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GWAS of longitudinal amyloid accumulation on ^{18}F-florbetapir PET in Alzheimer's disease implicates microglial activation gene *IL1RAP*

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Brain amyloid deposition is thought to be a seminal event in Alzheimer's disease. To identify genes influencing Alzheimer's disease pathogenesis, we performed a genome-wide association study of longitudinal change in brain amyloid burden measured by ^{18}F-florbetapir PET. A novel association with higher rates of amyloid accumulation independent from *APOE* (apolipoprotein E) $\varepsilon 4$ status was identified in *IL1RAP* (interleukin-1 receptor accessory protein; rs12053868-G; $P = 1.38 \times 10^{-9}$) and was validated by deep sequencing. *IL1RAP* rs12053868-G carriers were more likely to progress from mild cognitive impairment to Alzheimer's disease and exhibited greater longitudinal temporal cortex atrophy on MRI. In independent cohorts rs12053868-G was associated with accelerated cognitive decline and lower cortical ^{11}C-PBR28 PET signal, a marker of microglial activation. These results suggest a crucial role of activated microglia in limiting amyloid accumulation and nominate the IL-1/IL1RAP pathway as a potential target for modulating this process.

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Abbreviations: ADNI = Alzheimer's Disease Neuroimaging Initiative; GWAS = genome-wide association study; MCI = mild cognitive impairment; SNP = single nucleotide polymorphism; SUV(R) = standardized uptake value (ratio)

Introduction

Deposition of amyloid-β in the brain is thought to be a necessary early step in the development of Alzheimer's disease, a progressive and highly prevalent neurodegenerative disorder with substantial societal burdens (Karran *et al.*, 2011; Jack *et al.*, 2013*a*). Existing prospective studies suggest that brain amyloid accumulation occurs over decades, preceding the onset of clinical symptoms and subsequently contributing to clinical progression (Villemagne *et al.*, 2013; Doraiswamy *et al.*, 2014; Huijbers *et al.*, 2015). However, the mechanisms underlying amyloid accumulation and clearance in Alzheimer's disease are not fully understood.

Pathogenic mutations causing rare, early-onset forms of Alzheimer's disease have been described in three genes involved in amyloidogenesis, *APP* (amyloid precursor protein), *PSEN1* (presenilin 1), and *PSEN2* (presenilin 2) (Bettens *et al.*, 2013). For late-onset Alzheimer's disease, the strongest known genetic risk factor is the *APOE* ε4 allele (Corder *et al.*, 1993). Several mechanisms have been proposed relating *APOE* ε4 to enhanced aggregation and reduced clearance of brain amyloid (Kim *et al.*, 2009). However, *APOE* ε4 is neither necessary nor sufficient for development of amyloid pathology or incident Alzheimer's disease, suggesting that other contributing factors remain to be discovered.

With the development of radiotracers allowing for non-invasive *in vivo* detection of amyloid plaque burden in large samples (Clark *et al.*, 2012), amyloid PET has become an established endophenotype used in cross-sectional studies to relate genetic variants to Alzheimer's disease pathology (Swaminathan *et al.*, 2012; Rhinn *et al.*, 2013; Shulman *et al.*, 2013; Lim *et al.*, 2014; Ramanan *et al.*, 2014*b*). We hypothesized that genetic factors would also modulate the rate of amyloid accumulation over time. We therefore performed a genome-wide association study (GWAS) of longitudinal change in brain amyloid burden measured by ^{18}F-florbetapir PET to identify novel genetic influences on the pathogenesis and trajectory of Alzheimer's disease.

Materials and methods

Subjects and phenotypes

The Alzheimer's Disease Neuroimaging Initiative (ADNI, Weiner *et al.*, 2010), Indiana Memory and Aging Study (IMAS; Ramanan *et al.*, 2014*a*), Rush Memory and Aging Project (MAP, Bennett *et al.*, 2012*b*), and Religious Orders Study (ROS; Bennett *et al.*, 2012*a*) are longitudinal studies of older adults representing clinical stages along the continuum from normal ageing to Alzheimer's disease. All participants provided written informed consent, and study protocols were approved by each site's institutional review board.

^{18}F-Florbetapir PET imaging was performed at baseline and 2-year follow-up for participants enrolled in the ADNI GO and 2 phases. Image acquisition and preprocessing were performed as described previously (Jagust *et al.*, 2010). Tracer uptake was normalized to average uptake values from an atlas-based composite reference region expected not to exhibit amyloid pathology (composed of the cerebral white matter degraded to 0.7, brainstem, and whole cerebellum). This normalization yielded standardized uptake value ratio (SUVR) images (Schmidt *et al.*, 2014). As described previously, the mean SUVR for a customized composite region was obtained to represent a global cortical measure of amyloid burden at each time point (Risacher *et al.*, 2015). The annualized per cent change in global cortical SUVR at 2-year follow-up compared to baseline was used as the main quantitative phenotype for genetic analysis. Extreme outliers (annualized per cent change > three standard deviations from the sample mean) were excluded to limit the potential for spurious associations.

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For *post hoc* analyses, baseline amyloid status (positive versus negative) was determined for each participant as described previously (Risacher *et al.*, 2015).

^{11}C-PBR28 PET imaging was performed for a subset of IMAS participants as described previously (Yoder *et al.*, 2013). The sample analysed included cognitively normal older adults ($n = 7$), older adults with cognitive complaints in the absence of significant cognitive deficits ($n = 5$), participants with mild cognitive impairment (MCI, $n = 7$), and clinical Alzheimer's disease participants ($n = 6$). SUV images were created by normalizing each voxel by the injected dose of ^{11}C-PBR28 per total body weight. Mean SUV data were extracted for the frontal, parietal, temporal, limbic, and occipital lobes. The average SUV for these five regions was calculated to represent a global cortical index of activated microglia for use as a quantitative phenotype. *TSPO* (translocator protein, 18 kDa) rs6971 genotype was used to delineate participants with high, mixed, and low affinity states of the TSPO binding site, as ^{11}C-PBR28 is highly sensitive to these states (Kreisl *et al.*, 2013). For genetic analyses, participants with low affinity TSPO binding sites (rs6971-TT) were excluded and rs6971 genotype (CC versus TC) was included as a covariate (Yoder *et al.*, 2013).

For ADNI participants, structural MRI scans from baseline and 2-year follow-up visits were downloaded (www.adni.loni.usc.edu) and processed as described previously (Risacher *et al.*, 2010) using FreeSurfer, version 5.1. For each scan, mean thickness values from the left and right temporal cortex regions were averaged to create a measure of bilateral temporal cortex thickness. The annualized per cent change in bilateral temporal cortex thickness at 2-year follow-up compared to baseline was calculated for use in genetic analyses.

