Identification of retinoblastoma binding protein 7 (Rbbp7) as a mediator against tau pathology in Alzheimer's disease and related tauopathies.

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Introduction

- Clinically, AD is characterized by impairments in cognition, deficits in developing new memories, and a loss of long-term memories as the disease progresses [1].
- The hallmark neuropathologies of AD include extracellular plaques composed predominately of the amyloid-β (Aβ) peptide, intraneuronal tangles of hyperphosphorylated tau (NFT), & synaptic and neuronal loss [1].
- Recent work has highlighted the emerging role of the epigenome in tau pathogenesis, suggesting that dysregulation of epigenetic proteins may contribute to acetylation and hyperphosphorylation of cytoplasmic tau [2].
- Rbbp7 is responsible for chaperoning chromatin remodeling proteins to their nuclear histone substrates, including histone acetylases (HATs) and histone deacetylases (HDACs), and serving as a core component of several epigenetic complexes [3].
- Tau acetylation has been observed in the brains of patients with AD and is known to precede the accumulation of NFTs, suggesting that tau acetylation is an early event in tau-mediated neurodegeneration [4].
- The lysine acetyltransferase p300 has been shown to be aberrantly activated in tauopathies, directly acetylating tau at lysine 280 [5].
- Prior to this work, Rbbp7 has never been implicated in Alzheimer's disease, tau pathogenesis, or aging-associated cell death.

Objective

Here, we sought to understand the role of Rbbp7 in Alzheimer's disease pathogenesis by interrogating Rbbp7 expression in human post-mortem tissue and AD mouse models and modulating Rbbp7 expression *in-vitro* and *in-vivo*.

Rbbp7 expression is decreased in AD brain tissue and negative correlates with Braak stage

Fig. 1. Rbbp7 expression is decreased in AD brain tissue and negatively correlate with Braak stage. (a-c) AD patients show a significantly lower Rbbp7 expression in the middle temporal gyrus (MTG) that is neuronal specific compared to age-matched CTL. (d-e) AD patients show a marginally significant negative correlation between Rbbp7 mRNA expression and total Aβ plaque density and a significant negative correlation with Braak Score. (f) Linear regression analysis reveals that as Rbbp7 expression goes down, so does brain weight. Box plots: center line represents the median, the limits represent the 25th and 75th percentile, and the whiskers represent the minimum and maximum values of the distribution. *p<0.05, **p<0.01, ***p<0.001, ****p<0.00001.

Rbbp7 is reduced in AD mouse models with tau mutations and increased expression protects against cell death *in-vitro*

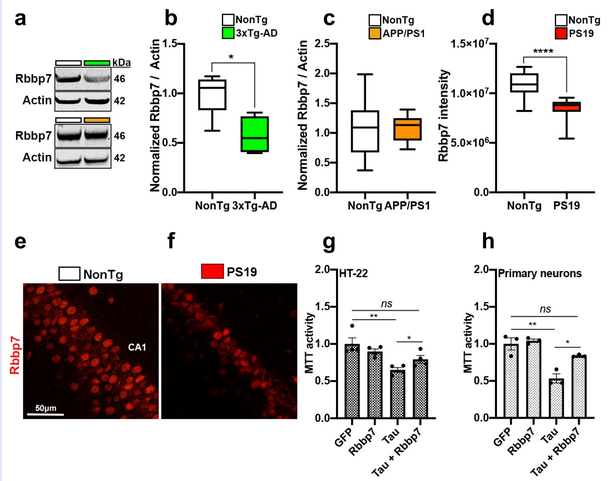


Fig. 2. Rbbp7 protein levels are reduced in AD mouse model with tau mutations and increased expression protects against cell death in vitro. (a) Representative immunoblot of Rbbp7 and loading control β-actin. (b) 3xTg-AD mice have significantly decreased Rbbp7 protein level compared to NonTg. (c) APP/PS1 mice show similar Rbbp7 levels compared to NonTg. (d) Rbbp7 levels in CA1 of PS19 mice are significantly lower compared to NonTg. (e, f) Hippocampal CA1 photomicrographs stained for Rbbp7. (g-h) Overexpression of Rbbp7 significantly reduces TauP301L-induced cytotoxicity in HT-22 immortalized hippocampal cells and in primary cortical neurons. Box plots: center line represents the median, the limits represent the 25th and 75th percentile, and the whiskers represent the minimum and maximum values of the distribution. Bar graphs are means ± SE. *p<0.05, **p<0.01, ****p<0.00001.

