

# A New Perspective of Lysosomal Cation Channel-Dependent Homeostasis in Alzheimer's Disease

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**Abstract** Studies have reported typically biophysical lysosomal cation channels including TPCs. Their plausible biological roles are being elucidated by pharmacological, genetic and conventional patch clamp procedures. The best characterized so far among these channels is the ML1 isoform of TRP. The reported TRPs and TPCs are bypass for cation fluxes and are strategic for homeostasis of ionic milieu of the acidic organelles they confine to.  $\text{Ca}^{2+}$  homeostasis and adequate acidic  $\text{pH}_L$  are critically influential for the regulation of a plethora of biological functions these intracellular cation channels perform. In lysosomal ion channel biology, we review: ML1 and TPC2 in  $\text{Ca}^{2+}$  signaling, ML1 and TPC2 in  $\text{pH}_L$  regulation. Using A $\beta$ 42 and tau proteins found along clathrin endolysosomal internalization pathway (Fig. 3), we proffer a mechanism of abnormal  $\text{pH}_L$  and ML1/TPC2-dependent cation homeostasis in AD.

**Keywords** Lysosomal Cation Channels · Cation homeostasis · AD ·  $\text{pH}_L$

## Introduction

Lysosomes are membrane-enclosed acidic organelles and contain acid hydrolases (Fig. 1). The acid hydrolases lend

lysosomes the ability to degrade and process biological materials including polysaccharides, proteins and lipids. The degradative ability is a function of 4.5–5.0 acidic pH in the lumen of the lysosome (Fig. 1). In this acidic optima, lysosomal acid hydrolases are optimally active [1]. Apart from the acid hydrolase contents, lysosomes house  $\text{Ca}^{2+}$  [2, 3], and cation channels [4–6]. Some of the cation channels among others are transient receptor potential mucolipin 1 (ML1) and two-pore channel 2 (TPC2). These channels are well known to perform multifarious functions and a single distinctive physiological function could not yet be completely ascribed to either of these channels despite huge intense research work.

Accordingly, it is been reported that the intracellular lysosomal cation channels play roles in autophagy [7], migration and cell polarity [8], morphogenesis [9], cancer, neurodegeneration [10], stroke/ischemia and even in aging through a coordinated action of  $\text{Ca}^{2+}$  homeostasis, membrane lipids and proteins. Other roles of the channels may include: neuronal communication [2, 6], membrane trafficking [11, 12], regulation of gene expression and nociception, temperature [13], and sensation controls, muscle contraction, and vaso-motor actions.

The regulatory mechanisms underlying the roles are being hugely investigated and are not clearly understood. Phosphoinositides [4, 5, 14, 15] and GTPases [16, 17] can potentiate ML1/TPC2 specific activation in the functions. The phosphoinositides are membrane-bound lipid that prescribes signature identify for cytoplasmic proteins and potentiates the activity of the cation channels with specificity and potency [14]. The GTPases are well known regulators of membrane trafficking that switch between active and inactive forms in a GTP/GDP-dependent manner respectively to regulate membrane trafficking. The classical switches underline a process that manifests discrete signaling to regulate the ML1/TPC2 activity in the functions. Furthermore, by working hand-in-

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hand in a combined coordinated fashion rather than just single entities in signaling processes, intracellular dependent activations by the GTPases, the  $\text{Ca}^{2+}$ -mediated and the phosphoinositides can fine-tune the channels mediated activities. Juxta-organelle levels of  $\text{Ca}^{2+}$  that fixes the spatio and temporary signal required for membrane trafficking is also a way. Another indispensable crucial means is mechanization of the structure and magnitude of lysosomal pH ( $\text{pH}_L$ ). The maintenance of  $\text{pH}_L$  is largely done by vacuolar-type ATPase (V-ATPase), albeit, either influx of cations or efflux of anions to truncate electrical potential that can be generated by proton accumulation through V-ATPase across lysosomal membrane.

