Published in final edited form as:

Neurotox Res. 2015 October ; 28(3): 232–238. doi:10.1007/s12640-015-9541-0.

GSK3 β Interactions with Amyloid Genes: An Autopsy Verification and Extension

Timothy J. Hohman¹, Lori Chibnik², William S. Bush³, Angela L. Jefferson¹, Phillip L. De Jaeger⁴, Tricia A. Thornton-Wells⁵, David A. Bennett⁶, and Julie A. Schneider⁶

¹ Vanderbilt Memory and Alzheimer's Center, Vanderbilt, University School of Medicine, Vanderbilt University, Medical Center, 2525 West End Ave, 12th Floor, Suite 1200, Nashville, TN 37203, USA

² Program in Translational NeuroPsychiatric Genomics, Departments of Neurology & Psychiatry, Institute for the Neurosciences, Brigham and Women's Hospital, Boston, MA, USA

³ Department Epidemiology & Biostatistics, Institute for Computational Biology, Case Western Reserve University, Cleveland, OH, USA

⁴ Department of Neurology, Harvard Medical School, Cambridge, MA, USA

⁵ Center for Human Genetics Research, Department of Molecular Physiology & Biophysics, Vanderbilt University Medical Center, Nashville, TN, USA

⁶ Rush Alzheimer's Disease Center, Department of Neurological Sciences, Rush University Medical Center, Chicago, IL, USA

Abstract

Glycogen synthase kinase 3 (GSK3) plays an important role in the pathophysiology of Alzheimer's disease (AD) through the phosphorylation of tau. Recent work has suggested that *GSK3 β* also plays a role in the amyloid pathway of AD through genetic interactions with *APP* and *APBB2* on in vivo measures of amyloid. This project extends the previously identified genotype interactions to an autopsy measure of amyloid, while also testing the same interactions leveraging gene expression data quantified in the prefrontal cortex. 797 participants (251 cognitively normal, 196 mild cognitive impairment, and 350 Alzheimer's disease) were drawn from the Religious Orders Study and Rush Memory and Aging Project. A mean score of amyloid load was calculated across eight brain regions, gene expression levels from frozen sections of the dorsolateral prefrontal cortex were quantified using RNA amplification, and expression signals were generated using Beadstudio. Three SNPs previously identified in genetic interactions were genotyped using the Illumina 1M genotyping chip. Covariates included age, sex, education, and diagnosis. We were able to evaluate 2 of the 3 previously identified interactions, of which the interaction between *GSK3 β* (rs334543) and *APBB2* (rs2585590) was found in this autopsy sample ($p = 0.04$).

Timothy J. Hohman, Timothy.J.Hohman@Vanderbilt.edu.

Electronic supplementary material The online version of this article (doi:10.1007/s12640-015-9541-0) contains supplementary material, which is available to authorized users.

Compliance with Ethical Standards

Conflict of interest The authors report no biomedical financial interests or potential conflicts of interest.

We observed a comparable interaction between *GSK3 β* and *APBB2* when comparing the highest tertile of gene expression to the lowest tertile, $t(1) = -2.03$, $p = 0.043$. These results provide additional evidence of a genetic interaction between *GSK3 β* and *APBB2* and further suggest that *GSK3 β* is involved in the pathophysiology of both of the primary neuropathologies of Alzheimer's disease.

Keywords

GSK3; Alzheimer's disease; Amyloid; Gene interaction; Gene expression; Genomics; Autopsy

