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# Interactions between *GSK3β* and amyloid genes explain variance in amyloid burden

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ABSTRACT

The driving theoretical framework of Alzheimer's disease (AD) has been built around the amyloid-β (Aβ) cascade in which amyloid pathology precedes and drives tau pathology. Other evidence has suggested that tau and amyloid pathology may arise independently. Both lines of research suggest that there may be epistatic relationships between genes involved in amyloid and tau pathophysiology. In the current study, we hypothesized that genes coding glycogen synthase kinase 3 (GSK-3) and comparable tau kinases would modify genetic risk for amyloid plaque pathology. Quantitative amyloid positron emission tomography data from the Alzheimer's Disease Neuroimaging Initiative served as the quantitative outcome in regression analyses, covarying for age, gender, and diagnosis. Three interactions reached statistical significance, all involving the *GSK3β* single nucleotide polymorphism rs334543—2 with *APBB2* (rs2585590, rs3098914) and 1 with *APP* (rs457581). These interactions explained 1.2%, 1.5%, and 1.5% of the variance in amyloid deposition respectively. Our results add to a growing literature on the role of GSK-3 activity in amyloid processing and suggest that combined variation in *GSK3β* and *APP*-related genes may result in increased amyloid burden.



## 1. Introduction

The pathologic cascade in Alzheimer's disease (AD) involves 2 primary lesions: amyloid-β (Aβ) plaques and neurofibrillary tangles made up of hyperphosphorylated tau. Genes involved in the production of Aβ cause autosomal dominantly inherited forms of AD (Price and Sisodia, 1998). The genetic etiology of late-onset AD is more complex and includes a great deal of missing heritability according to current approximations (Bertram et al., 2010).

The driving theoretical framework of AD over the past decade has been built around the Aβ cascade. The amyloid cascade hypothesis suggests that the disease process is initiated by Aβ formation leading to downstream pathologies and neurodegeneration (Hardy and Selkoe, 2002). Within such a framework, amyloid pathology precedes tau pathology and would have to drive its formation in some way. As nicely reviewed by Ittner and Götz (2010), there is substantial evidence that such a causal relationship does exist. However, other evidence has suggested that tau and amyloid pathology may arise independently, with upstream genetic interactions causing both pathologies through separate defects in distinct molecular pathways (Small and Duff, 2008). In either scenario, genes that confer risk for tau pathology may also confer risk for amyloid pathology through complex epistatic relationships. The current project sought to identify such interaction effects, primarily focusing on the tau kinases that have recently been implicated in both pathologic pathways.

One such kinase that has been implicated in both tau and amyloid pathology is glycogen synthase kinase 3 (GSK-3). GSK-3 has been implicated in tau hyperphosphorylation, subsequent neurodegeneration (Lucas et al., 2001) and amyloid accumulation (Martin et al., 2013). Moreover, GSK-3 appears to regulate Aβ production (Phiel et al., 2003), and silencing GSK-3 leads to reduced plaque and tangle formation in transgenic mouse models of AD (Hurtado et al., 2012). These findings have led to the GSK-3 hypothesis that suggests overactivity of GSK-3 can account for cognitive impairments, the pathologic cascade, and the neuroinflammatory response characteristic of AD (Hooper et al., 2008).

In addition to GSK-3, 2 other tau kinases have been implicated in both amyloid and tau pathology. As reviewed previously (Martin et al., 2013), knock-down of cyclin-dependent kinase 5 (CDK5) results in reduced tau pathology in transgenic AD models

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(Piedrahita et al., 2010), and the CDK5-related tau cascade appears to be activated by Aβ (Lopes et al., 2010). Dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) phosphorylates both tau and APP (Martin et al., 2013; Ryoo et al., 2008) and has been related to the pathologic cascades of tau and Aβ (Wegiel et al., 2011). In addition, there has been some evidence that phosphorylation of tau by DYRK1A leads to additional tau phosphorylation by GSK-3, ultimately resulting in hyperphosphorylation (Liu et al., 2008).

The aim of the current study was to identify epistatic relationships between genes coding tau kinases and genes previously associated with amyloid deposition. We hypothesized that genes coding GSK-3 and comparable tau kinases would modify genetic risk for amyloid plaque pathology.