Abnormalities in  $\text{pH}_L$  regulation, in  $\text{Ca}^{2+}$  second messenger signaling, in specific and potent activations by phosphoinositides and in on and off classical switches of GTPase, together can defect lysosomes function in the degradation of biological materials. Defects in the signaling networks associated with lysosomes potentially underlie how macromolecules accumulate in AD (Fig. 3). Accumulation once initiated can beget accumulation through consequent disruption of biochemical signaling. This is supported in part by a hypothesis which states that accumulation of materials that cannot be degraded by lysosomal hydrolytic enzymes causes a kind of lysosomal stress that can disrupt if not abolish completely biochemical signaling in the lysosome [18, 19].

Since luminal acidifications regulated by cation sources are necessary for the distribution and degradation of macromolecules in the endolysosomal pathway, we review ML1 and TPC2 in  $\text{Ca}^{2+}$  signaling, ML1 and TPC2 in  $\text{pH}_L$  acidification (Fig. 1). With the identification of  $\text{Ca}^{2+}$  permeable channels in the lysosomes, we opine a mechanism of  $\text{pH}_L$  and ML1/TPC2-mediated aberrant cation signaling in AD for the first time that potentially provides the basis for the famous AD amyloid hypothesis.

### The Channels in $\text{pH}_L$ Regulation

Because  $\text{pH}_L$  regulation is important in endolysosomal biogenesis and repair, fusion and fission, trafficking, cell growth, immunity, lipid storage disease, AD, Parkinson's disease and cystic fibrosis, we discussed how ML1 and TPC2 mediated abnormal  $\text{Ca}^{2+}$  release could dilute the structure and magnitude of  $\text{pH}_L$ . This is believed to be the initial bases upon which acid hydrolases that degrade macromolecules including A $\beta$  become defective. In turn, for instance, in lipid storage disease (LSD), defective acid hydrolases stimulates substrate accumulation of macromolecules. In the LSD, the role of ML1/TPC2 in such accumulation has been studied and in such accumulation there is near to complete compromised  $\text{Ca}^{2+}$  signaling.  $\text{Ca}^{2+}$  signaling per say is desperate and ubiquitous occurring in both LSD and AD. Aberrant  $\text{Ca}^{2+}$  homeostasis in luminal acidic organelle as found in NP are a key strategy leading to

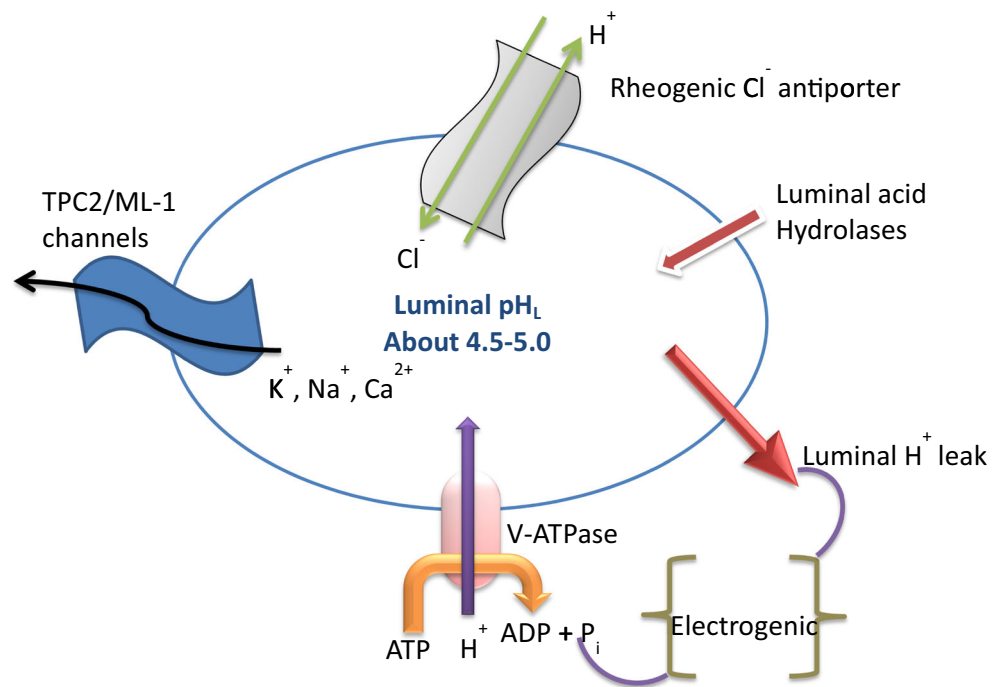
LSD [20]. In such homeostatic process,  $\text{PI}(3, 5)\text{P}_2$  could potentiate ML1/TPC2 dependent  $\text{Ca}^{2+}$  release but NAADP could not potentiate TPC2-dependent  $\text{Ca}^{2+}$  release (Wang et al. [15]). Also, in such homeostasis, and as direct evidence, NAADP and  $\text{PI}(3,5)\text{P}_2$  could potentiate TPC2 in cellular functions [21]. This resolves an existing controversy that NAADP does not activate TPC2 [7, 15] and that NAADP does activate TPC2 [6] for  $\text{Ca}^{2+}$  release. For ML1, much is not known about NAADP-dependent activation. Apparently, it appears that NAADP dependent endolysosomal ML1/TPC2 activation for  $\text{Ca}^{2+}$  release is restricted to TPC2.

