PERSPECTIVE

# Defective autophagy and Alzheimer's disease: is calcium the key?

**Presenilins and autophagy:** Presenilin 1 (PS1) and presenilin 2 (PS2) are homologous, multi-pass transmembrane proteins endowed with pleiotropic functions, ranging from the regulation of membrane trafficking to cell differentiation. Their catalytic activity within the γ-secretase complex, an aspartyl-protease responsible for the intramembrane cleavage of several different type I transmembrane proteins, has been intensively studied in the context of Alzheimer's disease (AD). Indeed, tens of autosomal dominant mutations in the *PSEN1* and *PSEN2* genes (encoding PS1 and PS2, respectively) have been associated with the rare familial forms of AD (FAD). FAD-PS1/PS2 mutants are known to alter the γ-secretase-dependent cleavage of the amyloid-precursor-protein, generating amyloid-β (Aβ) peptides, whose toxicity is thought to underlie AD onset and progression. Interestingly, in the last decade, besides their γ-secretase activity, both PS1 and PS2 have been implicated in the regulation of macroautophagy (hereafter called autophagy), a key cellular pathway in which different cell material (proteins, lipids, sugars, damaged organelles) is engulfed within double-membrane vesicles (autophagosomes) and targeted to lysosomes for degradation and recycling of the molecular constituents. Considered that impairment of autophagy can promote neurodegeneration, the finding that FAD-linked PSs perturb this process suggests it could be involved in AD pathogenesis.

**Presenilins and lysosomes (pH, number and $Ca^{2+}$ content):** The first evidence that PSs modulate autophagy came in 2004, when, associated with dysregulated cytosolic $Ca^{2+}$ signalling, the formation of large lysosomes with accumulation of α- and β-synuclein was observed in PS1 knock-out (KO) mouse neurons and fibroblasts (Wilson et al., 2004). Further studies consistently indicated that PS1 and PS2 are involved in the late steps of the autophagy flux, *i.e.*, the fusion of autophagosomes with lysosomes, which is critical to complete the degradative process. However, while consensus has been reached on this point, the underlying molecular mechanisms, as well as the effects of FAD-PS mutants on the process, still remain matter of intense debate. In particular, either impaired lysosomal acidification or altered lysosomal $Ca^{2+}$ signalling has been proposed. For instance, Lee et al. (2010) reported accumulation of enlarged autophagosomes in PS1-KO blastocysts and PS1-hypomorphic neurons, because of their defective clearance after fusion with lysosomes. The authors suggested that full-length PS1, by physically interacting with the V0a1 subunit of the proton pump V-ATPase, promotes the maturation of this latter protein and its targeting to lysosomes, thus allowing their physiological acidification. Indeed, defective lysosomal acidification was observed in PS1-KO cells and, importantly, in human fibroblasts from patients harbouring FAD-PS1 mutations, suggesting a loss-of-function mechanism for FAD-PS1 mutants (**Figure 1**). Interestingly, PS1 γ-secretase activity was not involved in the process. On the other hand, Neely et al. (2011) observed a similar autophagosome and lysosome accumulation upon ablation/depletion of either PS1 or PS2, but failed to detect a defective lysosomal acidification. Importantly, however, this group also concluded that PSs, independently of γ-secretase activity, are critical in the final steps of autophagy (**Figure 1**), as suggested by the finding that the addition of lysosomal inhibitors do not block further the flux. In line with this finding, Coen et al.(2012) reported that, in PS1 and PS2 double KO cells, the endo-lysosomal dysfunction was not due to defective lysosomal acidification, nor to changes in V0a1 maturation or sorting, but rather to a reduced lysosomal $Ca^{2+}$ content and/or release, affecting their fusion with autophagosomes (**Figure 1**). A normal lysosomal pH and V0a1 maturation in different PS-KO models was also found by Zhang et al. (2012) who reported, however, an altered expression of genes involved in lysosome biogenesis, known as the coordinated lysosomal expression and regulation (CLEAR) network. An attenuation of the CLEAR network was also described by Reddy et al. (2016) upon PSs depletion or in the presence of FAD-PS1 mutants. The consequent block in autophagy could be rescued by increasing nuclear $Ca^{2+}$ and the levels of Sestrin2, a protein whose expression depends on the $Ca^{2+}$-regulated CaMKIV/pCREB signalling pathway and that negatively regulates mTORC1 activity. Recently, Nixon and collaborators, in line with their previous finding (Lee et al., 2010), reported that the decreased lysosomal $Ca^{2+}$ content in PS1-KO cells, observed also by others (see above), was secondary to lysosome alkalinisation, inducing lysosomal transient receptor potential mucolipin 1 channel hyper-activation and $Ca^{2+}$ release (Lee et al., 2015). Rescue of lysosomal pH, but not of $Ca^{2+}$ content alone, was sufficient to unblock the final steps of the autophagy flux (Lee et al., 2015).

