Title:

Novel rare coding variants in *PLCG2*, *ABI3* and *TREM2* implicate microglial-mediated innate immunity in Alzheimer's disease.

Running:

Rare coding variation in *PLCG2*, *ABI3* and *TREM2* associate with Alzheimer's disease.

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Introduction (150 words) = 148

We identified rare coding variants associated with Alzheimer's disease (AD) in a 3-stage case-control study of 85,133 subjects. In stage 1, 34,174 samples were genotyped using a whole-exome microarray. In stage 2, we tested associated variants (P<1x10^{-4}) in 35,962 independent samples using *de novo* genotyping and imputed genotypes. In stage 3, we used *in silico* analysis to test the most significant stage 2 associations (P<5x10^{-8}) in a further 14,997 samples. We observed 3 novel genome-wide significant (GWS) AD associated coding variants; a protective variant in *PLCG2* (rs72824905/p.P522R, P=5.38x10^{-10}, OR=0.68, MAF$_{cases}$=0.0059, MAF$_{controls}$=0.0093), a risk variant in *ABI3* (rs616338/p.S209F, P=4.56x10^{-10}, OR=1.43, MAF$_{cases}$=0.011, MAF$_{controls}$=0.008), and a novel GWS variant in *TREM2* (rs143332484/p.R62H, P=1.55x10^{-14}, OR=1.67, MAF$_{cases}$=0.0143, MAF$_{controls}$=0.0089). These protein-coding changes are in genes highly expressed in microglia and highlight an immune-related protein-protein interaction network enriched for previously identified AD risk genes. Thus, the microglia-mediated innate immune response contributes directly to AD development.

Text (1500 words) = 1500

Late-onset AD (LOAD) has a significant genetic component (h^2=58-79%[1]). Nearly 30 common LOAD susceptibility loci[2–12] are known, and risk is significantly polygenic[13]. However, these loci explain only a proportion of disease heritability. Rare variants also contribute to disease risk[14–17]. Recent sequencing studies identified a number of genes with rare AD associated, and candidate susceptibility variants [9–11,18–24]. Our approach to rare-variant discovery is to use powerful samples and genome-wide micro-arrays targeting known exome variants, a cost-effective alternative to *de novo* sequencing with proven utility[25–29].

We applied a 3-stage design (SFigure1) using subjects from the International Genomics of Alzheimer's Project (IGAP)(Table1, STables1&2). In stage 1, 16,097 LOAD cases and 18,077 cognitively normal elderly controls were genotyped using the Illumina HumanExome microarray. Data from multiple consortia were combined in a single variant meta-analysis (Supplement) assuming an additive model. In total, 241,551 variants passed quality-control (STable3). Of these 203,902 were polymorphic, 26,947 were common (minor allele frequency (MAF)≥5%), and 176,955 were low frequency or rare (MAF<5%). We analyzed common variants using a logistic regression model in each sample cohort and combined data using METAL[30]. Rare and low frequency variants were analyzed using the score test and data combined with SeqMeta[31] (SFigure2).

We reviewed cluster plots for variants showing association (P<1x10^{-4}) and identified 43 candidate variants (STable4) exclusive of known risk loci (STable5). Stage 2 tested these for association in 14,041 LOAD cases and

21,921 controls, using *de novo* and imputation derived genotypes (Supplement). We carried forward single nucleotide variants (SNVs) with GWS associations and consistent directions of effect to stage 3 where genotypes for 6,652 independent cases and 8,345 controls were determined using the Haplotype Reference Consortium resource[32,33] (Supplement).

We identified four rare coding variants with GWS association signals with LOAD ($P<5x10^{-8}$)(Table2, STables6&7). The first with missense variant p.P522R ($P=5.38x10^{-10}$, OR=0.68) in *Phospholipase C Gamma 2* (*PLCG2*)(Table2, Figure1a, STable8, SFigure3). This variant is associated with decreased risk of LOAD, showing a MAF of 0.0059 in cases and 0.0093 in controls. The reference allele (p.P522) is conserved across several species (SFigure4). Gene-wide analysis showed nominal evidence for association at $P=1.52x10^{-4}$ (STables9&10) and we found no other independent association at this gene (SFigure5).

