Genetic interactions within inositol-related pathways are associated with longitudinal changes in ventricle size

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Abstract

The genetic etiology of late onset Alzheimer disease (LOAD) has proven complex, involving clinical and genetic heterogeneity and gene-gene interactions. Recent genome wide association studies (GWAS) in LOAD have led to the discovery of novel genetic risk factors; however, the investigation of gene-gene interactions has been limited. Conventional genetic studies often use binary disease status as the primary phenotype, but for complex brain-based diseases, neuroimaging data can serve as quantitative endophenotypes that correlate with disease status and closely reflect pathological changes. In the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort, we tested for association of genetic interactions with longitudinal MRI measurements of the inferior lateral ventricles (ILVs), which have repeatedly shown a relationship to LOAD status and progression. We performed linear regression to evaluate the ability of pathway-derived SNP-SNP pairs to predict the slope of change in volume of the ILVs. After Bonferroni correction, we identified four significant interactions in the right ILV (RILV) corresponding to gene-gene pairs *SYNJ2-PI4KA, PARD3-MYH2, PDE3A-ABHD12B* and *OR2L13-PRKG1* and one significant interaction in the left ILV (LILV) corresponding to *SYNJ2-PI4KA*. The SNP-SNP interaction corresponding to *SYNJ2-PI4KA was* identical in the RILV and LILV and was the most significant interaction in each (RILV: p=9.10×10^{-12}; LILV: p=8.20×10^{-13}). Both genes belong to the inositol phosphate signaling pathway which has been previously associated with neurodegeneration in AD and we discuss the possibility that perturbation of this pathway results in a down-regulation of the Akt cell survival pathway and, thereby, decreased neuronal survival, as reflected by increased volume of the ventricles.

Keywords

Alzheimer Disease; neuroimaging; atrophy; epistasis; Magnetic Resonance Imaging; ADNI; inositol

Introduction

Late onset Alzheimer's disease (LOAD) is a devastating, degenerative neurological disease that affects over 5 million people in the United States alone, an already substantial statistic that is expected to triple by 2050 (http://www.alz.org) [1]. The complex genetics of LOAD have proven difficult to unravel due to the disease's clinical and genetic heterogeneity. To date, ten genes have been confirmed by replication and meta-analysis to be associated with LOAD [2]. Of these genes, only *APOE* has a large effect with an odds ratio of about 3.7 for one copy of the high risk 4 allele; the remaining nine genes (*CR1, CLU, PICALM, BIN1, EPHA1, MS4A, CD33, CD2AP* and *ABCA7*) exhibit small effect sizes, with odds ratios closer to 1.2 [2]. In order to increase our power and biological interpretability of this complex disease, we expanded our analysis beyond the traditional approach of testing for single marker effects using binary disease status as the primary outcome. In this study, we include rich phenotypic information derived from magnetic resonance imaging (MRI) quantitative traits (QTs) as our outcomes, which addresses the problem of clinical heterogeneity, and we explicitly test for gene-gene interactions, which confronts the issue of genetic heterogeneity [3].

Interactions have shown significant associations in many other complex diseases such as schizophrenia [4], autism [5] and type 2 diabetes [6]. In LOAD, previous gene-gene interaction studies have implicated interactions between *CR1* and *APOE* [7], cholesterol trafficking genes [8,9], tau phosphorylation genes [10], and calcium signaling and axon guidance genes [11]. These studies demonstrate that important mechanistic insight can be garnered from investigating higher order genetic relationships in complex diseases like LOAD.

For LOAD and other brain-based diseases, brain structure derived from imaging modalities can be the source of relevant QTs or endophenotypes. Endophenotypes are biological measurements that are more proximal to genetic function and pathology than disease status [12] and can provide increased statistical power (and therefore decreased sample size requirements) over dichotomous outcome variables [13]. Many measurements of brain structures have been shown to correlate with LOAD status and to have greater sensitivity in detecting early pathological changes [14]. QTs from structural MRI have been used as endophenotypes in LOAD GWAS previously [15], and in this study, we extend that work by investigating associations of an endophenotype of LOAD with gene-gene interactions.

The lateral ventricles have repeatedly shown a relationship to Alzheimer's disease (AD) status and progression [16–19]. The lateral ventricles normally dilate over time with age, as brain tissue volume decreases, but in patients with mild cognitive impairment (MCI) or AD, the rate of ventricular dilation is much greater than in the normal aging population [20]. MRI measurements of lateral ventricle expansion correlate with disease status, with ventricular volumes and rates of dilation increasing from healthy controls (HC) to MCI and from MCI to AD [20]. The inferior horns of the lateral ventricles are surrounded by gray and white matter structures (corpus callosum, hippocampus, amygdala, caudate nucleus, deep white matter, and thalamus). These structures, particularly the hippocampus and amygdala, often deteriorate in AD, and patients with AD and MCI have significantly higher rates of tissue atrophy in these structures than normal aging adults [20], and ventricular dilation is inversely reflective of atrophy of these surrounding structures [21]. Ventricular dilation is evident 10 years before clinical symptoms, and dilation rate rapidly accelerates two years prior to initial MCI diagnosis, making longitudinal MRI measurement of ventricular dilation a plausible clinical trial biomarker for disease inclusion or progression criteria [20]. Because of the evidence demonstrating atrophy of brain structures surrounding the ILVs in LOAD

and because changes in these structures are reflected and magnified in the ILVs, we chose to investigate genetic associations with longitudinal change in volume of these structures.

