Dystrophic neurite accumulation of BACE1 in relation to its endosomal trafficking adaptors EHD1, EHD3, and BIN1 in 5XFAD model

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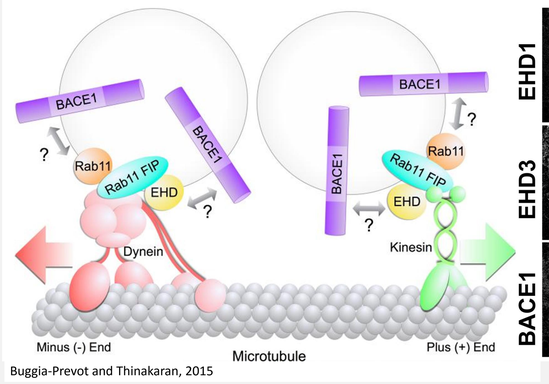
Abstract

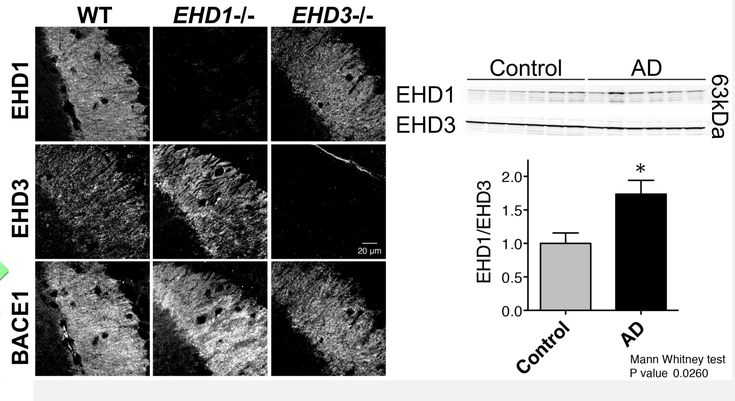
Accumulation of BACE1 in dystrophic neurites surrounding amyloid deposits is a shared pathological feature in human Alzheimer's disease (AD) and transgenic mouse models. Several hypotheses have been proposed to explain the amyloid deposit-associated increase in BACE1 levels, and how this presynaptic elevation of BACE1 might contribute to amyloid production and deposition. In the brain, BACE1 is normally enriched in axons and presynaptic terminals, especially in the mossy fibers of the hippocampus; however, the cause of the abnormal accumulation of BACE1 in dystrophic neurites remains to be elucidated. Polarized sorting of neuronal proteins to the axonal compartment and consequently their targeting to the presynaptic terminals are regulated by complex trafficking mechanisms, including endosomal membrane sorting. Multiple lines of evidence suggest that a deregulation of endosomal trafficking occurs early during AD pathogenesis. In this study, we examined the *in vivo* relationship between BACE1 and the endosomal adaptor proteins: Esp15 Homology Domain-containing proteins (EHD1 and EHD3) and Bridging INtegrator-1 (BIN1). Recently we reported that EHD proteins regulate BACE1 transcytosis in primary cultured hippocampal neurons. BIN1 has been identified as the second most significant risk factor gene for late-onset AD and loss of BIN1 expression has been reported to modify BACE1 trafficking. We examined endogenous BACE1 localization in mice with targeted deletion of *Ehd1* or *Ehd3* alleles. We found that neither loss of EHD1 or EHD3 expression altered BACE1 localization in the hippocampal mossy fibers. Moreover, we examined the localization of EHD1 and EHD3 proteins in the brains of 5XFAD mice. Although BACE1 was already accumulating in dystrophic neurites surrounding amyloid deposits in 2 month-old mice, EHD1 and EHD3 localization remained unchanged at the presynaptic terminals of mossy fibers of the hippocampus. EHD1 and EHD3 accumulated in dystrophic neurites surrounding amyloid deposits only occasionally when amyloid pathology was extensive in the brains of 5 month-old animals, and this was restricted to the hippocampus. These data suggested that aberrant localization of EHD1 or EHD3 is unlikely to be responsible for BACE1 accumulation in dystrophic neurites in 5XFAD mice. In order to clarify the role of BIN1 in AD, we examined BIN1 expression in 5XFAD mice. Our results show little overlap between BIN1 with BACE1 or LAMP1 at the dystrophic neurites. These findings raise the possibility that BIN1 might not have a role in the pathological accumulation of BACE1 in the brain. Our findings reveal new insights into the *in vivo* regulation of BACE1 in the context of AD pathogenesis.