Verbal episodic memory performance was assessed at baseline and 2-year follow-up for participants from ADNI, MAP and ROS using delayed recall of logical memory prose passages from the Wechsler Memory Scale-Revised. For genetic analyses, the 2-year difference in delayed recall score was used as the phenotype and baseline age, gender and education were included as covariates.

Genotyping and imputation

GWAS data for ADNI participants were obtained and processed as described previously (Ramanan *et al.*, 2014*b*). Briefly, genotyping was performed per manufacturer's protocol using blood genomic DNA samples and Illumina GWAS arrays (610-Quad, OmniExpress, or HumanOmni2.5-4v1). The single nucleotide polymorphisms (SNPs) characterizing *APOE* $\varepsilon2/\varepsilon3/\varepsilon4$ status (rs429358 and rs7412) were genotyped separately and merged with the array data sets as described previously (Saykin *et al.*, 2010, 2015). Genotype data underwent stringent quality control including identity checks, sample exclusion for call rate $<95\%$, and SNP exclusion for call rate $<95\%$, Hardy-Weinberg $P < 1 \times 10^{-6}$, or minor allele frequency (MAF) $<1\%$.

MaCH (Li *et al.*, 2010), Minimac (Howie *et al.*, 2012), and haplotype patterns from the 1000 Genomes Project reference panel were used to impute SNP genotypes not directly assayed by the GWAS arrays. Imputation was performed as described previously (Nho *et al.*, 2013; Ramanan *et al.*, 2014*b*). Following additional quality control (SNP call rate $< 95\%$, Hardy-Weinberg $P < 1 \times 10^{-6}$) and frequency filtering (MAF $< 5\%$), 6 112 217 genotyped and imputed SNPs were available for analysis. Six participant pairs exhibited significant relatedness (PI_HAT > 0.5) and therefore one individual from each pair was randomly selected for exclusion. For additional studies in IMAS, MAP and ROS, identical procedures were used to impute the specific SNPs required for analysis (Chibnik *et al.*, 2011; Ramanan *et al.*, 2014*a*).

Whole genome sequencing was obtained from blood genomic DNA samples for a subset of the ADNI sample. Sequencing was performed using the Illumina HiSeq2000 system through paired-end read chemistry and read lengths of 100 base pairs. The resulting Illumina GSEQ files were converted into FASTQ files for introductory evaluation using FastQC (Andrews, 2010). Initial alignment to the reference human genome (NCBI build 37.72) for bases with Phred quality > 15 was completed using the Burrows-Wheeler Alignment tool (Li and Durbin, 2009). Suspicious reads were locally realigned and the Illumina base calling quality scores were recalibrated to account for effects of sequencing technology and machine cycle. These realigned reads were written to a BAM file to be used for multi-sample variant calling using the GATK HaplotypeCaller (DePristo *et al.*, 2011). ANNOVAR (Wang *et al.*, 2010*b*) was used to annotate variants passing recommended quality criteria (Van der Auwera *et al.*, 2013). Participants with poor quality variant calls (concordance rate $< 99\%$ for SNPs genotyped through both sequencing and the Illumina HumanOmni2.5-4v1 array) were excluded from further analysis.

To limit potential effects of population stratification, all genetic analyses were restricted to non-Hispanic white participants as determined by multidimensional clustering using PLINK. To verify appropriate control for population structure, EIGENSTRAT was used to generate principal component eigenvectors for use as covariates in *post hoc* analyses.

Statistical analysis

GWAS was performed using linear regression under an additive genetic model in PLINK. Baseline age and gender were included as covariates in the GWAS. A conservative significance threshold ($P < 5 \times 10^{-8}$) was used based on a Bonferroni correction of one million independent tests (Pe'er *et al.*, 2008). Manhattan and Q-Q plots were generated with Haploview and regional association plots were generated with LocusZoom. The genome partitioning algorithm GCTA (Yang *et al.*, 2011) was used to estimate the proportion of phenotypic variance explained by all SNPs in the GWAS. Power calculations and curves were obtained using GWAPower (Feng *et al.*, 2011).

Significant associations were further investigated using sequence data from a subset of the GWAS sample. Common variants in *IL1RAP*, defined as having MAF $\geqslant 1 / \sqrt{(2n)} = 0.034$ (Ionita-Laza *et al.*, 2013), were analysed using linear regression under an additive genetic model in PLINK. SKAT (Ionita-Laza *et al.*, 2013) was used to perform association testing of low-frequency and rare *IL1RAP* variants (MAF < 0.034). Pairwise linkage disequilibrium calculations were obtained for selected SNP pairs using PLINK.

Complementary approaches were used to extend the GWAS findings. GATES (KGG software version 2.5) (Li *et al.*, 2011) was used to calculate a summary *P*-value for each gene (including a default $\pm$ 5 kb window to account for putative regulatory regions) based on its size, linkage disequilibrium

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structure and constituent GWAS SNP associations. GSA-SNP (Nam *et al.*, 2010; Ramanan *et al.*, 2012*a*) was used to identify biological pathways exhibiting enrichment of association in the GWAS. Pathway definitions from three resources (Biocarta, KEGG and Reactome) were downloaded from the Molecular Signatures Database, version 4.0 and analysis was restricted to pathways containing 5–100 genes to limit the potential for size-influenced spurious associations (Ramanan *et al.*, 2012*b*). Pathways with false discovery rate (FDR)-corrected $P < 0.05$ were considered as significant.

Statistical Parametric Mapping 8 (Wellcome Trust Centre for Neuroimaging) was used to perform voxel-wise analysis of the effect of *IL1RAP* rs12053868 on longitudinal change in ^{18}F-florbetapir PET amyloid burden. A two-way ANCOVA was performed using rs12053868 genotype and scan visit (baseline versus 2-year follow-up) as the independent variables and age, gender, baseline diagnosis, *APOE* ε4 status (positive versus negative), and time between PET scans as covariates. To specify an additive model, we *a priori* (based on the GWAS results) coded the analysis vector as [positive change in AA] < [positive change in GA] < [positive change in GG], corresponding to a vector of [−1, 0, −1, 1, −1, 2]. A grey matter mask was used and results were displayed at a voxel-wise threshold of $P < 0.001$ (uncorrected) with minimum cluster size (k) = 175 voxels. These voxel-wise parameters were selected to approximately correspond to a cluster-wise threshold of $P < 0.05$ (FDR-corrected). Only the GG > GA > AA results are shown, as no significant clusters were observed from the reciprocal model of AA > GA > GG.