While correction for multiple testing in single-marker genome-wide association analysis is highly burdensome, due to combinatorics, in genome-wide interaction analyses it is essentially prohibitive except perhaps for very large datasets. However, interaction analyses limited to known candidate genes are unduly constrained by information from previously published studies. Alternative strategies may instead conduct intelligent variable selection based on prior biological knowledge assembled from a wide variety of scientific disciplines. In this study, we selected genes participating in common biological pathways for investigation of gene-gene interactions associated with the endophenotypes of ILV atrophy rate. By doing so, we aimed to increase the biological plausibility of interactions that are novel to AD, while decreasing computational burden. We hypothesized that novel gene-gene interactions would be significantly associated with the dilation of the ILVs and that the novel interactions will generate new or altered hypotheses regarding the etiology of this disease.

Materials and Methods

Data used in the preparation of this article were obtained from the ADNI database (adni.loni.ucla.edu). The ADNI was launched in 2003 by the National Institute on Aging (NIA), the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the Food and Drug Administration (FDA), private pharmaceutical companies and non-profit organizations, as a $60 million, 5-year public-private partnership. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. Determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness, as well as lessen the time and cost of clinical trials.

The Principal Investigator of this initiative is Michael W. Weiner, MD, VA Medical Center and University of California – San Francisco. ADNI is the result of efforts of many co-investigators from a broad range of academic institutions and private corporations, and subjects have been recruited from over 50 sites across the U.S. and Canada. The initial goal of ADNI was to recruit 800 adults, ages 55 to 90, to participate in the research, approximately 200 cognitively normal older individuals to be followed for 3 years, 400 people with MCI to be followed for 3 years and 200 people with early AD to be followed for 2 years. For up-to-date information, see ww.adni-info.org. Further information on ADNI can be found here [22].

Subjects

Participants were enrolled based on the criteria outlined in the ADNI-1 protocols (http://www.adni-info.org/Scientists/AboutADNI.aspx). Information on ADNI subject protocols can be found here [23]. To minimize population stratification, only Caucasian subjects who had both genotype data and T1-weighted MRI data were included. Demographic data are presented in Table 1.

Genotyping

Genotyping was performed by the ADNI Genetics Core using the Illumina Infinium Human-610-Quad BeadChip. Further information about the ADNI Genetics Core efforts can be found here [24]. ADNI quality control (QC) steps included removing copy number

variant probes, strand checking, base pair position checking, and allele specificity checking [25]. Further QC was performed using PLINK software (version 1.07; [26]), excluding SNPs with a genotyping efficiency < 95%, out of Hardy Weinberg Equilibrium ($p<1\times10^{-6}$), or with a minor allele frequency (MAF) of < 5%. Subjects were excluded if they had a call rate < 95%, if there was a reported versus genetic sex inconsistency, or if relatedness was established (PI_HAT > 0.5). After QC, 515,839 SNPs and 730 subjects remained available for discovery analyses.

Analysis of Imaging Data

Structural T1-weighted MRI scans were acquired on subjects at baseline, 12, 24, and 36 month follow-up appointments as per the ADNI protocol [27]. Further information on ADNI's MRI protocols can be found here [27,28]. Cortical reconstruction and volumetric segmentation of these images were performed with the FreeSurfer [29] image analysis suite version 4.3 by the ADNI consortium which has been described in detail elsewhere [30]. An early version of the longitudinal image processing framework was used to process the sequential scans [31]. Volumes of the RILV and LILV were calculated in FreeSurfer (in mm^3) for every scan available for each individual in the dataset and slopes of change in RILV and LILV volume over time were calculated in SAS 9.3 (SAS Institute Inc., Cary, NC) using mixed model regression (PROC MIXED) to leverage the longitudinal data available in ADNI-1. We used the slopes of change of the LILV and RILV as our primary outcome measurement (mm^3/year) and included a measurement of intracranial volume (ICV in mm^3) as a covariate in all volume analyses, which was also defined with FreeSurfer.

SNP-SNP Interaction Analysis

Genotype data that passed QC were analyzed in a pathway-based interaction analysis using the publicly available InterSNP program [32]. We took advantage of InterSNP's pathway based option that tests SNP-SNP pairs between genes that belong to a common biological pathway derived from the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/). This option decreases the number of tests performed and also increases the interpretability of an interaction based on *a priori* information. SNP-SNP interaction effects were explored using a dominant model and a linear regression framework for QTs [32]. A total of 130,512,955 SNP-SNP interaction pairs were tested for each region of interest (ROI), and SNP-SNP pairs were considered significant after Bonferroni correction accounting for the number of pairs tested ($p < 3.83\times10^{-10}$). All SNPs were entered into the model as binary variables (minor allele absent or present) to attenuate the problem of data sparsity commonly confronted in interaction analyses.

Model covariates included: baseline age (in years), sex, education (in years), *APOE* status (number of 4 risk alleles) and last diagnosis recorded as of January 2013 (1= Normal, 2=MCI, 3=AD). Each of these covariates was chosen to avoid confounding, with the goal of identifying interactions which explain variance *beyond* these known risk factors. The brain is known to atrophy over time even in normal aging [33], and the rate of change differs by sex [34]. Education level is correlated with age of disease onset [35]. *APOE* is a very strong genetic risk factor predisposing patients to LOAD, with even normal subjects who are carriers of the risk allele showing greater neurodegeneration before any symptom onset [36]. And finally, atrophy rates are known to trend with disease status, wherein AD>MCI>HC [16–18,37].