Notwithstanding, it is noteworthy that both ML1 and TPC2 are strategic machineries for modulating amounts of  $\text{Ca}^{2+}$  necessary to elicit cellular functions. Abnormal  $\text{Ca}^{2+}$  effluxes by these channels can increase luminal alkalization (Fig. 1). Increase in intraluminal alkalization reduces luminal  $\text{pH}_L$  acidification [22]. This negates a stringent requirement for a functional  $\text{pH}_L$  system within an acidic pH optima ranging between 4.5 and 5.0. Accordingly, if the NAADP-dependent endolysosomal activation is restricted to the TPC2 or to both TPC2 and ML1 even, a common effect would be to elicit lysosomal  $\text{Ca}^{2+}$  release. If this adds to  $\text{Ca}^{2+}$ -induced release by other second messengers like cyclic nucleotides, this would further increase lysosomal  $\text{Ca}^{2+}$  release. Hence, to the luminal side of the acidic organelles, NAADP-induced  $\text{Ca}^{2+}$  release do not only deplete luminal  $\text{Ca}^{2+}$  but causes intraluminal alkalization as well [23].

Indeed, ML1 and TPC2 are potential sources for regulating  $\text{pH}_L$  (Fig. 1). TPC2 knock-out macrophage showed  $\text{pH}_L$  alkalization [7] and mucolipin 1 V, a genetic disorder caused by mutation of ML1 caused either increase [24] or decrease [25] in  $\text{pH}_L$ . Another strong potential candidate of  $\text{pH}_L$  regulation is P2X4. Besides, acidification of lysosomal lumen is done basically by a vacuolar-type ATPase (V-ATPase). V-ATPase is an electrogenic rotary proton transport motor that uses the energy of ATP hydrolysis to move protons across membranes [26] (Fig. 1). Mass movement of proton across the membrane generates a positive net charge — ‘a positive feedback mechanism’ in the lumen. This mechanism, if left unchecked, can destroy the V-ATPase activity and attenuate the extent of the  $\text{pH}_L$  acidification (Fig. 1). To have a physiological condition, in other words to eliminate the positive feedback process, a counter-ion conductive process must exist together with the V-ATPase to dispel the deleterious positive feedback process that can exhaust cellular energy contents. It is been thought that either the efflux of cations or the influx of anion in opposite direction could salvage the positive feedback process (Fig. 1).

Studies in human and mice osteoclast (Osmt1) revealed chloride as the principal counter-ion that dispels the lysosomal lumen of the deleterious positive feedback electrical potential difference [27]. However,  $\text{pH}_L$  is not abnormal in cells that do not have either Cl-7 or Osmt1 [28, 29]. It became unclear what

**Fig. 1** Structure and magnitude of a mechanized pH<sub>L</sub>. A stringent requirement for a functional luminal acid hydrolases is low pH<sub>L</sub> of 4.5 to 5.0, maintained by ATP-mediated proton pump and leak hypothesis. Either pump in or leak out of H<sup>+</sup> is electrogenic requiring a counter mechanism (ML1/TPC2-mediated cation efflux or anion influx that may not be the rheogenic Cl<sup>-</sup> antiporter) to banish a positive net charge inside the lumen generated by ATP hydrolysis to regulate V-ATPase, the principle regulator of pH<sub>L</sub>