Additional analyses were performed using IBM SPSS Statistics, Version 22.0. Following the GWAS, *post hoc* models including additional covariates were used to assess the robustness of the association of *IL1RAP* rs12053868 with higher rates of amyloid accumulation. Baseline ^{18}F-florbetapir PET SUVR and the square of this value were both included among the additional covariates in these *post hoc* analyses to account for the sigmoidal relationship of cortical amyloid PET burden to time (Jack *et al.*, 2013*b*). Consistent with previous data (Jack *et al.*, 2013*b*), the rate of amyloid accumulation as a function of baseline amyloid burden displayed an inverted U relationship (Supplementary Fig. 1). A one-way ANCOVA was used to assess the effect of rs12053868 genotype (AA versus GA/GG) on annualized per cent change in bilateral temporal cortex thickness, including baseline age, gender, total intracranial volume, and MRI scanner type (1.5 T versus 3.0 T field strength) as covariates. A subsequent two-way ANCOVA was performed to further explore the potential interaction of rs12053868 genotype with baseline diagnosis (cognitively normal versus MCI versus Alzheimer's disease). Logistic regression was used to test the association of rs12053868 genotype (AA versus GA/GG) with progression from MCI to Alzheimer's disease, including baseline age and gender as covariates. The associations of rs12053868 with ^{11}C-PBR28 PET SUV and longitudinal change in memory performance were tested using linear regression under an additive genetic model. As described above, baseline age, gender, and *TSPO* rs6971 genotype were included as covariates in the ^{11}C-PBR28 PET analysis. Baseline age, gender, and education were included as covariates in the memory analysis. METAL (Willer *et al.*, 2010) was used to perform inverse-variance weighted meta-analysis of the within-cohort memory studies.

Results

Longitudinal change in brain amyloid PET burden in ADNI participants

Primary phenotype (annualized per cent change in global cortical amyloid burden) and GWAS data passing stringent quality control were available for 495 ADNI participants (Table 1). Baseline age and gender were included as covariates in all analyses. The annualized per cent change in cortical amyloid burden was approximately normally distributed across the full sample (Supplementary Fig. 2). Mean annualized rates of amyloid accumulation were higher in Alzheimer's disease (1.36%; $n = 41$) than in MCI (0.79%; $P = 0.02$; $n = 294$) or cognitively normal (0.66%; $P = 5.47 \times 10^{-3}$; $n = 160$) participants.

APOE ε4 is associated with higher rates of amyloid accumulation

Because of its well-known association with Alzheimer's disease, prior to GWAS we investigated the effect of the *APOE* locus on longitudinal change in amyloid burden. Genotypes for *APOE* rs429358 and rs7412 were obtained for all but one participant. *APOE* ε4 carriers showed larger increases in amyloid burden over time compared to non-carriers ($P = 9.00 \times 10^{-6}$; Cohen's $d = 0.42$; Fig. 1A). *APOE* ε2/ε3 participants displayed lower rates of amyloid accumulation compared to ε3/ε3 ($P = 0.01$; Cohen's $d = 0.41$), ε3/ε4 ($P = 1.42 \times 10^{-5}$; Cohen's $d = 0.75$), and ε4/ε4 ($P = 1.57 \times 10^{-4}$; Cohen's $d = 0.84$) participants (Fig. 1B).

GWAS of longitudinal change in amyloid PET burden

To identify additional genetic modulators of longitudinal change in amyloid burden, we performed a GWAS testing 6 112 217 SNPs, with baseline age and gender included as covariates (Fig. 2). No evidence of systematic inflation of *P*-values was observed ($\lambda = 1.016$; Supplementary Fig. 3).

A genome-wide significant association was identified on chromosome 3 for rs12053868, an intronic SNP in *IL1RAP* ($P = 1.38 \times 10^{-9}$; Fig. 3A). The rs12053868-G allele was associated with higher rates of amyloid accumulation compared to the major (A) allele (Fig. 3B). A large effect size was observed in homozygous GG participants (Cohen's $d = 1.20$) equivalent to an odds ratio of 8.79 (Borenstein, 2009). Using stepwise linear regression with forward selection, this SNP explained 7.1% of the phenotypic variance in addition to, and independent from, the 3.4% explained by *APOE* ε4 status (Supplementary Fig. 4). This association remained genome-wide significant ($P = 5.80 \times 10^{-9}$) after the inclusion of *APOE* ε4 status, baseline diagnosis, years of education, baseline cortical

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amyloid PET burden (modelled as described in the 'Materials and methods' section), and the first three principal components from population structure analysis. No significant interactions were identified between rs12053868 and any of these factors, or age or gender. The effect of the G allele on higher rates of amyloid accumulation was present in both amyloid-negative and amyloid-positive participants as classified by the baseline PET scan (Supplementary Fig. 5). Using voxel-wise analysis to further characterize the spatial effect of this SNP in the brain, we again observed an association of rs12053868-G with higher rates of amyloid accumulation, with significant clusters for this effect observed primarily in the bilateral frontal, medial, and lateral parietal, and lateral temporal lobes, as well as throughout the posterior and anterior cingulate cortex (Fig. 3C).

Suggestive associations with longitudinal change in amyloid burden ($P < 5 \times 10^{-6}$) were also identified (Fig. 2 and Table 2). These included additional SNPs in *IL1RAP*, as well as SNPs on other chromosomes within or near *KCNG1* (potassium voltage-gated channel, subfamily G, member 1), *UBR3* (ubiquitin protein ligase E3, component n-recognin 3, putative), and *JAM2* (junctional adhesion molecule 2). Variants in *BIN1* (bridging integrator 1) and *CASS4* (cas scaffolding protein family member 4) which were identified in recent Alzheimer's disease case-control GWAS (Hollingworth *et al.*, 2011; Naj *et al.*, 2011; Lambert *et al.*, 2013) displayed uncorrected $P < 0.05$ in our analysis (Supplementary Table 1). Collectively, all SNPs tested in the GWAS (including the *IL1RAP* and *APOE* SNPs described previously) were estimated to explain 34% of the phenotypic variance based on a genome partitioning analysis (Yang *et al.*, 2011).

