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BIN1 localization is distinct from Tau tangles in Alzheimer's disease

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Abstract

BIN1 is the second most significant Alzheimer's disease (AD) risk factor gene identified through genome-wide association studies. *BIN1* is an adaptor protein that can bind to several proteins including c-Myc, clathrin, adaptor protein-2 and dynamin. *BIN1* is widely expressed in the brain and peripheral tissue as ubiquitous and tissue-specific alternatively spliced isoforms that regulate membrane dynamics and endocytosis in multiple cell types. The function of *BIN1* in the brain and the mechanism(s) by which AD-associated *BIN1* alleles increase the risk for the disease are not known. *BIN1* has been shown to interact with Tau and two studies reported a positive correlation between *BIN1* expression and neurofibrillary tangle pathology in AD. However, an inverse correlation between *BIN1* expression and Tau propagation has also been reported. Moreover, there have been conflicting reports on whether *BIN1* is present in tangles. A recent study characterized predominant *BIN1* expression in mature oligodendrocytes in the gray matter and the white matter in rodent, and the human brain. Here, we have examined *BIN1* localization in the brains of patients with AD using immunohistochemistry and immunofluorescence techniques to analyze *BIN1* cellular expression in relation to cellular markers and pathological lesions in AD. We report that *BIN1* immunoreactivity in human AD is not associated with neurofibrillary tangles or senile plaques. Moreover, our results show that *BIN1* is not expressed by resting and activated microglia, astrocytes, or macrophages in human AD. In accordance with a recent report, low-level *de novo* *BIN1* expression can be observed in a subset of neurons in the AD brain. Further investigations are warranted to understand the complex cellular mechanisms underlying the observed correlation between *BIN1* expression and the severity of tangle pathology in AD.

Keywords

Alzheimer's Disease; *BIN1*; Oligodendrocytes; Tangle; Tau Pathology

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Additional Information

Methods and Supplementary Material

Please see <https://sciencematters.io/articles/201611000018>.

Ethics Statement

Not Applicable.

Objective

Given the significant association between BIN1 variants and late-onset AD as well as an interest in BIN1 as a potential target for AD therapeutics, we sought to carefully evaluate BIN1 immunoreactivity in AD brain, in relation to tau tangles.

Introduction

The *BIN1* gene is located within the second most significant late-onset Alzheimer's disease (LOAD) susceptibility locus identified via genome-wide association studies (Karch 2015^[1]) (Bertram 2007^[2]). BIN1 (Bridging INtegrator-1) is a member of the BAR (Bin/Amphiphysin/Rvs) adaptor family proteins that regulate membrane dynamics in the context of endocytosis and membrane remodeling (Prokic 2014^[3]). Alternate splicing of *BIN1* generates multiple transcripts encoding ubiquitous and tissue-specific isoforms, which differ in their tissue distribution, subcellular localization, and function. BIN1 is predominantly expressed in mature oligodendrocytes and white matter tracts in rodent, and the human brain (De Rossi_2016^[4]) (Adams 2016^[5]). BIN1 can directly bind to Tau and the altered expression of *Drosophila* Amph (the fly BIN1 homolog) significantly modifies the human Tau-induced rough eye phenotype, leading to the suggestion that BIN1 mediates LOAD risk by modulating Tau pathology (Chapuis 2013^[6]) (Sottejeau 2015^[7]) (Zhou 2014^[8]). In support, the levels of BIN1 isoform 9, which is mainly expressed by oligodendrocytes and enriched in the white matter (De Rossi_2016^[4]), was found to correlate with the extent of neurofibrillary tangle pathology in the brains of patients with LOAD (Holler, 2014^[9]). In contrast, a recent study demonstrated an inverse correlation between BIN1 expression and propagation of Tau pathology in cultured hippocampal neurons (Calafate 2016^[10]). Moreover, there have been conflicting reports on the presence of BIN1 immunoreactivity in neurofibrillary tangles. One study reported that the BIN1 immunostaining strongly colocalized with tangle-bearing neurons (Holler, 2014^[9]) whereas others found occasional or no overlap between BIN1-immunoreactivity and Tau tangles (Adams 2016^[5]) (Chapuis 2013^[6]). BIN1 has also been reported to regulate BACE1 trafficking and A β production (Miyagawa 2016^[11]).