Introduction

Glycogen synthase kinase 3 (GSK3) has been increasingly investigated in the field of Alzheimer's disease (AD) over the past few years (Hooper et al. 2008), including ongoing work applying GSK3 inhibitors in the context of AD treatment (del Ser et al. 2013a, b). GSK3 has been implicated in the pathophysiology of both primary pathologies in AD, amyloid- β plaques, and neurofibrillary tangles made up of hyperphosphorylated tau. Specifically, the β isoform of GSK3 (GSK3 β) is a tau protein kinase (Ishiguro et al. 1993) and relates to both tau hyperphosphorylation and subsequent neurodegeneration (Lucas et al. 2001). More recently, work in transgenic mice has suggested that treatment targeting GSK3 results in partial reversal of tangle pathology and prevention of disease progression early in the disease cascade (Hernandez et al. 2013). In relation to amyloid, GSK3 has been implicated in A β production (Phiel et al. 2003) and accumulation (Martin et al. 2013). Thus, it has been suggested that genetic interactions with GSK3 upstream of both amyloid and tau pathology may play a causal role in AD pathogenesis (Small and Duff 2008).

Previously, we evaluated the possibility of upstream interactions between amyloid and tau genes by investigating interactions between genes in the amyloid pathway with GSK3 in relation to an in vivo measure of amyloid deposition using data from the Alzheimer's Disease Neuroimaging Initiative (ADNI; Hohman et al. 2014). We identified 3 interactions all involving the SNP rs334543 just upstream of *GSK3 β* . However, the ADNI dataset did not allow for the opportunity to evaluate how the identified SNP–SNP interactions related to pathologic burden at autopsy, nor whether the genotypes identified were associated with levels of gene expression in the brain. This manuscript extends our previous work in the following ways. First, we evaluated the identified SNP–SNP interactions in relation to an autopsy measure of amyloid burden to provide additional verification of the observed effect in an independent sample. Second, we tested whether the genotypes at the relevant SNPs are related to gene expression in the prefrontal cortex. Finally, we tested whether we observe the same gene–gene interactions when leveraging gene expression rather than genotypes in statistical models predicting amyloid burden. Collectively, this work will further clarify the role of GSK3 in both the primary pathologies of AD, while also highlighting the means by which the seemingly independent amyloid and tau pathways may intersect through upstream genetic interactions.

Materials and Methods

Subjects

Participants were drawn from two cohort studies of aging and dementia: the Religious Orders Study (ROS) and the Rush Memory and Aging Project (MAP; Bennett et al. 2012a, b). The Religious Orders Study began in 1994 and involves older Catholic nuns, priests, and brothers recruited from >40 groups across the United States. The Rush Memory and Aging Project began in 1997 and involves older lay persons recruited from retirement communities, subsidized housing facilities, and social service agencies in the Chicago metropolitan area. Persons in both studies enrolled without dementia and agreed to annual clinical evaluations and organ donation at death. Written informed consent was obtained in each study after procedures were fully explained, and both studies were approved by the institutional review board of Rush University Medical Center. All participants also signed a Uniform Anatomical Gift Act for organ donation. The analysis of the data was approved by the institutional review board of Vanderbilt University Medical Center.

Clinical Evaluation

All participants underwent a comprehensive clinical and neuropsychological evaluation that has been outlined previously (Bennett et al. 2002, 2006a). A board certified neuropsychologist provided an opinion on the presence and severity of cognitive impairment based on a participants neuropsychological test performance. Clinical evaluation of AD using this information, and relevant information from the medical history and physical examination, was based on the criteria of the joint working group of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (McKhann et al. 1984). A diagnosis of mild cognitive impairment was made for those individuals judged to have cognitive impairment by the neuropsychologist but did not meet clinical criteria for dementia.

Autopsy Procedures and Neuropathologic Measures

Details on autopsy and neuropathology procedures have been published previously (Bennett et al. 2012). Briefly, brains were removed and weighed, hemispheres were cut coronally into 1-cm slabs, and uniform examination procedures were conducted using slabs and/or pictures from both hemispheres. For the present analyses, we used quantification of amyloid load calculated across eight brain regions (entorhinal cortex, CA1/subiculum of the hippocampus, superior frontal cortex, dorsolateral prefrontal cortex, inferior temporal cortex, angular gyrus, anterior cingulate cortex, and occipital cortex), the details of which have been published previously (Bennett et al. 2006b). These regions were cut into 20 μ m to identify and assess average amyloid load using an N-terminus-directed monoclonal antibody (10D5, dilution = 1:300, Elan, Dublin, Ireland; Beta-Amyloid, 17–24 (4G8), dilution = 1:9000, Leica Biosystems, Buffalo Grove, IL; DAKO, dilution = 1:50, Carpinteria, CA). Quantification of amyloid- β load was accomplished by image processing in an automated, multistage computational image analysis protocol. Twenty-four percent of each cortical region (~ 75 images) was sampled to determine the average percent is occupied by A β per region. The value included intracortical vascular amyloid. The unbiased random sampling technique has been outlined previously (Bennett et al. 2004), and the algorithm has been

