INPP5D inhibition attenuates amyloid pathology through the regulation of microglial functions

Peter B. Lin , Andy Tsai, Disha Soni, Audrey Lee-Gosselin, Kwangsik Nho, Bruce Lamb, Adrian L. Oblak
Indiana University School of Medicine, Indianapolis Indiana

INDIANA UNIVERSITY SCHOOL OF MEDICINE

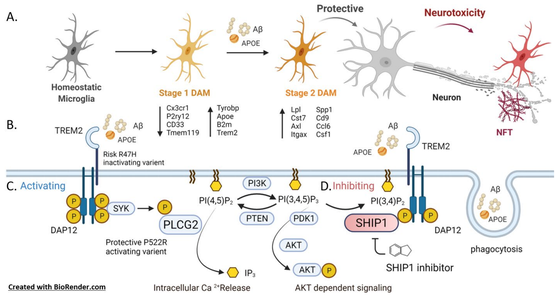
BACKGROUND

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by accumulated beta-amyloid (Aβ) deposits and robust microgliosis. Recent genome-wide association studies have identified genetic risk factors in late-onset AD (LOAD) which are largely microglia. Among the risk factors, Inositol polyphosphate-5-phosphatase D (INPP5D) confers an increased risk of developing AD and is associated with increased plaque deposition. As a microglia-specific lipid phosphatase, INPP5D negatively regulates signaling via several microglial cell surface receptors, including TREM2; however, the impact of INPP5D inhibition on AD pathology remains unclear.

METHODS

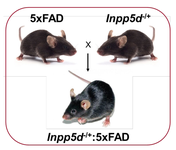
To determine the impact of Inpp5d on disease pathogenesis and microglial phenotypes, we utilized the 5xFAD model expressing Inpp5d haplodeficiency.

HYPOTHESIS



SHIP1 inhibition will increase PLCγ2/AKT-mediated signaling and increase the protective phagocytic activity of microglia to clear extracellular neurotoxins before they accumulate and drive the neuroinflammation that causes neurotoxicity. **A.** Microglia respond to Aβ and APOE with protective or neurotoxic phenotypes depending on their microenvironment. **B.** TREM2 binds Aβ and APOE, which activate microgliosis. Inactivating TREM2 variants increase risk. **C.** DAP12 mediated activation through SYK and PLCγ2. Activating PLCG2 variant is protective. **D.** SHIP1 completes with SYK and limits PIP3-dependent PLCγ2 and AKT signaling downstream from TREM2.

ANIMAL MODEL

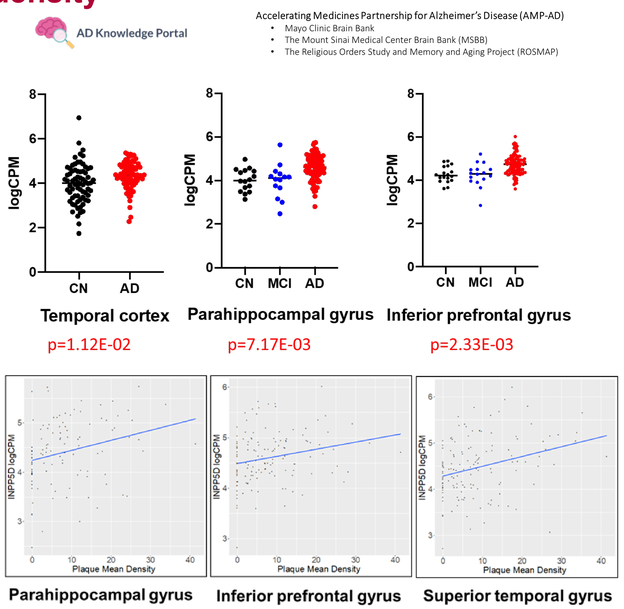


5xFAD animal model

- APP: KM670/671NL (Swedish), I716V (Florida), V717I (London)
- PSEN1: M146L, L286V

