO and PS1/2 KO MEFS, PS1-FAD human fibroblast lines, PS1 KO neurons, and PS1/APP mice [30, 41]. The roles of the V0a1 subunit and of PS1 in lysosome acidification have been confirmed in recent studies of PS1 ablated cells or mutant PS1 mouse models and other systems [30, 38–40, 42] though Coen et al. has proposed that neither of these components is involved in lysosomal pH regulation [43]. The importance of this PS1 chaperone function in AD pathogenesis is underscored by the ability of pharmacological inhibitors of vATPase to induce identical AD-related autophagy dysfunction in normal cells and by the complete reversal this phenotype in PS1 deficient cells by pharmacologically normalizing lysosomal acidification [41]. The connection of PS1 loss of function to wnt signaling was recently demonstrated in which the loss of PS1 led to an increase in nuclear  $\beta$ -catenin and phosphorylated LRP6 due to increased wnt activity, all of which was attributed to defective acidification of the multivesicular body [38].

### **Defective lysosome acidification – an emerging factor in pathogenesis of degenerative diseases**

Interestingly, disruptions of normal v-ATPase activity appear to be important for the progression of other diseases. Mutations within the  $\alpha 3$  subunit of the v-ATPase cause misfolding and ER retention of this subunit, leading to osteopetrosis, a bone disease that may be accompanied by neurodegeneration [44]. Mice lacking the  $\alpha 3$  subunit also have impaired acidification of lysosomes in macrophages [45]. In a rare familial form of Parkinson's disease, the loss of function of ATPase13A2 (Park9), believed to be involved in lysosome acidification, leads to impaired autophagy and neurodegeneration [46]. Mutations in leucine-rich repeat kinase 2 (LRRK2) increase the activity of the protein and lead to late onset familial Parkinson's disease. Among other pathological effects that have been attributed to this protein kinase, increase in LRRK2 activity leads to pathologically increased autophagy induction and defective lysosomal acidification [47]. Reduced v-ATPase activity and abnormally elevated lysosomal pH due to a deficiency of VMA21, an integral membrane protein required for the assembly of the vATPaseV0 complex in the ER, leads to x-linked myopathy, a form of muscle fiber degeneration [48].

Although lysosomal pH is predominantly maintained through the activity of the vacuolar-ATPase (v-ATPase), other ion channels localized to the lysosomal membrane are suspected to be important in maintaining or regulating pH shifts during lysosomal proteolysis and may be particularly important when the intraluminal lysosomal environment is disturbed in disease states. The chloride channel, CLC7, has been implicated in fine-tuning lysosomal pH in conjunction with v-ATPase [49] and other evidence has suggested an involvement of calcium channels, mucolipin (TRPML1) and TPC2, in lysosomal pH regulation [50–52]. Lysosomal storage disorders exhibit elevated lysosomal pH as a part of the progression of the disease. Elevated lysosomal pH has been detected in several forms of neuronal ceroid lipofuscinosis, including juvenile NCL (Batten disease) and infantile NCL leading to altered lysosomal enzyme activity [53].

## Calcium dysregulation in EOAD – the important contribution of defective lysosomal acidification

PS1 holoprotein has also been implicated in mediating the gamma-secretase-independent effects of AD-causing PS1 mutations (or PS1 deletion) in dysregulating calcium homeostasis at several different subcellular levels, including the ER [54–57], the mitochondrial-associated membranes (MAM)[58] and lysosomes[43, 59] (Figure 2). Calcium homeostasis in the ER is normally maintained by regulating calcium efflux through ryanodine receptors (RyR) and inositol 1,4,5-triphosphate receptors (IP<sub>3</sub>R), and by regulating the import of calcium through the sarco-/endoplasmic reticulum Ca<sup>2+</sup> ATPase (SERCA) channel [60]. Although the mechanism(s) are unresolved, loss of function of PS1 leads to ER calcium dysregulation. Exaggerated calcium release by IP<sub>3</sub>R in response to IP<sub>3</sub> was initially reported [61] and confirmed by other groups in different cell types [57, 62, 63] and recent studies into the underlying mechanism of the IP<sub>3</sub>R mediated calcium release linked the exaggerated release to a gain-of-function mechanism in which mutations in PS1 stimulate IP<sub>3</sub>R gating [64]. The expression of ryanodine receptors is also upregulated in the brains of transgenic mice harboring PS1 mutations [65] as well as in PC12 cells expressing PS1 mutations and in primary cells from PS1 KI mice [63]. This increase in RyR channels mediates calcium release [63, 65]. Additionally, PS1 may act as a passive calcium leak channel within the ER: mutations within the PS1 holoprotein are proposed to disrupt the leak function leading ER stores to overfill, thereby over-activating IP<sub>3</sub>R and increasing calcium release [22, 66]. Another group of investigators, however, was unable to observe changes in ER calcium fill or leak rates in cells lacking PS or expressing FAD linking PS mutations [24] and the basis for the observed differences is not yet resolved. The removal of calcium from the cytosol back to the ER is controlled by the SERCA pump, but in cells lacking PS1, PS2, or PS1/2, SERCA activity is diminished leading to increased cytosolic calcium. A similar effect has been seen in cells expressing either PS2 or FAD-PS2 [67], although others have observed increased SERCA function leading to the overfilling of ER calcium stores in cells overexpressing PS2 or AD-linked mutant PS1 [68]. Despite these uncertainties about the precise mechanisms, there is consensus in the field that ER calcium is dysregulated in response to loss of PS1 function [56, 60].