## 2. Methods and materials

Data used in the preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). The ADNI was launched in 2003 by the National Institute on Aging, the National Institute of Biomedical Imaging and Bioengineering, the US Food and Drug Administration, private pharmaceutical companies, and nonprofit 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), Positron Emission Tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early AD. Determination of sensitive and specific markers of early AD progression is intended to aid researchers and clinicians to develop new treatments and monitor their effectiveness and to 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 coinvestigators from a broad range of academic institutions and private corporations, and subjects have been recruited from >50 sites across the United States and Canada. The initial goal of ADNI was to recruit 800 adults, aged 55–90, 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.

### 2.1. Subjects

Demographic data are presented in Table 1. Participants were enrolled according to the criteria outlined in the ADNI protocol (http://www.adni-info.org/Scientists/AboutADNI.aspx) and the ADNI2/ADNI-GO protocols (http://adni.loni.ucla.edu/wp-content/uploads/2008/07/ADNI_Go_Protocol.pdf; http://adni.loni.ucla.edu/wp-content/uploads/2008/07/ADNI2_Protocol_FINAL_20100917.pdf). For the present project, analyses were restricted to Caucasian subjects who had both genotype data and PET data.

### 2.2. Genotyping

We used data from all subjects who received a PET scan in the ADNI-2/GO protocol. Some of those subjects were genotyped in ADNI-1 on the Illumina Infinium Human-610-Quad BeadChip (Shen et al., 2010), and some were genotyped in ADNI-2/GO on the Illumina OmniQuad array (Potkin et al., 2009). For the present analyses, we looked at candidate single nucleotide polymorphisms (SNPs) that were present on both chips and combined both data sets to maximize our power.

Quality control was performed using PLINK software (version 1.07) (Purcell et al., 2007) excluding SNPs with a genotyping efficiency <98%, a minor allele frequency of <10%, or deviation from Hardy-Weinberg equilibrium (HWE) $<1e^{-6}$. Subjects were excluded if they had a call rate <90%, if there was a reported versus genetic sex inconsistency, or if relatedness to another sample was established (PI_HAT >0.5).

### 2.3. Single nucleotide polymorphism selection

For tau genes, we chose to focus on tau kinase genes that had been implicated in amyloid processing, as outlined in the Introduction. These included *GSK3β*, *GSK3A*, *CDK5*, and *DYRK1A*. For amyloid genes, we chose to focus on the 3 genes involved in dominantly inherited forms of AD (*APP*, *PSEN1*, *PSEN2*) as well as those genes that had previously shown either SNP- or gene-level associations with amyloid deposition measured with PET, including *ABCG1*, *APBB2*, *DHCR24*, *SOAT1*, and *BCHE* (Ramanan et al., 2013; Swaminathan et al., 2012).

SNPs that annotated to these genes were selected using the Illumina annotation file, which is freely available at http://www.switchtoi.com/annotationfiles.ilmn. We only used SNPs that were genotyped in both ADNI-1 and ADNI-2/GO and were annotated to these genes, resulting in 193 SNPs used in analyses (Supplementary Table 1). Of note, there were no SNPs that passed quality control and were annotated to *GSK3A* or *SOAT1*.

### 2.4. Quantification of amyloid deposition

Amyloid deposition was quantified using an $^{18}$F-AV-45 tracer as has been described extensively elsewhere (Landau and Jagust,