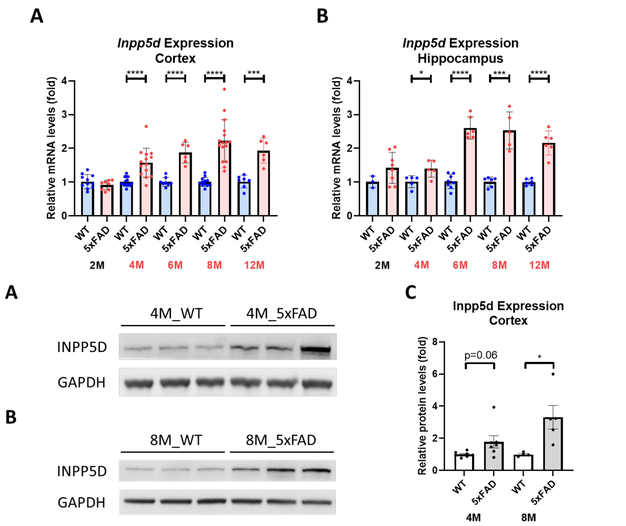
- Develop amyloid pathology around ~2 months of age
- Cognitive deficits observed by 6 months of age

INPP5D expression is increased in LOAD and is correlated with amyloid plaque density



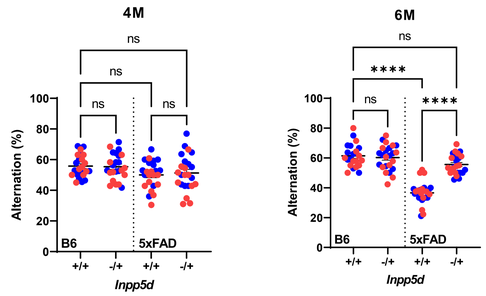
Association of INPP5D expression with amyloid plaque mean density. The scatter plots show the positive association between INPP5D expression and plaque mean density in parahippocampal gyrus, inferior frontal gyrus, and superior temporal gyrus from the MSBB cohort.

Inpp5d gene and protein expression are increased in 5xFAD mice.

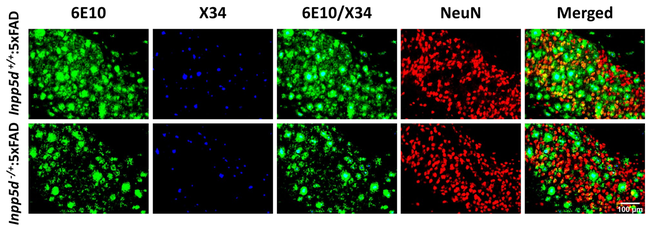


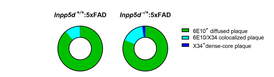
Inpp5d levels are increased in 5xFAD mice Gene and protein levels of Inpp5d were assessed in cortical and hippocampal lysates from 5xFAD mice. Gene expression levels of Inpp5d were significantly increased in both cortex (A) and hippocampus (B) at 4, 6, 8, and 12 months of age (n=6–15 mice). There were significant changes in Inpp5d protein levels in the cortex at 8 months of age and an increased trend in the cortex at 4 months of age (n=4–7; C and D). Increased Inpp5d levels were abolished with PLX5622 treatment (E), and restored after switching PLX diet to normal diet (F) (n=3–10). *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001, ns not significant.

Inpp5d deficiency mitigates the behavioral deficits in 5xFAD mice.



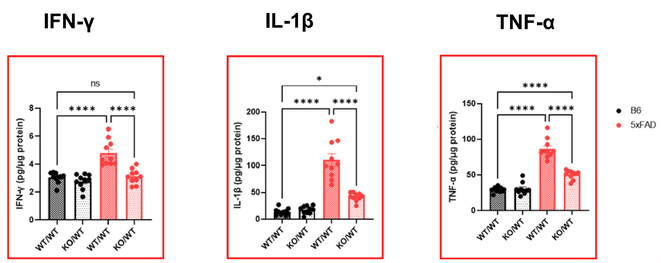
Inpp5d deficiency alters plaque phenotypes and cytokine production in 5xFAD mice.





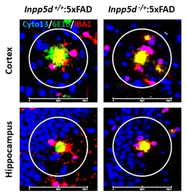
	Inpp5d+/+:5xFAD	Inpp5d-/-:5xFAD
6E10+ diffused plaque (%)	88.76 ± 7.82	81.28 ± 8.06 **
6E10/X34 colocalized plaque (%)	11.00 ± 0.77	16.53 ± 1.11 *
X34+ dense-core plaque (%)	0.24 ± 0.04	2.20 ± 0.27 ***

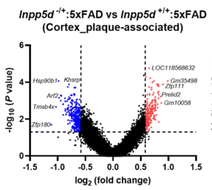
Immunostaining for the diffused plaques (6E10, green), dense-core plaques (X34, blue), and neurons (NeuN, red) in the subiculum of 7.5-month-old Inpp5d$^{KO/WT}$:5xFAD and Inpp5d$^{WT/WT}$:5xFAD mice. (b) The ratio of plaque phenotypes in the subiculum was determined (n = 3-4 per genotype).