applied in the context of other pathologies that are expressed ubiquitously throughout the brain (Mitchell et al. 2000).

Genotyping, Quality Control, and SNP Selection

DNA was extracted from whole blood, lymphocytes, or frozen postmortem brain tissue. Genotyping was performed using the Illumina 1M chip (Shulman et al. 2013). For the current analysis, we evaluated the three SNPs from the interactions observed previously in ADNI, rs2585590, rs457581, and rs334543 (Hohman et al. 2014). A second SNP annotated to *APBB2* in the original analysis was not available in this sample. No SNPs were removed during quality control, which included exclusions if the genotyping rate was <0.01 , the minor allele frequency was <0.01 , or if deviation from Hardy–Weinberg equilibrium was observed ($p < 0.000001$).

Quantification of Gene Expression

RNA expression levels for *GSK3 β* and *APBB2* were obtained for the ROS/MAP from frozen sections of the dorsolateral prefrontal cortex that was manually dissected from postmortem brain tissue. Details of RNA extraction, processing and data quality control and normalization have been previously published (Lim et al. 2014). In brief, RNA was isolated using the RNeasy lipid tissue kit (Qiagen, Valencia, CA) and was reverse transcribed and biotin-UTP labeled using the Illumina® TotalPrep™ RNA Amplification Kit from Ambion (Illumina, San Diego, CA). Expression signals were generated using the Beadstudio software suite (Illumina, San Diego, CA). Standard control and normalization methods were employed to account for technical variability due to differences in hybridization dates and stabilize the variance for the purpose of statistical analyses.

Statistical Analyses

All statistical analyses were performed in R (version 2.15.2; <http://www.r-project.org/>). Our threshold for statistical significance was set a priori at $\alpha < 0.05$. Participant characteristics were compared using a one-way ANOVA for continuous variables and a Kruskal–Wallis test for categorical variables.

Autopsy Extension of Previously Identified SNP–SNP Interactions—For the gene–gene interaction analysis, we set amyloid load as a quantitative outcome in a linear regression model.

Amyloid load was square root transformed to better approximate a normal distribution (Bennett et al. 2006b). We used additive coding for the SNPs and included age at death, diagnosis, sex, and education as covariates. The SNP $\times$ SNP interaction was our term of interest.

Gene–Gene Expression Follow-Up Analysis—For the gene–gene expression analysis, we evaluated the replicated gene–gene effects from the autopsy extension (see autopsy extension interaction analysis above). First, we evaluated whether the SNPs of interest were expression quantitative trait loci (eQTL) for the relevant gene using a one-way ANOVA. Second, we tested whether gene expression was related to amyloid load using

linear regression including the same covariates used in the SNP–SNP interaction analysis. Given the genotype interaction observed, we also evaluated expression using tertiles in order to better compare high expression to low expression. In the tertile analysis, the lowest tertile was set as the referent in the linear regression model. Third, we evaluated the previously observed gene–gene interaction using expression levels instead of genotypes. We ran this analysis both treating expression as a continuous variable and as a categorical variable (i.e., tertiles with the lowest tertile set as the referent). We also re-ran analyses evaluating amyloid levels derived from the prefrontal cortex (middle frontal gyrus and superior frontal gyrus) to assess whether the expression derived from prefrontal cortex was specifically associated with amyloid levels in the prefrontal cortex. Finally, because our measure of amyloid is somewhat confounded by the presence of cerebral amyloid angiopathy (CAA), we chose to run secondary analyses evaluating all significant associations in relation to CAA using binary logistic regression with the presence of CAA set as a binary outcome variable.