The second novel association is missense change p.S209F ($P=4.56x10^{-10}$, OR=1.43) in *B3 domain-containing transcription factor ABI3* (*ABI3*). The p.F209 variant shows consistent evidence for increasing LOAD risk across all stages, with a MAF of 0.011 in cases and 0.008 in controls (Table2, Figure1b, STable11, SFigure6). The reference allele is conserved across multiple species (SFigure7). Gene-wide analysis showed nominal evidence of association ($P=5.22x10^{-5}$)(STables9&10). The *B4GALNT2* gene, adjacent to *ABI3,* contained an independent suggestive association (SFigure8), but this failed to replicate in subsequent stages ($P_{combined}=1.68x10^{-4}$)(STable6).

Following reports of suggestive association with LOAD[34], we report the first evidence for GWS association at *TREM2* coding variant p.R62H ($P=1.55x10^{-14}$, OR=1.67), with a MAF of 0.0143 in cases and 0.0089 in controls (Table2, Figure1c, STable12, SFigures9&10). We also observed evidence for the

previously reported[9,11] *TREM2* rare variant p.R47H (Table2). These variants are not in linkage disequilibrium (STable13) and conditional analyses confirmed that p.R62H and p.R47H are independent risk variants (SFigure11). Gene-wide analysis showed a GWS association (P_{SKAT}=1.42x10^{-15})(STables9&10). Removal of p.R47H and p.R62H variants from the analysis diminished the gene-wide association but the signal remains interesting (P_{SKAT-O}=6.3x10^{-3}, P_{Burden}=4.1x10^{-3}). No single SNV was responsible for the remaining gene-wide association (STable12, SFigure11) suggesting that there are additional risk variants in *TREM2*. We previously reported a common variant LOAD association near *TREM2*, in a GWAS of cerebrospinal fluid tau and P-tau[35]. We have also observed a different suggestive common variant signal in another LOAD case-control study (P=6.3x10^{-7})[2].

We undertook a pathway analysis of the eight gene clusters previously identified as enriched for common variants[36] (Supplement, STable14). Here we considered only rare variants (MAF<1%) and used Fisher's method to combine gene-wide p-values for all genes in each cluster. After correction, we observed enrichment for immune response (P=8.64x10^{-3}), cholesterol transport (P=3.84x10^{-5}), hemostasis (P=2.10x10^{-3}), Clathrin/AP2 adaptor complex (P=9.20x10^{-4}) and protein folding (P=0.02).

Previous analysis of normal brain co-expression networks identified 4 gene modules that are enriched for common variants associated with LOAD risk[2,36]. These 4 modules are enriched for immune response genes. There are 151 genes present in 2 or more of these 4 modules that show strong enrichment (P=4.0x10^{-6}) for LOAD-associated common variants[2]. Gene-set analysis of these 151 genes showed significant association with rare variants (MAF<1%)(STable14, P=1.17x10^{-6}). From these, we identified a subset of 56

genes, including *PLCG2*, *ABI3* and *TREM2*, connected by high-quality protein-protein interactions[37] (Figure2a)(Supplement). This subset is strongly enriched for association signals from both the previous common variant analysis (P=5.0x10^{-6}, STable15) and this rare variant gene-set analysis (P=1.08x10^{-7}, STable14). The remaining 95 genes do not show enrichment for association in this study or in the previous study (STables14&15), suggesting that the 56-gene (STable16) network is driving the enrichment observed in the common-variant GWAS.

