PLCG2 as a Risk Factor for Alzheimer's Disease.

Andy P. Tsai

Stark Neurosciences Research Institute

Chuanpeng Dong

Indiana University School of Medicine

Christoph Preuss

Jackson Laboratory

Miguel Moutinho

Stark Neurosciences Research Institute

Peter Bor-Chian Lin

Stark Neurosciences Research Institute

Nicole Hajicek

University of North Carolina at Chapel Hill

John Sondek

University of North Carolina at Chapel Hill

Stephanie J. Bissel

Indiana University School of Medicine

Adrian L. Oblak

Indiana University School of Medicine

Gregory W. Carter

Jackson Laboratory

Yunlong Liu

Indiana University School of Medicine

Gary E. Landreth

Indiana University School of Medicine

Bruce T. Lamb

Stark Neurosciences Research Institute

Kwangsik Nho (✉ knho@iupui.edu)

Indiana University School of Medicine

Research

Keywords: Alzheimer's disease (AD), Microglia, PLCG2, M28L, P522R, Risk variant

Posted Date: June 30th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-38216/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

PLCG2 as a Risk Factor for Alzheimer's Disease.

Andy P. Tsai¹, Chuanpeng Dong², Christoph Preuss³, Miguel Moutinho¹, Peter Bor-Chian Lin¹,
Nicole Hajicek⁴, John Sondek⁴, Stephanie J. Bissel^{1,6}, Adrian L. Oblak^{1,7}, Gregory W. Carter³,
Yunlong Liu², Gary E. Landreth^{1,5}, Bruce T. Lamb^{1,6}, Kwangsik Nho^{7*}

¹. Stark Neurosciences Research Institute, IUSM, Indianapolis, IN, USA. tandy@iu.edu (A.P.T);
mmoutinh@iu.edu (M.M); pblin@iu.edu (P.B.L); sbissel@iu.edu (S.J.B); aoblak@iupui.edu
(A.L.O); glandret@iu.edu (G.E.L); btlamb@iu.edu (B.T.L)

². Department of Medical and Molecular Genetics, Center for Computational Biology and
Bioinformatics, IUSM, Indianapolis, IN, USA. cpdong@iu.edu (C.D); yunliu@iu.edu (Y.L)

³. The Jackson Laboratory, Bar Harbor, ME, USA. Christoph.Preuss@jax.org (C.P);
Gregory.Carter@jax.org (G.W.C)

⁴. Department of Pharmacology, The University of North Carolina at Chapel Hill, Chapel Hill, NC,
USA. nhajicek@email.unc.edu (N.H); sondek@med.unc.edu (J.S)

⁵. Department of Anatomy and Cell Biology, IUSM, Indianapolis, IN, USA. glandret@iu.edu
(G.E.L)

⁶. Department of Medical and Molecular Genetics, IUSM, Indianapolis, IN, USA. sbissel@iu.edu
(S.J.B); btlamb@iu.edu (B.T.L)

⁷. Department of Radiology & Imaging Sciences, IUSM, Indianapolis, IN, USA.
aoblak@iupui.edu (A.L.O); knho@iupui.edu (K.N)

Corresponding Author: *For correspondence contact Kwangsik Nho, Department of Radiology
& Imaging Sciences, IUSM, Indianapolis, IN, USA, Methodist GH4101, RADY, Indianapolis, IN,
46202, USA. Phone: +1-317-963-7503; Email: knho@iupui.edu

Abstract

Background

Alzheimer's disease (AD) is characterized by robust microgliosis and phenotypic changes that accompany disease pathogenesis. Indeed, genetic variants in microglial genes are linked to risk for late-onset AD (LOAD). Phospholipase C γ 2 (PLCG2) participates in the transduction of signals emanating from immune cell-surface receptors that regulate the inflammatory response and is selectively expressed by microglia in the brain. A rare variant in *PLCG2* (P522R) was previously found to be protective against LOAD. Here, we performed association analysis to identify a new genetic variation in *PLCG2* that is associated with elevated risk for LOAD.

Methods

Using whole genome sequencing (N=1,894) and RNA-Seq (N=1,077) data from the AMP-AD cohort, we investigated whether a missense variant in *PLCG2* (M28L) was associated with risk for LOAD. We have examined the homology model and space-filling model of PLCG2 generated with PyMOL to investigate the protein structure of PLCG2 with substitutions of LOAD risk and protective variants in *PLCG2*. Gene expression analysis and expression quantitative trait loci (eQTL) of *PLCG2* were conducted. We also evaluated the relationship between *PLCG2* expression levels and amyloid plaque density and expression levels of microglia specific markers (*AIF1* and *TMEM119*). Age, sex, and *APOE* ϵ 4 carrier status were used as covariates. Finally, we investigated the longitudinal changes *PLCG2* expression in the 5XFAD mouse model of AD and its relationship to amyloid pathology progression.

Results

A rare missense variant in *PLCG2* (M28L) confers increased AD risk ($p=0.047$; OR=1.164 [95% CI=1.002-1.351]). *PLCG2* is highly expressed in the brain and was significantly up-regulated in the parahippocampal gyrus, superior temporal gyrus, and inferior temporal gyrus in LOAD. Higher *PLCG2* expression levels were associated with increased brain amyloid

deposition. The findings were validated in 5xFAD mice, showing a disease progression-dependent increase in *Plcg2* expression with amyloid pathology. Furthermore, eQTL analysis identified several variants as associated with increased *PLCG2* expression levels in the brain and other organs.

Conclusions

Our results provide further evidence that *PLCG2* and the M28L variant confers increase risk for LOAD and may play an important role in AD pathophysiology.

Keywords: Alzheimer's disease (AD), Microglia, *PLCG2*, M28L, P522R, Risk variant

Background

Late-onset Alzheimer's disease (LOAD) is the most common form of AD, with symptoms typically arising after age 65 [1]. Age, sex, and the apolipoprotein $\epsilon 4$ (*APOE* $\epsilon 4$) allele are the three greatest risk factors for LOAD [2-4]. The risk for LOAD doubles every five years after age 65, and over 60% of AD patients are female [2, 5]. The mechanisms underlying the development of LOAD are incompletely understood, but recent large-scale genome-wide association studies (GWAS) have identified that more than 30 genetic variants are associated with LOAD [6, 7].