Table 1 Selected sample characteristics

	ADNI	IMAS[a]	MAP[b]	ROS[b]
Participants, *n*	495	25	178	190
Gender				
Male	274 (55%)	8 (32%)	54 (30%)	68 (36%)
Female	221 (45%)	17 (68%)	124 (70%)	122 (64%)
Age at baseline (years)	73.0 (7.8)	70.6 (7.3)	80.8 (6.2)	76.0 (6.8)
Education (years)	16.4 (2.7)	16.2 (2.6)	15.0 (3.0)	18.3 (3.2)

Values are *n* (percentage) or mean (SD).
[a]^{11}C-PBR28 PET subsample.
[b]Memory analysis subsample.

Gene- and pathway-based GWAS extensions

Using gene-based GWAS analysis, *IL1RAP* displayed genome-wide significant association with longitudinal change in amyloid burden [$P < 2.17 \times 10^{-6}$ (0.05/23 000 genes)]. Additional genes not initially uncovered through single SNP analysis displayed strong gene-level associations (Table 3). We also identified 83 biological pathways displaying enrichment of GWAS association, including numerous pathways related to cell adhesion and the complement system (Supplementary Table 2).

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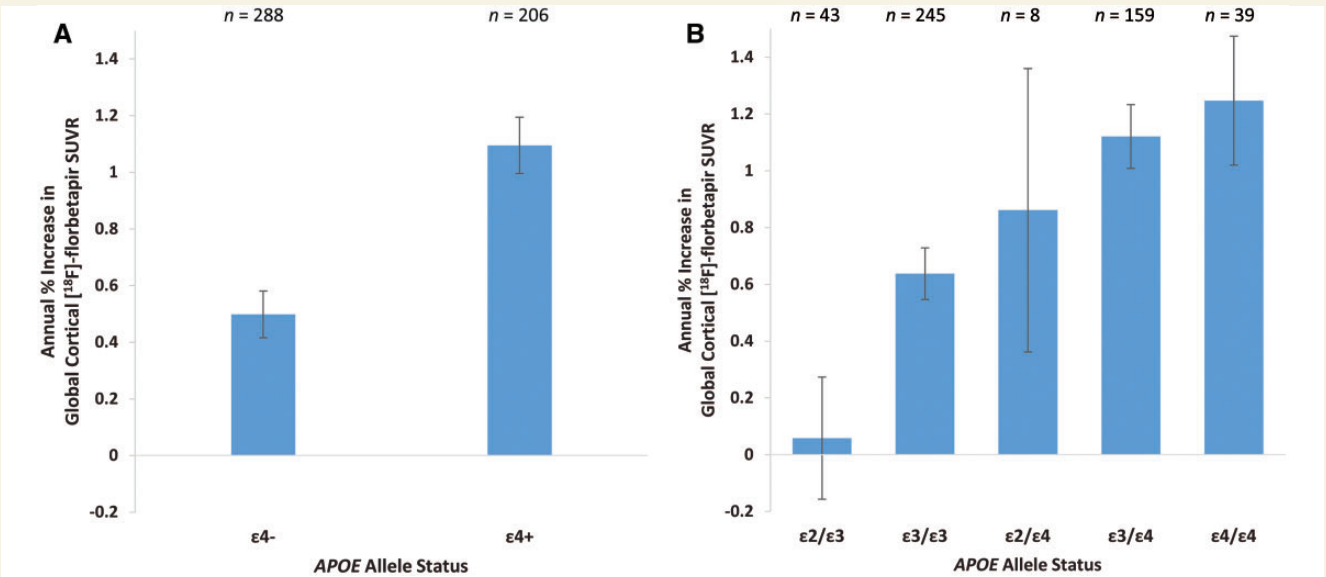

Figure 1 Effect of the *APOE* locus on 2-year change in cortical amyloid PET burden. Mean annualized per cent change in global cortical ^{18}F-florbetapir SUVR (adjusted for age and gender) ± standard errors are displayed based on (**A**) *APOE* ε4 status and (**B**) *APOE* ε2/ε3/ε4 status. (**A**) *APOE* ε4 carriers exhibited larger increases in brain amyloid PET burden compared to non-carriers ($P = 9.00 \times 10^{-6}$). (**B**) *APOE* ε2/ε3 participants displayed lower rates of amyloid accumulation compared to ε3/ε3 ($P = 0.01$), ε3/ε4 ($P = 1.42 \times 10^{-5}$), and ε4/ε4 ($P = 1.57 \times 10^{-4}$) participants.

Deep sequence analysis of *IL1RAP*

To further investigate the *IL1RAP* locus, we analysed gene sequence data for a subset of the GWAS sample ($n = 435$). Following stringent quality control, 1311 base pairs with at least one alternative allele present in the sample were available for analysis. Testing of 406 common variants confirmed a peak association with higher rates of amyloid accumulation for rs12053868 ($P = 8.18 \times 10^{-9}$; Cohen's $d = 1.18$ for GG versus AA). Although six additional common variants displayed strong association ($P < 5 \times 10^{-4}$), pairwise linkage disequilibrium calculations (Supplementary Table 3) and conditional analyses suggested that these SNPs, while not complete proxies for rs12053868, were not independent from rs12053868. Using SKAT (Ionita-Laza *et al.*, 2013), we identified a collective association of low-frequency and rare variants with rate of change in amyloid burden ($P = 7.74 \times 10^{-3}$).

IL1RAP rs12053868 is associated with a marker of cortical microglial activation

IL1RAP encodes a necessary component of the IL-1 (interleukin-1) receptor complex and its downstream signalling pathway (Gabay *et al.*, 2010). IL-1 is a potent pro-inflammatory cytokine known to promote activation of microglia, the resident phagocytes in the brain (Ghosh *et al.*, 2013; Doens and Fernandez, 2014). Recent reports have suggested that microglia may be crucial in clearing brain amyloid and limiting plaque growth (Chakrabarty *et al.*, 2015; Condello *et al.*, 2015; Johansson *et al.*, 2015). We hypothesized that the *IL1RAP* SNP associated with higher rates of amyloid accumulation would also be associated with lower microglial activation. We tested this *in vivo* in IMAS using PET and ^{11}C-PBR28, a radioligand considered to be a marker of microglial activity (Brown *et al.*, 2007). Controlling for age, gender, and *TSPO* rs6971 genotype, *IL1RAP* rs12053868-G was associated with lower cortical ^{11}C-PBR28 signal, indicative of lower cortical microglial activation ($P = 0.031$; Cohen's $d = 1.33$; Supplementary Fig. 6).