Results

Participant Characteristics

Demographic and clinical characterization of the sample is presented in Table 1. We restricted our analyses to Caucasian individuals to reduce the risk of spurious genetic effects due to population stratification. The genotype dataset included 797 participants (251 NC, 196 MCI, 350 AD). As expected, AD participants were older ($p < 0.001$), had a higher percentage of *APOE* $\epsilon 4$ carriers ($p < 0.001$), and a higher percent burden of amyloid at autopsy ($p < 0.001$) compared to MCI and NC participants. The gene expression dataset included 436 participants (143 NC, 114 MCI, 179 AD). AD participants showed lower levels of *GSK3 β* expression ($p = 0.049$) and higher levels of *APBB2* expression ($p = 0.006$) in the prefrontal cortex compared to NC and MCI participants.

Autopsy Extension of Previous SNP–SNP Interactions

Results are presented in Table 2. Consistent with our previous results, we did not observe a significant main effect of any of the SNPs on amyloid burden; however, we did observe an interaction between *GSK3 β* (rs334543) and *APBB2* (rs2585590) on amyloid burden ($t(1) = 2.03$, $p = 0.04$). This interaction was in a consistent direction with our previously observed finding in ADNI (Fig. 1). We did not observe this same interaction on the presence of CAA ($t(1) = 1.32$, $p = 0.184$). We did not find the previously observed interaction between *GSK3 β* (rs334543) and *APP* (rs457581; $t(1) = -0.98$, $p = 0.33$). Therefore, in our follow-up gene expression analyses we only evaluated *APBB2* and *GSK3 β* .

In eQTL analyses, rs334543 did not show an association with expression levels of *GSK3 β* ($p = 0.147$), and rs2585590 was not related to *APBB2* expression ($p = 0.76$).

When evaluating main effects of gene expression on amyloid burden, we observed a significant effect of *APBB2* ($t(1) = 3.06$, $p = 0.002$) but not *GSK3 β* ($p = 0.20$). Neither gene was associated with the presence of CAA ($p > 0.05$).

Finally, when evaluating the gene–gene interaction using levels of expression, we did not observe a significant interaction between *APBB2* and *GSK3 β* when treated as continuous

variables ($t(1) = -1.03$, $p = 0.301$) but did observe a significant interaction between the highest tertile relative to the lowest tertile ($t(1) = -2.03$, $p = 0.043$). As seen in Fig. 2, high levels of *GSK3 β* expression were protective in those individuals with high levels of *APBB2* expression. When restricting analyses to amyloid levels derived from the prefrontal cortex the interaction remained statistically significant both in the middle frontal gyrus ($t(1) = -2.47$, $p = 0.014$) and the superior frontal gyrus ($t(1) = -2.07$, $p = 0.039$). When evaluating the same interaction on the presence of CAA, we did not observe a statistically significant association ($t(1) = -0.79$, $p = 0.427$).

Discussion

The present manuscript extends a previously observed SNP–SNP interaction using an autopsy measure of amyloid pathology, confirming that among minor allele carriers of rs2585590 the minor allele of rs334543 appears protective against high levels of amyloid deposition. Moreover, in a smaller sample of subjects with gene expression data, we were able to provide preliminary evidence that high levels of *GSK3 β* expression in the prefrontal cortex appear to be protective against amyloid deposition in the presence of high levels of *APBB2* expression.