**Table 1**
Demographic information

| | Baseline clinical diagnosis[a] | | |
|---|---|---|---|
| | Normal control | Mild cognitive impairment | Alzheimer's disease |
| ADNI-1 data set | | | |
| Number of patients | 68 | 54 | 41 |
| Number of APOE-ε4 carriers | 15 | 18 | 26 |
| Number of females | 32 | 17 | 15 |
| Mean baseline age (SD) | 81.10 (5.01) | 79.41 (7.35) | 77.05 (6.54) |
| Mean years of education (SD) | 16.07 (3.03) | 15.54 (3.19) | 16.15 (2.88) |
| Mean SUVR[b] AV-45 (SD) | 1.07 (0.16) | 1.19 (0.25) | 1.32 (0.25) |
| ADNI-2/GO data set | | | |
| Number of patients | 109 | 239 | 25 |
| Number of APOE-ε4 carriers | 26 | 104 | 17 |
| Number of females | 53 | 103 | 9 |
| Mean baseline age (SD) | 74.83 (5.57) | 71.82 (7.44) | 74.20 (10.06) |
| Mean years of education (SD) | 16.45 (2.59) | 16.04 (2.64) | 15.80 (2.77) |
| Mean SUVR[b] AV-45 (SD) | 1.11 (0.20) | 1.19 (0.22) | 1.38 (0.21) |
| Combined Data set | | | |
| Number of patients | 177 | 293 | 66 |
| Number of APOE-ε4 carriers | 41 | 122 | 43 |
| Number of females | 85 | 120 | 24 |
| Mean baseline age (SD) | 77.24 (6.16) | 73.22 (7.98) | 75.97 (8.09) |
| Mean years of education (SD) | 16.31 (2.76) | 15.95 (2.75) | 16.02 (2.82) |
| Mean SUVR[b] AV-45 (SD) | 1.10 (0.19) | 1.19 (0.23) | 1.34 (0.23) |

[a] Normal control 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). Subjects with mild cognitive impairment had a MMSE score between 24 and 30, objective memory impairment, subjective memory impairment, and a CDR score of 0.5. Subjects with Alzheimer's disease met clinical criteria for dementia, had an MMSE of between 20 and 26, and had CDR score of 0.5 or 1.

[b] SUVR, standardized uptake value ratio for amyloid tracer.

2011). The mean standardized uptake value ratio (SUVR) measure was calculated across the cingulate (including anterior and posterior regions), frontal, temporal (including middle and lateral regions), and lateral parietal (including the precuneus and supramarginal gyrus) cortices, and divided by the reference region (cerebellar gray matter).

### 2.5. Single nucleotide polymorphism-single nucleotide polymorphism interaction analysis

Interaction analyses were run using SAS version 9.3 (http://www.sas.com). Mean SUVR was set as the quantitative outcome measure in a general linear regression model (PROC GLM). Covariates included age, gender, and diagnosis. We included the main effect of each SNP (1 from an amyloid-related gene and 1 from a tau-kinase gene) and the interaction term. A full additive model was used for SNP terms, meaning each SNP was coded as 0, 1, 2 based on the number of minor alleles. A total of 4175 tests were run, evaluating all SNP-SNP interactions between tau and amyloid genes. Correction for multiple comparisons using the false discovery rate procedure (FDR $<0.05$, PROC MULTTEST) and the Bonferroni procedure (family-wise error $<0.05$, PROC MULTTEST) was performed across all 4175 analyses.

### 2.6. Post hoc hierarchical linear regression

Following the identification of significant interactions, we used hierarchical linear regression in IBM SPSS 20 (http://www-01.ibm.com/software/analytics/spss) to quantify the amount of variance in amyloid deposition accounted for by these interaction terms. Our first step included age, diagnosis, and gender. Next, we included *APOE* status and the SNP main effects from the 2 candidate genes. Finally we included the SNP-SNP interaction term to see how much additional variance was explained by the interaction term beyond these known predictors of amyloid deposition.

### 2.7. Post hoc binary logistic regression

The variable quantifying amyloid load in the current analyses was not normally distributed within or across diagnostic groups. Although linear regression is known to be fairly robust to deviations from normality, we chose to validate our findings using binary logistic regression. A binary variable differentiating amyloid positive versus amyloid negative individuals was derived using a previously identified and accepted cut point of mean SUVR $>1.11$ (Landau and Jagust, 2011). This variable was set as a binary outcome measure in a logistic regression model using the same parameters as those in the original SNP-SNP interaction analysis above. Binary logistic regression was only run as a post hoc examination of the significant interactions identified in the primary analysis.

## 3. Results

### 3.1. Single nucleotide polymorphism-single nucleotide polymorphism interaction results

Three SNP-SNP interactions reached statistical significance when correcting for multiple comparisons (Table 2). One *GSK3β* SNP (rs334543) was involved in all 3 interactions, 2 with SNPs annotated to *APBB2* (rs2585590, rs3098914) and 1 with an SNP annotated to *APP* (rs457581). We also evaluated whether the observed effects were consistent across the 2 genotyping platforms. All interactions showed an effect across the 2 chips, although the *APP* × *GSK3β* interaction only showed a trend level association in the ADNI-1 subsample (Table 2).