Results & Discussion

We performed immunohistochemical analysis on adjacent brain sections of individuals with or without AD using three BIN1 antibodies (pAb BSH3, mAb 2F11, and mAb 99D) and markers of AD pathology. Figures A and B show the distribution and density of senile plaques and neurofibrillary tangles in the entorhinal cortex of a patient with AD [immunostained using antibodies against A β (mAb 4G8) and Tau (Tau-2)], in relation to BIN1 cellular distribution [immunostained using pAb BSH3 and mAb 2F11]. As described in a recent study (De Rossi_2016^[4]), we have observed widespread BIN1 immunoreactivity in oligodendrocytes and processes throughout the neuropil, and more intense staining of the white matter. However, the pattern of BIN1 immunoreactivity did not fit the profiles of Tau immunostaining (Figures A and B). Whereas the Tau-2 antibody labeled a number of tangles and neuropil threads in the entorhinal cortex, BIN1 antibodies labeled profuse punctate structures and cell soma that had no resemblance to tangles (Figure C). At closer inspection,

only weak BIN1 immunoreactivity was found associated with the cytoplasm of neurons (red arrows in Figure C). Robust BIN1 immunoreactivity was found in oligodendrocytes (yellow arrows in Figure C), which are smaller than neurons and can be labeled by antibodies against the oligodendrocyte marker TPPP/p25 (Figure D). Thus, BIN1 immunoreactivity is not associated with neurofibrillary tangles or neuropil threads.

We also examined BIN1 distribution in relation to senile plaques in the brains of patients with AD. Similar to what was recently reported in control brains(De Rossi_2016^[4]), immunohistochemical analysis of advanced AD brain tissue revealed the strong BIN1 immunoreactivity in oligodendrocytes and processes throughout the neuropil. However, in areas with the high senile plaque density, we observed an absence of BIN1 immunoreactivity in the areas that corresponded to amyloid deposits (Figure B). This finding is consistent with a recently published report(Adams 2016^[5]).

To explore BIN1 cellular expression in the context of inflammatory response to AD pathology, we performed immunostaining of adjacent sections with antibodies against BIN1 and cellular markers of microglia (Iba1), activated microglia/macrophages (CD68), and astrocytes (GFAP). A comparison of Iba1 immunostaining with that of BIN1 revealed that the overall pattern of BIN1 immunoreactivity did not fit the overall distribution profiles of microglia, activated microglia/macrophages, and astrocytes, suggesting that these reactive cell types in pathological AD brain do not express BIN1 (Figure B and not shown). Inspection at higher magnification revealed clusters of Iba1-positive microglia with readily discernible ramified processes near senile plaques. However, BIN1 immunoreactivity was not associated with cells that resembled Iba1-positive microglia (Figure D). This finding is consistent with a recent report showing little evidence for microglial expression of BIN1 in the human (non-AD) and mouse brain(De Rossi_2016^[4]). Moreover, a comparison of the morphology of cells positive for CD68 and GFAP revealed no similarity with BIN1 immunoreactivity, suggesting the lack of BIN1 expression in reactive microglia, macrophages, and astrocytes (Figure D). These results suggest that BIN1 is unlikely to be involved in the inflammatory processes associated with pathogenesis in AD. Our findings that BIN1 protein is not detected in ramified microglia, reactive microglia, and macrophages are notable because BIN1 mRNA expression in microglia and macrophages acutely isolated from mouse and human brain are reported in RNA-seq transcriptome databases(Zhang 2014^[11])(Zhang 2016^[12]).

In order to confirm the above findings, we performed immunofluorescence staining of BIN1 and Iba1 along with Thioflavin S staining. We observed a clearance of BIN1 within the area of neurofibrillary tangles stained by Thioflavin S (Figure E). A number of Iba-1 positive microglia were found near the tangles but they were negative for BIN1 immunostaining. These results from immunofluorescence labeling are in agreement with the observations made by immunohistochemical staining of serial sections described above.