Importantly, approximately 40% of the identified genes are immune- and microglia-related, suggesting that microglia are involved in modulating AD pathology [8]. Among these microglia-related genetic factors, a rare variant in *PLCG2* (phospholipase C γ 2), P522R (rs72824905-G), was found to be associated with reduced LOAD risk [9]. *PLCG2* is a membrane-associated enzyme that catalyzes the conversion of phospholipid PIP2 (1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate) to IP3 (1D-myo-inositol 1,4,5-trisphosphate) and DAG (diacylglycerol), which plays a crucial role in cell-surface receptor signal transduction [10]. Specifically, *PLCG2* is necessary for immune cell function and is highly expressed in

microglia. Genomic deletions (exon 19, Δ 19, or exon 20 through 22, Δ 20-22) or somatic mutations (R665W or S707Y) within the regulatory domain of *PLCG2* resulted in constitutive activation and resistance to treatment by inhibition of its upstream activator, BTK (Bruton's tyrosine kinase), in leukemia [11, 12]. Interestingly, a missense M28L variant in *PLCG2* that does not alter the constitutive activity of the enzyme following Rac2 GTPase stimulation has been identified in BTK-inhibitor-resistant patients [13]. BTK inhibition reduces phagocytosis and inhibits the uptake of synaptic structures in rodent microglia, indicating a role for the BTK-*PLCG2* pathway in microglial function [14].

Here, we report that expression levels of *PLCG2* are increased in AD, and that the M28L variant is associated with elevated LOAD risk. Similarly, *Plcg2* is highly expressed in the brain of an AD mouse model. Importantly, upregulated expression of *PLCG2* is associated with increased amyloid plaques, which was validated in an amyloid mouse model. Elucidating the different effects of *PLCG2* variants on LOAD risk will expand our understanding of the function of microglia in AD pathophysiology.

Methods

Human participants and RNA-Seq

RNA-Seq and whole-genome sequencing data were obtained from the Accelerating Medicines Partnership for Alzheimer's Disease (AMP-AD) Consortium, where all individuals used in the analysis were participants of the Mayo Clinic Brain Bank cohort, the Mount Sinai Medical Center Brain Bank (MSBB) cohort, and the Religious Orders Study and Memory and Aging Project (ROSMAP) cohort.

In the Mayo Clinic RNA-Seq dataset [15], RNA was isolated from the temporal cortex (TCX) and cerebellum (CER). The RNA-Seq-based whole transcriptome data were generated from human samples of 151 TCX (71 cognitively normal older adult controls (CN) and 80 LOAD) and 151 CER (72 CN and 79 LOAD). LOAD had a Braak score of ≥ 4.0 and met

neuropathological criteria for AD, while CN had a Braak score of ≤ 3.0 and without neurodegenerative diagnoses. The quality of the samples was selected to be RNA integrity number (RIN) ≥ 5.0 for inclusion in the study.

In the MSBB dataset [16], RNA was isolated from the top two most vulnerable regions of LOAD, parahippocampal gyrus (PHG) and inferior frontal gyrus (IFG), and the ranked 7th and 14th most vulnerable brain regions of LOAD, superior temporal gyrus (STG) and frontal pole (FP).

The assessment of dementia and cognitive status was conducted using a clinical dementia rating scale (CDR) [17]. LOAD had a CDR score of ≥ 0.5 , while mild cognitive impairment (MCI) had a CDR score of 0.5, and CN had a CDR score of 0 without any significant memory concern of their daily activity. This study included 108 participants (16 CN, 14 MCI, and 78 AD) for PHG, 137 participants (21 CN, 18 MCI, and 98 LOAD) for STG, 136 participants (18 CN, 16 MCI, and 102 LOAD) for IFG, and 153 participants (22 CN, 20 MCI, and 111 LOAD) for FP.

In the ROSMAP dataset [18], RNA-Seq was performed on dorsolateral prefrontal cortex from 241 participants (86 CN and 155 LOAD). The diagnosis is based on the assessment of clinical diagnosis of cognitive status, which followed the criteria of the joint working group of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association.

Whole Genome Sequencing (WGS)

Pre-processed whole-genome sequencing data were obtained from the Accelerating Medicines Partnership for Alzheimer's Disease (AMP-AD) Consortium [15, 16, 18-20]. Whole-genome sequencing libraries were prepared using the KAPA Hyper Library Preparation Kit per the manufacturer's instructions. Libraries were sequenced on an Illumina HiSeq X sequencer using pair-end read chemistry and read lengths of 150bp. The paired-end 150bp reads were aligned to the NCBI reference human genome (GRCh37) using the Burrows-Wheeler Aligner

(BWA-MEM) [21] and processed using the GATK best practices workflow that marks potential duplicates, locally realigns any suspicious reads, and re-calibrates the base-calling quality scores using Genome Analysis Toolkit (GATK) [22]. The resulting BAM files were analyzed to identify variants using the HaplotypeCaller module of GATK for multi-sample variant callings [23].

Statistical analysis

To investigate the diagnosis group difference of *PLCG2* expression between CN, MCI, and LOAD, we used the *limma* software [24] to perform a differential expression analysis [18]. Age, sex, and *APOE* ϵ 4 carrier status were used as covariates. To investigate the relationship between *PLCG2* expression levels and amyloid plaque density or expression levels of microglia specific markers (*AIF1* and *TMEM119*), we used general linear models with *PLCG2* expression levels as a dependent variable and plaque density or microglia specific markers as well as age, sex, and *APOE* ϵ 4 carrier status as explanatory variables. The regression was performed with the "glm" function from the stats package in R (version 3.6.1).

In the mouse study, statistical analyses were performed using GraphPad Prism (Version 8.4.2). Experiments at the 2-, 4-, 6- and 8-month time points were performed independently, so statistical comparisons between wild-type and 5xFAD AD mice were performed by unpaired t-test. Graphs represent the mean, and error bars denote the SEM.

Mice

All wild-type and 5XFAD mice used in this study were maintained on the C57BL/6J background and purchased from Jackson Laboratory (JAX MMRRC Stock# 034848). All mice were bred and housed in specific-pathogen-free conditions. Both male and female mice were used in this study.

Mouse RNA isolation for qPCR

Mice were anesthetized with Avertin and perfused with ice-cold PBS. The cortical and hippocampal regions from the hemisphere were micro-dissected and stored at -80-degree C.