Significant SNP-SNP interactions were annotated to their gene-gene pairs using dbSNP (Homo sapiens, updated in build 137) and their common pathways using KEGG. InterSNP calculates contingency tables which can be seen in Supplemental Table 1. The difference in R^2 for these models was calculated in SPSS as $R^2 = R^2_{\text{(full model with interaction term)}}-$

R^2 (reduced model without interaction term). Visualization of interaction effects was created in SPSS as well, showing rate of change by allelic combination (Figure 1A/B, Figure 2).

Results

Pathway Based Interaction Analysis

Demographic information is presented in Table 1. The study sample included 187 NC, 191 MCI, and 352 AD subjects. The number of *APOE-* 4 carriers is enriched in the AD and MCI subjects. The slopes of the RILV and LILV increased from NC to MCI to AD subjects. We identified four Bonferroni-corrected significant SNP-SNP interactions in the RILV corresponding to the gene-gene pairs: *SYNJ2-PI4KA, PARD3-MYH2, PDE3A-ABHD12B*, and *OR2L13-PRKG1,* in order of significance. None of the SNPs involved had significant main effects (uncorrected p 0.01).

One Bonferroni-corrected significant SNP-SNP interaction was discovered in the LILV corresponding to *SYNJ2-PI4KA*. The specific SNP-SNP interaction corresponding to *SYNJ2-PI4KA* was the same in both the RILV and LILV and was the top hit in both hemispheres (RILV: $p=9.10\times10^{-12}$; LILV: $p=8.20\times10^{-13}$). This gene-gene pair belongs to the metabolic, the inositol phosphate metabolism and the phosphatidylinositol signaling system pathways in KEGG (hsa001100, hsa00562 and hsa04070, respectively), and neither gene is involved in additional KEGG pathways. The effect of this interaction was in the same direction for both hemispheres ($_{LILV}=352.44$ and $_{RILV}=351.88$) and explained >4% of the variance in both the RILV and LILV ($R^2_{RILV}= .043$, $R^2_{LILV}=.046$). As seen in Figure 1A and 1B, having the minor allele for both genes corresponded to an increased rate of change in both ILVs. This interaction was consistent across subjects with MCI and AD diagnoses (Figure 2). Contingency tables showing sample sizes by genotype combination are presented in Supplemental Table 1.

The *PDE3A-ABHD12B* interaction that was significantly associated at the Bonferroni-corrected level with change in the RILV did not pass Bonferroni correction in the LILV ($p=1.59\times10^{-8}$). The other SNP-SNP interactions associated with the RILV did not show a strong trend in the LILV. As a post-hoc analysis, we looked at the effect of the significant interactions between *SYNJ2-PI4KA, PARD3-MYH2, PDE3A-ABHD12B*, and *OR2L13-PRKG1* on the average volume of RILV and LILV combined ($p=1.52\times10^{-13}$, 3.49×10^{-8}, 1.34×10^{-10}, 1.85×10^{-7}, respectively).

Discussion

In this study, we focused on the ILVs, which are used frequently as a source of quantitative endophenotypes for LOAD [16–18,37], and we limited hypothesis testing to SNP-SNP pairs within KEGG pathways. Our quantitative trait and pathway-based interaction analysis yielded several interesting candidate gene-gene interactions, one of which was significantly associated with change in both the right and left ILV. Using existing biological knowledge, we were able to deduce a plausible biological context for these significant interactions.

The SNP-SNP interaction rs9295289-rs178051 corresponds to the gene-gene pair *SYNJ2-PI4KA* and was significantly associated with change in the RILV and LILV, and with the average rate of change in the combined ILV. Below we present evidence suggesting that the biological mechanism for this statistical interaction may involve the perturbation of the phosphatidylinositol (PI) signaling system that results in a down-regulation of the Akt cell survival pathway, resulting in decreased neuronal survival as reflected by increased volume of the ventricles.

Both *SYNJ2* and *PI4KA* are involved in the synthesis of 1-Phosphatidyl-1D-*myo*-inositol-4P (PIP) (Figure 3). *SYNJ2* (MIM: 609410) encodes synaptojanin-2, which is a ubiquitously expressed inositol polyphosphate 5-phosphatase that dephosphorylates 1-Phosphatidyl-1D-*myo*-inositol-4,5P_2 (PI-4,5P_2) to PIP and *PI4KA* (MIM: 600286) encodes PI 4-kinase, which phosphorylates phosphatidylinositol (PI) to PIP (Figure 3).