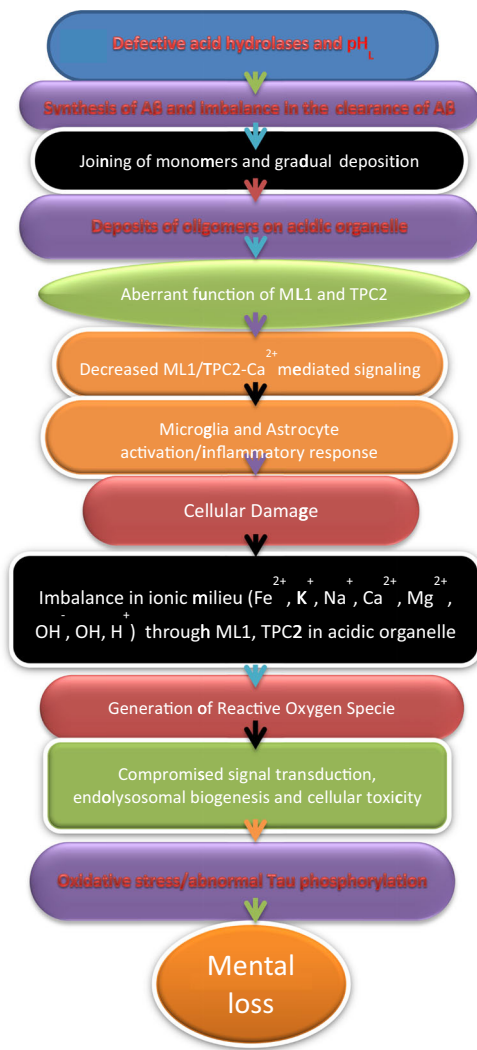
specific biological roles the Cl<sup>-</sup> channels can perform. Surprisingly, Cl<sup>-</sup> is emerging as a likely Cl<sup>-</sup>/H<sup>+</sup> antiporter in the lysosomal membrane instead [30]. To address this controversy, [29] constructed Cl<sup>-</sup> mutant named “Cl<sup>-</sup> uncouple” with glutamate-to-alanine substitution to switch this Cl<sup>-</sup> from an ion antiporter to a Cl<sup>-</sup> channel. The uncouple breaks up Cl<sup>-</sup>/H<sup>+</sup> antiporter and separates Cl<sup>-</sup> influx from H<sup>+</sup> exchange and allows for the flow of Cl<sup>-</sup> anions, not supporting the translocation of protons across lysosomal membrane. Their elegant data showed not much differences in lysosome acidification, membrane potential, and steady-state pH between the uncouple Cl<sup>-</sup> mice and the wild-type. Similarly, Grinstein’s group in 2010 made similar documentation and implicated rather lysosomal cations (Na<sup>+</sup> and K<sup>+</sup>) much more to regulate pH<sub>L</sub>. The source of these cations in the acidic organelle awaits identification.

### ML1/TPC2-Dependent Cation Homeostasis and pH<sub>L</sub> Regulation as an Emerging Aspect of Neurodegenerative Diseases

We discussed how the structure and magnitude of pH<sub>L</sub> could be diluted or regulated. Since the discovery of lysosome, the role of lysosome in disease processes has been of a great interest and importance. The nature of gradual (persistent) to full disease states (permanent) or clinical syndromes, emanating from lysosomal and cell dysfunctions has remained poorly understood. We hypothesize the role of ML1/TPC2-mediated Ca<sup>2+</sup> signaling and pH<sub>L</sub> regulation in AD (Fig. 2). AD is a neurodegenerative disorder characterized by cognitive