Rbbp7-AAV injected into the CA1 of the hippocampus infects neurons

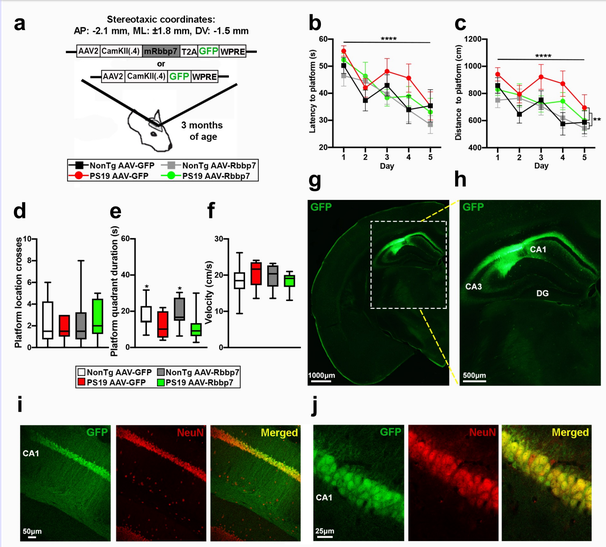


Fig. 3. AAVs into CA1 of the hippocampus infects neurons. (a) The adeno-associated viruses (AAVs) bilaterally injected into CA1 of the hippocampus. [anterior-posterior (AP); medial-lateral (ML); dorsal-ventral (DV)]. (b-c) Escape latency and distance traveled in the five-day learning phase of the Morris water maze. (d-f) Results of the day 6 probe trails of the MWM. (g, h) Low and high magnification photomicrographs depicting the spread of AAV with the GFP reporter in the hippocampus. (i, j) Low and high magnification hippocampal sections stained against neuronal marker NeuN and the GFP reporter. Box plots: center line represents the median, the limits represent the 25th and 75th percentile, and the whiskers represent the minimum and maximum values of the distribution. **** p<0.0001.

Rbbp7 overexpression in the CA1 of the hippocampus increases Rbbp7 levels and reduces neuronal loss in PS19 mice

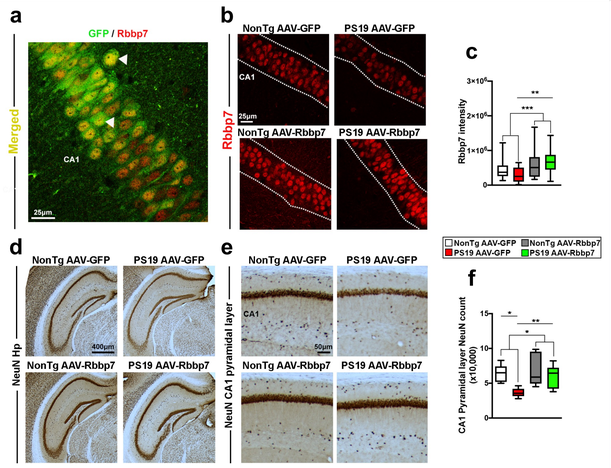


Fig. 4 AAV-Rbbp7 injected into CA1 of the hippocampus increases Rbbp7 levels and reduces neuronal loss in PS19 mice. (a) High magnification photomicrograph depicting neurons labeled with the GFP reporter and Rbbp7. (b) Hippocampal CA1 photomicrographs stained for Rbbp7. (c) Quantitative analysis of Rbbp7 intensity reveals a significant increase in AVV-Rbbp7 injected mice. PS19 AAV-Rbbp7 have a higher expression of Rbbp7 than PS19 AAV-GFP mice. (d, e) Low and high magnification of hippocampal sections stained for NeuN. (f) Quantitative analysis of neuronal counts using unbiased stereology in the CA1 of the hippocampus. PS19 AAV-Rbbp7 mice show a significantly higher number of NeuN+ cells compared to PS19 AAV-GFP and cell count number did not differ compared to NonTg mice. Box plots: center line represents the median, the limits represent the 25th and 75th percentile, and the whiskers represent the minimum and maximum values of the distribution. *p<0.05, **p<0.01.