### 3.2. Post hoc hierarchical linear regression

Gender, age, and diagnosis were entered into the model first and accounted for 12% of variance in amyloid deposition. Next, APOE status was entered into the model and accounted for an additional 18% of variance. Four separate hierarchical linear regression models were run across the 4 significant interactions. (We did not include all interactions in one model.) In each case, we added in the genetic main effects first and then the genetic interaction term to determine the variance associated with the interaction term alone. For *APPB2* (rs3098914) × *GSK3β* (rs334543), the nonsignificant ($p > 0.05$) SNP main effects accounted for 0.5% of variance, and the interaction term accounted for 1.5% of variance (2% of variance for the main effects and interaction combined). For *APBB2* (rs2585590) × *GSK3β* (rs334543), the nonsignificant ($p > 0.05$) SNP main effects accounted for 0.4% of variance, and the interaction accounted for 1.2% of variance (1.7% for the main effects and interaction combined). For *APP* (rs457581) × *GSK3β* (rs334543), the nonsignificant ($p > 0.05$) SNP main effects accounted for 0.4% of variance, and the interaction term accounted for 1.5% of variance (1.9% for the main effect and interaction combined). Finally, all 3 interactions remained statistically significant when performing binary logistic regression as outlined in Methods and Materials (Table 2).

## 4. Discussion

This project has identified 3 interactions with 1 *GSK3β* SNP (rs334543) that suggest *GSK3β* may indeed modify risk for amyloid deposition within specific genetic contexts. Given the role of GSK-3 in the neuroinflammatory response system and its suggested role in both amyloid and tau phosphorylation, it is not surprising that the genetic relationship to amyloid load in the present cohort is complex. Our results suggest that combined variation in *GSK3β*- and *APP*-related genes may result in increased amyloid burden.

### 4.1. Glycogen synthase kinase3β (rs334543) single nucleotide polymorphism function

All interactions in the current analyses involved rs334543. As reported in Haploreg (Ward and Kellis, 2012), this SNP is 20 kb 5′ from *GSK3β*; it acts as a strong enhancer in a variety of cell lines, including epithelial cells, skeletal muscle myoblasts, and lung fibroblasts among others; it has been shown to bind with 4 transcription factors in ENCODE tracks, including FOXA, POL2, and STAT3; and it has been shown to alter the p300 regulatory motif p300_known1 identified using position weigh matrix techniques. This suggests that this SNP is in a highly active genetic region and may regulate gene expression or otherwise play an active role in *GSK3β* function. In addition, rs334543 is in a DNase-I hypersensitivity uniform peak in an astrocyte cell line, suggesting this SNP may be functionally active in the brain (Rosenbloom et al., 2013).

### 4.2. Glycogen synthase kinase3B and amyloid burden

The first interaction in which the minor allele in *GSK3β* (rs334543) was related to high levels of amyloid deposition was a *GSK3β x APP* interaction. Although only 6 subjects were homozygote carriers of both the *APP* and *GSK3*β minor alleles (Fig. 1), none of the subjects were statistical outliers in amyloid deposition with each

**Table 2**
SNP-SNP interaction analysis

| Gene | SNP | Combined data set | | | | | | | | ADNI-1 data set | | ADNI2/GO data set | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MAF | T[a] | $R^{2}$[b] | *p* value | FWE[c] | FDR[d] | $\chi^{2}$[e] | *p* value | $t$[a] | *p* value | $t$[a] | *p* value |
| *APPB2* | rs2585590 | 0.46 | **4.41** | **0.015** | $\mathbf{1.2 \times 10^{-5}}$ | **0.052** | **0.036** | **11.14** | $\mathbf{1.0 \times 10^{-3}}$ | **3.21** | **0.002** | **3.17** | **0.002** |
| ×*GSK3β* | ×rs334543 | 0.31 | | | | | | | | | | | |
| *APPB2* | rs3098914 | 0.24 | **−4.25** | **0.012** | $\mathbf{2.5 \times 10^{-5}}$ | 0.107 | **0.036** | **10.63** | $\mathbf{1.0 \times 10^{-3}}$ | **−2.51** | **0.013** | **−3.37** | **0.001** |
| ×*GSK3β* | ×rs334543 | 0.31 | | | | | | | | | | | |
| *APP* | rs457581 | 0.25 | **4.25** | **0.015** | $\mathbf{2.5 \times 10^{-5}}$ | 0.107 | **0.036** | **13.86** | $\mathbf{2.0 \times 10^{-4}}$ | 1.85 | 0.066 | **3.51** | **0.001** |
| ×*GSK3β* | ×rs334543 | 0.31 | | | | | | | | | | | |