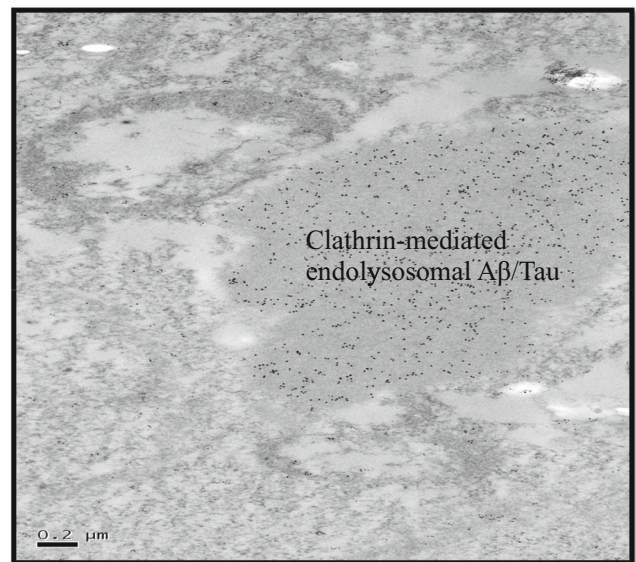
decline. Amyloid-beta (Aβ) and Tau proteins are well known proteins of AD pathology. An unpublished data shifts our knowledge of the independent roles of Tau and Aβ in AD pathology towards them being interacting partners in AD formation. Also, in this study, heavy accumulation of amyloid-beta and Tau was observed along the clathrin-endolysosomal degradation pathway (Fig. 3). The clathrin endolysosomal pathway represents a means of internalization of macromolecules for delivery to the lysosomes for degradation and recycling. By this way, defective signaling networks within the lysosomes can potentiate accumulation of internalized macromolecules in the lysosomes (Fig. 3). Accumulated materials particularly Aβ<sub>42</sub> and Tau in AD—the product of defective acidic hydrolases may be in part due to alteration in the pH<sub>L</sub>. Abnormal ML1/TPC2-induced Ca<sup>2+</sup> release (Fig. 2) do not only delete luminal Ca<sup>2+</sup> but also causes intraluminal alkalinization that deranges the pH<sub>L</sub>. Conversely, accumulation when initiated will beget accumulation through the dysfunctional ML1/TPC2-mediated Ca<sup>2+</sup> release. This illustrates the substrate (escalated) accumulation within various parts of autophagosomal-endosomal-lysosomal- system (Fig. 3).

In this line, membrane-inserted portion of Aβ<sub>42</sub> accumulated in lysosomes may destabilize the lysosomal membrane and induce neurotoxicity [33]. A model for pathological features of AD by ML1/TPC2-mediated Ca<sup>2+</sup> signaling via deranged pH<sub>L</sub> and Aβ/Tau accumulation shows how this membrane-inserted (deposited) portion of Aβ<sub>42</sub> could cause cellular toxicity and final mental loss in AD (Fig. 2). Membrane-inserted portion of Aβ<sub>42</sub> could induce altered membrane trafficking of juxtaorganellar Ca<sup>2+</sup> in cells. Defects



**Fig. 2** A model for pathological features of ML1/TPC2-mediated aberrant  $\text{Ca}^{2+}$  signaling in AD: each arrow starting from defective acid hydrolases by abnormal  $\text{pH}_i$  forms a step in the pathogenesis of AD representing the mechanisms of A $\beta$ /Tau-ML1/TPC2-mediated aberrant  $\text{Ca}^{2+}$  signaling and their interactive roles. The basis of this model is predicated on ML1/TPC2 as a global road network for modulating ionic milieu in AD. This is since ML1 are non-selective permeable to  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ ,  $\text{Na}^+$  [31] and  $\text{Fe}^{2+}$  [32]. TPC2 are also selective permeable cation channel to  $\text{Na}^+$  [7, 15]. ML1 is particularly permeable to  $\text{Fe}^{2+}$  and the role of  $\text{Fe}^{2+}$  in the formation ROS is indispensable. Targeting ML1 selective permeability to  $\text{Fe}^{2+}$  pharmacologically could provide means of ameliorating AD

in membrane trafficking, accumulation of macromolecules and altered  $\text{Ca}^{2+}$  homeostasis are common typical features in NP form of LSD [4, 5, 20, 34]. For the AD and as observed in Fig. 3, and illustrated in Fig. 2, A $\beta$ 42 [33] and tau accumulate in the endolytic pathway. This sort of accumulations as already tested in NP form of LSD [4, 5, 20] but not in AD, are partly seriously evident in the eclectic hypothesis which states that primary accumulated materials cause a sort of lysosomal stress which can impair common biological and/or specific signaling pathways in the lysosomes [18]. Although in



**Fig. 3** An ultra-structure of intracellular 24 h 10um beta Nu1-Tau-5000x in the clathrin endolysosomal internalization pathway illustrating high accumulation of A $\beta$  (bigger in size) and Tau (smaller in size) likely mediated by abnormal ML1/TPC2- $\text{Ca}^{2+}$  dependent signaling

AD, A $\beta$  and Tau proteins are well known to accumulate, sphingomyelin lipids are not completely understood to accumulate.