**PS2, cytosolic $Ca^{2+}$ signal and autophagosomes:** Whether lysosomal $Ca^{2+}$ content is consistently altered in AD and, importantly, whether these possible alterations have an impact on the reported defects in autophagy is however unclear. Recently, we reported that, in different FAD-PS2 cell models, including primary cortical neurons from FAD-PS2-N141I transgenic mice and primary human fibroblasts from a FAD-PS2-N141I patient, autophagosomes accumulate because of a defective fusion with lysosomes (Fedeli et al., 2019). This is in line with previous observations by other groups, predominantly focused on FAD-PS1 mutants (see above), and further suggests that an impaired autophagosome-lysosome fusion is likely a conserved pathogenic mechanism in AD (**Figure 1**). However, we failed in detecting any alteration in lysosome acidification or number, or in lysosomal $Ca^{2+}$ levels. Rather, we found that the defect depends on a reduced cytosolic $Ca^{2+}$ signal, in turn associated with a lower endoplasmic reticulum (ER) $Ca^{2+}$ content, consistently observed in our FAD-PS2-based models. Specifically, FAD-PS2 dampens ER and cis-medial Golgi $Ca^{2+}$ levels by reducing SERCA-pump activity (Greotti et al., 2019). Upon cell stimulations triggering ER $Ca^{2+}$ release, lower cytosolic $Ca^{2+}$ signals are generated. In turn, this latter defect impacts on the recruitment of the small GTPase RAB7 to autophagosomes, but not to lysosomes. Indeed, cell treatments with molecules capable to buffer cytosolic $Ca^{2+}$, such as BAPTA-AM and EGTA-AM, mimicked the FAD-PS2-induced effects on both RAB7-recruitment to autophagosomes and autophagosome-lysosome fusion, an event, this latter, critically regulated by the extent of RAB7 association with both organelles (Gutierrez et al., 2004). The mechanism by which cytosolic $Ca^{2+}$ modulates RAB7 distribution remains however to be investigated. It is possible that the activity of some $Ca^{2+}$-sensitive RAB7 partners, capable to regulate its sorting, such as

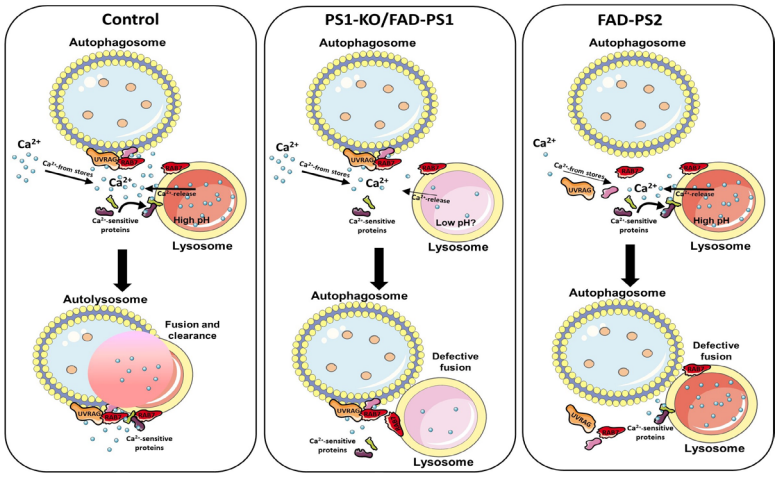


**Figure 1 Possible common mechanism underlying the effect of FAD-PS mutants on autophagy.**
PS1 ablation and FAD-PS1 mutants reduce lysosomal $Ca^{2+}$ content and, possibly, pH. The defective $Ca^{2+}$ release from lysosomes, upon specific stimuli, could affect the sorting of specific $Ca^{2+}$-sensitive proteins, necessary to regulate and complete the fusion process between autophagosomes and lysosomes. On the other hand, FAD-PS2 mutants reduce cytosolic $Ca^{2+}$ signals by depleting ER $Ca^{2+}$ content, possibly affecting the activity of $Ca^{2+}$-modulated proteins (such as UVRAG) that control RAB7 sorting. In turn, the impaired RAB7 recruitment to autophagosomes affects fusion, leading to their accumulation. FAD: Familial Alzheimer's disease; PS: presenilin; ER: endoplasmic reticulum; KO: knock out.