Note: *p* values < .05 are shown in bold.
Key: MAF, minor allele frequency.
[a] *t* value for SNP × SNP interaction term.
[b] $R^2$ value represents the change in $R^2$ when introducing the SNP × SNP interaction term into the post hoc hierarchical linear regression model.
[c] FWE (family wise error) represents the *p* value when correcting for multiple comparisons using the Bonferroni procedure (4175 tests).
[d] FDR (false discovery rate procedure) represents the *p* value when correcting for multiple comparisons using the FDR procedure.
[e] $\chi^2$ value for SNP × SNP interaction term in the post hoc binary logistic regression model using amyloid positive/negative status as outcome.

falling between 1.4 and 1.8 mean SUVR. Previous research has suggested that *GSK3β* might play a role in APP processing. A GSK-3 blocker, lithium, has been shown to decrease amyloid burden in APP mice, and the effect appears to be driven by GSK3β, given that genetic modification of *GSK3β* mimics this effect (Su et al., 2004). Additional evidence has suggested that modulation of GSK3β activity reduces APP phosphorylation and amyloid load (Rockenstein et al., 2007). In the present study, the effect of *GSK3β* was only present in carriers of the *APP* (rs457581) minor allele, and neither SNP showed a main effect in conferring risk for amyloid when the interaction term was excluded. This may suggest that slight increases in GSK3β activity are only related to a negative outcome when *APP* is overexpressed as well, perhaps via the increased phosphorylation of APP suggested previously (Rockenstein et al., 2007). Regardless of the exact mechanism, the observed interaction adds additional support to a *GSK3β-APP* relationship that appears to have a meaningful impact on risk for amyloid burden in vivo.

The other 2 significant interactions were between *GSK3β* and *APBB2*. In both cases, the strongest effect of *GSK3β* was present in homozygous carriers of the A allele for these SNPs (Figs. 2 and 3), although the A allele was actually the major allele for rs2585590 (54% frequency). These 2 *APBB2* SNPs are in low linkage disequilibrium in the 1000 Genomes data set ($r^2 = 0.27$, $D' = 1$), leaving open the possibility that the same signal is driving both effects. However, the 2 SNPs did appear to differ slightly in terms of their interaction effect (Figs. 2 and 3). The interaction with rs2585590 was particularly interesting because it appeared that *GSK3β* homozygous minor allele carriers showed especially low amyloid burden in homozygous carriers of the *APBB2* A allele and especially high amyloid burden in homozygous carriers of the G allele. We would suggest that this may be due to the role GSK-3 plays in both *APP* processing and the neuroinflammatory response system. In certain scenarios, the increased cytokine production and microglial response driven by GSK-3 (Woodgett and Ohashi, 2005) might have beneficial effects by decreasing amyloid load through microglial phagocytosis (Rogers et al., 2002). However, when GSK-3 activity is overactive in the presence of overexpressed *APP*, any beneficial effects of an early pro-inflammatory response fail to clear amyloid fast enough, ultimately resulting primarily in the damaging side effects of neuroinflammation and failure to substantially reduce the aggregation of amyloid deposits.

Such a hypothetical model is particularly relevant to the *APBB2* × *GSK3β* interaction because *APBB2* appears to drive the intracellular production of both APP and GSK-3. The gene product of *APBB2* is a member of the FE65 protein family, which interacts with the amyloid intracellular domain (AICD) and ultimately has an effect on APP processing (McLoughlin and Miller, 2008). Moreover, the AICD-FE65 interaction appears to have an effect on GSK3 activity in that the AICD modulates (increases) GSK-3 activity, but only when bound by FE65 (Ryan and Pimplikar, 2005). In the present results, variation in *APBB2* may ultimately influence the probability of FE65 binding to the AICD and thus influence whether *APP* becomes overexpressed and whether GSK-3 becomes overactive. When FE65 binding to AICD is reduced (perhaps in homozygous carriers of the A allele in either of these 2 *APBB2* SNPs), the slightly increased *GSK3β* expression related to minor allele status in rs334543 is actually beneficial. However, when the FE65-AICD complex is more prevalent, perhaps in rs2585590 G/G carriers, the slightly increased *GSK3β* expression becomes damaging in the presence of increased APP, and the additional GSK-3 activity driven by the FE65-AICD complex.