The results of the SNP–SNP replication provide additional evidence that the effect of *GSK3 β* on amyloid deposition may depend largely on the genetic context. Consistent with our previous finding, we observed a protective, dose-dependent effect of the *GSK3 β* (rs334543) SNP in homozygous carriers of the *APBB2* (rs2585590) SNP. However, we did not observe the complete reversal of the effect in homozygous carriers of the G allele as previously observed (Fig. 1). One possible explanation for this discrepancy is the difference in the samples evaluated. The present cohort has a much larger percentage of ADs compared to sample previously evaluated in ADNI (44 % AD in ROS/MAP v. 12 % AD in the ADNI Flortetapir sample) and a much lower percentage of patients with MCI (24 % in ROS/MAP v. 54 % in ADNI Flortetapir sample). Unfortunately, we did not have a sufficient sample size in either dataset to provide a comprehensive evaluation of SNP–SNP–diagnosis interactions in our models, as the three way interaction left contingency table cells with as few as 1 subject in both ADNI and ROS/MAP. Larger samples are needed to evaluate how this observed genetic interaction differs across diagnostic categories. Stratified figures are presented in Supplementary Fig. 1 from both ADNI and ROS/MAP to clarify how the observed interaction may differ across diagnostic categories. However, the strongest observed effect in homozygous carriers of the rs2585590 A allele appears to be consistent across datasets and across diagnostic categories. It should also be noted that in the current analysis, we used actual neuropathological measures of amyloid pathology, whereas in ADNI we only looked at a proxy measure of amyloid using PET imaging. The neuropathological metric should be more sensitive and less susceptible to artifact, so although the effect sizes differ, the consistency of the observed interaction across in vivo and ex vivo measures provides strong support for the validity of the observed interaction effects.

This manuscript has provided some clarification on the manner of the interaction between *GSK3 β* and *APBB2* by demonstrating that the elevated amyloid burden observed in those

individuals with the highest levels of *APBB2* expression is attenuated in those who also show the highest levels of *GSK3 β* . Given the role the protein product of *APBB2* plays in *APP* processing (McLoughlin and Miller 2008), it is not surprising that we observed a strong main effect of *APBB2* expression levels on amyloid burden. However, the results are important in that they provide additional information about the context in which the beneficial effects of *GSK3 β* are observed. Previously, we had speculated based on the genotype results that *GSK3 β* may be beneficial in the presence of low levels of *APBB2*, which would lead to a reduced effect of the amyloid intracellular domain (AICD) in driving both *APP* expression and *GSK3* expression (Ryan and Pimplikar 2005). Yet, the present results seem to indicate that it is in the context of high levels of *APBB2* expression that *GSK3 β* appears to be most beneficial. This makes some intuitive sense given that the AICD only drives *GSK3* expression when the FE65 protein is present, which suggests that the added expression of *GSK3* may be precisely what results in a beneficial effect.

Given that higher levels of *APBB2* are associated with higher levels of amyloid at autopsy, and that higher levels of *GSK3 β* are associated with lower levels of amyloid in the presence of high *APBB2*, it is not surprising that participants with AD showed higher levels of *APBB2* and lower levels of *GSK3 β* when compared to normal controls (Table 1). Interestingly, similar to what we observed in the SNP analyses, the protective effect of *GSK3 β* appears across diagnostic categories (Supplementary Fig. 1), suggesting that the high levels of *GSK3 β* in the presence of *APBB2* may promote reduced levels of amyloid regardless of disease stage. In the case of AD, perhaps the small reductions in amyloid related to *GSK3 β* are insufficient to stave off the disease process entirely, particularly when additional risk factors for amyloid deposition are present.

One limitation of the current study is that both of the observed interaction effects are quite modest. One possible explanation is that our power was somewhat restricted because we were not able to explore genetic interaction effects in the most relevant manner: stratified by diagnostic status. It should also be noted that the expression levels of *GSK3 β* and *APBB2* in the prefrontal cortex were considerably lower than expression of other genes. This leaves open the possibility that stronger expression interaction effects may be observed in regions like the hippocampus, particularly given recent evidence that beneficial effects of *GSK3 β* blockers are driven by reductions in hippocampal *GSK3 β* expression and not cortical expression in adult rats (Mendes et al. 2009). Moreover, we were unable to specifically address the direct relationship between gene expression and amyloid deposition within each region sampled to quantify amyloid, leaving open the possibility that gene-amyloid effects may differ across regions. From our results, we can only conclude that *GSK3 β* and *APBB2* in the prefrontal cortex interact on a global level of amyloid in the brain. Future work evaluating the association between these genes and AD pathology across relevant brain regions may clarify whether the observed effect has some regional specificity, and whether the strength of the effect is highest in brain regions implicated in the earliest disease-related changes.