1. Loss of EHD1 or EHD3 expression does not affect axonal localization of BACE1 in hippocampal mossy fibers

We first examined the requirement for EHD1 and EHD3 for BACE1 expression and axonal localization in the brains of mice. We immunostained the brains of EHD1 or EHD3 KO mice using antibodies against BACE1, EHD1, and EHD3. Our results showed that the **loss of either EHD1 or EHD3 expression does not affect the steady-state axonal localization of BACE1 in mossy fibers**. In addition, the levels of BACE1 were similar in the absence of EHD1 or EHD3 expression. These results suggested that neither EHD1 nor EHD3 plays an essential role in the physiological expression of BACE1, and it is possible that the loss of one EHD is compensated by the other EHD.



Buggia-Prevot and Thinakaran, 2015

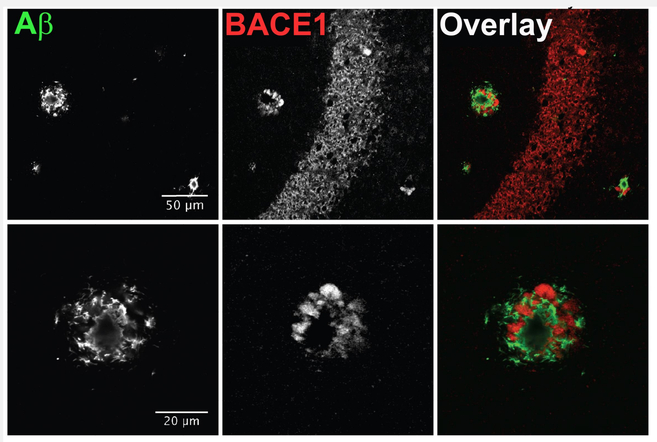


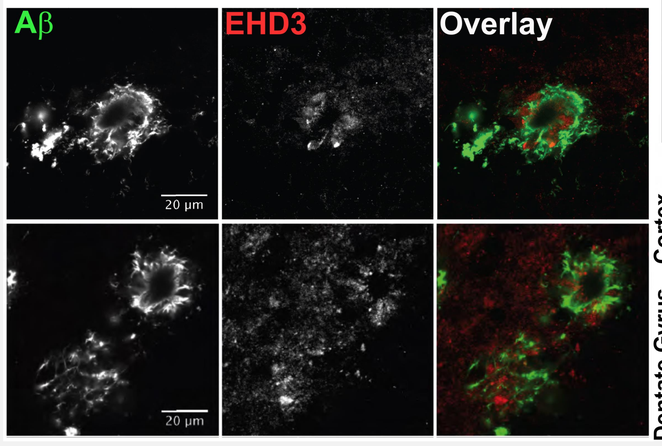
We next explored whether EHD expression is altered in the brains of patients with or without Alzheimer's disease. We compared the expression levels of EHD1 and EHD3 in the brain of AD patients compared to age-matched controls. Our results show a significant **increase in EHD1 levels in the brains of patients with AD**. The functional implication of this increase is not known.

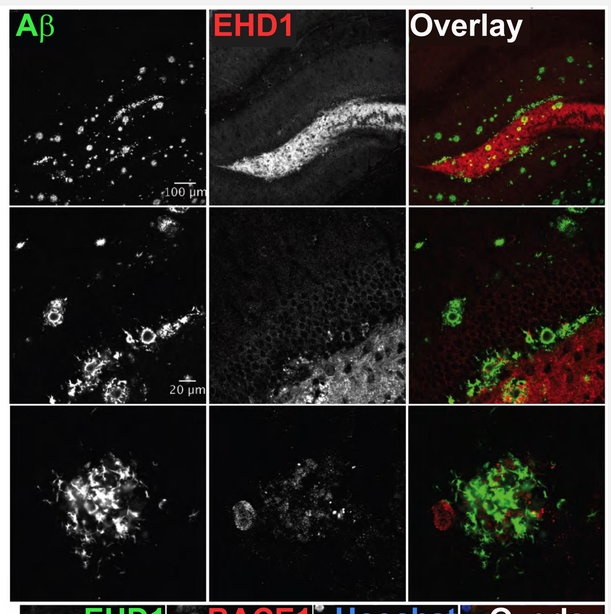
2. BACE1 accumulation is not correlated to EHDs