Although the ER contains the largest stores of calcium, with concentrations in the low millimolar range [69] lysosomes are not far behind with calcium concentrations in the range of 500–600 μM [70]. Recently, efflux of calcium from lysosomes in PS1/2KO MEF was reported, although the underlying mechanism was not investigated (Coen et al. 2012). A subsequent study confirming this phenomenon also revealed reduced levels of the lysosome-associated NAADP-dependent calcium channel, TPC2 [59], although it remains unclear how diminished amounts of a calcium efflux channel would explain elevated lysosomal calcium efflux. An earlier study using the lysosomal pH modulators chloroquine and bafilomycin, however, provides an attractive possible basis for calcium dysregulation in PS1 loss of function conditions by linking lysosomal calcium efflux to changes in lysosomal pH [71]. The possibility that decreased lysosomal calcium is a direct consequence of elevated lysosomal pH is indeed consistent with our recent findings [41] that raising lysosomal pH in wild-type blastocysts not only reproduces the autophagy defects seen in PS1 mutant or deficient cells but also induces the abnormal lysosomal calcium efflux. Importantly, reversing lysosomal pH deficits in PS1 deleted cells rapidly rescues both the lysosomal calcium abnormalities and the autophagy phenotype. By contrast, reversing the calcium efflux abnormalities alone neither corrects the acidification defect nor the autophagy deficits in PS1 deleted cells, indicating that lysosomal calcium dysregulation is a secondary consequence of pH elevation. These studies therefore link two γ-secretase independent effects of PS1 loss of function. They imply that vATPase deficiency is the common basis for

these deficits and suggest that calcium efflux from lysosomes may be a significant contributor to the overall defect in cytosolic calcium elevation seen in PS1-deficient cells.

Altered ER calcium homeostasis reflected in a depletion of ER stores leading to elevated cytosolic calcium is common among several lysosomal storage disorders and it is possible that the mechanisms are similar to those proposed in the case of PS1 loss of function. Enhanced release of calcium by the ryanodine receptor has been observed in models of Gaucher's disease due to the accumulation of glycosylceramide [72–74]. The ER calcium stores are depleted in GM1 gangliosidosis due to the interaction of GM1 with IP<sub>3</sub>R [75] and downregulation of SERCA pumps has been observed in Sandhoff disease [74] and in Neimann-Pick type A [76] due to interactions with ganglioside GM2 resulting in elevation of cytosolic calcium [77]. Lowered amounts of lysosomal calcium have also been observed in Neimann-Pick type C due to accumulation of sphingosine [78].

### **PS1 mediated calcium dysregulation— a pathway to accelerated calpain – dependent neurofibrillary degeneration in FAD**