This manuscript also has multiple strengths. The validation of a previously identified SNP provides support of the interaction effect, and more importantly demonstrates that the observed effect carries over from a biomarker of amyloid deposition to measures of amyloid

at autopsy. The availability of gene expression data further strengthens our finding by providing some hints at mechanism and clarifying future directions to better understand the role of *GSK3 β* in the pathophysiological cascade in AD. Future work should focus on how the role of *GSK3 β* on disease risk and resilience changes across the course of the dementia spectrum.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the study participants and staff of the Rush Alzheimer's Disease Center. This research was supported in part by the Pharmaceutical Research and Manufacturers of America Foundation Fellowship in Translational Medicine and Therapeutics, by the Alzheimer's Association IIRG-08-88733, K24AG046373, K12HD043483, R01AG034962, R01HL111516, P30AG10161, R01AG15819, R01AG17917, U01AG46152, and the Vanderbilt Memory & Alzheimer's Center. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

- Bennett D, Wilson R, Schneider J, Evans D, Beckett LA, Aggarwal N, Bach J. Natural history of mild cognitive impairment in older persons. *Neurology*. 2002; 59(2):198–205. [PubMed: 12136057]
- Bennett DA, Schneider JA, Wilson RS, Bienias JL, Arnold SE. Neurofibrillary tangles mediate the association of amyloid load with clinical Alzheimer disease and level of cognitive function. *Arch Neurol*. 2004; 61(3):378. [PubMed: 15023815]
- Bennett DA, Schneider JA, Aggarwal NT, Arvanitakis Z, Shah RC, Kelly JF, Treinkman AD, et al. Decision rules guiding the clinical diagnosis of Alzheimer's disease in two community-based cohort studies compared to standard practice in a clinic-based cohort study. *Neuroepidemiology*. 2006a; 23:169–176. [PubMed: 17035694]
- Bennett DA, Schneider JA, Tang Y, Arnold SE, Wilson RS. The effect of social networks on the relation between Alzheimer's disease pathology and level of cognitive function in old people: a longitudinal cohort study. *Lancet Neurol*. 2006b; 5(5):406–412. doi:10.1016/S1474-4422(06)70417-3. [PubMed: 16632311]
- Bennett DA, Schneider JA, Arvanitakis Z, Wilson RS. Overview and findings from the religious orders study. *Curr Alzheimer Res*. 2012a; 9(6):628. [PubMed: 22471860]
- Bennett DA, Schneider JA, Buchman AS, Barnes LL, Boyle PA, Wilson RS. Overview and findings from the Rush Memory and Aging Project. *Curr Alzheimer Res*. 2012b; 9(6):646. [PubMed: 22471867]
- Bennett DA, Wilson RS, Boyle PA, Buchman AS, Schneider JA. Relation of neuropathology to cognition in persons without cognitive impairment. *Ann Neurol*. 2012c; 72(4):599–609. doi: 10.1002/ana.23654. [PubMed: 23109154]
- del Ser T, Lovestone S, Boada-Rovira M, Dubois B, Hüll M, Rinne J, León T. A phase II randomized, double-blind, parallel group, 26-week study of GSK-3 inhibitor tideglusib in Alzheimer's disease (ARGO trial). *Alzheimer's Dement*. 2013a; 9(4):P689–P690.
- del Ser T, Steinwachs KC, Gertz HJ, Andrés MV, Gómez-Carrillo B, Medina M, León T. Treatment of Alzheimer's disease with the GSK-3 inhibitor tideglusib: a pilot study. *J Alzheimers Dis*. 2013b; 33(1):205–215. [PubMed: 22936007]
- Hernandez F, Lucas JJ, Avila J. GSK3 and tau: two convergence points in Alzheimer's disease. *J Alzheimers Dis*. 2013; 33:S141–S144. [PubMed: 22710914]
- Hohman TJ, Koran ME, Thornton-Wells TA. Interactions between GSK3B and amyloid genes explain variance in amyloid burden. *Neurobiol Aging*. 2014; 35(3):460–465. [PubMed: 24112793]