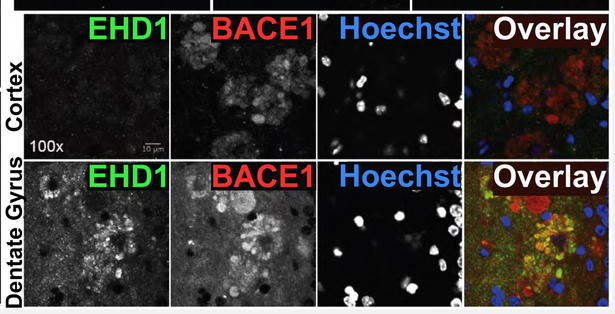
The reason why BACE1 abnormally accumulates in dystrophic neurites is still unknown. Theories include **trafficking defects** and local stress-related protein translation. Since endosomal abnormalities are thought to manifest **even before overt amyloid deposition occurs** in the brains of AD patients, we decided to examine EHD localization in the well-characterized 5XFAD mouse model.

We performed a longitudinal study to assess change over time in the levels of expression and cellular/subcellular localization of EHDs in the 5XFAD mice. Specifically, we performed immunofluorescence staining to determine whether a concurrent accumulation of EHD proteins with BACE1 is seen in dystrophic neurites surrounding the amyloid deposits. Our results showed that **EHDs do accumulate to some extent near amyloid deposits in BACE1-positive nerve terminals**, but **only within the mossy fibers**. However, **BACE1-positive dystrophic neurites in the cortex do not accumulate EHDs**.