Rbbp7 overexpression reduces tau acetylation, phosphorylation and is negatively associated with p300 in PS19 mice

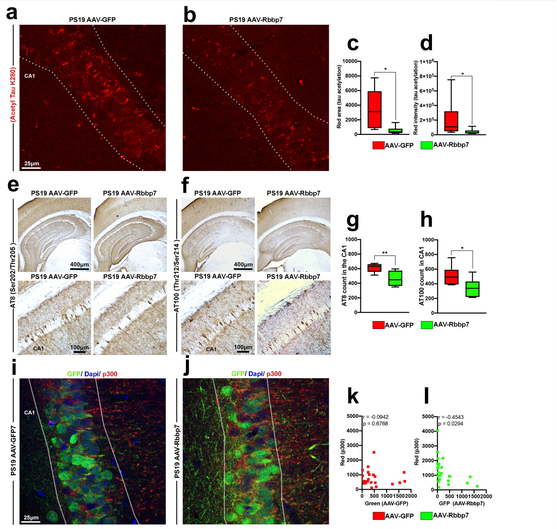


Fig. 5. AAV-Rbbp7 reduces tau acetylation, phosphorylation and is negatively associated with p300 in PS19 mice. (a-b) High magnification representative photomicrographs of hippocampal sections stained for Acetyl Tau K280. Dotted lines depict CA1 area analyzed. (c-d) Quantitative analysis of Acetyl Tau K280 area and intensity in CA1 of the hippocampus. (e-f) Low and high magnification representative photomicrographs of hippocampal sections stained for AT8 and AT100. (g) Quantitative analysis of AT8+ cell count in CA1 of the hippocampus. (h) Quantitative analysis of AT100+ cell count in CA1 of the hippocampus. (i-j) High magnification representative photomicrographs of hippocampal sections stained for the GFP reporter and p300. (k-l) Linear correlation of GFP and p300 in CA1 of the hippocampus for the PS19 AAV-GFP and PS19 AAV-Rbbp7 mice. Box plots: center line represents the median, the limits represent the 25th and 75th percentile, and the whiskers represent the minimum and maximum values of the distribution. *p<0.05; **p<0.01.

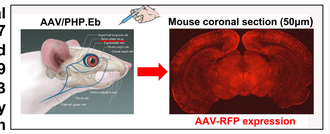
Conclusions

- Our results show that Rbbp7 is downregulated in post-mortem brain tissue of AD patients and is negatively associated with Braak stage.
- We find a positive correlation between Rbbp7 and brain weight in post-mortem AD brains, suggesting that reduced Rbbp7 may account for brain atrophy seen in these patients [6].
- A similar effect was found in the 3xTg-AD and PS19 mouse models of AD, which contain mutations leading to tau pathology; APP/PS1 mice that lack tau mutations show comparable levels of Rbbp7 to NonTg, further highlighting an association between Rbbp7 and pathogenic tau.
- HT-22 hippocampal cells and primary cortical neurons transfected with pathological TauP301L are more susceptible to cell death, and Rbbp7 co-transfection protected them from death.
- Our results show that overexpression of Rbbp7 in the CA1 region of the hippocampus, an area heavily affected by tau acetylation, phosphorylation and neuronal loss, rescues these deficits in the PS19 mice [7].
- We find a negative correlation between AAV-Rbbp7 and p300.

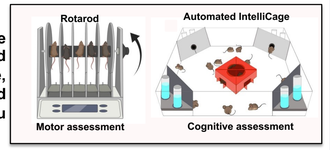
Collectively, our results illustrate a novel role of Rbbp7 in protecting against tau pathology, which has significant therapeutic implications for AD and related tauopathies.

Future Work

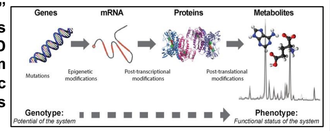
- To determine whether a global upregulation of neuronal Rbbp7 rescues cognitive deficits and reduces tau pathology in PS19 mice, we will use the AAV/PHP.eB serotype to globally overexpresses neuronal Rbbp7 in NonTg and PS19 mice.



- Mice will be assessed using the rotarod, Morris water maze and the automated IntelliCage, followed by an unbiased stereological analysis of tau pathology and neuronal loss.



- We will employ a "multi-omics" approach to characterize Rbbp7's protein-protein interactions in AD and reveal the downstream epigenomic and transcriptomic changes associated with Rbbp7's protection against tau pathology.



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Acknowledgments and more information about our research

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