Protective variant in PLCγ2 mitigates Alzheimer's disease-associated pathologies via enhancing beneficial microglia functions

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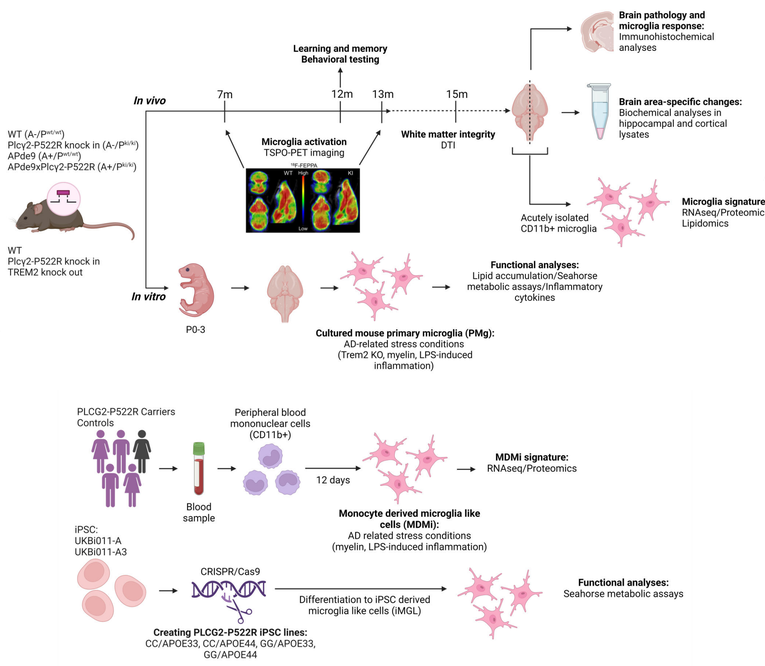
Aims, research questions and working hypothesis

PLCγ2-P522R (phospholipase C gamma 2, proline 522 to arginine) variant reduces the risk for late onset Alzheimer's disease (LOAD). In this study, our goal was to investigate the protective functions of PLCγ2-P522R variant in a mouse model of AD as well as to assess the underlying mechanisms at the molecular and cellular level using mouse and human microglia models.

Study outline and methods



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Results

PLCγ2-P522R variant reduces brain β-amyloid plaque burden of APP/PS1 mouse brain

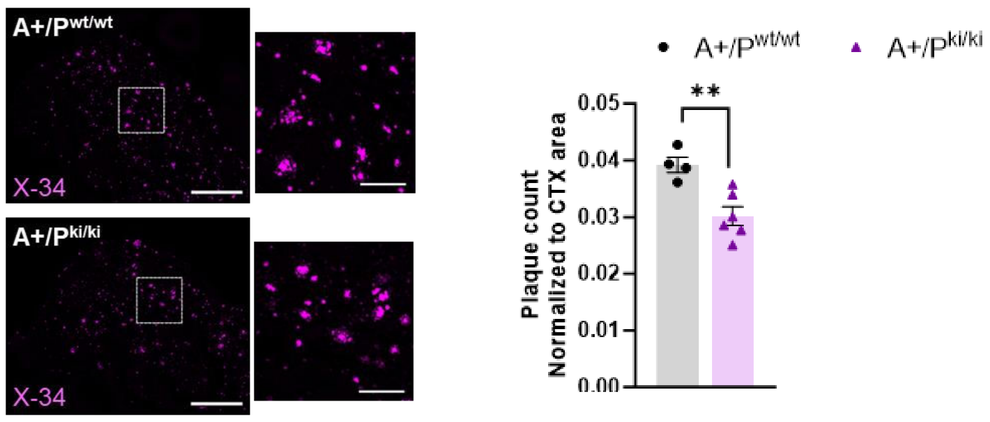

Figure 1. X-34- positive β-amyloid plaque count (**p=0.005) is decreased in the entorhinal cortex of the APP/PS1xPLCγ2-P522R (A+/P ki/ki) mice as compared to the APP/PS1 (A+/P wt/wt) mice. n(A+/P wt/wt)=4, n(A+/P ki/ki)=6.

PLCγ2-P522R variant increases microglia clustering around β-amyloid plaques leading to reduced β-amyloid plaque-associated neuronal dystrophy