However, lipid rafts are dynamic assemblies of proteins and lipids floating freely within the liquid-disordered bilayer of cellular membranes were cholesterol [35]. Indirect relationships that exist between cholesterol and sphingomyelin metabolism are strongly related to A $\beta$  [36]. There are clues indicating that A $\beta$  level changes in response to blood cholesterol content, and clinical progression of AD is commonly associated with hypercholesterolemia in the brain [37]. Lipids and proteins accumulation can transmute  $\text{pH}_i$  optima outside 4.5–5.0, the functional conventional ranges. Outside the conventional range, the functional integrity of the acid hydrolases, necessary for synthesis and breakdown of biological materials along the clathrin-endolytic pathway (Fig. 3) is disrupted.

The cascade process of AD formation has well been studied. Because this cascade is necessary for understanding the diagnosis, the treatment and the management modes of AD, it deserves further attention. Unveiling the mechanism further is both fascinating and complex. Experimental and theoretical approaches such as in vivo and in vivo electrophysiology, numerical modelling, pharmacological and genetic have been useful. From the experimental and theoretical point of view, we proposed the role of  $\text{pH}_i$  regulation and ML1/TPC2 cation-dependent homeostasis in the accumulation of macromolecules in AD (Figs. 2 and 3). Previously, we stated that accumulated biological materials potentially damages signaling. Accumulated A $\beta$ 42 and tau in Fig. 3 can affect the relay of ML1/TPC2-mediated  $\text{Ca}^{2+}$  signaling and  $\text{pH}_i$  regulated functions. As shown in the model for pathological features

of ML1/TPC2-mediated aberrant  $\text{Ca}^{2+}$  signaling (Fig. 2), it is therefore, appreciable that the phenotypes of this would include: depletion of signal transduction [38, 39], oxidative stress, inflammation [40], and transport defect.

With the identification of memory decline process in pre-clinical setting of HIV infection, where  $\text{A}\beta$  accumulation was known to cause memory impairment, the merits of this hypothesis are emerging. TRPs and TPCs are bypass for cation fluxes and are strategic for homeostasis of ionic milieu of the acidic organelles they confine to. By evoking  $\text{Ca}^{2+}$  efflux from the lysosome with ML1 potent agonist, the structure and magnitude of  $\text{pH}_L$  (Fig. 1) was mechanized for and luminal acidification promoted [41]. This consequently cleared sphingomyelin and the accumulated  $\text{A}\beta$  from the lysosome. This in part is indicative of the model for pathological features of ML1/TPC2-mediated aberrant  $\text{Ca}^{2+}$  signaling in AD (Fig. 2) as a therapeutic target for improving memory decline through sphingomyelin and the  $\text{A}\beta$  clearance. However, this setting where  $\text{A}\beta$  predominantly accumulates intracellularly maybe distinct from core AD conditions where extracellular senile plaques recapitulate the feature.

It is underlying in the AD cascade presented in Fig. 2 that acid hydrolases do not instantly become defective in the synthesis and breakdown of macromolecules including  $\text{A}\beta_{42}$ . Abnormal  $\text{pH}_L$  is the ‘stimulus’ for defective acid hydrolases, and regulation of this stimulus by ML1/TPC2 (Fig. 1) has been demonstrated [7, 24, 25]. This stimulus provides an excellent background upon which the famous amyloid hypothesis can be predicated, implying further a role of ML1/TPC2 in the cascade process of AD formation. It therefore beholds an era of lysosomal cation channel functions that will advance our current understanding of the cascade process leading to AD formation.