Altered ER and lysosomal calcium homeostasis leads to an increase in cytosolic calcium levels [54], one consequence of which is the activation of calpain 1 ( $\mu$ -calpain) and calpain 2 (m-calpain), proteases that are highly expressed in the central nervous system [79] (Figure 2). Targets of calpain cleavage include cytoskeletal proteins, signal transduction proteins, synaptic proteins, and transcription factors [80]. Abnormally increased activities of both calpain 1 and calpain 2 have been linked to AD [81–83] with calpain 1 actions possibly concentrated at synapses [82] and calpain 2 bound to neurofibrillary tangles (NFTs) and more activated within the neurites of neurons at risk for developing NFTs [83]. Calpain over-activation is well-established to have many deleterious effects, among them being the pathological activation of CDK5 (through the cleavage of p35 to p25) [84], ERK1/2 [85], and GSK3 $\beta$  [86] and consequent hyperphosphorylation of tau leading to NFT formation. Calpains can also cleave IP<sub>3</sub>R making it constitutively active which may further disrupt ER calcium homeostasis in PS1-related EOAD [87]. The pathogenic role of calpains in AD is further indicated by the marked depletion in AD brain of calpastatin [88], the specific endogenous inhibitor of calpains [89, 90]. Moreover, overexpressing calpastatin reduced calpain activity, tau phosphorylation, ERK activation, and amyloid plaque load in either of two different mouse models of AD [91, 92]. A synthetic calpain inhibitor, BDA-410, improved spatial memory, fear conditioning, and synaptic plasticity associated with increased CREB phosphorylation in APP/PS1 transgenic mice [93]. Finally, we have recently observed that calpastatin overexpression in the JNPL3 tauopathy mouse model leads to decreased tau phosphorylation, increased life span, and loss of motor neurons (McBrayer, MK unpublished results). Because ER and lysosomal calcium dysregulation is altered in PS1 FAD and PS1 KO cell lines [43, 59], it is not surprising that we have also found calpains to be robustly activated in PS1 KO blastocysts and PS1-FAD fibroblasts as reflected in marked increase in calpain-cleaved spectrin and significantly decreased calpastatin levels (McBrayer, MK unpublished results).

### **Conclusions ---- lysosomal and calcium dysfunction in sporadic AD**

While calcium dysregulation may be initially driven by presenilin mutations in FAD, additional cellular calcium derangements are also seen in the more prevalent late-onset forms of AD [94] and likely have a multifactorial origin, including contributions from excitotoxicity, ischemia, and A $\beta$  neurotoxicity [95, 96]. It is tempting to speculate that the notable disruption of lysosomal function in AD [97, 98] may give rise to additional lysosomal-related calcium dysregulation. Interestingly, a further connection has been suggested by Yamashima between calpain activation and lysosomal dysfunction leading to

lysosomal membrane rupture, which involves increased calpain-mediated cleavage of carbonylated HSP70.1. HSP70.1 has roles as both a protein chaperone and stabilizer of lysosomal membrane integrity. Calpain-mediated loss of HSP70.1, coupled with other factors impairing autophagic clearance [98], are proposed to lead to lysosomal membrane disruption and likely to diverse downstream pathological events [95]. Calpain activation is observed in patients with sAD and recent research indicates that calcium homeostasis is also disrupted in sAD[99–101]. EOAD and sAD likely have different initiation points leading to dysregulated lysosome dysfunction and calcium dysregulation but ultimately share pathological outcomes, including calpain over-activation, that should prove to be useful drug targets for AD therapy.

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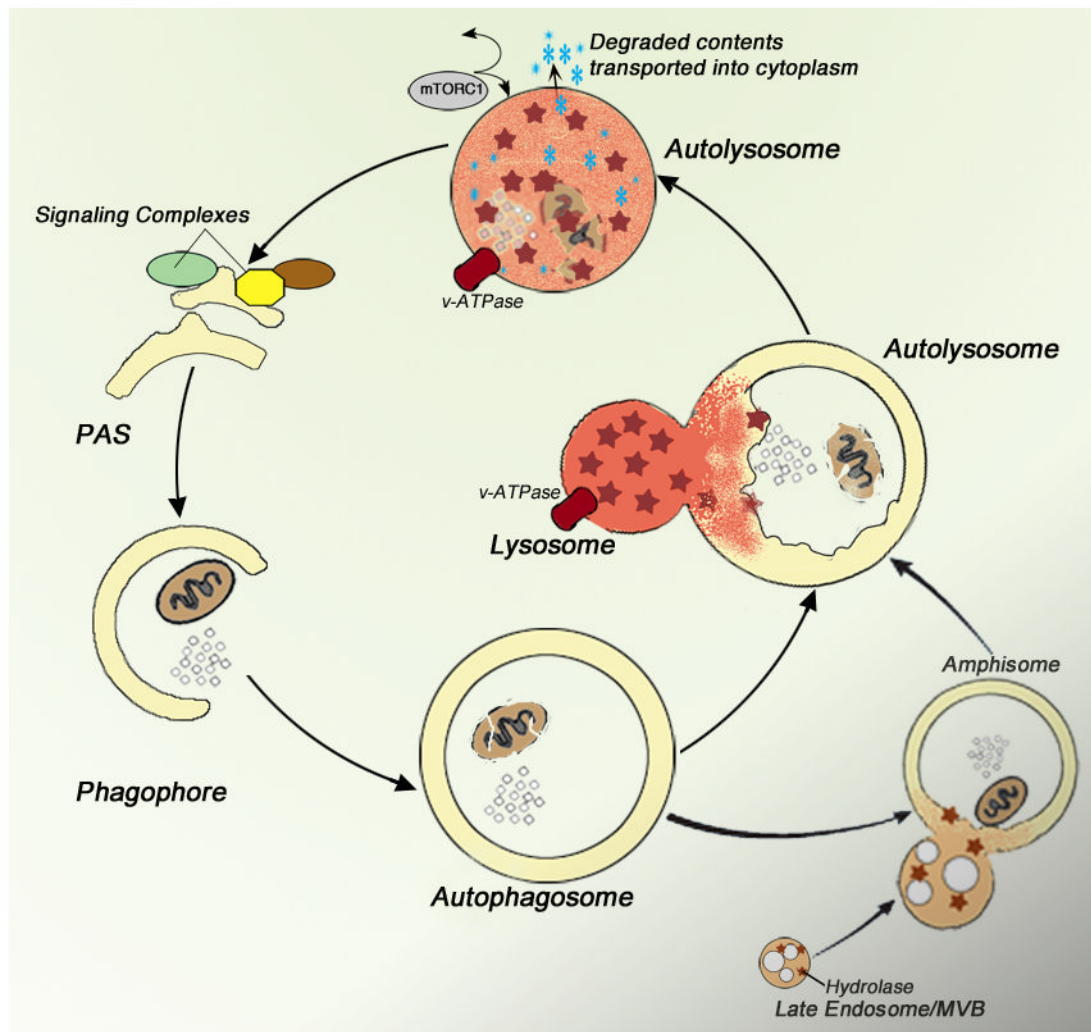