Frozen brain tissue was homogenized in buffer containing 20 mM Tris-HCl (pH=7.4), 250 mM sucrose, 0.5 mM EGTA, 0.5 mM EDTA, RNase-free water, and stored in an equal volume of RNA-Bee (Amsbio, CS-104B) at -80-degree C until proceeding to RNA extraction. RNA was isolated by using chloroform extraction and purified by using the Purelink RNA Mini Kit (Life Technologies). The cDNA was prepared from 750 ng of RNA by using high-capacity of RNA-to-cDNA kit (Applied Biosystems), and the qPCR was performed by using the StepOne Plus Real-Time PCR system (Life Technologies) with Taqman Gene Expression Assay (Mm01242530_m1 from the Life Technologies). The relative gene expression was determined by using $\Delta\Delta CT$ and assessed relative to GAPDH (Mm99999915_g1). A Student's t test was performed for qPCR assays, comparing the results of wild-type and 5XFAD animals.

Expression quantitative trait loci (eQTL) analysis

To perform an eQTL analysis of *PLCG2* expression levels, we used common SNPs (± 20 kb from the gene boundary; minor allele frequency (MAF) > 5%) from whole-genome sequencing. Only non-Hispanic Caucasian participants were selected. In addition, age and sex were used as covariates. The results of the eQTL analysis were plotted with LocusZoom [25].

Homology Modeling

The model of *PLCG2* structure containing all residues from amino acid 14-1190 was built by using the template of *PLCG1* model [26]. The cartoons with substitutions of M28L and P522R were generated with PyMol (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC).

Results

The *PLCG2* M28L Variant Confers a Higher Risk for LOAD.

Using previously published large-scale GWAS summary statistics [6], we identified that the missense variant M28L in *PLCG2* is associated with LOAD. The minor allele (T) of M28L in *PLCG2* significantly increased risk for LOAD ($p=0.047$, OR=1.164 [95% CI=1.002-1.351])

(**Table 1**). PLCG2 possesses a set of core domains common to most other isoforms of PLC, including an N-terminal PH domain, two pairs of EF hands, a catalytic TIM barrel composed of the X- and Y-boxes, and a C2 domain (**Fig. 1a**). The PLCG isozymes elaborate on this core with a unique array of regulatory domains, including a split PH (sPH) domain, two SH2 domains, and an SH3 domain. Two substitutions that modulate risk for LOAD, M28L, and P522R, were mapped onto a protein structure homology model of PLCG2 [27] (**Fig. 1b**). M28 is buried in the core of the PH domain but is not visible in a space-filling model (**Fig. 1c**). The PH domain is essential for membrane association of PLCG2.

***PLCG2* Expression Levels are Increased in LOAD.**

We performed gene expression analysis using RNA-Seq data generated from seven brain regions. The demographic information of the participants included in this study is summarized in **Table 2**. We found that *PLCG2* was over-expressed in LOAD in the temporal cortex (logFC=0.27, p=4.56E-02; **Fig. 2a**), parahippocampal gyrus (logFC=0.55, p=1.74E-03; **Fig. 2b**), superior temporal gyrus (logFC=0.46, p=2.55E-02; **Fig. 2c**), and inferior prefrontal gyrus (logFC=0.36, p=1.38E-02; **Fig. 2d**) with age and sex as covariates (**Table 3**). However, we did not find any diagnosis group differences in the cerebellum (**Fig. 2e**), frontal pole (**Fig. 2f**), and dorsolateral prefrontal cortex (**Fig. 2g**) (**Table 3**). With age, sex, and *APOE* ϵ 4 carrier status as covariates, *PLCG2* remained over-expressed in LOAD in the parahippocampal gyrus (logFC=0.57, p=2.02E-03), superior temporal gyrus (logFC=0.42, p=4.95E-02), and inferior prefrontal cortex (logFC=0.33, p=2.60E-02) (**Table 3**). In the parahippocampal gyrus, superior temporal gyrus, and inferior prefrontal gyrus, *PLCG2* was over-expressed in LOAD with and without *APOE* ϵ 4 carrier status as an additional covariate. We investigated whether *PLCG2* expression levels were associated with expression levels of microglia-specific marker genes (*AIF1* and *TMEM119*). Our analysis revealed that expression levels of *AIF1* and *TMEM119* were significantly associated with *PLCG2* expression levels in the frontal pole (*AIF1*: β =0.258, p=1.09E-06; *TMEM119*: β =0.518, p=1.28E-15), superior temporal gyrus (*AIF1*: β =0.33,

p=8.98E-07; *TMEM119*: β =0.71, $p<2E-16$), parahippocampal gyrus (*AIF1*: β =0.419, $p=5.43E-07$; *TMEM119*: β =0.806, $p<2E-16$), and inferior prefrontal gyrus (*AIF1*: β =0.257, $p=6.15E-06$; *TMEM119*: β =0.582, $p=6.64E-15$) (**Table 4**).

Increased *PLCG2* Expression Levels are Associated with Amyloid Plaque Density in the Human Brain.

We performed an association analysis of *PLCG2* expression levels with mean amyloid plaque densities measured in four brain regions. Expression levels of *PLCG2* in the human brain was associated with amyloid plaques in three brain regions (**Table 4**). Increased *PLCG2* expression was associated with increased amyloid plaques in the parahippocampal gyrus (β =0.027, $p=5.22E-05$; **Fig. 3a**), superior temporal gyrus (β =0.026, $p=2.02E-04$; **Fig. 3b**), and inferior prefrontal cortex (β =0.017, $p=2.73E-03$; **Fig. 3c**), but not in the frontal pole (β =0.01, $p=0.08$; **Fig. 3d**).

***PLCG2* Expression Levels are Increased in a Mouse Model of Amyloid Pathology.**

Plcg2 expression was increased in a mouse model of amyloid pathology, which is in accordance with the results from our analysis of human LOAD. In the 5xFAD mice, *Plcg2* expression was increased in the cortex (**Fig. 4a**) and hippocampus (**Fig. 4b**) of four-, six -and eight-month-old mice (4-month: 1.39-fold in the cortex, 1.37-fold in the hippocampus; 6-month: 2.37-fold in the cortex, 1.96-fold in the hippocampus; and 8-month: 2.43-fold in the cortex and 2.67-fold in the hippocampus) (**Fig. 4**). Furthermore, our analysis showed a disease progression-dependent increase in *Plcg2* expression in mice with amyloid pathology (**Fig. 4**).