In multiple cohorts, PIP levels were observed to be reduced in the temporal cortices of patients with LOAD [39,40]. Further, PI kinase activity was decreased while PIP kinase activity remained stable, suggesting that PI kinases, like that encoded by *PI4KA*, have a specific functional relevance to LOAD [39,41]. PIP is important because it can be phosphorylated to form 1-Phosphatidyl-1D-*myo*-inositol-3,4P_2 (PI-3,4P_2), which activates the protein Akt, also known as Protein Kinase B (PKB) [42]. Akt/PKB has proliferative and anti-apoptotic cell response function [42] and has been shown to regulate neuronal survival [43], protect from the neurotoxic effects of AD associated amyloid beta protein [44], and mediate neuronal cell death when its activation is inhibited [45]. Thus, a decrease in PIP synthesis could disrupt a vital mechanism of neuroprotection. Variation in either *SYNJ2* or *PI4KA* might modulate the efficiency of PIP synthesis, but perhaps disruption of both routes of PIP synthesis is required to have detrimental effects seen in our study as increased ventricle dilation.

Evidence that *PI4KA* is involved in LOAD derives primarily from its role in the synthesis of PIP. However, there is some additional literature linking *SYNJ2* to cognitive function, which would lend further support for a role in Alzheimer disease. SYNJ2 is localized at nerve terminals in the brain [46]. It is differentially expressed in hippocampal sub-regions of the marmoset primate [47] and shows decreased expression in the human temporal cortex in persons with major depressive disorder [48]. *SYNJ2* has been associated with cognitive abilities in two independent elderly cohorts [49]. Finally, haploinsufficiency of *SYNJ2* due to a microdeletion on 6q is associated with a syndrome that presents with microencephaly, developmental delay and agenesis of the corpus callosum [50].

Thus, the genetic interactions associated with ILV atrophy rate in this study may be mapping variants in *SYNJ2* and *PI4KA* that interact to decrease synthesis of PIP and its phosphorylated form (PI-3,4P_2), which is required for activation of the neuroprotective, Akt-mediated, cell survival signaling pathway.

In conclusion, by using a pathway based approach, we identified four SNP-SNP interactions significantly associated with AD related quantitative endophenotypes, one of which was significantly associated with bilateral volume change of the inferior lateral ventricles. Focusing on this interaction, we used existing biological knowledge of within-pathway interactions and proposed a plausible biological context for this statistical interaction, which suggests that volume change in the LOAD brain might be mediated by alterations of the inositol signaling pathway, leading to deficits in neuroprotective mechanisms.

The most common variable selection strategy for interaction studies only selects SNPs with main effects to test for interactions. It is important to emphasize that such an approach would not have discovered any of the Bonferroni-significant interactions presented here, highlighting the strength of the pathway-based approach we took.

In the future, the interactions found in this study should be replicated in independent datasets to confirm the SNP-SNP associations. Furthermore, functional analyses could help clarify the basis of these statistical genetic interactions and provide greater specificity for identification of targets for clinical intervention or diagnosis. Further molecular studies on the relationship between *SYNJ2* and *PI4KA* and the PI / PI-3,4P_2 / Akt balance are warranted to draw definite conclusions about the relationship between risk variants *SYNJ2*

and *PI4KA* causing downstream decreases in PI-3,4P$_2$, Akt mediated cell survival signaling and, ultimately, increased neurodegeneration.

The present results must be interpreted within the framework of our statistical models. In all cases, we included covariates related to disease status and progression including age, sex, education, diagnosis and *APOE* status. Thus, all significant interactions are explaining variance beyond known predictors of risk, and while the contributions of these interactions appear to be meaningful, the implications should not be extended without considering the variance accounted for by the other factors in our model. The interactions in this study represent dominant effects (carriers versus non-carriers), and the results were interpreted accordingly.