- Hooper C, Killick R, Lovestone S. The GSK3 hypothesis of Alzheimer's disease. *J Neurochem*. 2008; 104(6):1433–1439. [PubMed: 18088381]
- Ishiguro K, Shiratsuchi A, Sato S, Omori A, Arioka M, Kobayashi S, Imahori K. Glycogen synthase kinase 3 beta is identical to tau protein kinase I generating several epitopes of paired helical filaments. *FEBS Lett*. 1993; 325(3):167–172. [PubMed: 7686508]
- Lim AS, Srivastava GP, Yu L, Chibnik LB, Xu J, Buchman AS, De Jager PL. 24-hour rhythms of DNA methylation and their relation with rhythms of RNA expression in the human dorsolateral prefrontal cortex. *PLoS Genet*. 2014; 10(11):e1004792. [PubMed: 25375876]
- Lucas JJ, Hernandez F, Gomez-Ramos P, Moran MA, Hen R, Avila J. Decreased nuclear [beta]-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3[beta] conditional transgenic mice. *EMBO J*. 2001; 20(1/2):27–39. [PubMed: 11226152]
- Martin L, Latypova X, Wilson CM, Magnaudeix A, Perrin ML, Yardin C, Terro F. Tau protein kinases: involvement in Alzheimer's disease. *Ageing Res Rev*. 2013; 12(1):289–309. [PubMed: 22742992]
- McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease Report of the NINCDS-ADRDA Work Group* under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984; 34(7):939. [PubMed: 6610841]
- McLoughlin DM, Miller CCJ. The FE65 proteins and Alzheimer's disease. *J Neurosci Res*. 2008; 86(4):744–754. [PubMed: 17828772]
- Mendes CT, Mury FB, de Sá Moreira E, Alberto FL, Forlenza OV, Dias-Neto E, Gattaz WF. Lithium reduces Gsk3b mRNA levels: implications for Alzheimer Disease. *Eur Arch Psychiatry Clin Neurosci*. 2009; 259(1):16–22. [PubMed: 18932008]
- Mitchell TW, Nissanov J, Han LY, Mufson EJ, Schneider JA, Cochran EJ, Arnold SE. Novel method to quantify neuropil threads in brains from elders with or without cognitive impairment. *J Histochem Cytochem*. 2000; 48(12):1627–1638. [PubMed: 11101631]
- Phiel CJ, Wilson CA, Lee VMY, Klein PS. GSK-3[alpha] regulates production of Alzheimer's disease amyloid-[beta] peptides. *Nature*. 2003; 423(6938):435–439. [PubMed: 12761548]
- Ryan KA, Pimplikar SW. Activation of GSK-3 and phosphorylation of CRMP2 in transgenic mice expressing APP intracellular domain. *J Cell Biol*. 2005; 171(2):327–335. [PubMed: 16230462]
- Shulman JM, Chen K, Keenan BT, Chibnik LB, Fleisher A, Thiyyagura P, Corneveaux JJ. Genetic susceptibility for Alzheimer disease neuritic plaque pathology. *JAMA Neurol*. 2013; 70(9):1150–1157. [PubMed: 23836404]
- Small SA, Duff K. Linking Aβeta and tau in late-onset Alzheimer's disease: a dual pathway hypothesis. *Neuron*. 2008; 60(4):534–542. [PubMed: 19038212]

Fig. 1.