Common Single Nucleotide Polymorphisms in *PLCG2* are Associated with *PLCG2* Expression Levels.

We performed an expression quantitative trait loci analysis (eQTL) analysis of *PLCG2* expression levels using common SNPs, including ± 20 kb from the gene boundary; minor allele frequency (MAF) > 5%, from whole-genome sequencing. The eQTL analysis identified several variants that are associated with *PLCG2* expression levels. In particular, rs4420523 was most

significantly associated with *PLCG2* expression levels in the superior temporal gyrus ($p=2.43E-06$, **Fig. 5a**). Individuals with minor alleles of rs4420523 (C) have higher *PLCG2* expression levels compared to those without minor alleles (**Fig. 5b**). The eQTL association of rs4420523 was replicated in the temporal cortex of the MAYO cohort (**Fig. 5c and Fig. 5d**). Furthermore, using the GTEx eQTL database, we found that rs4420523 is significantly associated with *PLCG2* expression levels in several brain regions and other organs (**Fig. 5e**).

Discussion

In this study, we report the first evidence that a missense variant (M28L) in *PLCG2* is associated with increased risk for LOAD. The results indicated that different genetic variants in *PLCG2* may be either advantageous or deleterious in LOAD, suggesting a potentially important role of this gene in modifying AD risk. The M28L variant was previously found to be associated with leukemia and mechanistically linked to disease pathogenesis by its resistance to inhibition of its upstream kinase activator BTK [13]. *PLCG2* activity can be regulated by several mechanisms, including phosphorylation by kinases such as BTK [28, 29], PH domain-dependent regulation [30-32], SH2 domain-containing components regulation [33], and autoregulation of the intrinsic inhibition region [33, 34]. The methionine 28 is buried within the N-terminal PH domain, and the substitution likely leads to a less well-packed PH domain with a concomitant loss of domain stability. The instability of the PH domain may affect the protein-protein interactions that regulate enzymatic activity. Additionally, the conformational change may disrupt *PLCG2*-BTK or other protein interactions, leading to ibrutinib resistance. *PLCG2* is selectively expressed by microglia in the brain [35], and the M28L mutation likely induces a dysfunctional microglial phenotype with significant implications in AD pathology. Conversely, the P552R *PLCG2* variant has been found to reduce AD risk [9, 36-39]. Therefore, determining how the M28L and P522R variants affect enzyme activity and microglial phenotype will provide valuable insights into the role of *PLCG2* in AD.

Furthermore, we found that *PLCG2* expression increases in several brain regions of LOAD patients, which correlates with brain amyloid plaque density and expression levels of microglial markers *AIF1* and *TMEM119* [40-42]. These results highlight an important relationship between amyloid plaques and *PLCG2* expression, which is further supported by increased levels of *Plcg2* throughout disease progression in the 5xFAD mice, a well-studied model of amyloid pathology [34]. *PLCG2* expression is also upregulated in the amyloid models TgCRND8 and App^{NL-G-F/NL-G-F} mice [43]. Interestingly, microglia surrounding cortical plaques in TgCRND8 mice express *Plcg2* [35], further implicating *PLCG2* in microglial response to A β . Nonetheless, further studies are necessary to investigate the role of *PLCG2* in plaque-associated microglia.

eQTL analysis was performed to gain insight into the relationship between genetic variants and *PLCG2* expression levels. The SNP most significantly associated with *PLCG2* expression levels was rs4420523, an intronic variant. Within the same haplotype blocks [44], about ten variants that significantly increased *PLCG2* expression were strongly correlated with rs4420523, including a synonymous *PLCG2* variant, rs1143688. Further studies to investigate whether increased gene expression by the *PLCG2* variants is related to AD pathogenesis are of great interest.

Limitations

Further analysis of the *PLCG2* M28L variant using human datasets is limited due to the very low frequency of this variant. Specifically, it was not possible to investigate the interaction of the *PLCG2* M28L variant with *PLCG2* expression, amyloid plaques, and *APOE* ϵ 4. Therefore, larger cohorts are required to confirm our observation and perform a more comprehensive study of the role of the *PLCG2* M28L variant in microglia-mediated AD pathology.

Conclusions

In conclusion, we identified a novel association between a missense variant in *PLCG2* (M28L) and an increased risk for LOAD. Additionally, we showed that *PLCG2* expression was increased in several brain regions of LOAD patients and strongly correlated with brain amyloid

burden in LOAD patients and AD animal models. Our results provide further evidence that *PLCG2* may play an important role in AD pathophysiology. Future studies investigating how the M28L and P522R variants in *PLCG2* modulate its function and microglia phenotypes in AD could lead to the identification of potential therapeutic strategies focused on PLCG2 activity.

List of abbreviations

AD: Alzheimer's disease, PLCG2: phospholipase C γ 2, LOAD: late-onset Alzheimer's disease, APOE ϵ 4: apolipoprotein ϵ 4 (APOE ϵ 4) allele, OR: odds ratio, CI: confidence interval, PyMol: The PyMOL molecular graphics system, N: amino-terminus, C: carboxyl-terminus, PH: pleckstrin homology domain, EF: EF hand motif, TIM: TIM barrel, sPH: split PH domain, nSH2: n-terminus Src Homology 2 domain, cSH2: c-terminus Src Homology 2 domain, SH3: SRC Homology 3 domain, C2: C2 domain, CN: cognitively normal, MCI: mild cognitive impairment, RIN: RNA integrity number, logFC: log fold-change, logCPM: log counts per million, NES: normalized effect size.

Declarations

Ethics approval and consent to participate

Animal used in the study were housed in the Stark Neurosciences Research Institute Laboratory Animal Resource Center, Indiana University School of Medicine and all experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC).

Consent for publication

All participants were properly consented for this study.

Availability of data and materials

RNA-Seq and whole-genome sequencing data were obtained from the Accelerating Medicines Partnership for Alzheimer's Disease (AMP-AD) Consortium (<https://adknowledgeportal.synapse.org>). The datasets analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

312 The authors declare no competing interests exist.

313 **Funding**

314 This work was supported by NIA grant RF1 AG051495 (B.T.L and G.E.L), NIA grant RF1
315 AG050597 (G.E.L), NIA grant U54 AG054345 (B.T.L et al.), NIH grant R01 GM057391 (J.S),
316 NIA grant R03 AG063250 (K.N), and NIH grant NLM R01 LM012535 (K.N)

317 **Authors' contributions**

318 A.P.T, J.S, S.J.B, A.L.O, G.W.C, Y.L, G.E.L, B.T.L, and K.N designed the study. A.P.T,
319 C.D, C.P, M.M, P.B.L, N.H, and K.N performed the experiments and analyzed the data. A.P.T,
320 M.M, G.E.L, and K.N wrote the manuscript. All authors discussed the results and commented on
321 the manuscript.