IL1RAP rs12053868-G carriers exhibit greater temporal cortex atrophy over 2 years

Amyloid deposition has been associated with increased rates of brain atrophy in cognitively normal older adults and in Alzheimer's disease (Storandt *et al.*, 2009; Chetelat *et al.*, 2010; Dore *et al.*, 2013). We hypothesized that

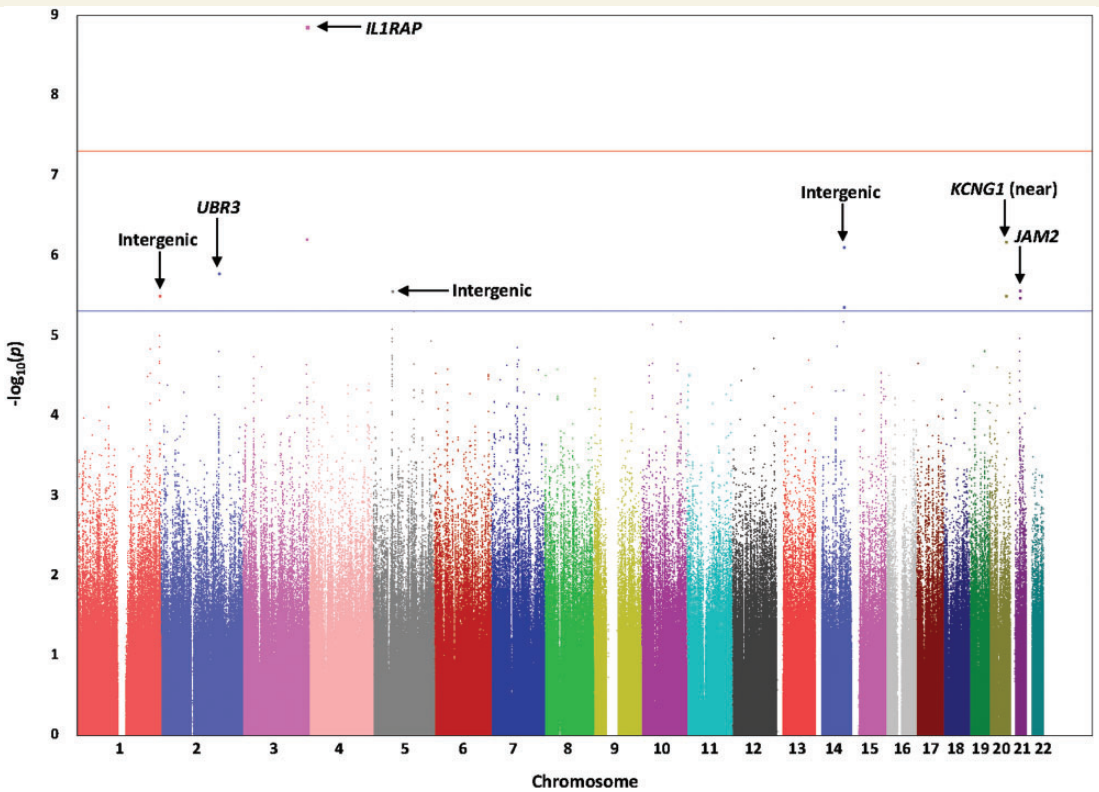

Figure 2 Manhattan plot for the GWAS of longitudinal change in cortical amyloid PET burden. Observed $-\log_{10}$ *P*-values (*y*-axis) are displayed for all tested SNPs on each autosomal chromosome (*x*-axis). A genome-wide significant association ($P < 5 \times 10^{-8}$; red line) with longitudinal change in global cortical amyloid burden measured by ^{18}F-florbetapir PET was identified on chromosome 3 within *IL1RAP*. Suggestive associations ($P < 5 \times 10^{-6}$; blue line) were identified on additional chromosomes.

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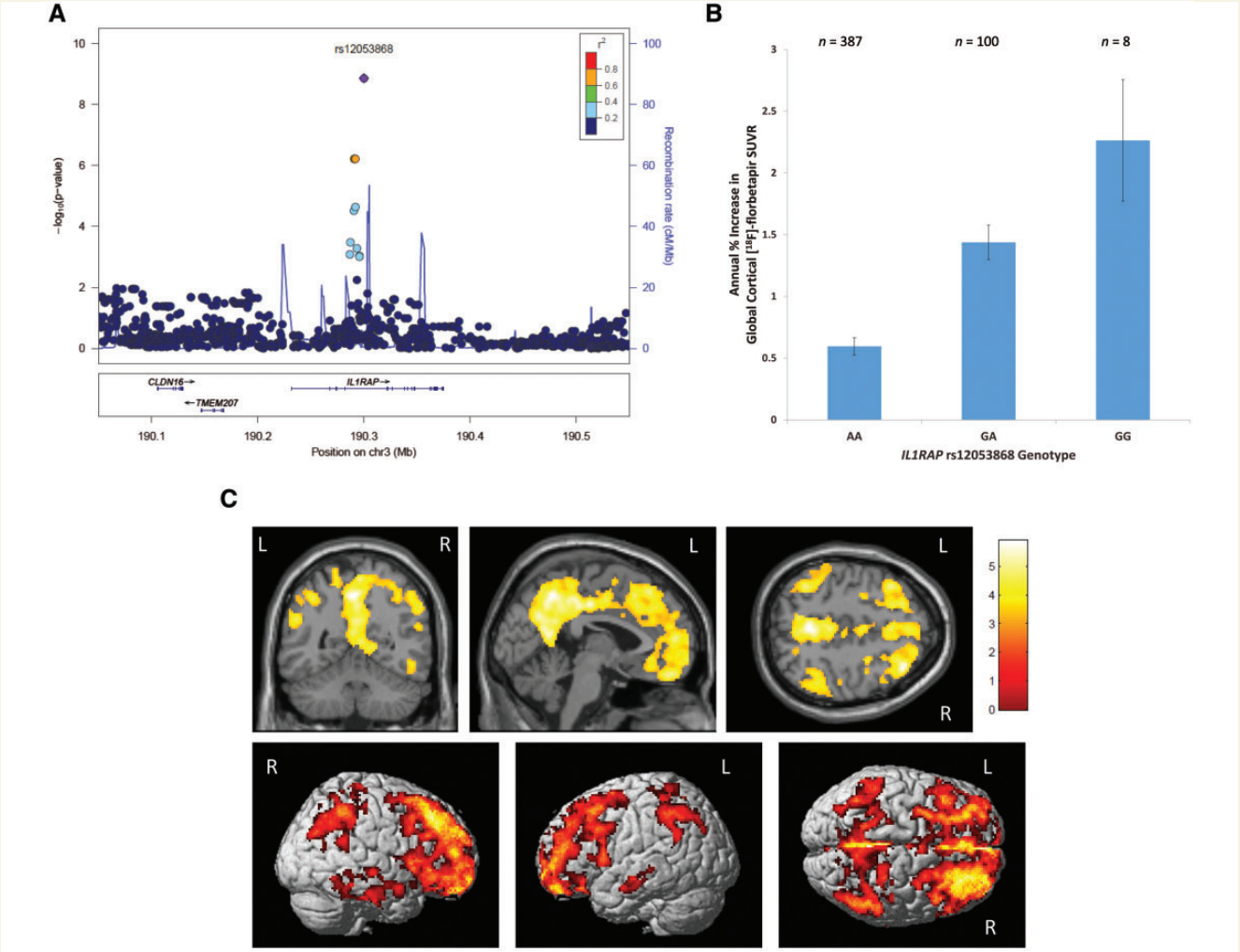