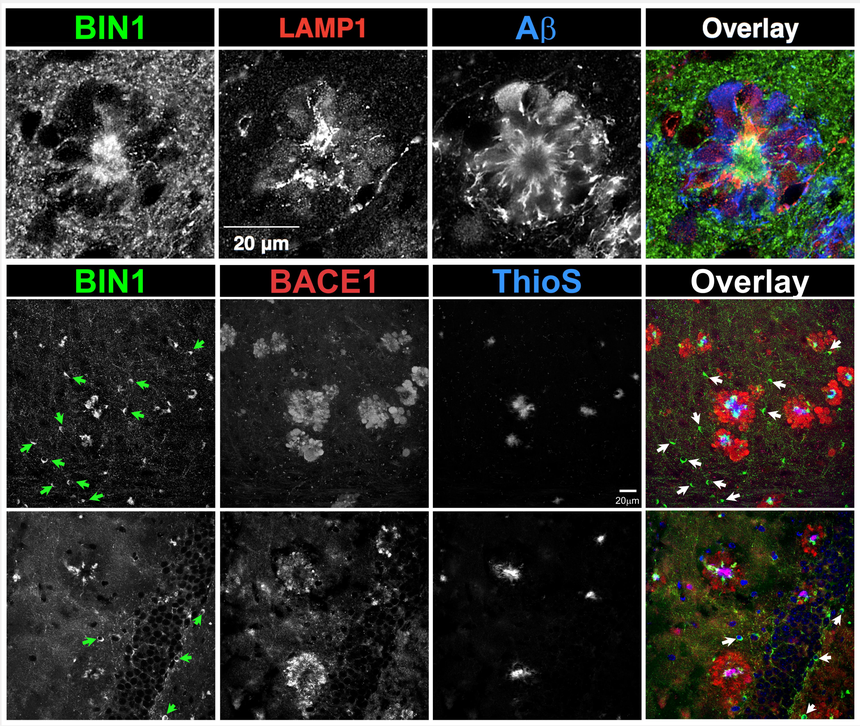








3. BIN1 as new candidate for BACE1 accumulation at the deposits

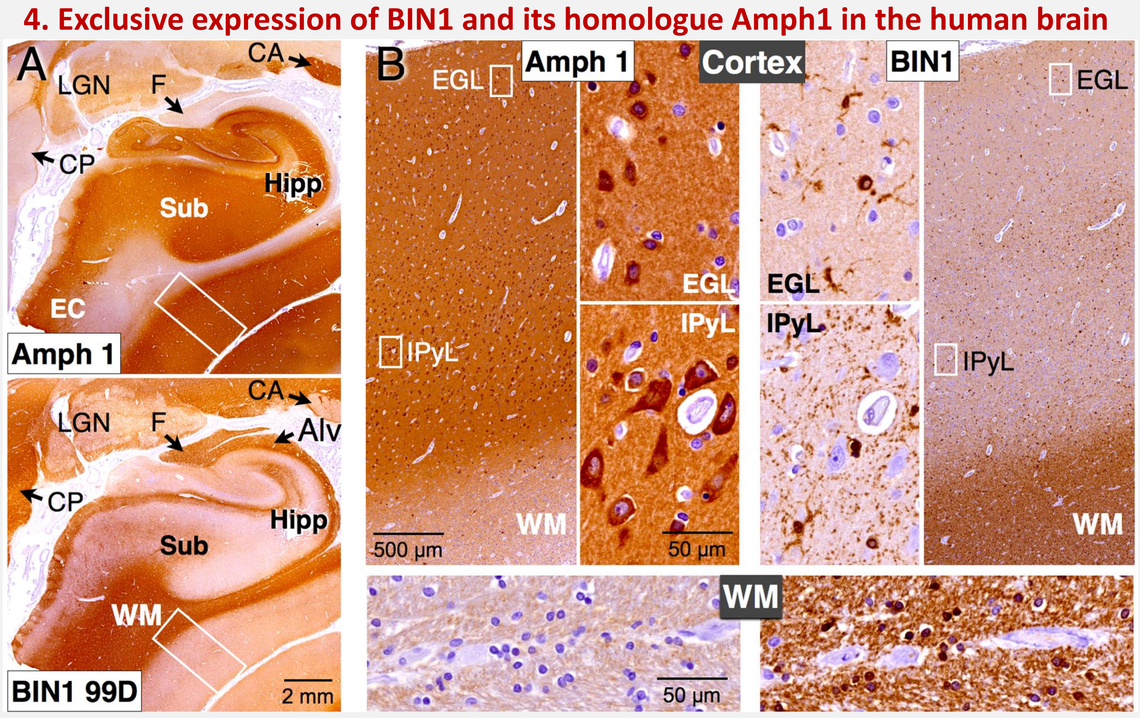


Next, we turned our attention to BIN1, another adaptor involved in endosomal trafficking and identify as a risk factor for LOAD. In the 5XFAD model, **BIN1 does not accumulate in dystrophic neurites**. Intense BIN1 (green) and LAMP1 (red) immunostaining was observed near amyloid deposits but in distinct structures.

A co-immunostaining of BIN1 (green) and BACE1 (red) along with ThioflavinS surprisingly showed intense **BIN1 immunoreactivity within the deposits**. However, BIN1 was not found to co-localize with BACE1 in dystrophic neurites, indicating that BIN1 is unlikely to play a role in the abnormal accumulation of BACE1 near amyloid deposits.

The lack of BIN1 accumulation in dystrophic neurites is not surprising because, unlike its neuronal homology Amphiphysin 1, BIN1 is predominantly expressed in oligodendrocytes (arrows).