322 **Acknowledgments**

323 We thank Louise Pay for critical comments on the manuscript and Alison Goate (Icahn
324 School of Medicine), Thomas Wingo (Emory University), and Aliza Wingo (Emory University) for
325 discussion.

326 References

- 327 1. Isik AT: **Late onset Alzheimer's disease in older people.** *Clin Interv Aging* 2010,
328 5:307-311.
- 329 2. Riedel BC, Thompson PM, Brinton RD: **Age, APOE and sex: Triad of risk of**
330 **Alzheimer's disease.** *J Steroid Biochem Mol Biol* 2016, **160**:134-147.
- 331 3. Reiman EM, Arboleda-Velasquez JF, Quiroz YT, Huentelman MJ, Beach TG, Caselli RJ,
332 Chen Y, Su Y, Myers AJ, Hardy J *et al*: **Exceptionally low likelihood of Alzheimer's**
333 **dementia in APOE2 homozygotes from a 5,000-person neuropathological study.**
334 *Nat Commun* 2020, **11**(1):667.
- 335 4. Kim J, Basak JM, Holtzman DM: **The role of apolipoprotein E in Alzheimer's disease.**
336 *Neuron* 2009, **63**(3):287-303.
- 337 5. Corrada MM, Brookmeyer R, Paganini-Hill A, Berlau D, Kawas CH: **Dementia**
338 **incidence continues to increase with age in the oldest old: the 90+ study.** *Ann*
339 *Neurol* 2010, **67**(1):114-121.
- 340 6. Kunkle BW, Grenier-Boley B, Sims R, Bis JC, Damotte V, Naj AC, Boland A, Vronskaya
341 M, van der Lee SJ, Amlie-Wolf A *et al*: **Genetic meta-analysis of diagnosed**
342 **Alzheimer's disease identifies new risk loci and implicates Abeta, tau, immunity**
343 **and lipid processing.** *Nat Genet* 2019, **51**(3):414-430.
- 344 7. Efthymiou AG, Goate AM: **Late onset Alzheimer's disease genetics implicates**
345 **microglial pathways in disease risk.** *Mol Neurodegener* 2017, **12**(1):43.
- 346 8. Karch CM, Goate AM: **Alzheimer's disease risk genes and mechanisms of disease**
347 **pathogenesis.** *Biol Psychiatry* 2015, **77**(1):43-51.
- 348 9. Sims R, van der Lee SJ, Naj AC, Bellenguez C, Badarinarayan N, Jakobsdottir J, Kunkle
349 BW, Boland A, Raybould R, Bis JC *et al*: **Rare coding variants in PLCG2, ABI3, and**
350 **TREM2 implicate microglial-mediated innate immunity in Alzheimer's disease.** *Nat*
351 *Genet* 2017, **49**(9):1373-1384.
- 352 10. Hernandez D, Egan SE, Yulug IG, Fisher EM: **Mapping the gene that encodes**
353 **phosphatidylinositol-specific phospholipase C-gamma 2 in the human and the**
354 **mouse.** *Genomics* 1994, **23**(2):504-507.
- 355 11. Ombrello MJ, Remmers EF, Sun G, Freeman AF, Datta S, Torabi-Parizi P, Subramanian
356 N, Bunney TD, Baxendale RW, Martins MS *et al*: **Cold urticaria, immunodeficiency,**
357 **and autoimmunity related to PLCG2 deletions.** *N Engl J Med* 2012, **366**(4):330-338.
- 358 12. Woyach JA, Furman RR, Liu TM, Ozer HG, Zapatka M, Ruppert AS, Xue L, Li DH,
359 Steggerda SM, Versele M *et al*: **Resistance mechanisms for the Bruton's tyrosine**
360 **kinase inhibitor ibrutinib.** *N Engl J Med* 2014, **370**(24):2286-2294.
- 361 13. Walliser C, Hermkes E, Schade A, Wiese S, Deinzer J, Zapatka M, Desire L, Mertens D,
362 Stilgenbauer S, Gierschik P: **The Phospholipase Cgamma2 Mutants R665W and**
363 **L845F Identified in Ibrutinib-resistant Chronic Lymphocytic Leukemia Patients Are**
364 **Hypersensitive to the Rho GTPase Rac2 Protein.** *J Biol Chem* 2016, **291**(42):22136-
365 22148.
- 366 14. Keaney J, Gasser J, Gillet G, Scholz D, Kadiu I: **Inhibition of Bruton's Tyrosine**
367 **Kinase Modulates Microglial Phagocytosis: Therapeutic Implications for**
368 **Alzheimer's Disease.** *J Neuroimmune Pharmacol* 2019, **14**(3):448-461.
- 369 15. Allen M, Carrasquillo MM, Funk C, Heavner BD, Zou F, Younkin CS, Burgess JD, Chai
370 HS, Crook J, Eddy JA *et al*: **Human whole genome genotype and transcriptome data**
371 **for Alzheimer's and other neurodegenerative diseases.** *Sci Data* 2016, **3**:160089.
- 372 16. Wang M, Beckmann ND, Roussos P, Wang E, Zhou X, Wang Q, Ming C, Neff R, Ma W,
373 Fullard JF *et al*: **The Mount Sinai cohort of large-scale genomic, transcriptomic and**
374 **proteomic data in Alzheimer's disease.** *Sci Data* 2018, **5**:180185.