TREM2, *ABI3* and *PLCG2* have a common expression pattern in human brain cortex, with high expression in microglia cells and limited expression in neurons, oligodendrocytes, astrocytes and endothelial cells (Figure2b, SFigure12)[38]. Other known LOAD loci with the same expression pattern include *SORL1,* the *MS4A* gene cluster, and *HLA-DRB1*. *PLCG2*, *ABI3*, and *TREM2* are up-regulated in LOAD human cortex and in two APP mouse models. However, when corrected for levels of other microglia genes, these changes in expression appear to be related to microgliosis (STables17&18).

PLCG2 (SFigure13) encodes a transmembrane signaling enzyme (PLCγ2) that hydrolyses the membrane phospholipid PIP2 (1-phosphatidyl-1D-myo-inositol 4,5-bisphosphate) to secondary messengers IP3 (myo-inositol 1,4,5-trisphosphate) and DAG (diacylglycerol). IP3 is released into the cytosol and acts at the endoplasmic reticulum where it binds to ligand-gated ion channels to increase cytoplasmic Ca^{2+}. DAG remains bound to the plasma membrane where it activates two major signaling molecules, protein kinase C (PKC) and Ras guanyl nucleotide-releasing proteins (RasGRPs), which initiate the NF-κB and mitogen-activated protein kinase (MAPK) pathways. While the IP3/DAG/Ca+2 signaling pathway is active in many cells and tissues, in brain,

PLCG2 is primarily expressed in microglial cells. *PLCG2* variants also cause Antibody Deficiency and Immune Dysregulation (PLAID) and Autoinflammation and PLAID (APLAID)[39]. Genomic deletions (PLAID) and missense mutations (NPLAID) affect the cSH2 autoinhibitory regulatory region. The result is a complex mix of loss and gain of function in cellular signalling[39].

Functional annotation (STable19) suggests *ABI3* (SFigure14) plays a role in the innate immune response via interferon-mediated signaling[40]. *ABI3* is co-expressed with *INPP5D* (P=2.2x10^{-10}), a gene previously implicated in LOAD risk[2]. ABI3 plays a significant role in actin cytoskeleton organization through participation in the WAVE2 complex[41], a complex that regulates multiple pathways leading to T-cell activation[42].

TREM2 encodes a transmembrane receptor present in the plasma membrane of brain microglia (SFigure15). TREM2 protein forms an immune-receptor-signaling complex with DAP12. Receptor activation results in activation of Syk and ZAP70 signaling which in turn activates PI3K activity and influences PLCɣ2 activity[43]. In microglia, TREM2-DAP12 induces an M2-like activation[44] and participates in recognition of membrane debris and amyloid deposits resulting in microglial activation and proliferation[45–47]. When *TREM2* knockout (KO) or *TREM2* heterozygous KO mice are crossed with *APP*-transgenics that develop plaques, the size and number of microglia associated with plaques are markedly reduced[46,47]. *TREM2* risk variants are located within exon 2, which is predicted to encode the conserved ligand binding extracellular region of the protein. Any disruption in this region may attenuate or abolish TREM2 signaling, resulting in the loss or decrease in TREM2 function[47].

This 56-gene interaction network identified here is enriched in immune response genes and includes *TREM2*, *PLCG2*, *ABI3*, *SPI1*, *INPP5D*, *CSF1R*, *SYK*

and *TYROBP* (Figure2a). *SPI1* is a central transcription factor in microglial activation state that has a significant gene-wide association with AD[5] and is in the proximity of GWS signals identified by IGAP[2]. Loss-of function mutations in *CSF1R* cause hereditary diffuse leukoencephalopathy with spheroids, a white matter disease related to microglial dysfunction[48]. Activated microglial cells surround plaques[49,50], a finding consistently observed in AD brain and AD transgenic mouse models[51]. In AD mouse model brain, synaptic pruning associates with activated microglial signalling[52]. Pharmacological targeting of CSF1R inhibits microglial proliferation and shifts the microglial inflammatory profile to an anti-inflammatory phenotype in murine models[53]. SYK regulates Aβ production and tau hyperphosphorylation[54], is affected by the INPP5D/CD2AP complex[55] encoded by two LOAD associated genes[2], and mediates phosphorylation of PLCG2[56]. Notably, the anti-hypertensive drug Nilvadipine, currently in a phase III AD clinical trial, targets *SYK* as well as *TYROBP,* a hub gene in an AD-related brain expression network[38],that encodes the TREM2 complex protein DAP12.