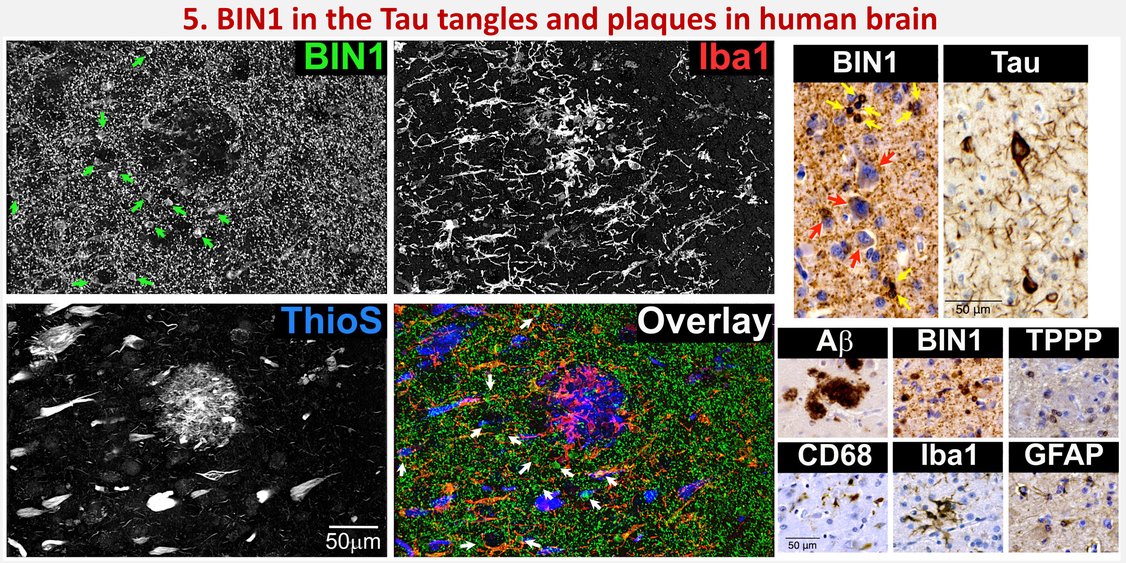
4. Exclusive expression of BIN1 and its homologue Amph1 in the human brain



A) Immunohistochemical labeling of adjacent sections of the hippocampus and parahippocampal gyrus reveals near complementary localization of BIN1 and Amphiphysin 1. Whereas **Amphiphysin 1 is mainly present in the gray matter, BIN1 labeling is prominent in the white matter.** B) In the cortex Amphiphysin 1 and BIN1 labeling of morphologically distinct neurons and oligodendrocytes, respectively, is quite obvious. In all areas, Amphiphysin 1 stains the neuronal soma and the neuropil whereas BIN1 stains smaller cells and profuse branched processes. Very little BIN1 immunoreactivity is found in neurons. Amph 1 = Amphiphysin 1; WM = white matter; Alv = alveus; Hipp = hippocampus; Sub = subiculum; EC = entorhinal cortex; CA = caudate nucleus; CP = cerebral peduncle; F = fimbria; LGN = lateral geniculate nucleus; EGL = external granular layer; IPyL = internal pyramidal layer; DG = dentate gyrus; CA2-CA4 = Cornu Ammonis areas 2–4; MF = mossy fibers; PML = polymorphic layer; GCL = granule cell layer; ML = molecular layer; SO = stratum oriens; SP = stratum pyramidale; SR = stratum raidatum

De Rossi et al, 2016

5. BIN1 in the Tau tangles and plaques in human brain



To confirm the above results in human AD, we examined the link between BIN1 and the hallmarks of Alzheimer's disease: the amyloid plaques and the neurofibrillary tangles. For this we immunostained human brain sections using antibodies against BIN1, Tau, b-amyloid, and cellular markers.

Our results show that BIN1 immunostaining was absent **within senile plaques and did not overlap with Tau-immunoreactive neurofibrillary tangles or neuropil threads**. Moreover, we found no evidence for BIN1 expression by ramified microglia, reactive microglia, macrophages, or astrocytes in the human brain.

Conclusions

Our study demonstrates that:

1) EHD1 and EHD3 are unlikely responsible for physiological BACE1 expression or pathological accumulation of BACE1 in dystrophic neurites near amyloid deposits.

2)BACE1 in dystrophic neurites did not co-localize with BIN1 in 5XFAD mice.

3) BIN1 immunoreactivity does not seem to be related to amyloid or Tau pathology in the brains of patients with AD.

References

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De Rossi et al, Mol Neurodegener. 2016 Aug 3;11(1):59, PMID: 27488240

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