17. Morris JC: **The Clinical Dementia Rating (CDR): current version and scoring rules.** *Neurology* 1993, **43**(11):2412-2414.
18. Bennett DA, Buchman AS, Boyle PA, Barnes LL, Wilson RS, Schneider JA: **Religious Orders Study and Rush Memory and Aging Project.** *J Alzheimers Dis* 2018, **64**(s1):S161-S189.
19. Carrasquillo MM, Zou F, Pankratz VS, Wilcox SL, Ma L, Walker LP, Younkin SG, Younkin CS, Younkin LH, Bisceglia GD *et al*: **Genetic variation in PCDH11X is associated with susceptibility to late-onset Alzheimer's disease.** *Nat Genet* 2009, **41**(2):192-198.
20. De Jager PL, Ma Y, McCabe C, Xu J, Vardarajan BN, Felsky D, Klein HU, White CC, Peters MA, Lodgson B *et al*: **A multi-omic atlas of the human frontal cortex for aging and Alzheimer's disease research.** *Sci Data* 2018, **5**:180142.
21. Li H, Durbin R: **Fast and accurate long-read alignment with Burrows-Wheeler transform.** *Bioinformatics* 2010, **26**(5):589-595.
22. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M *et al*: **A framework for variation discovery and genotyping using next-generation DNA sequencing data.** *Nat Genet* 2011, **43**(5):491-498.
23. Nho K, West JD, Li H, Henschel R, Bharthur A, Tavares MC, Saykin AJ: **Comparison of Multi-Sample Variant Calling Methods for Whole Genome Sequencing.** *IEEE Int Conf Systems Biol* 2014, **2014**:59-62.
24. Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK: **limma powers differential expression analyses for RNA-sequencing and microarray studies.** *Nucleic Acids Res* 2015, **43**(7):e47.
25. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, Boehnke M, Abecasis GR, Willer CJ: **LocusZoom: regional visualization of genome-wide association scan results.** *Bioinformatics* 2010, **26**(18):2336-2337.
26. Hajicek N, Keith NC, Siraliev-Perez E, Temple BR, Huang W, Zhang Q, Harden TK, Sondek J: **Structural basis for the activation of PLC-gamma isozymes by phosphorylation and cancer-associated mutations.** *Elife* 2019, **8**.
27. Laskowski RA, Stephenson JD, Sillitoe I, Orengo CA, Thornton JM: **VarSite: Disease variants and protein structure.** *Protein Sci* 2020, **29**(1):111-119.
28. Hajicek N, Charpentier TH, Rush JR, Harden TK, Sondek J: **Autoinhibition and phosphorylation-induced activation of phospholipase C-gamma isozymes.** *Biochemistry* 2013, **52**(28):4810-4819.
29. Peng Q, Malhotra S, Torchia JA, Kerr WG, Coggeshall KM, Humphrey MB: **TREM2- and DAP12-dependent activation of PI3K requires DAP10 and is inhibited by SHIP1.** *Sci Signal* 2010, **3**(122):ra38.
30. Hurley JH, Grobler JA: **Protein kinase C and phospholipase C: bilayer interactions and regulation.** *Curr Opin Struct Biol* 1997, **7**(4):557-565.
31. Falasca M, Logan SK, Lehto VP, Baccante G, Lemmon MA, Schlessinger J: **Activation of phospholipase C gamma by PI 3-kinase-induced PH domain-mediated membrane targeting.** *EMBO J* 1998, **17**(2):414-422.
32. Matsuda M, Paterson HF, Rodriguez R, Fensome AC, Ellis MV, Swann K, Katan M: **Real time fluorescence imaging of PLC gamma translocation and its interaction with the epidermal growth factor receptor.** *J Cell Biol* 2001, **153**(3):599-612.
33. Wang J, Sohn H, Sun G, Milner JD, Pierce SK: **The autoinhibitory C-terminal SH2 domain of phospholipase C-gamma2 stabilizes B cell receptor signalosome assembly.** *Sci Signal* 2014, **7**(343):ra89.

34. Everett KL, Bunney TD, Yoon Y, Rodrigues-Lima F, Harris R, Driscoll PC, Abe K, Fuchs H, de Angelis MH, Yu P *et al*: **Characterization of phospholipase C gamma enzymes with gain-of-function mutations**. *J Biol Chem* 2009, **284**(34):23083-23093.
35. Magno L, Lessard CB, Martins M, Lang V, Cruz P, Asi Y, Katan M, Bilsland J, Lashley T, Chakrabarty P *et al*: **Alzheimer's disease phospholipase C-gamma-2 (PLCG2) protective variant is a functional hypermorph**. *Alzheimers Res Ther* 2019, **11**(1):16.
36. van der Lee SJ, Conway OJ, Jansen I, Carrasquillo MM, Kleineidam L, van den Akker E, Hernandez I, van Eijk KR, Stringa N, Chen JA *et al*: **A nonsynonymous mutation in PLCG2 reduces the risk of Alzheimer's disease, dementia with Lewy bodies and frontotemporal dementia, and increases the likelihood of longevity**. *Acta Neuropathol* 2019, **138**(2):237-250.
37. Kleineidam L, Chouraki V, Prochnicki T, van der Lee SJ, Madrid-Marquez L, Wagner-Thelen H, Karaca I, Weinhold L, Wolfsgruber S, Boland A *et al*: **PLCG2 protective variant p.P522R modulates tau pathology and disease progression in patients with mild cognitive impairment**. *Acta Neuropathol* 2020.
38. Conway OJ, Carrasquillo MM, Wang X, Bredenberg JM, Reddy JS, Strickland SL, Younkin CS, Burgess JD, Allen M, Lincoln SJ *et al*: **ABI3 and PLCG2 missense variants as risk factors for neurodegenerative diseases in Caucasians and African Americans**. *Mol Neurodegener* 2018, **13**(1):53.
39. Dalmaso MC, Brusco LI, Olivar N, Muchnik C, Hanses C, Milz E, Becker J, Heilmann-Heimbach S, Hoffmann P, Prestia FA *et al*: **Transethnic meta-analysis of rare coding variants in PLCG2, ABI3, and TREM2 supports their general contribution to Alzheimer's disease**. *Transl Psychiatry* 2019, **9**(1):55.
40. Hopperton KE, Mohammad D, Trepanier MO, Giuliano V, Bazinet RP: **Markers of microglia in post-mortem brain samples from patients with Alzheimer's disease: a systematic review**. *Mol Psychiatry* 2018, **23**(2):177-198.
41. Kaiser T, Feng G: **Tmem119-EGFP and Tmem119-CreERT2 Transgenic Mice for Labeling and Manipulating Microglia**. *eNeuro* 2019, **6**(4).
42. Satoh J, Kino Y, Asahina N, Takitani M, Miyoshi J, Ishida T, Saito Y: **TMEM119 marks a subset of microglia in the human brain**. *Neuropathology* 2016, **36**(1):39-49.
43. Castillo E, Leon J, Mazzei G, Abolhassani N, Haruyama N, Saito T, Saido T, Hokama M, Iwaki T, Ohara T *et al*: **Comparative profiling of cortical gene expression in Alzheimer's disease patients and mouse models demonstrates a link between amyloidosis and neuroinflammation**. *Sci Rep* 2017, **7**(1):17762.
44. Ward LD, Kellis M: **HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants**. *Nucleic Acids Res* 2012, **40**(Database issue):D930-934.