UVRAG, could be affected by FAD-PS2 expression (Fedeli et al., 2019) (**Figure 1**). Moreover, the FAD-PS2-induced alterations may rely on either bulk, widespread reductions of cytosolic $Ca^{2+}$ rises, or localized events at the level of $Ca^{2+}$ micro-domains. Indeed, upon stimulated ER $Ca^{2+}$ release, high-concentration $Ca^{2+}$ micro-domains originate near the mouth of $Ca^{2+}$-releasing channels. The amplitude and extent of these "$Ca^{2+}$ hot-spots" is critically determined by ER $Ca^{2+}$ content. Importantly, the fact that both BAPTA-AM (which quickly buffers $Ca^{2+}$ also in micro-domains) and EGTA-AM (which buffers bulk cytosolic $Ca^{2+}$ rises, but less efficiently $Ca^{2+}$ hot-spots, because of its lower $K_{on}$) mimic the FAD-PS2-induced effects on autophagy (Fedeli et al., 2019) may suggest that the former possibility (*i.e.*, a widespread mechanism) could be involved. We cannot however completely exclude a minimal activity of EGTA-AM also on $Ca^{2+}$ micro-domains, leaving therefore open the possibility that more restricted, localized events are also in play. For instance, $Ca^{2+}$ micro-domains, generated in proximity of lysosomal membrane, have been suggested to regulate autophagy. Specifically, upon starvation, $Ca^{2+}$ release from lysosomes through transient receptor potential mucolipin 1 channels occurs, inducing a localized calcineurin activation, transcription factor EB dephosphorylation and translocation into the nucleus, where it upregulates the expression of lysosomal and autophagy genes (Medina et al., 2015).

Overall, evidence has accrued indicating that, in AD, the final steps of the autophagy flux and, specifically, the process of autophagosome-lysosome fusion, followed by the clearance of their luminal material, are impaired. PS-mutation-specific mechanisms have been proposed, but consensus has been reached on the fact that γ-secretase activity is not involved. For FAD-PS1 mutants, contradictory results are present with respect to lysosomal acidification, but the majority of studies converge on a reduced $Ca^{2+}$ content within lysosomes. On the other hand, much less investigated have been the effects of FAD-PS2 or FAD-amyloid-precursor-protein mutants. Our data suggest that, in different FAD-PS2 models, neither lysosomal pH nor $Ca^{2+}$ content are significantly altered, but a reduced cytosolic $Ca^{2+}$ signal underlies the defective autophagosome-lysosome fusion, specifically by affecting RAB7 recruitment to autophagosomes. Therefore, though some specificities remain, $Ca^{2+}$ dysregulation is emerging as a possible common hallmark potentially implicated in the observed autophagy alterations in AD (**Figure 1**).

Interestingly, PS2-containing γ-secretase complexes have been described selectively targeted to late endosomes and lysosomes, while PS1-containg enzymes resulted more broadly distributed within the cell (Sannerud et al., 2016). Although the effects of FAD-PSs on $Ca^{2+}$ signaling and autophagy are independent of their enzymatic activity, it is possible that γ-secretase, besides catalyzing the cleavage of different type I transmembrane substrates, constitutes a scaffold for the recruitment/assembling/sorting of different autophagy-related proteins. In this context, the specific intracellular distribution of PS2 compared to PS1 may be responsible for their partially different roles in the autophagy pathway.

More than one hundred FAD-linked PSs mutations, distributed in different domains of the protein, are currently known, and a key question is to understand their common link with $Ca^{2+}$ dysregulation and the onset of neurodegeneration. It is possible that different FAD-PS mutants converge on an altered assembly and/or maturation of PS proteins within the γ-secretase complex, thus affecting its proper sorting and, consequently, its functional, but enzymatic-independent, interaction with $Ca^{2+}$-modulated factors involved in autophagy regulation. Intriguingly, along this line, a subset of FAD-PS1 mutants has been demonstrated to phenocopy PS2, shifting PS1 localization to lysosomes (Sannerud et al., 2016).

Finally, further studies will be necessary to check whether similar defects are commonly observed also in sporadic AD or, on the contrary, are specific in the context of FAD-PS cases. Surely, defective autophagy could be related to the progressive neurodegeneration typical of AD. In this scenario, understanding the mechanisms underlying these alterations is critical to design possible therapeutic interventions, and targeting specific $Ca^{2+}$ signalling pathways appears an intriguing possibility that deserve additional investigations.

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