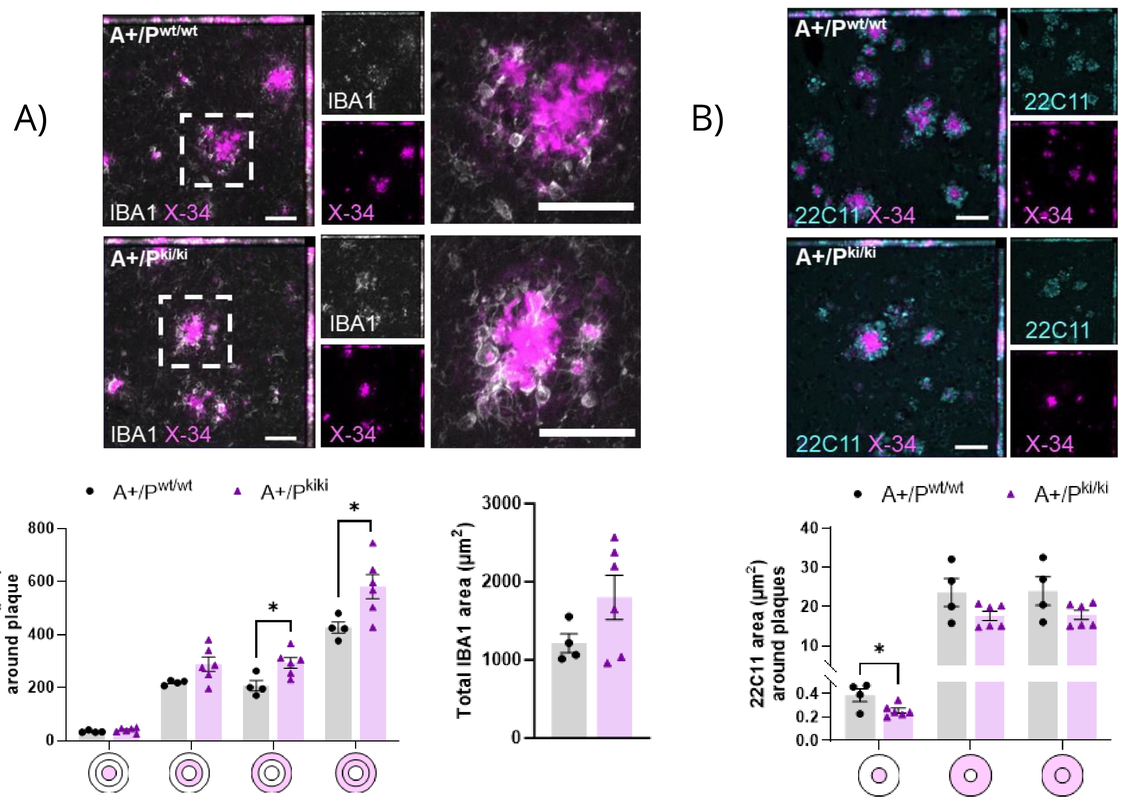

Figure 2. A) Area (µm2) of IBA1-positive microglia is increased at 20-40 µm (*p=0.017) and 0-40 µm (*p=0.03) distances from the plaque outline in the entorhinal cortex of the APP/PS1xPLCγ2- P522R (A+/P ki/ki)mice as compared to the APP/PS1 (A+/P wt/wt)mice. Simultaneously, a trend towards an increase in total IBA1 area is observed. n(A+/P wt/wt)=4, n(A+/P ki/ki)=6. B) Area (µm2) of 22C11-labeled dystrophic neurites around β-amyloid plaques strongly correlates with plaque size (r=0.8113, ****p<0.0001). 22C11-positive area shows a decrease within β-amyloid plaque area (p=0.033) and a similar decreasing trend within the 0-14 µm radius from the β-amyloid plaque outline in the entorhinal cortex of the A+/P ki/ki mice as compared to the A+/P wt/wt mice when normalized to the plaque size. n(A+/P wt/wt)=4, n(A+/P ki/ki)=6.

PLCγ2-P522R variant decreases lipid droplets, upregulates fatty acid metabolism and mitochondrial function-related pathways, and downregulates inflammatory targets in PLCγ2-P522R KI mouse microglia