We identified three rare coding variants in *PLCG2, ABI3* and *TREM2* with GWS associations with LOAD that are part of a common innate immune response. Our network analysis also suggests that the adaptive immune system may be involved in AD pathogenesis, but further work is needed to strengthen this conclusion. Our findings show that the microglial response in LOAD is directly part of a causal pathway leading to disease and is not simply a downstream consequence of neurodegeneration[46,47,57,58]. PLCɣG2, as an enzyme, represents the first classically drug-able target to emerge from LOAD genetic studies.

Bibliography

1. Gatz, M. *et al.* Role of genes and environments for explaining Alzheimer disease. *Arch. Gen. Psychiatry* **63,** 168–174 (2006).
2. Lambert, J. C. *et al.* Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat. Genet.* **45,** 1452–1458 (2013).
3. Harold, D. *et al.* Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. *Nat. Genet.* **41,** 1088–1093 (2009).
4. Lambert, J.-C. *et al.* Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. *Nat. Genet.* **41,** 1094–1099 (2009).
5. Escott-Price, V. *et al.* Gene-wide analysis detects two new susceptibility genes for Alzheimer's disease. *PloS One* **9,** e94661 (2014).
6. Hollingworth, P. *et al.* Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat. Genet.* **43,** 429–435 (2011).
7. Naj, A. C. *et al.* Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat. Genet.* **43,** 436–441 (2011).
8. Ruiz, A. *et al.* TOWARD FINE MAPPING AND FUNCTIONAL CHARACTERIZATION OF GENOME-WIDE ASSOCIATION STUDY-IDENTIFIED LOCUS RS74615166 (TRIP4) FOR ALZHEIMER'S DISEASE. *Alzheimers Dement. J. Alzheimers Assoc.* **10,** P257–P258 (2014).
9. Jonsson, T. *et al.* Variant of TREM2 Associated with the Risk of Alzheimer's Disease. *N. Engl. J. Med.* **368,** 107–116 (2013).
10. Jonsson, T. *et al.* A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* **488,** 96–99 (2012).
11. Guerreiro, R. *et al.* TREM2 Variants in Alzheimer's Disease. *N. Engl. J. Med.* **368,** 117–127 (2013).

12. Seshadri, S. *et al.* Genome-wide analysis of genetic loci associated with Alzheimer disease. *JAMA* **303,** 1832–1840 (2010).
13. Escott-Price, V. *et al.* Common polygenic variation enhances risk prediction for Alzheimer's disease. *Brain J. Neurol.* **138,** 3673–3684 (2015).
14. Bodmer, W. & Bonilla, C. Common and rare variants in multifactorial susceptibility to common diseases. *Nat. Genet.* **40,** 695–701 (2008).
15. Pritchard, J. K. Are rare variants responsible for susceptibility to complex diseases? *Am. J. Hum. Genet.* **69,** 124–137 (2001).
16. Schork, N. J., Murray, S. S., Frazer, K. A. & Topol, E. J. Common vs. rare allele hypotheses for complex diseases. *Curr. Opin. Genet. Dev.* **19,** 212–219 (2009).
17. Surakka, I. *et al.* The impact of low-frequency and rare variants on lipid levels. *Nat. Genet.* **47,** 589–597 (2015).
18. Vardarajan, B. N. *et al.* Coding mutations in SORL1 and Alzheimer disease. *Ann. Neurol.* **77,** 215–227 (2015).
19. Vardarajan, B. N. *et al.* Rare coding mutations identified by sequencing of Alzheimer disease genome-wide association studies loci. *Ann. Neurol.* **78,** 487–498 (2015).
20. Steinberg, S. *et al.* Loss-of-function variants in ABCA7 confer risk of Alzheimer's disease. *Nat. Genet.* **47,** 445–447 (2015).
21. Logue, M. W. *et al.* Two rare AKAP9 variants are associated with Alzheimer's disease in African Americans. *Alzheimers Dement. J. Alzheimers Assoc.* **10,** 609–618.e11 (2014).
22. Jun, G. *et al.* PLXNA4 is associated with Alzheimer disease and modulates tau phosphorylation. *Ann. Neurol.* **76,** 379–392 (2014).
23. Hunkapiller, J. *et al.* A rare coding variant alters UNC5C function and predisposes to Alzheimer's disease. *Alzheimers Dement. J. Alzheimers Assoc.* **9,** P853 (2013).