We limited our studies to interactions within pathways, but interactions between genes across pathways may be related to disease risk as well and warrant further exploration. By using KEGG pathways, we also biased our results toward mechanisms that are well-studied. As a result, there may be other novel interactions in other realms of biology that this strategy did not discover. While the sample size utilized in this study is considered large for imaging studies, it is still modest compared with most case-control genetic association studies being conducted at this time, and this is a limitation of the current study as well. We advocate for similar analyses in other complex neurological or neuropsychiatric disorders to improve our understanding of the mechanisms underlying genetic risk for disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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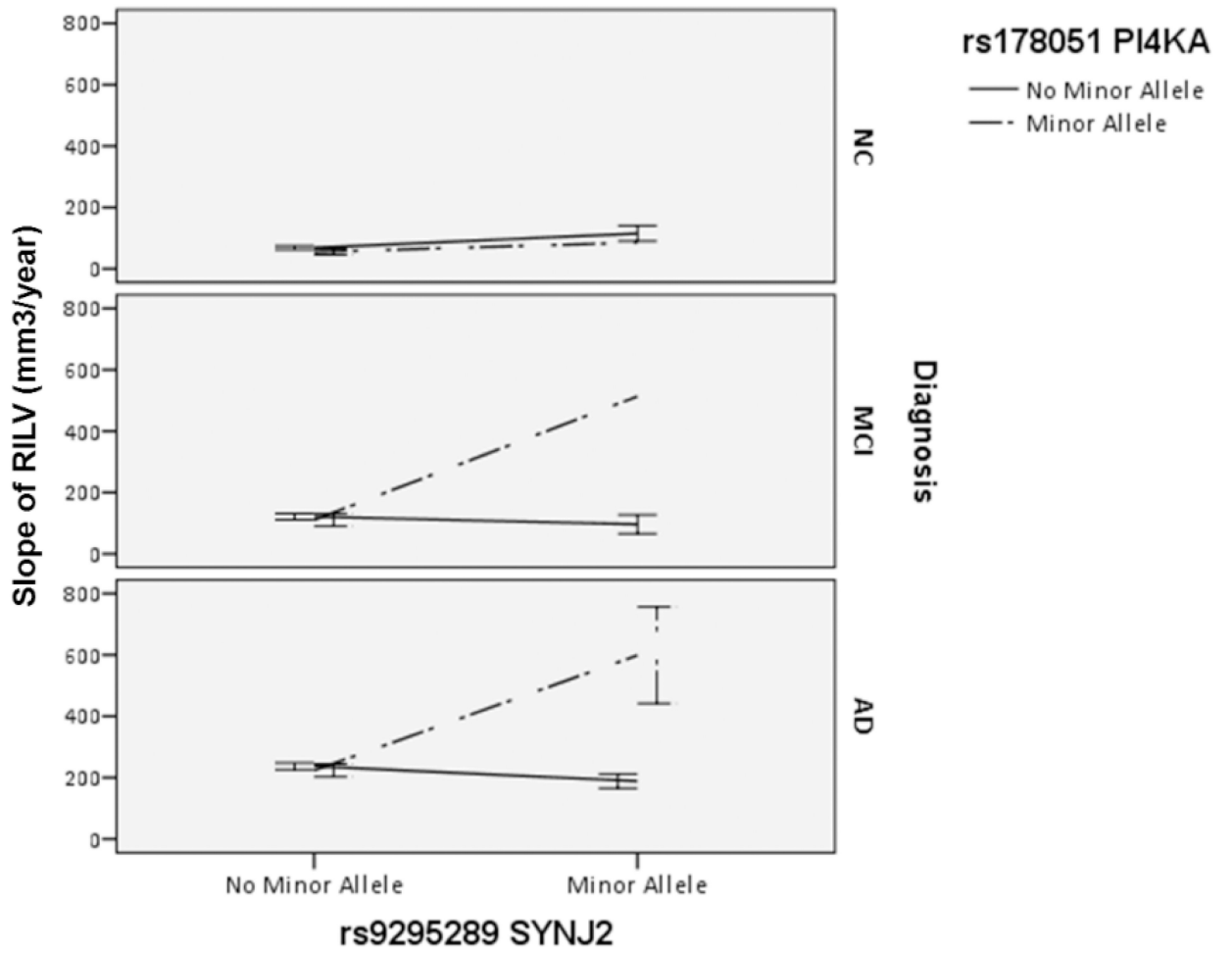