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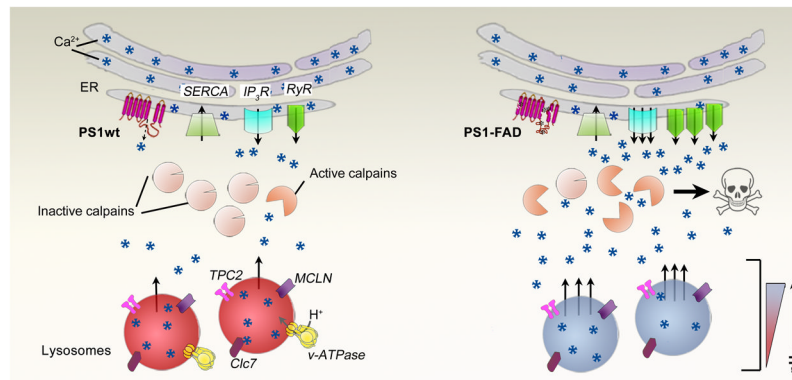
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**Figure 1.**

An overview of macroautophagy (autophagy). Multiple signaling pathways and protein modification assemblies initiate formation of an isolation membrane which is then elongated around the selected substrate or region of cytoplasm (phagophore). The inner and outer bi-layers of the isolation membrane close to form a double membrane-limited autophagosome. Lysosomes fuse with either an autophagosome or an amphisome created by autophagosome– endosome fusion, yielding autolysosomes. The contents of the autolysosome are degraded by hydrolytic enzymes activated within a highly acidic environment by the proton pump, v-ATPase. Digestion yields basic metabolites which are released into the cytoplasm to be used for new synthesis or as sources of energy and can also modulate TORC1 activity and other aspects of autophagy induction [26, 102]. MVB, multivesicular body; PAS, preautophagosomal structure (adapted from Nixon, 2013).



**Figure 2.**

Diagram of calcium dysregulation in PS1wt (left) versus PS1-FAD conditions (right). In the ER, PS1-FAD induces an upregulation of RyR expression, induces a gain of function in IP<sub>3</sub>R, enhances SERCA function, and inhibits passive leak function of PS1. At the lysosomes, PS1-FAD elevates lysosomal pH by blocking vATPase maturation in the ER and delivery to lysosomes leading to increased calcium efflux through TPC2 and/or MCLN. Consequently, cytosolic calcium levels are increased leading to calpain activation. All of which contributes to impairments of cellular function and neurodegeneration (depicted by skull and crossbones). Black asterisks are symbolic of PS1-FAD mutations. Arrows depict movement of calcium. TPC2, two-pore calcium channel; MCLN, mucolipin1; CLC7, chloride channel protein 7