24. Wetzel-Smith, M. K. *et al.* A rare mutation in UNC5C predisposes to late-onset Alzheimer's disease and increases neuronal cell death. *Nat. Med.* **20,** 1452–1457 (2014).
25. Richards, A. L. *et al.* Exome arrays capture polygenic rare variant contributions to schizophrenia. *Hum. Mol. Genet.* (2016). doi:10.1093/hmg/ddv620
26. Wessel, J. *et al.* Low-frequency and rare exome chip variants associate with fasting glucose and type 2 diabetes susceptibility. *Nat. Commun.* **6,** 5897 (2015).
27. Igartua, C. *et al.* Ethnic-specific associations of rare and low-frequency DNA sequence variants with asthma. *Nat. Commun.* **6,** 5965 (2015).
28. Tachmazidou, I. *et al.* A rare functional cardioprotective APOC3 variant has risen in frequency in distinct population isolates. *Nat. Commun.* **4,** 2872 (2013).
29. Huyghe, J. R. *et al.* Exome array analysis identifies new loci and low-frequency variants influencing insulin processing and secretion. *Nat. Genet.* **45,** 197–201 (2013).
30. Willer, C. J., Li, Y. & Abecasis, G. R. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* **26,** 2190–2191 (2010).
31. R Development Core Team. *R: A language and environment for statistical computing*. (R Foundation for Statistical Computing).
32. Das, S. *et al.* Imputation server: next generation genotype imputation service. *Nat. Genet.*
33. McCarthy, S. *et al.* A reference panel of 64,976 haplotypes for genotype imputation. *bioRxiv* 035170 (2015). doi:10.1101/035170
34. Jin, S. C. *et al.* Coding variants in TREM2 increase risk for Alzheimer's disease. *Hum. Mol. Genet.* **23,** 5838–5846 (2014).
35. Cruchaga, C. *et al.* GWAS of cerebrospinal fluid tau levels identifies risk variants for Alzheimer's disease. *Neuron* **78,** 256–268 (2013).