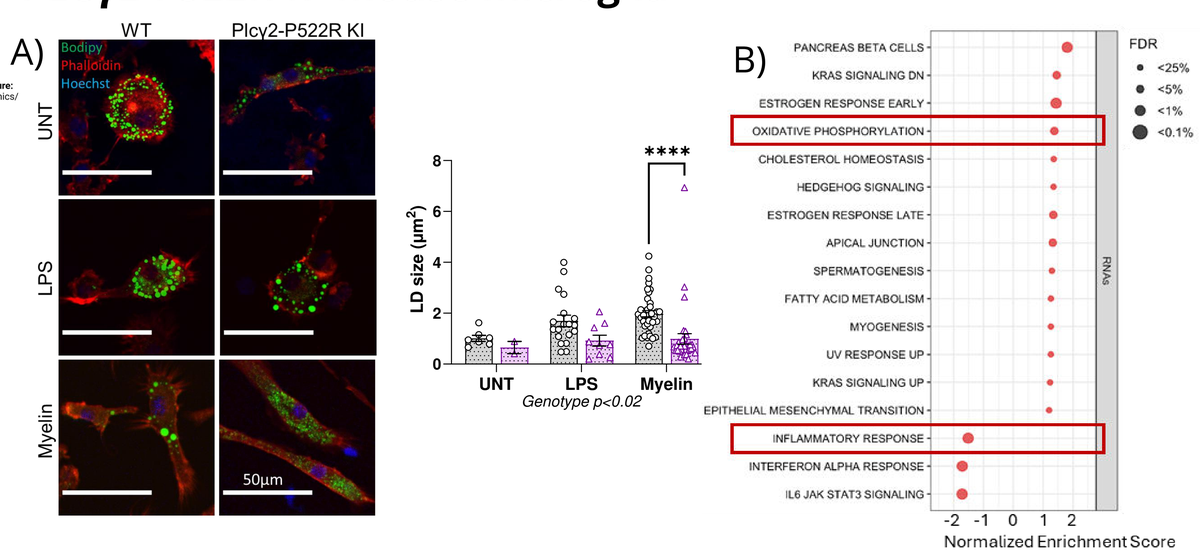

Figure 3. A) The percentage of lipid droplet (LD)-positive cells is increased in mouse primary microglia upon 24-h lipopolysaccharide (LPS) and 48-h myelin treatments in comparison to untreated cells (UNT). The percentage of LD-positive cells do not differ between wildtype (WT) and PLCγ2-P522R knock-in (KI) microglia. PLCγ2-P522R KI decreases the size (µm2) of individual LDs in myelin-treated conditions (****p<0.0001). n(analyzed images) WT UNT=11, LPS=22, myelin=42; KI PLCγ2-P522R UNT=6, LPS=12, myelin=35. B) A dot plot of normalized enrichment scores for enriched and depleted gene sets in enrichment analyses by GSEA for gene (MSigDB Hallmarks, FDR<0.25) expression in CD11b+ microglia isolated from 13-month-old PLCγ2-P522R KI and wildtype (WT) mice.

Mitochondrial function is improved in human microglia models of the PLCγ2-P522R variant carriers

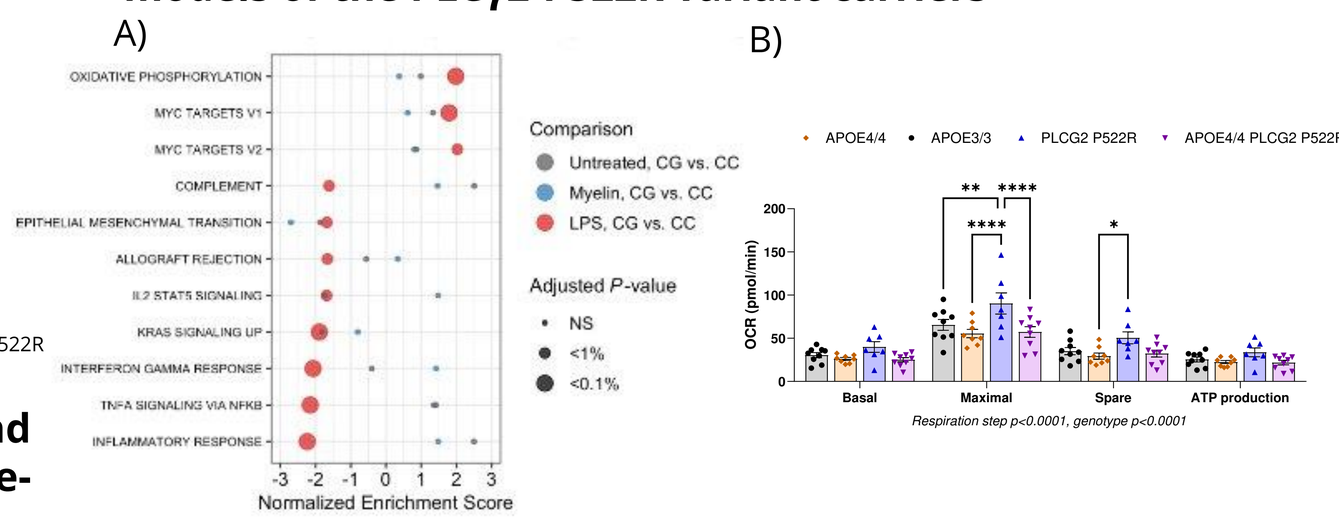

Figure 4. A) A dot plot of normalized enrichment scores for enriched and depleted gene sets in enrichment analyses by GSEA for gene (Hallmarks, FDR<0.25) expression in MDMi cells of CG and CC individuals upon UNT, LPS, and myelin treatments. B) Mitochondrial oxygen consumption rate (OCR, maximal respiration and spare respiratory capacity) is increased in homozygous PLCγ2-P522R (GG) induced pluripotent stem cell derived microglia (iMGL) as compared to isogenic controls (CC) having APOE33, but not APOE44 genetic background. iMGL n=1 line per group, 7-9 technical replicates per line.

Significance and impact on the field

- These findings suggest that PLCγ2-P522R variant exerts protection against AD-associated β-amyloid and neuronal pathologies via enhancing microglial barrier formation around β-amyloid plaques and suppressing pro-inflammatory activation
- Observed changes in fatty acid metabolism and mitochondrial flexibility as well as the downregulation of genes involved in inflammatory signaling pathways suggest that these protective effects of the PLCγ2-P522R variant are mediated through an anti-ageing mechanism
- New molecular mechanisms leading to the development of novel biomarkers and therapeutic targets for disease-modifying therapies

Next steps and future challenges

- Characterization of PLCγ2-P522R-driven mechanisms upon different stress conditions associated with aging and AD, with special emphasis on pathways and functions associated with lipid metabolism and mitochondrial function