### 4.3. Strengths and limitations

This article has highlighted a potential gene-gene interaction in support of the GSK-3 hypothesis of AD. The strengths of the current analyses include the well-characterized sample, a validated, disease-relevant quantitative phenotype, and consistent interaction effects observed across 2 independent cohorts.

However, this study is not without limitations. Although consistent effects were observed across independent subsets in ADNI, a true replication sample from an additional data source with GWAS and PET data will be necessary to confirm our findings. This is especially relevant given the relative low cell counts present when performing interaction analyses, although the consistency in the present analysis in the fewer minor allele homozygotes is encouraging. Our 2 subsamples also included slight differences in clinical characteristics. The ADNI-1 sample in our analysis included fewer MCI and mild MCI subjects relative to ADNI-2/GO. In the case of the *APP* × *GSK3β* interaction, the trend level effect in ADNI-1 may indeed be driven by the discrepancy in MCI subjects relative to the other categories. The observed effects do appear to be driven by an effect in the MCI cohort in all cases when stratifying across diagnostic categories, but a larger sample of each group is necessary to test for a 3-way interaction (diagnosis × *APP* × *GSK3β*). Moreover, because all AV-45 PET scans were conducted as part of ADNI-GO (about 6 years after ADNI-1 was initiated), subjects who were genotyped in ADNI-1 and received an AV-45 PET scan had been in the study longer and might have introduced a form of survivor bias into the ADNI-1 subsample.

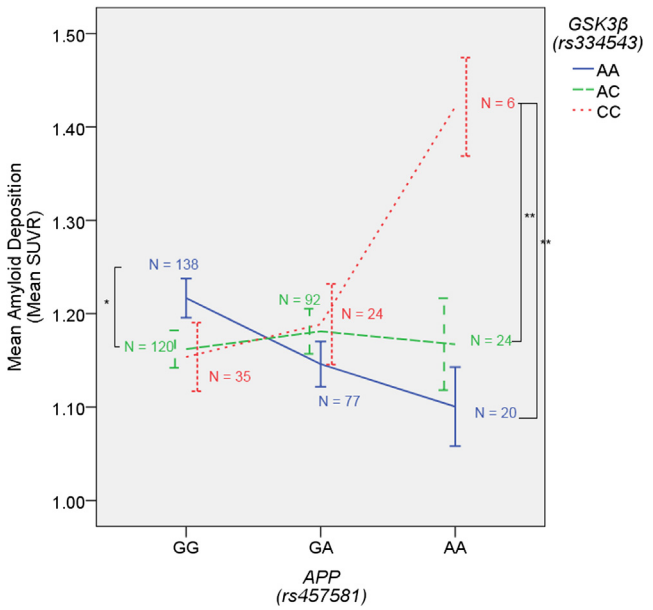


**Fig. 1.** GSK3β × *APP* on amyloid deposition. The *GSK3β* (rs334543) minor allele is associated with higher amyloid burden in homozygous carriers of the *APP* (rs457581) minor allele. Error bars represent the standard error. ** $p < 0.005$ (1-tailed).

The other main weakness of this study is the lack of data demonstrating the function of the implicated SNPs. Although bioinformatics methods were used to annotate findings based on known or predicted function, additional molecular experiments verifying the functional relationships between these genes, and these SNPs more specifically, is warranted.