### The Channels in $\text{Ca}^{2+}$ Signaling

We opined the role of ML1/TPC2-mediated cation signaling in substrate accumulations within autophagosomal-endosomal-lysosomal system in AD for the first time. This sort of signaling has been reported in neurodegenerative LSD. We reflected on the seminal works that have reported ML1 and TPC2 mediated  $\text{Ca}^{2+}$  signaling in such processes. This is not to ‘reinvent the wheel’ but to signal awareness of lysosomal cation channels mediated homeostasis in AD. First for ML1, the biophysical properties of ML1 are being studied. However, the literature regarding their properties is somewhat conflicting. ML1 was said to be a nonselective outward rectifying monovalent cation channel [42]. In contrast, ML1 is a nonselective inward rectifying cation channel [43] for excellent review, [4, 5, 20] and thus permeable to  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , [31],  $\text{Fe}^{2+}/\text{Mn}^{2+}$  [32], and  $\text{Zn}^{2+}$ . It opens and closes-gated, and is activated and localized in the lysosomes (Dong

et al. [4, 5]). The localization and the biophysical nature of ML1 can be tangled with the distinct roles ML1 can perform. Understanding ML1 gating probability, permeability, and ML1 localization could be necessary for underpinning ML1 distinctive functions.  $\text{Ca}^{2+}$  signaling in itself is desperately a most influential requirement for many biological functions like the cell-cell communication, memory formation and/or loss, neuronal excitability and contraction and relaxation of muscles.

ML1 provides bypass for  $\text{Ca}^{2+}$  fluxes in cells and becomes a powerful strategic domain for juxta-organelle  $\text{Ca}^{2+}$  homeostasis necessary for gene transcription, apoptosis, and membrane trafficking in both normal and disease states including the Alzheimer’s. For instance, ML1 mutation causes mucopolipidosis type IV (ML1V). ML1V is associated with enlarged vacuoles, lipid accumulations and altered  $\text{Ca}^{2+}$  homeostasis and membrane trafficking. To test that these phenotypes are associated with ML1V and to demonstrate the physiological importance of ML1 activated dependent  $\text{Ca}^{2+}$  signaling, [20], constructed a single wavelength lysosome-targeted genetically encoded  $\text{Ca}^{2+}$  indicator, GCaMP3-ML1 for ML1. This construct was potentiated by a small chemical compound, a synthetic agonist for ML1, generated through high-throughput screening [44]. Using this construct and lysosomal whole-patch clamp [32, 45], that is now reliable for accessing the electrical behavior of lysosomes, Shen and Colleagues showed that  $\text{Ca}^{2+}$ -dependent trafficking selectively compromised in NP was mediated by abnormal lipid storage that can inhibit ML1  $\text{Ca}^{2+}$ -mediated release.

This documentation precisely defined the source of  $\text{Ca}^{2+}$  and repudiates a previous report [22], that implicated only lysosomal  $\text{Ca}^{2+}$  stores as the source of  $\text{Ca}^{2+}$  signaling selectively compromised in NP. The source of the unidentified  $\text{Ca}^{2+}$  channels from the lumen of acidic organelles and vesicles [46], that can mediate  $\text{Ca}^{2+}$  release is the ML1 [4, 5, 34]. Though considering how difficult it may be to clamp intracellular  $\text{Ca}^{2+}$ , a perfectly firm condition to rule out the participation of  $\text{Ca}^{2+}$  from the lysosomal  $\text{Ca}^{2+}$  stores may have not been adequately ensured in the [20] documentation. Perhaps, both sources may be necessary to provide  $\text{Ca}^{2+}$  clues required in the cell functions. Identification of the other factors is obligatory on ML1 genes expression and molecular identity of ML1 in membrane trafficking.