Table 1: *PLCG2* M28L confers a higher risk of LOAD

<i>PLCG2</i> M28L (rs61749044)
p=0.047
OR=1.164 [95% CI=1.002-1.351]

OR *odds ratio*, CI *confidence interval*

Fig. 1 *PLCG2* M28L variant in PH domain

Fig 1. *PLCG2* M28L variant in PH domain.

Domain architecture of *PLCG2* drawn to scale. Genomic deletions (exon 19, Δ19, or exon 20-22, Δ20-22) or somatic mutations (R665W or S707Y) in *PLCG2* within the regulatory domains are shown in the domain architecture. *PLCG2* M28L (risk) and P522R (protective) variants are shown in the domain architecture (a) and mapped onto the structure of *PLCG2* (magenta spheres) in both homology model (b) and space-filling model (c).

N amino-terminus, C carboxyl-terminus, PH pleckstrin homology domain, EF EF hand motif, TIM TIM barrel, sPH split PH domain, nSH2 n-terminus Src Homology 2 domain, cSH2 c-terminus Src Homology 2 domain, SH3 SRC Homology 3 domain, C2 C2 domain

Fig. 1

Table 2. Demographic information of the participants included in this study

Brain region	TCX		PHG			STG			IFG			FP			CER		DLPFC	
Diagnosis	CN	AD	CN	MCI	AD	CN	MCI	AD	CN	MCI	AD	CN	MCI	AD	CN	AD	CN	AD
No. of participants	71	80	16	14	78	21	18	98	18	16	102	22	20	111	72	79	86	155
Sex (Female/Male)	35/36	49/31	11/5	7/7	54/25	16/5	11/7	65/33	12/6	7/9	69/33	16/6	11/9	75/36	35/37	47/32	47/39	109/46
Mean age at death (SD), years	82.7 (8.5)	82.6 (7.7)	83.5 (8.9)	80.6 (10.8)	85.5 (6.1)	83.8 (8.1)	82.1 (10.4)	84.5 (6.7)	83.2 (8.6)	80.8 (10.5)	85.4 (6.0)	83.1 (7.7)	83.2 (9.8)	85.4 (5.9)	82.3 (8.3)	82.5(7.7)	83.4 (5.9)	88.2 (3.1)
Mean RIN (SD)	7.7 (1.0)	8.6 (0.6)	7.1 (0.9)	6.7 (0.7)	6.5 (0.9)	6.4 (1.0)	6.4 (0.8)	6.3 (0.9)	8.7 (1.4)	8.8 (1.6)	8.0 (1.8)	6.8 (0.9)	7.0 (1.0)	6.8 (0.9)	7.7 (1.0)	8.4 (0.7)	7.3 (1.0)	6.9 (0.9)
APOE genotype (ε4+/ε4-)	62/9	38/42	14/2	9/5	57/21	17/4	12/6	65/33	16/2	8/8	72/30	19/3	13/7	70/41	62/10	38/41	77/9	91/64

TCX temporal cortex, PHG parahippocampal gyrus, STG superior temporal gyrus, IFG inferior temporal gyrus, FP frontal pole, CER cerebellum, DLPFC dorsolateral prefrontal cortex, CN cognitively normal , AD Alzheimer’s disease, MCI mild cognitive impairment, RIN RNA integrity number, APOE ε4+/- carriers and non-carriers of the APOE ε4 allele

Fig 2. Relative quantification of the *PLCG2* expression in the studied participants

Fig 2. Relative quantification of *PLCG2* expression in the studied participants. Gene expression is shown as logCPM values. (a) Temporal cortex (TCX)-Mayo, (b) Parahippocampal gyrus (PHG)-MSBB, (c) Superior temporal gyrus (STG)-MSBB, (d) Inferior prefrontal gyrus (IFG)-MSBB, (e) Cerebellum (CER)-Mayo, (f) Frontal pole (FP)-MSBB, (g) Dorsolateral prefrontal cortex (DLPFC)-ROSMAP

CN cognitively normal , AD Alzheimer's disease, MCI mild cognitive impairment

Table 3. *PLCG2* expression levels were increased in LOAD

Brain Regions	TCX	PHG			STG			IFG		
Covariate	Age and Sex	Age and Sex			Age and Sex			Age and Sex		
Contrast	CN vs. AD	CN vs. MCI	MCI vs. AD	CN vs. AD	CN vs. MCI	MCI vs. AD	CN vs. AD	CN vs. MCI	MCI vs. AD	CN vs. AD
logFC	0.27056314	0.07196964	0.473066378	0.550085612	0.127793252	0.34416553	0.45549039	0.076802391	0.29211474	0.35622471
p-value	4.56E-02	9.99E-01	6.74E-02	1.74E-03	1.00E+00	1.29E-01	2.55E-02	1.00E+00	5.72E-02	1.38E-02
Covariate	Age, Sex, and <i>APOE</i> $\epsilon 4$ status	Age, Sex, and <i>APOE</i> $\epsilon 4$ status			Age, Sex, and <i>APOE</i> $\epsilon 4$ status			Age, Sex, and <i>APOE</i> $\epsilon 4$ status		
Contrast	CN vs. AD	CN vs. MCI	MCI vs. AD	CN vs. AD	CN vs. MCI	MCI vs. AD	CN vs. AD	CN vs. MCI	MCI vs. AD	CN vs. AD
logFC	0.248525854	0.02041513	0.492722546	0.569868694	0.097484106	0.329619141	0.415487533	0.007469606	0.300240428	0.33069001
p-value	1.03E-01	1.00E+00	8.71E-02	2.20E-03	1.00E+00	1.73E-01	4.95E-02	1.00E+00	5.96E-02	2.60E-02

Brain Regions	CER	FP			DLPFC
Covariate	Age and Sex	Age and Sex			Age and Sex
Contrast	CN vs. AD	CN vs. MCI	MCI vs. AD	CN vs. AD	CN vs. AD
logFC	0.207937827	-0.188185731	0.178098774	0.02365209	-0.080816649
p-value	9.34E-02	9.85E-01	4.56E-01	9.67E-01	4.53E-01
Covariate	Age, Sex, and <i>APOE</i> $\epsilon 4$ status	Age, Sex, and <i>APOE</i> $\epsilon 4$ status			Age, Sex, and <i>APOE</i> $\epsilon 4$ status
Contrast	CN vs. AD	CN vs. MCI	MCI vs. AD	CN vs. AD	CN vs. AD
logFC	0.2365597	-0.228761625	0.18490708	0.01861041	-0.094639191
p-value	8.46E-02	9.53E-01	4.73E-01	9.82E-01	4.53E-01

Table 3 shows the *p*-value for the gene expression analysis performed by *limma* using RNA-Seq data from AMP-AD Consortium.