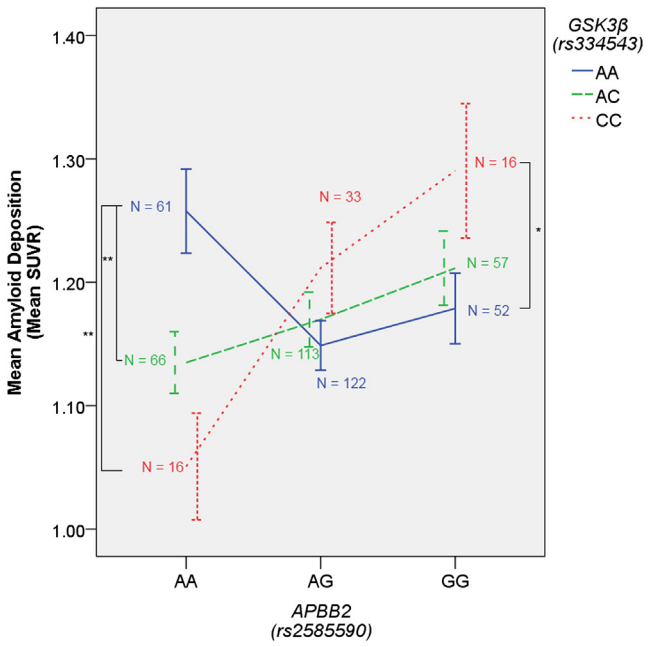


**Fig. 2.** *GSK3β* × *APBB2* (rs2585590) on amyloid deposition. The *GSK3β* (rs334543) minor allele is associated with higher amyloid burden in homozygous carriers of the *APBB2* (rs2585590) minor allele, and lower amyloid burden in homozygous carriers of the *APBB2* major allele. Error bars represent the standard error. * $p < 0.05$ (1-tailed), ** $p < 0.005$ (1-tailed).

### 4.4. Future directions

The biologically plausible mechanism implicated in this study suggests some possible avenues for future exploration. Functional analyses focusing on rs334543 may help clarify the role this SNP plays in GSK-3 activity—and ultimately better elucidate the role of GSK-3 activity in APP processing and the neuroinflammatory response to amyloid deposition. Additional genetic analyses incorporating tau protein levels as measured in cerebrospinal fluid may also shed some light on the complex relationship between amyloid and tau pathology. This work has identified a candidate genetic interaction between *GSK3β* and 2 genes involved in amyloid pathophysiology—*APP* and *APBB2*. Our results were consistent across the sub−data sets of ADNI, but future work replicating these interactions in an independent data source is warranted.

## Disclosure statement

The authors declare no competing financial interests.

## Acknowledgements

Data used in preparation of this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.ucla.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in analysis or writing of this report. A complete listing of ADNI investigators can be found at http://adni.loni.ucla.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf.

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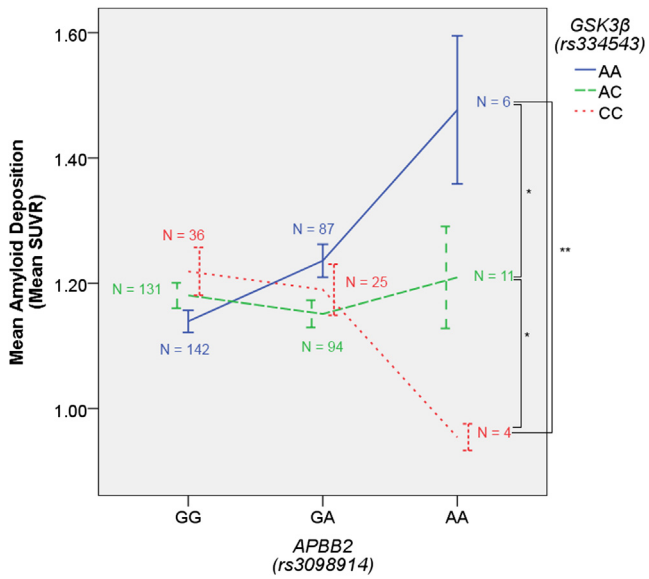


**Fig. 3.** *GSK3β* × *APBB2* (rs3098914) on amyloid deposition. The *GSK3β* (rs334543) minor allele is associated with lower amyloid burden in homozygous carriers of the *APBB2* (rs3098914) minor allele. Error bars represent the standard error. * $p < 0.05$ (1-tailed), ** $p < 0.005$ (1-tailed).

collection and analysis, decision to publish, or preparation of the manuscript.

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The data contained have not been previously published and will not be submitted elsewhere while under consideration at Neurobiology of Aging. Appropriate approvals were received from the local institutional review boards. All authors have reviewed the contents of the manuscript, approve of its contents, and verify the accuracy of the data.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.neurobiolaging.2013.08.032.

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