Membrane trafficking is the movement of membrane materials between endomembrane compartments. It is necessary for transport of proteins, lipids and other macromolecules in form of cargo sorting to various destinations during endolysosomal biogenesis. Membrane fusion and fission that characterize endolysosomal biogenesis is tightly regulated by both phosphoinositides ( $\text{PIP}_2$ ) and  $\text{Ca}^{2+}$  signaling [4, 5, 14, 16]. Cells that lack  $\text{PI}(3,5)\text{P}_2$  have enlarged vacuole as those that lack ML1. Using enlarged vacuoles, gotten from cells pretreated with vacuolin-1, [4, 5] demonstrated that ML1

regulates membrane trafficking by converting electrical signals regarding PI(3,5)P<sub>2</sub> levels into specific and potent cues that modulate Ca<sup>2+</sup> homeostasis. Consistently, a silent plasma membrane ML1 (ML1 exocytosed from the lysosome into the plasma membrane or inside-out membrane patch) was activated by direct decrease in PI(4,5)P<sub>2</sub> levels or by direct increase in PI(3,5)P<sub>2</sub> levels [14]. This suggests that PIP<sub>2</sub> isoforms can either be an activator or an inhibitor of the ML1 mediated Ca<sup>2+</sup> release in membrane trafficking.

Second, the ML1 counterpart, TPC2 as a lysosomal cation channel is not left out in the configurational frame work of intracellular Ca<sup>2+</sup> dependent homeostasis. Unlike the ML1, TPC2 is a cation-selective channel. Like ML1, TPC2 is associated with acidic organelles in human cells [6, 47, 48]. Despite this information, the exact knowledge concerning the distribution and localization of TPC2 has remained yet unclear. This may be due to availability of inadequate TPCs antibodies that can distinguish subcellular localization of TPC2.

TPC2 is also important in clamping Ca<sup>2+</sup> signaling in membrane trafficking, signal transduction, gene transcription and in AD. To elucidate the role of human lysosomal TPC2-mediated Ca<sup>2+</sup> release, [6] used flash photolysis of caged-nicotinic acid adenine dinucleotide phosphate (NAADP), intracellular NAADP dialysis, fluorescence Ca<sup>2+</sup> indicator—fluo3 and measurement of Ca<sup>2+</sup>-activated cation currents to demonstrate that lysosomal hTPC2 cell mediates Ca<sup>2+</sup> release. Significantly, this activation was extremely potent at <1 mM concentration of NAADP [49]. While this proposed NAADP dependent TPC2 activation, others [7, 14–16] proposed phosphoinositide isoforms dependent specific TPC2 activation. In the light of this controversy, [21] recently presented the first direct evidence that TPC2 can also be activated by NAADP as well as by PI(3,5)P<sub>2</sub>. Thus, confirming TPC2 potentiation by NAADP dependent Ca<sup>2+</sup> release.

## Conclusion

It is our conviction that the context of AD mechanism will be revolutionized by lysosomal cation channels in the near future, and the definition of the exact physiological functions of ML1 and TPC2 in the plethora of cellular functions they could perform is much closer to reality as intense research is ongoing towards underpinning their importance, physiological properties, and molecular identity. At present, their gating probability, activation mechanisms and subcellular localizations are not completely understood. Formation of antibodies with better immunogenicity to detect endogenously ML1/TPC2 channels would greatly help in defining their exact subcellular localizations. Also, identification of more of their genes and their synthetic agonists/antagonist can help towards addressing outstanding controversies and gap in knowledge about their physiological properties. ML1 and TPC2-

mediated Ca<sup>2+</sup> dependent signaling are necessary for but not limited to: neurodegeneration, membrane trafficking, neurotransmission, cell polarity, morphogenesis, migration apoptosis, gene transcription/regulation, autophagy, cancers including those of the skin, sensation to temperature and taste, ischemia/stroke and aging. The exact gradient of Ca<sup>2+</sup> generated by ML1/TPC2 NAADP, PIP<sub>2</sub>, and GTPases-dependent Ca<sup>2+</sup> release to potentiate the functions is poorly understood. This may be in part due to ubiquitous nature of Ca<sup>2+</sup> signaling, effect of pH<sub>L</sub>, temperature and ionic strengths on the amount of intracellular Ca<sup>2+</sup>.

ML1 and TPC2 as road network for Ca<sup>2+</sup> fluxes are also permeable to other cations and could provide a source for pH<sub>L</sub> regulation that maintains diverse biological functions of acid hydrolases. Abnormal function of these channels as a source of Ca<sup>2+</sup>-dependent regulatory machineries, abnormal accumulation of macromolecules through defective lysosome, defective hydrolase functions and alkalinized lysosomal lumen partly underpin features of neurodegenerative and LS diseases, ischemia/stroke, and aging.

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