Figure 3 Association and effect of *IL1RAP* rs12053868-G on longitudinal change in cortical amyloid PET burden. (**A**) All SNPs within 250 kb of rs12053868 are plotted based on their GWAS $-\log_{10}$ *P*-values, NCBI build 37 genomic position, and recombination rates calculated from the 1000 Genomes Project reference data. The colour scale of r^2 values is used to label SNPs based on their degree of linkage disequilibrium with rs12053868. Genes in the region are labelled with arrows denoting 5'-to-3' orientation. (**B**) Mean annualized per cent change in global cortical ^{18}F-florbetapir SUVR (adjusted for age and gender) ± standard errors are displayed based on rs12053868 genotype. The minor allele (G) of rs12053868 was associated with a 0.8% increase per allele copy per year in cortical amyloid PET burden. The association of rs12053868 was genome-wide significant under additive ($P = 1.38 \times 10^{-9}$) and dominant ($P = 5.26 \times 10^{-9}$) genetic models. (**C**) Selected cross-sectional slices (*top*) and surface renderings (*bottom*) from voxel-wise analysis of the effect of rs12053868 on longitudinal amyloid accumulation measured by ^{18}F-florbetapir PET. The colour scale indicates regions where the rs12053868-G allele was associated with higher rates of amyloid accumulation (GG > GA > AA). All comparisons are displayed at a voxel-wise threshold of $P < 0.001$ (uncorrected) with minimum cluster size (k) = 175 voxels (approximately corresponding to a cluster-wise threshold of FDR-corrected $P < 0.05$). Where applicable, the left and right cerebral hemispheres are labelled for orientation. As displayed, the most significant clusters (identifying regions where rs12053868-G exhibited the greatest effect on rates of amyloid accumulation) were observed in the bilateral frontal lobes, medial parietal lobes, lateral parietal lobes, lateral temporal lobes, and the anterior and posterior cingulate cortex.

IL1RAP rs12053868-G would be associated with higher rates of atrophy in Alzheimer's disease-specific regions (the bilateral temporal cortex) (Dore *et al.*, 2013). Using structural MRI in a subset of the GWAS sample ($n = 358$), rs12053868-G carriers exhibited greater declines in temporal cortex thickness compared to non-carriers ($P = 0.035$; Cohen's $d = 0.28$; Supplementary Fig. 7A). This effect was observed across all diagnostic groups (Supplementary Fig. 7B) and remained significant ($P = 0.042$) after the inclusion of diagnosis ($P < 0.001$) as an independent predictor variable.

IL1RAP rs12053868-G carriers exhibit greater likelihood of progression from MCI to Alzheimer's disease

Amyloid deposition in MCI is a predictor of clinical progression to Alzheimer's disease (Huijbers *et al.*, 2015).

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Table 2 Peak associations ($P < 5 \times 10^{-6}$) for the GWAS of longitudinal change in amyloid PET burden

Chromosome	SNP	Gene symbol	Gene name	MAF[a]	β (SE)[b]	R^{2c}	P	Gen/Imp[d]
3	rs12053868	*IL1RAP*	Interleukin-1 receptor accessory protein	0.12	0.84 (0.14)	0.071	1.38×10^{-9}	437/58
3	rs3773970	*IL1RAP*	Interleukin-1 receptor accessory protein	0.13	0.67 (0.13)	0.049	6.19×10^{-7}	494/1
3	rs3773973	*IL1RAP*	Interleukin-1 receptor accessory protein	0.13	0.67 (0.13)	0.049	6.19×10^{-7}	436/59
3	rs147346019	*IL1RAP*	Interleukin-1 receptor accessory protein	0.13	0.67 (0.13)	0.049	6.19×10^{-7}	0/495
20	rs10470013	Near *KCNG1*	Potassium voltage-gated channel, subfamily G, member 1	0.11	0.73 (0.14)	0.049	6.65×10^{-7}	438/57
14	rs79110742	Intergenic		0.06	0.94 (0.19)	0.048	7.74×10^{-7}	0/495
2	rs13012722	*UBR3*	Ubiquitin protein ligase E3 component n-recognin 3 (putative)	0.49	−0.45 (0.09)	0.045	1.67×10^{-7}	0/495
21	rs8129913	*JAM2*	Junctional adhesion molecule 2	0.43	0.43 (0.09)	0.043	2.75×10^{-6}	0/495
21	rs11087971	*JAM2*	Junctional adhesion molecule 2	0.43	0.43 (0.09)	0.043	2.75×10^{-6}	0/495
5	rs11744848	Intergenic		0.13	−0.63 (0.13)	0.043	2.80×10^{-6}	0/495
1	rs10737896	Intergenic		0.21	−0.51 (0.11)	0.043	3.19×10^{-6}	0/495
1	rs7534801	Intergenic		0.21	−0.51 (0.11)	0.043	3.19×10^{-6}	495/0
20	rs6096218	Near *KCNG1*	Potassium voltage-gated channel, subfamily G, member 1	0.11	0.69 (0.15)	0.043	3.20×10^{-6}	494/1
21	rs4817054	*JAM2*	Junctional adhesion molecule 2	0.43	0.43 (0.09)	0.043	3.34×10^{-6}	0/495
14	rs8023225	Intergenic		0.08	0.77 (0.17)	0.042	4.41×10^{-6}	494/1

[a]Minor allele frequency in the GWAS sample.
[b]β (unstandardized) effect size from the GWAS (with standard error indicated in parentheses), denoting the annualized percent change in global cortical ^{18}F-florbetapir SUVR conferred by one copy of the minor allele.
[c]Proportion of phenotypic variance explained (not necessarily uniquely) by the SNP, including age and gender as covariates.
[d]Gen = number of participants for which the SNP was genotyped on a GWAS array (ADNI participants were genotyped on one of three Illumina GWAS arrays which each had different genomic coverages); Imp = number of participants for which the SNP was imputed.