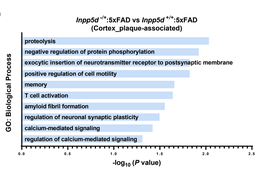


Immunostaining for the plaques (X34, blue), activated microglia (Iba1, green), and homeostatic microglia (P2ry12, red) in the cortex of 7.5-month-old Inpp5d$^{KO/WT}$:5xFAD and Inpp5d$^{WT/WT}$:5xFAD mice. The microglia engagement in the cortex and hippocampus was determined Cytokine production in the cortex of 7.5-month-old animals using the MSD ELISA assay. Statistical analysis was performed by student's t-test for microglia phenotypes. Data are expressed as mean values ± SEM (*P < 0.05, ***P < 0.001, and ****P < 0.0001).

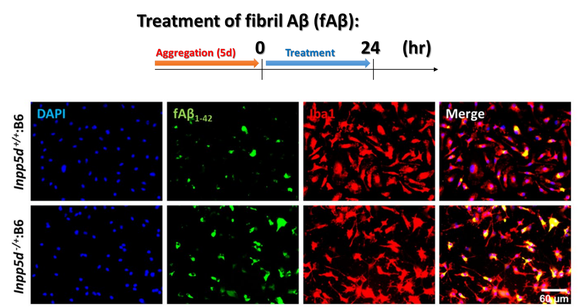
Distinct transcriptomic profiles altered by Inpp5d deficiency in 5xFAD mice.

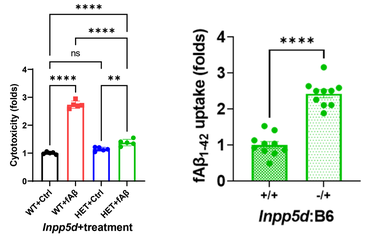






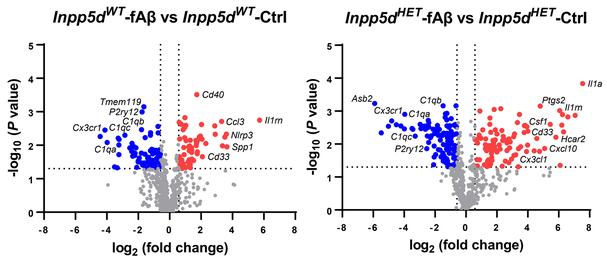
Inpp5d haplodeficiency increases the capacity of Aβ uptake and reduces Aβ induced cytotoxicity.





Immunofluorescence of primary mouse microglia from Inpp5d$^{KO/WT}$ and Inpp5d$^{WT/WT}$ mice incubated with aggregates of fluorescently labeled Aβ1-42 (10 μM, green) for 30 mins. Cells were stained with 4-,6-diamidino-2-phenylindole for nuclei (DAPI, blue) and Iba1 for microglia (red). Quantification of Aβ1-42 uptake by analyzing fluorescence per cell (n = 5 per condition).

Differential gene expression analysis revealed distinct neuroinflammatory phenotypes



CONCLUSIONS & FUTURE WORK

- *INPP5D* expression is upregulated in brains of human LOAD subjects and 5xFAD mice.
- Reduced *Inpp5d* expression mitigates plaque burdens and Aβ levels in 5xFAD mice and protected against behavioral deficits induced by amyloid pathology.
- *Inpp5d* deficiency alters the plaque phenotypes by increasing the microglial engagements to plaques, which resulting in reduced pro-inflammatory cytokines release.
- *Inpp5d* deficiency increases SYK phosphorylation, but not ERK and AKT phosphorylation.
- *Inpp5d* deficiency reduced fibril Aβ-induced cytotoxicity in primary microglia.
- *Inpp5d* deficiency alters the fibril Aβ-induced immune response.
- **We currently have two INNP5D inhibitors we have screened and are utilizing our pipeline to test the efficacy in AD mice.**

FUNDING

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