SNP-SNP replication result and previous finding. The original ADNI finding is presented in (a). The ROS/MAP replication is presented in (b). APBB2 (rs2585590) is along the *x*-axis and the lines are grouped by the GSK3β (rs334543) genotype. In **a**, the *y*-axis is mean amyloid burden calculated using PET imaging. In **b**, the *y*-axis is mean amyloid burden calculated at autopsy. *Error bars* represent the standard error of the mean. The rs334543 C/C genotype is associated with lower levels of amyloid burden among carriers of the rs2585590 A/A genotype (*red line, left column in both panels*) (Color figure online)

Fig. 2.

Gene-gene interaction using gene expression. Mean amyloid burden is along the y-axis. APBB2 expression tertiles are along the x-axis, and lines are grouped by GSK3 β expression tertiles. Gene expression was quantified in the prefrontal cortex. Error bars represent the standard error of the mean. The highest tertile of GSK3 β expression (red line) is associated with lower levels of amyloid deposition among those in the highest tertile of APBB2 expression (right column) (Color figure online)

Table 1

Sample characteristics

Genotype dataset	Baseline clinical diagnosis			Overall sample	<i>p</i> value
	Normal control	Mild cognitive impairment	Alzheimer's disease		
Sample size, <i>n</i>	251	196	350	797	n/a
APOE ε4 carriers (%)	16 %	24 %	36 %	27 %	<0.001[#]
Females (%)	62 %	61 %	67 %	64 %	0.349 [#]
Age at death (years)	86 ± 6	88 ± 6	89 ± 6	88 ± 6	<0.001
Education (years)	16 ± 4	16 ± 4	16 ± 4	16 ± 4	0.993
Amyloid burden, mean	2.75 ± 3.31	3.86 ± 4.36	5.27 ± 4.43	4.13 ± 4.23	<0.001
Gene expression dataset					
Sample size, <i>n</i>	143	114	179	436	n/a
APOE ε4 carriers (%)	18 %	24 %	36 %	27 %	0.001[#]
Females (%)	59 %	63 %	67 %	63 %	0.429 [#]
Age at death (years)	86 ± 6	89 ± 6	90 ± 6	89 ± 6	<0.001
Education (years)	16 ± 3	16 ± 4	16 ± 4	16 ± 4	0.829
GSK3β expression	6.79 ± 1.64	6.38 ± 1.69	6.23 ± 1.45	6.46 ± 1.59	0.006
APBB2 expression	6.43 ± 1.70	6.63 ± 1.64	6.90 ± 1.80	6.68 ± 1.74	0.049
Amyloid burden, mean	2.50 ± 3.07	3.08 ± 3.84	4.93 ± 3.86	3.65 ± 3.77	<0.001

[#] Kruskal–Wallis Test with 2 degrees of freedom

Table 2Results of $GSK3\beta \times APBB2$ interaction analyses on amyloid deposition

	Linear regression results			
	β	<i>N</i>	<i>t</i> stat	<i>p</i> value
SNP main effects				
<i>GSK3β</i> (rs334543)	0.01	797	0.19	0.850
<i>APBB2</i> (rs2585590)	0.01	799	0.23	0.816
<i>APP</i> (rs457581)	0.08	797	1.21	0.226
SNP–SNP interactions				
<i>GSK3β</i> (rs334543) $\times$ <i>APBB2</i> (rs2585590)	0.18	797	2.03	0.043
<i>GSK3β</i> (rs334534) $\times$ <i>APP</i> (rs457581)	−0.09	797	−0.97	0.329
Gene expression main effects				
<i>GSK3β</i>	−0.04	436	−1.28	0.200
<i>APBB2</i>	0.08	436	3.06	0.002
Gene–gene expression interactions				
<i>GSK3β</i> $\times$ <i>APBB2</i>	−0.017	436	−1.04	0.301
<i>GSK3β</i> (3rd tertile) $\times$ <i>APBB2</i> (3rd tertile)	−0.62	436	−2.03	0.043

Boldface signifies effects that are significant at $p < 0.05$