We hypothesized that rs12053868-G would be associated with a greater likelihood of progression from MCI to Alzheimer's disease. Within the GWAS sample, 269/294 participants diagnosed with MCI at baseline had diagnosis information at 2-year follow-up, including 42 who progressed to clinical Alzheimer's disease and 227 who did not. Using logistic regression with age and gender included as covariates, rs12053868-G carriers were more likely to convert to Alzheimer's disease within the follow-up period than non-carriers [$P = 0.025$, odds ratio (OR) = 2.32 (1.11–4.87)] (Supplementary Fig. 8).

IL1RAP rs12053868 is associated with accelerated cognitive decline in high risk individuals

Memory impairment is the cardinal early symptom of Alzheimer's disease (Ballard *et al.*, 2011) and amyloid deposition is known to be related to memory impairment and longitudinal cognitive decline (Sperling *et al.*, 2013; Villemagne *et al.*, 2013). We hypothesized that rs12053868-G would be associated with accelerated decline of memory in participants at high risk for amyloid pathology (defined as being *APOE* ε4 positive or having a baseline diagnosis of clinical Alzheimer's disease). In a meta-analysis of 579 participants from three independent cohorts (ADNI, MAP and ROS), rs12053868-G was associated with faster 2-year decline in verbal episodic memory performance ($P = 7.72 \times 10^{-4}$), with each copy of the G allele adding approximately one-quarter of a standard deviation to the rate of decline (Supplementary Fig. 9). The heterogeneity statistic ($I^2 = 0$, $P = 0.37$) indicated no significant heterogeneity across the individual cohort studies used for meta-analysis (Higgins *et al.*, 2003).

Discussion

To our knowledge, this is the first reported GWAS of longitudinal change in brain amyloid load measured by ^{18}F-florbetapir PET. Our findings support *IL1RAP* as a novel potential Alzheimer's disease target and highlight the use of amyloid PET as a valuable Alzheimer's disease endophenotype, particularly in a longitudinal framework.

IL1RAP encodes a necessary and potentially rate-limiting component of the pro-inflammatory IL-1 signalling pathway (Gabay *et al.*, 2010). Activation of this pathway requires binding of IL1RAP to the IL-1/IL-1 receptor complex (Wang *et al.*, 2010*a*). *IL1RAP* splice variants, including one isoform expressed only in the CNS as well as a different soluble variant, exert inhibitory effects on the IL-1 pathway (Smith *et al.*, 2009). More broadly, the IL-1 pathway and its component genes have long been foci of interest in genetic and other studies of the Alzheimer's disease spectrum (Green *et al.*, 2002; Wang *et al.*, 2005; Tsai *et al.*, 2010; Latz *et al.*, 2013). These studies, and recent findings from Alzheimer's disease mouse models revealing that IL-1 overexpression leads to increased plaque-associated activated microglia, decreased amyloid burden, and

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Table 3 Peak GATES gene-based associations with longitudinal change in amyloid PET burden

Chromosome	Gene symbol	Gene name	GATES *P*
3	*IL1RAP*	Interleukin-1 receptor accessory protein	9.45×10^{-8}
20	*PSMA7*	Proteasome subunit, alpha type, 7	1.33×10^{-4}
20	*LSM14B*	SCD6 homolog B (S. cerevisiae)	1.46×10^{-4}
21	*JAM2*	Junctional adhesion molecule 2	1.59×10^{-4}
19	*APOC1*	Apolipoprotein C1	1.74×10^{-4}
2	*UBR3*	Ubiquitin protein ligase E3 component n-recognin 3 (putative)	1.75×10^{-4}
19	*APOE*	Apolipoprotein E	2.26×10^{-4}
10	*PNLIPRP1*	Pancreatic lipase-related protein 1	2.33×10^{-4}
19	*TOMM40*	Translocase of outer mitochondrial membrane 40 homolog (yeast)	3.10×10^{-4}
3	*MIR6828*	MicroRNA 6828	3.12×10^{-4}
14	*SNAPC1*	Small nuclear RNA activating complex, polypeptide 1	3.47×10^{-4}
12	*BRI3BP*	Brain protein 13 binding protein	3.82×10^{-4}
20	*SS18L1*	Synovial sarcoma translocation gene on chromosome 18-like 1	4.09×10^{-4}
3	*CLDN11*	Claudin 11	4.98×10^{-4}

increased tau phosphorylation (Prinz *et al.*, 2011; Ghosh *et al.*, 2013) highlight the potentially crucial roles of the IL-1/IL1RAP pathway in modulating Alzheimer's disease pathology.

Microglial activation pathways are leading candidates for promoting amyloid clearance and limiting plaque development. Variants in *TREM2* (triggering receptor expressed on myeloid cells 2) thought to impair microglial phagocytic function have been associated with increased Alzheimer's disease risk (Guerreiro *et al.*, 2013; Jonsson *et al.*, 2013) and large-scale pathway and network analyses have also implicated activated microglia in Alzheimer's disease pathogenesis (Jones *et al.*, 2010; Zhang *et al.*, 2013). Along with recent studies relating loss of microglial function to worsening amyloid pathology (Bradshaw *et al.*, 2013; Chakrabarty *et al.*, 2015; Condello *et al.*, 2015; Johansson *et al.*, 2015), the discovery that *IL1RAP* is associated with higher rates of amyloid accumulation and lower signal of a PET marker for microglial activation provides strong reinforcement for this hypothesis.