Two studies have previously suggested a link between BIN1 expression and neurofibrillary tangle pathology in AD(Chapuis 2013^[6])(Sottejeau 2015^[7])(Holler, 2014^[9]). The findings that Tau can bind to BIN1 in vitro, and can co-immunoprecipitate with the brain-specific BIN1 isoform 1 support this notion(Chapuis 2013^[6])(Sottejeau 2015^[7])(Zhou 2014^[8]). In

contrast, a recent study demonstrated an inverse correlation between BIN1 levels and propagation of Tau pathology(Calafate 2016^[10]). Earlier studies on the localization of BIN1 immunoreactivity and neurofibrillary tangles in AD brain described discordant findings(Chapuis 2013^[6])(Holler, 2014^[9]). However, a detailed immunohistochemical analysis of a large set of brain tissue at different stages of AD progression demonstrated a lack of correlation between tangles and BIN1 immunoreactivity in neurons and even a negative correlation between neurofibrillary tangle pathology and BIN1 immunoreactivity in the neuropil(Adams 2016^[5]). Our results presented here are in accordance to this later report as we found a lack of overlap between neurofibrillary tangles and BIN1 immunoreactivity by immunofluorescence analysis. Our negative finding is not due to the complexity of BIN1 alternate splicing, as four different BIN1 antibodies, including two that are capable of reacting to all BIN1 isoforms, failed to stain neurofibrillary tangles in our study (Figure C and data not shown). Finally, it is notable that the levels of the brain-specific BIN isoform 1, which was found to specifically associate with Tau in AD brain(Zhou 2014^[8]), is significantly decreased in AD brain(De Rossi_2016^[4])(Holler, 2014^[9]). Thus, additional studies are needed to fully understand the significance of the positive correlation between Tau pathology and the levels of the ubiquitous BIN1 isoform 9(Holler, 2014^[9]), which in the brain appears to be mainly expressed by oligodendrocytes(De Rossi_2016^[4]) and elucidate how BIN1 mechanistically participates as a risk factor in late-onset AD.

Conclusions

Our study clarifies BIN1 cellular expression in relation to neurofibrillary tangle pathology in AD brain. Specifically, our results show no overlap between BIN1 immunoreactivity and neurofibrillary tangle pathology in AD brain. Moreover, similar to the findings from non-AD human brain, BIN1 is predominantly expressed in oligodendrocytes in AD brain, and the highest BIN1 immunoreactivity is associated with the white matter. Although a low-level of BIN1 expression is observed in a subset of neurons in the AD brain, it is relatively minor in relation to BIN1 expression in oligodendrocytes. Finally, our study reports the lack of BIN1 expression in the brain inflammatory cells in the AD brain.

Limitations

Epitope masking and poor permeation of tissue sections limit the successful detection of antigens in fixed human brain tissue. While we have used optimized epitope retrieval methods to overcome this limitation and unmask a variety of antigens, there is the risk that some antibodies may not react or only poorly reacts with epitopes denatured by heat-induced epitope retrieval methods.

Conjectures

In light of the significant changes in the levels of BIN1 isoforms in AD brain described in previous studies, it is important to determine whether the levels of brain-specific BIN1 isoform 1 or the ubiquitous BIN1 isoforms 9 and 10 correlate with neurofibrillary tangle pathology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure. Analysis of the cellular expression of BIN1 in AD brain

A and B) Serial sections were immunostained with antibodies against Aβ (mAb 4G8), Tau (Tau2), BIN1 (pAb BSH3 and mAb 2F11), or Iba1 to visualize the overall distribution of BIN1 in relation to markers of AD pathology and microglia. The boxed regions in panels A are shown at a higher magnification in B. The asterisks indicate the blood vessels used as landmarks to align the adjacent sections.

C) Adjacent immunostained sections show weak BIN1 immunoreactivity in neurons (red arrows) in a region of the brain with Tau tangles (right panel). Note, the strong labeling of oligodendrocyte soma (yellow arrows) and profuse punctate processes that show no resemblance to Tau tangles or neuropil threads. Tau tangles (right panel). Note the strong

labeling of oligodendrocyte soma (yellow arrows) and profuse punctate processes that show no resemblance to Tau tangles or neuropil threads.

D) The higher-magnification panels from serial sections illustrate that BIN1-immunoreactive cells do not fit the profile of microglia, astrocyte, or macrophage but resemble mature oligodendrocytes marked by TPPP immunostaining.

E) Immunofluorescence staining of BIN1 and Iba1 along with Thioflavin S in the AD brain shows an exclusion of BIN1 within the tangles and an absence of BIN1 expression in microglia. The boxed area is shown at higher magnification in the lower panels.