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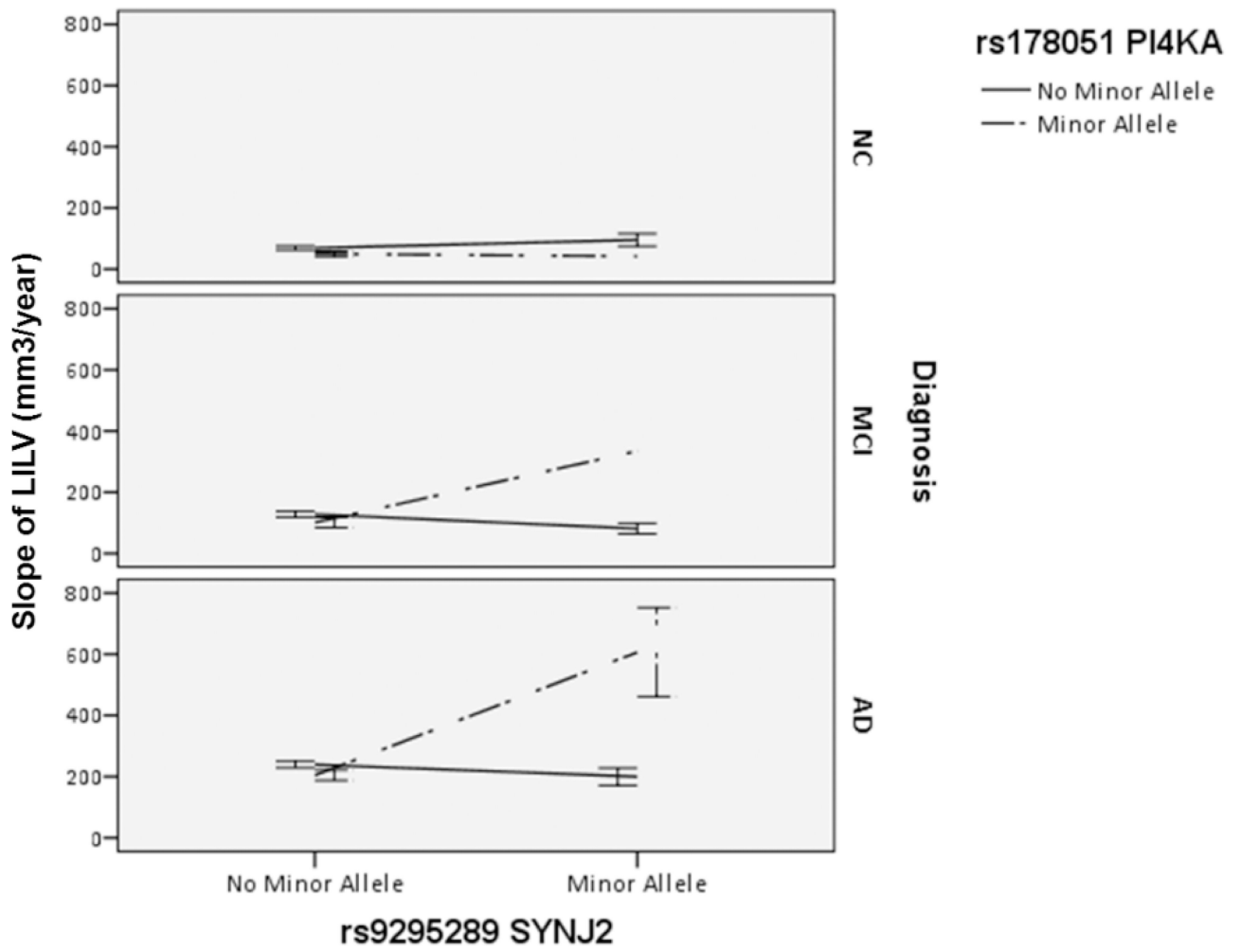
SYNJ2-PI4KA Interaction & RILV Slope
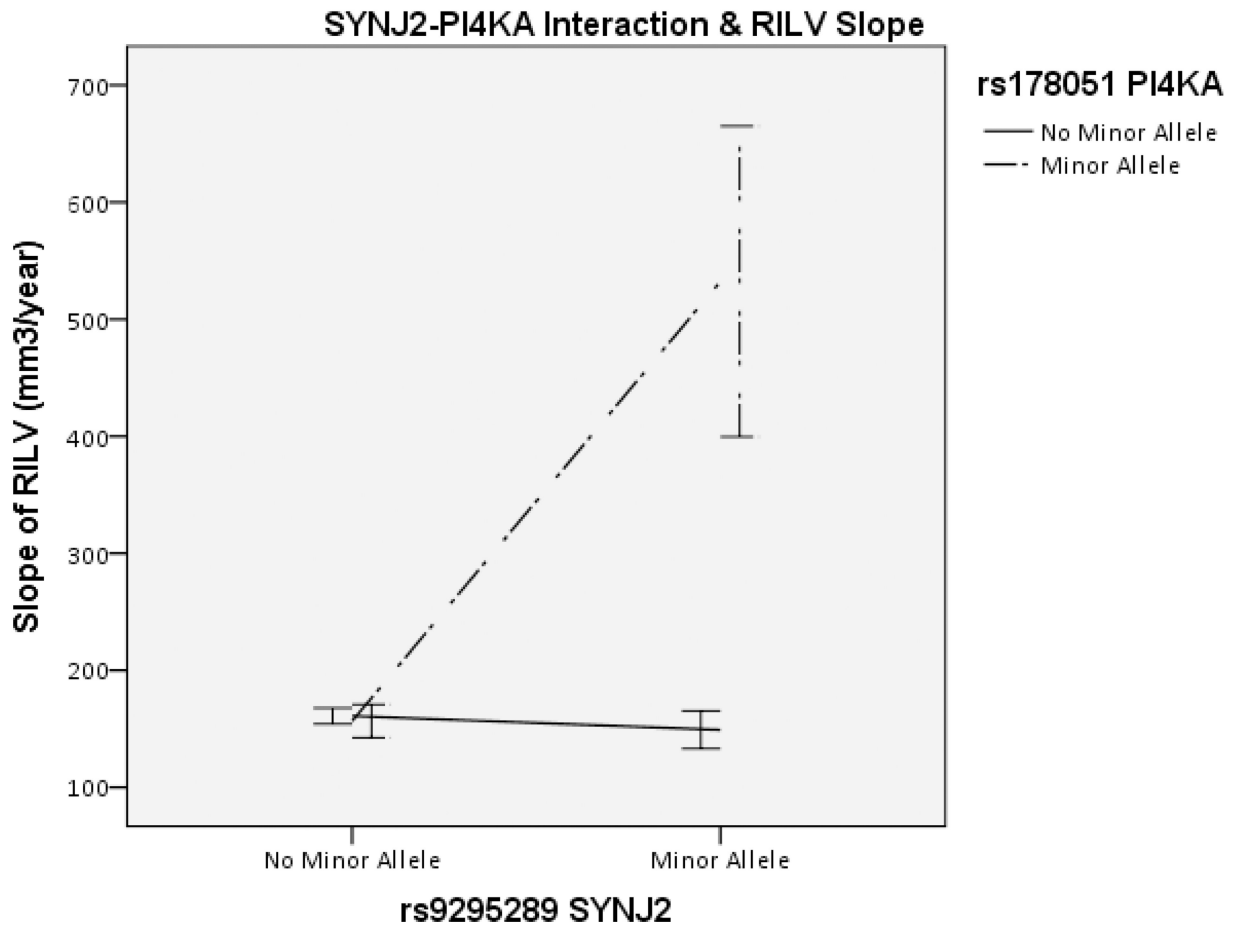
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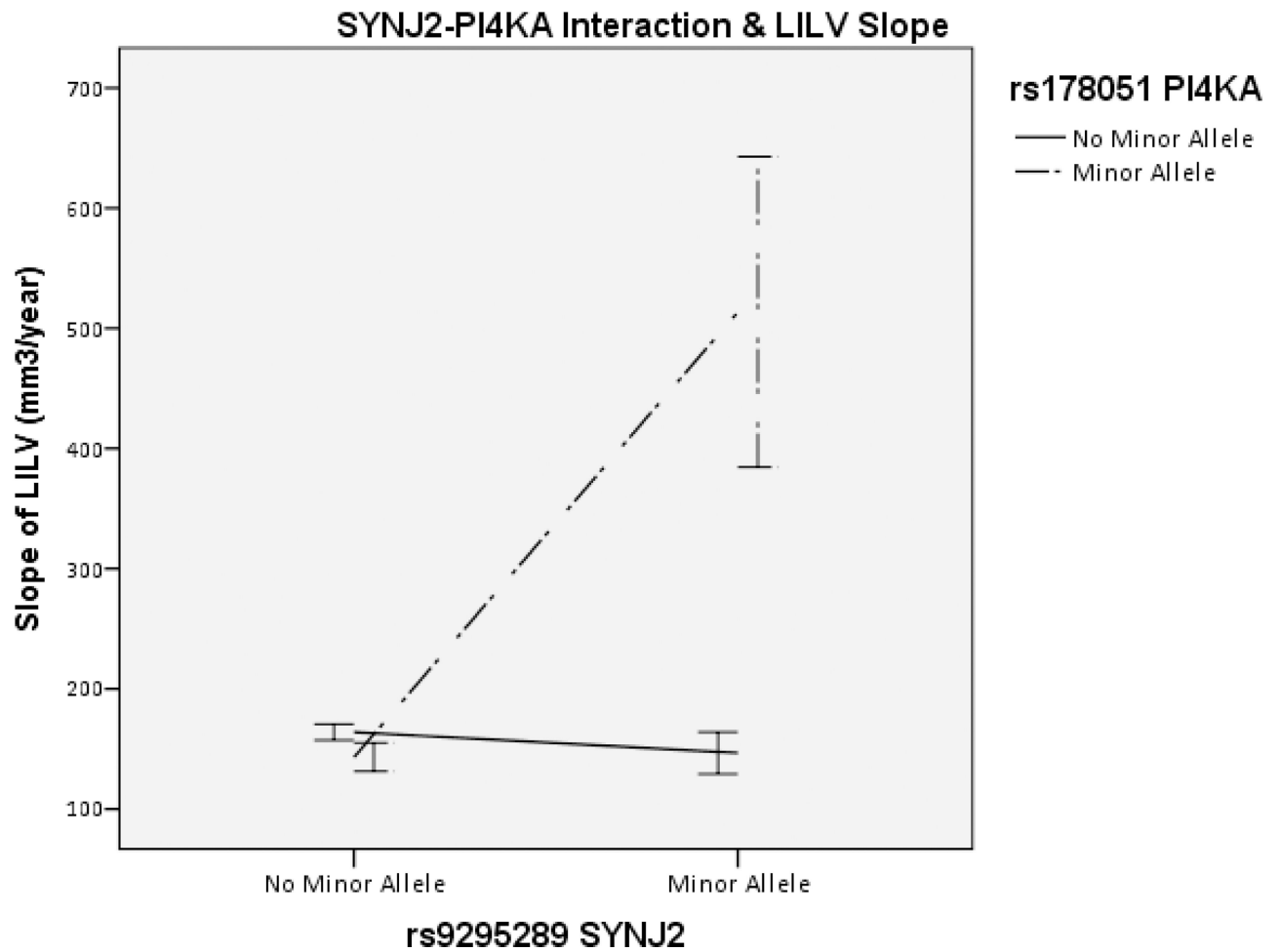
Figure 1.
Effect of SYNJ2 and PI4KA interaction on Right (A) and Left (B) Inferior Lateral Ventricle (RILV, LILV respectively). Bars represent one standard error.
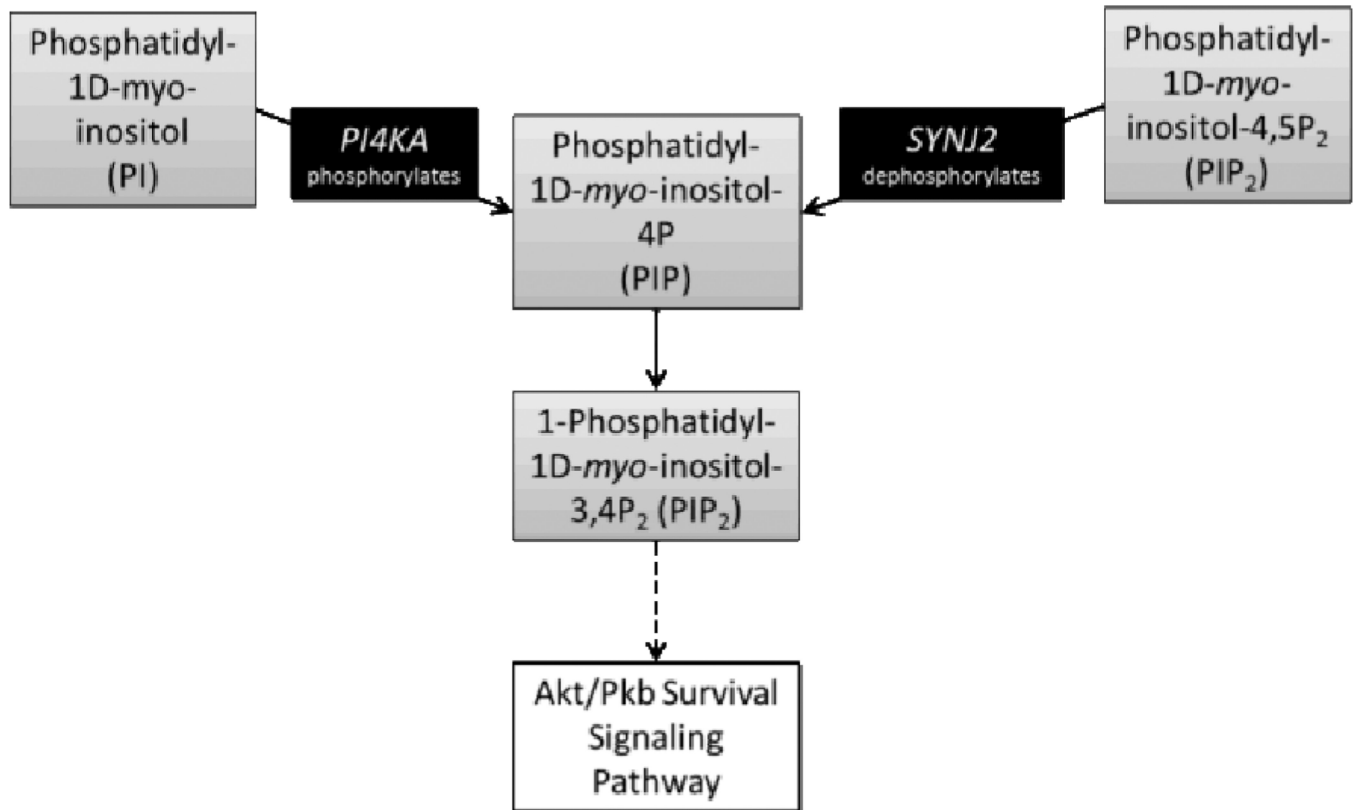
A.