36. Zhang, Y. *et al.* Purification and Characterization of Progenitor and Mature Human Astrocytes Reveals Transcriptional and Functional Differences with Mouse. *Neuron* **89,** 37–53 (2016).
37. Milner, J. D. PLAID: a Syndrome of Complex Patterns of Disease and Unique Phenotypes. *J. Clin. Immunol.* **35,** 527–530 (2015).
38. Fairfax, B. P. *et al.* Innate Immune Activity Conditions the Effect of Regulatory Variants upon Monocyte Gene Expression. *Science* **343,** 1246949 (2014).
39. Sekino, S. *et al.* The NESH/Abi-3-based WAVE2 complex is functionally distinct from the Abi-1-based WAVE2 complex. *Cell Commun. Signal. CCS* **13,** (2015).
40. Nolz, J. C. *et al.* The WAVE2 Complex Regulates Actin Cytoskeletal Reorganization and CRAC-Mediated Calcium Entry during T Cell Activation. *Curr. Biol. CB* **16,** 24–34 (2006).
41. Xing, J., Titus, A. R. & Humphrey, M. B. The TREM2-DAP12 signaling pathway in Nasu-Hakola disease: a molecular genetics perspective. *Res. Rep. Biochem.* **5,** 89–100 (2015).
42. Neumann, H. & Takahashi, K. Essential role of the microglial triggering receptor expressed on myeloid cells-2 (TREM2) for central nervous tissue immune homeostasis. *J. Neuroimmunol.* **184,** 92–99 (2007).
43. Painter, M. M. *et al.* TREM2 in CNS homeostasis and neurodegenerative disease. *Mol. Neurodegener.* **10,** 43 (2015).
44. Ulrich, J. D. *et al.* In vivo measurement of apolipoprotein E from the brain interstitial fluid using microdialysis. *Mol. Neurodegener.* **8,** 13 (2013).
45. Wang, Y. *et al.* TREM2 lipid sensing sustains the microglial response in an Alzheimer's disease model. *Cell* **160,** 1061–1071 (2015).

46. International Genomics of Alzheimer's Disease Consortium (IGAP). Convergent genetic and expression data implicate immunity in Alzheimer's disease. *Alzheimers Dement. J. Alzheimers Assoc.* **11,** 658–671 (2015).

47. Jensen, L. J. *et al.* STRING 8—a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Res.* **37,** D412–D416 (2009).

48. Rademakers, R. *et al.* Mutations in the colony stimulating factor 1 receptor (CSF1R) gene cause hereditary diffuse leukoencephalopathy with spheroids. *Nat. Genet.* **44,** 200–205 (2012).

49. Perlmutter, L. S., Barron, E. & Chui, H. C. Morphologic association between microglia and senile plaque amyloid in Alzheimer's disease. *Neurosci. Lett.* **119,** 32–36 (1990).

50. Wisniewski, H. M., Wegiel, J., Wang, K. C. & Lach, B. Ultrastructural studies of the cells forming amyloid in the cortical vessel wall in Alzheimer's disease. *Acta Neuropathol. (Berl.)* **84,** 117–127 (1992).

51. Schwab, C., Klegeris, A. & McGeer, P. L. Inflammation in transgenic mouse models of neurodegenerative disorders. *Biochim. Biophys. Acta BBA - Mol. Basis Dis.* **1802,** 889–902 (2010).

52. Hong, S. *et al.* Complement and microglia mediate early synapse loss in Alzheimer mouse models. *Science* aad8373 (2016). doi:10.1126/science.aad8373

53. Olmos-Alonso, A. *et al.* Pharmacological targeting of CSF1R inhibits microglial proliferation and prevents the progression of Alzheimer's-like pathology. *Brain* awv379 (2016). doi:10.1093/brain/awv379

54. Paris, D. *et al.* The Spleen Tyrosine Kinase (syk) Regulates Alzheimer's Aβ Production and Tau Hyperphosphorylation. *J. Biol. Chem.* jbc.M114.608091 (2014). doi:10.1074/jbc.M114.608091

55. Bao, M. *et al.* CD2AP/SHIP1 complex positively regulates plasmacytoid dendritic cell receptor signaling by inhibiting the E3 ubiquitin ligase Cbl. *J. Immunol. Baltim. Md 1950* **189,** 786–792 (2012).

56. Kurosaki, T. & Tsukada, S. BLNK: Connecting Syk and Btk to Calcium Signals. *Immunity* **12,** 1–5 (2000).

57. Wang, Y. *et al.* TREM2-mediated early microglial response limits diffusion and toxicity of amyloid plaques. *J. Exp. Med.* **213,** 667–675 (2016).

58. Yuan, P. *et al.* TREM2 Haplodeficiency in Mice and Humans Impairs the Microglia Barrier Function Leading to Decreased Amyloid Compaction and Severe Axonal Dystrophy. *Neuron* **90,** 724–739 (2016).