TCX temporal cortex, *PHG* parahippocampal gyrus, *STG* superior temporal gyrus, *IFG* inferior temporal gyrus, *FP* frontal pole, *CER* cerebellum, *DLPFC* dorsolateral prefrontal cortex, *CN* cognitively normal, *AD* Alzheimer's disease, *MCI* mild cognitive impairment, *logFC* log fold-change

Table 4. *PLCG2* expression levels were associated with amyloid plaque density and expression levels of microglia specific markers

Brain Regions (MSBB)	Frontal Pole			Parahippocampal Gyrus			Superior Temporal Gyrus			Inferior Frontal Gyrus		
	β	SE	<i>p</i> -value	β	SE	<i>p</i> -value	β	SE	<i>p</i> -value	β	SE	<i>p</i> -value
Plaque Mean Density	0.01	0.005	8.45E-02	0.027	0.006	5.22E-05	0.026	0.006	2.02E-04	0.017	0.006	2.73E-03
<i>AIF1</i>	0.258	0.051	1.09E-06	0.419	0.078	5.43E-07	0.33	0.063	8.98E-07	0.257	0.054	6.15E-06
<i>TMEM119</i>	0.518	0.057	1.28E-15	0.806	0.066	<2E-16	0.71	0.064	<2E-16	0.582	0.065	6.64E-15

Table 4 shows the β coefficient (β), standard error (SE), and *p*-value for the association analysis between *PLCG2* expression levels and amyloid plaque density or expression levels of microglia specific markers, *AIF1* and *TMEM119* by general linear models.

Fig 3. Associations of *PLCG2* expression with amyloid plaque mean density

Fig 3. Associations of *PLCG2* expression with amyloid plaque mean density.

The scatter plots show the positive association between *PLCG2* expression and plaque mean density in (a) parahippocampal gyrus, (b) superior temporal gyrus and (c) inferior prefrontal gyrus, and (d) frontal pole from MSBB cohort.

c

IFG

d

FP

Fig 4. *Plcg2* expression levels were increased in a mouse model of amyloid pathology.

Fig 4. *Plcg2* expression levels were increased in a mouse model of amyloid pathology.

Plcg2 RNA levels were assessed in cortical and hippocampal lysates from 5xFAD mice. There were significant changes in the *PLCG2* gene expression in both cortex (a) and hippocampus (b) at 4 months, 6 months, and 8 months of age (n=4-8 mice). **p<0.01; ***p<0.001

Fig. 5 eQTL analysis of *PLCG2*

a

b

Superior temporal gyrus

Fig 5. eQTL analysis of *PLCG2*.

SNP positions on chromosome 16 and their color-coded association with *PLCG2* expression from (a) superior temporal gyrus and (c) temporal cortex are plotted by LocusZoom. The SNP (rs4420523) with lowest p -value is indicated. The *PLCG2* expression levels of participants with or without rs4420523 are showed as both eQTL residual expression and logCPM value from superior temporal gyrus (b) and temporal cortex (d). The numbers of participants are shown below the genotypes. The results of single-tissue eQTL analysis for rs4420523 are presented in (e).

NES normalized effect size, chr16 chromosome 16, eQTL expression quantitative trait loci analysis, Major allele A, Minor allele C

c **d**

e

Figures

Figure 1

PLCG2 M28L variant in PH domain. Domain architecture of PLCG2 drawn to scale. Genomic deletions (exon 19, $\Delta 19$, or exon 20-22, $\Delta 20-22$) or somatic mutations (R665W or S707Y) in PLCG2 within the regulatory domains are shown in the domain architecture. PLCG2 M28L (risk) and P522R (protective) variants are shown in the domain architecture (a) and mapped onto the structure of PLCG2 (magenta spheres) in both homology model (b) and space-filling model (c). N amino-terminus, C carboxyl-terminus, PH pleckstrin homology domain, EF EF hand motif, TIM TIM barrel, sPH split PH domain, nSH2 nterminus

Src Homology 2 domain, cSH2 c-terminus Src Homology 2 domain, SH3 SRC Homology 3 domain, C2 C2 domain

Figure 2

Relative quantification of PLCG2 expression in the studied participants. Gene expression is shown as logCPM values. (a) Temporal cortex (TCX)-Mayo, (b) Parahippocampal gyrus (PHG)-MSBB, (c) Superior temporal gyrus (STG)-MSBB, (d) Inferior prefrontal gyrus (IFG)-MSBB, (e) Cerebellum (CER)-Mayo, (f) Frontal pole (FP)-MSBB, (g) Dorsolateral prefrontal cortex (DLPFC)-ROSMAP CN cognitively normal , AD Alzheimer's disease, MCI mild cognitive impairment

Figure 3

Associations of PLCG2 expression with amyloid plaque mean density. The scatter plots show the positive association between PLCG2 expression and plaque mean density in (a) parahippocampal gyrus, (b) superior temporal gyrus and (c) inferior prefrontal gyrus, and (d) frontal pole from MSBB cohort.

Figure 5

eQTL analysis of PLCG2. SNP positions on chromosome 16 and their color-coded association with PLCG2 expression from (a) superior temporal gyrus and (c) temporal cortex are plotted by LocusZoom. The SNP (rs4420523) with lowest p-value is indicated. The PLCG2 expression levels of participants with or without rs4420523 are showed as both eQTL residual expression and logCPM value from superior temporal gyrus (b) and temporal cortex (d). The numbers of participants are shown below the genotypes. The results of single-tissue eQTL analysis for rs4420523 are presented in (e). NES normalized effect size, chr16 chromosome 16, eQTL expression quantitative trait loci analysis, Major allele A, Minor allele C