B.

Figure 2.
Effect of SYNJ2 and PI4KA interaction on Right (A) and Left (B) Inferior Lateral Ventricle (RILV, LILV respectively) across diagnoses. Bars represent one standard error.

Figure 3.
Phosphatidylinositol and Akt/PKB Survival Signalling Pathway, adapted from the KEGG
Phosphatidylinositol signaling system (hsa04070, found at http://www.genome.jp/dbget-bin/
www_bget?hsa04070)

Table 1

Demographic Information

	Clinical Diagnosis[#]		
	Normal Control	Mild Cognitive Impairment	Alzheimer's Disease
Number of Patients	187	191	352
Number of *APOE*- 4 Carriers	45	81	231
Number of Females	85	61	146
Mean Baseline Age (*SD^*)	75.98 (5.61)	75.79 (7.09)	75.13 (7.33)
Mean Years of Education (*SD^*)	16.11 (2.79)	15.73 (3.01)	15.32 (2.97)
Mean RILV slope *(*SD^*)	70.55 (77.37)	119.85 (123.15)	237.67 (185.37)
Mean LILV slope *(*SD^*)	68.52 (78.63)	121.65 (110.92)	237.55 (179.6)

Clinical Diagnosis[#]

<u>Normal Control</u> subjects had a Mini-Mental Status Examination (MMSE) score between 24 and 30, a Clinical Dementia Rating (CDR) score of 0, and were not depressed (Geriatric Depression Scale score < 6).

<u>Mild Cognitive Impairment</u> subjects had a MMSE score between 24 and 30; objective memory impairment, subjective memory impairment, and a CDR score of 0.5

<u>Alzheimer's Disease</u> subjects met clinical criteria for dementia, had an MMSE of between 20 and 26, and had CDR score of .5 or 1.

*SD^* standard deviation.

*LILV/RILV** Left/Right inferior lateral ventricles.

Table 2

Full results with Bonferroni corrected Significant SNP-SNP interactions

| ROI | Gene 1 | | | Gene 2 | | | Interaction Term | | |
---	SNP	Gene	Main Effect p-value	SNP	Gene	Main Effect p-value	pvalue	R²	t
	rs9295289	*SYNJ2*	**0.10**	**rs178051**	*PI4KA*	**0.04**	**9.10E-12**	**0.04**	**7**
RILV	rs11596284	*PARD3*	0.30	rs3744566	*MYH2*	0.89	3.20E-10	0.04	6
	rs11614805	*PDE3A*	0.78	rs7154732	*ABHD12B*	0.91	3.60E-10	0.04	6
	rs11590865	*OR2L13*	0.01	rs1922127	*PRKG1*	0.19	3.60E-10	0.04	6
LILV	**rs9295289**	*SYNJ2*	**0.14**	**rs178051**	*PI4KA*	**0.34**	**8.20E-13**	**0.05**	**7**

ROI: region of interest. RILV: Right inferior lateral ventricle. LILV: Left inferior lateral ventricle. Chr: Chromosome. SNP: reference SNP number of first SNP in SNP-SNP pair. Gene: gene corresponding to SNP. Main: Main effect of SNP on ROI. p: nominal p value. : Bonferroni corrected p-value. : beta for SNP-SNP interaction term. $R^2= R^2$ for full model $-R^2$ for reduced model (without interaction term). t: t-statistic for interaction term. Bolded interaction was significant at Bonferroni corrected levels in both the RILV and LILV.