IL1RAP is a known therapeutic target for leukaemia (Barreyro *et al.*, 2012; Askmyr *et al.*, 2013) and chronic inflammatory diseases such as rheumatoid arthritis (Gabay *et al.*, 2010; Dinarello, 2011). Pathway analysis of a large Alzheimer's disease case-control GWAS (Harold *et al.*, 2009) ($n = 11\,789$) identified association of immune-related pathways with a significant contribution from *IL1RAP*, including a top Alzheimer's disease risk SNP (rs4571225; $P = 1.26 \times 10^{-5}$) which is not a proxy for, but is in moderate linkage disequilibrium with rs12053868 ($r^2 = 0.003$; $D' = 0.63$) (Jones *et al.*, 2010). An intergenic SNP (rs9877502) 290 kb downstream of *IL1RAP* also displayed genome-wide significant association with cross-sectional CSF tau levels (Cruchaga *et al.*, 2013), but this SNP is not in linkage disequilibrium with rs12053868 ($r^2 = 0.003$; $D' = 0.10$). Prior to our study, *IL1RAP* rs12053868 had not been previously reported in an Alzheimer's disease genetic association study. Our new association for this SNP may reflect a relative specificity for amyloid accumulation versus the more heterogeneous case-control status (Kendler and Neale, 2010), increased power obtained via endophenotype analysis (Potkin *et al.*, 2009), or previous suggestive association below reporting thresholds.

IL1RAP is highly expressed in the brain but seems to be downregulated in prefrontal cortex with increasing age (Kang *et al.*, 2011; Primiani *et al.*, 2014). Although it is not a coding SNP, rs12053868 may be associated with decreased *IL1RAP* expression in the cortex and hippocampus based on preliminary data (Supplementary Fig. 10). There is substantial precedent for non-coding SNPs to have functional effects (Kapranov *et al.*, 2007; Consortium, 2012; De Jager *et al.*, 2014), and intronic *IL1RAP* SNPs have previously been associated with plasma levels of soluble IL1RAP, including a top SNP (rs724608; $P = 8.81 \times 10^{-13}$) which is in moderate linkage disequilibrium with rs12053868 ($r^2 = 0.005$; $D' = 0.45$) (Lourdusamy *et al.*, 2012). However, functional genomics studies in brain tissue will be needed to further characterize the *IL1RAP* locus and its potential impact on Alzheimer's disease pathogenesis.

Following the GWAS discovery, we related *IL1RAP* rs12053868-G to other longitudinal Alzheimer's disease endophenotypes. In particular, the observed effect of rs12053868 on clinical progression in MCI argues for further study of the impact of *IL1RAP* on clinical trajectories in pre-MCI states. This result also suggests that in combination with *APOE*, other known Alzheimer's disease risk genes, and family history, *IL1RAP* might be useful for risk enrichment in clinical trial design and risk stratification in study analysis or as part of personalized genetic susceptibility tests for Alzheimer's disease onset or progression.

Using gene sequence data from a subset of the GWAS sample, we identified a pooled association of low-frequency

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and rare *IL1RAP* variants with the rate of amyloid accumulation. Although larger samples will facilitate assessment of the effects of individual rare variants, this finding bolsters the initial GWAS discovery of *IL1RAP*, as genes truly related to disease pathogenesis are likely to contain associated common and rare variants (Zuk *et al.*, 2014).

Suggestive associations were identified through GWAS and may have reached genome-wide significance with a larger sample. These included SNPs in *JAM2*, adjacent to *APP* on chromosome 21. Mutations in *APP* are among the causes of early-onset Alzheimer's disease (Bettens *et al.*, 2013) and a rare variant in *APP* was found to be protective against late-onset Alzheimer's disease (Jonsson *et al.*, 2012). SNPs near *APP* were also associated with amyloid plaque burden in a neuropathological study of post-mortem brain tissue (Shulman *et al.*, 2013). Notably, the top SNP from that study (rs2829887) is in strong linkage disequilibrium with the top *JAM2* SNP (rs8129913) from our analysis of longitudinal amyloid PET ($r^2 = 0.57$; $D' = 0.91$). In addition, *JAM2* ($P = 1.59 \times 10^{-4}$) and *APP* ($P = 0.048$) each displayed uncorrected gene-level $P < 0.05$ in our study. These suggestive findings argue for further investigation of the *JAM2-APP* locus to clarify the potential functional gene(s) and causal variant(s) related to amyloid pathology.

Complementary gene- and pathway-based analyses were used to test for collective effects of multiple variants within shared functional units (Ramanan *et al.*, 2012*b*). Gene-based analysis uniquely identified additional candidates for further study, and enrichment of GWAS association was identified in pathways related to the complement system, cell adhesion, and *Notch* transcription, as well as the IL-1 pathway overall. Activation of cell adhesion and complement receptors are crucial for microglia to recognize, aggregate around, and ultimately clear amyloid deposits (Ramanan and Saykin, 2013; Doens and Fernandez, 2014). Notch has key roles in regulating neuronal plasticity but these activities depend on its initial cleavage by γ-secretase, the enzyme also responsible for generating amyloid-β (Mattson, 2003).

All SNPs tested in the GWAS were estimated to collectively explain 34% of the variance in 2-year change in brain amyloid PET burden, a considerable proportion given the modest sample used for GWAS. Although amyloid deposition and clearance are dynamic processes with unknown heritability, our findings indicate that the rate of amyloid accumulation has a substantial genetic component and suggest that additional genetic variants, as well as gene–gene and gene–environment interactions, may be discovered in future using larger samples and complementary analytical approaches.

This work has several limitations. Although we leveraged publicly available ADNI genetics and longitudinal amyloid PET data to perform this original study, our sample size had limited power for a GWAS (Supplementary Fig. 11). The future availability of comparable data from larger samples will allow for suitable replication testing and additional discovery. Functional genomics experiments not performed here, including microglial immunohistochemistry and analyses of *IL1RAP* knockout and antibody- and siRNA-based knockdown models, will also be needed to characterize our novel finding. In particular, studies of brain tissue, rather than blood genomic DNA, will be better able to assess for epigenetic and transcriptomic events that may elucidate the mechanistic relationship between *IL1RAP* and amyloid accumulation. Further, while it could not be appropriately addressed with presently available data, analyses of serial CSF samples would help assess whether *IL1RAP* impacts soluble and oligomeric forms of amyloid. Finally, candidate PET radiotracers selective for tau aggregation in the brain are also now in clinical trials (Villemagne *et al.*, 2015), and if validated, would aid investigation of the potential relationship between the IL-1/IL1RAP pathway and tau pathology.

In conclusion, we discovered a new association of *IL1RAP* rs12053868 with higher rates of amyloid accumulation on longitudinal ^{18}F-florbetapir PET and we related this SNP to other Alzheimer's disease endophenotypes, including clinical progression, cognitive decline, temporal cortex atrophy on MRI, and lower signal of a PET marker of microglial activation. The biological roles of *IL1RAP* in amyloid deposition and clearance, particularly in relation to microglial function, merit further investigation and may have significant implications for risk stratification and therapeutic development in Alzheimer's disease.

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Supplementary material

Supplementary material is available at *Brain* online.

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